Print ISSN 0391-4097
Online ISSN 1720-8386

JOURNAL OF ENDOCRINOLOGICAL INVESTIGATION

Vol. 36, Suppl. to No. 5, 2013

XXXVI NATIONAL CONGRESS OF THE ITALIAN SOCIETY OF ENDOCRINOLOGY

Padova, PadovaFiere
June 5-8, 2013

On-line submission
http://mc.manuscriptcentral.com/jei
Electronic versions of articles on www.jendocrinolinvest.it

JOURNAL OF ENDOCRINOLOGICAL INVESTIGATION

Official Journal of
Italian Society of Endocrinology

Affiliated Societies

Italian Society of Andrology and Sexual Medicine
Italian Society of Pediatric Endocrinology and Diabetology
Italian Association of Clinical Endocrinologists
Italian Association of Thyroidology

BUSINESS MATTERS

Publication data
The **Journal of Endocrinological Investigation** is published monthly for the Italian Society of Endocrinology by *Editrice Kurtis s.r.l.* Supplements are published occasionally.

Subscriptions
One-year personal subscription rates
Print+Online: - 11 issues from subscription: € 210.00*
- 11 issues from subscription + online archives from 2002: € 270.00*
Online: - 11 issues from subscription: € 150.00
- 11 issues from subscription + online archives from 2002: € 210.00
One-year subscription rates for institutions
Print+Online: 11 issues from subscription + online archives from 2002: € 660.00*
Online: 11 issues from subscription + online archives from 2002: € 450.00
*Shipping charges not included in the subscription price: Italy: € 37.00; European Union Countries: € 61.00; Extra-European Union Countries: € 72.00.
Back issues
Personal use: € 30.00• per issue
Institutions: € 70.00• per issue
•Shipping charges not included in the price: regular mail: Italy: € 7.00, European Union Countries: € 15.00; Extra-European Union Countries: € 18.00; express courier: please contact Editrice Kurtis. Supplements are not included in the subscription.
Changes of address - Allow 60 days for all changes to become effective. All communications should include both old and new addresses (with postal codes). Claims are accepted and journals replaced on condition that subscription department is notified of nonreceipt within 3 months of issue date. All business matters, including correspondence and remittances relating to subscriptions and advertising should be sent to: subscriptions@kurtis.it

EDITRICE KURTIS s.r.l. - Via Arluno, 4 - 20010 Casorezzo (MI), Italy
Tel. +39 02.48202740 - Fax +39 02.48201219 - Internet: http://www.kurtis.it

President/Publisher
Canzio Fusé (Milano)

Managing Director
Alessia Fusé (Milano)

Privacy Policy
Manual and electronic handling and processing of personal data necessary for the shipping of this journal and other medical-scientific material is managed in compliance with Italian law 196/03, Art. 13. The data processing is also compliant with Italian law 196/03, Art. 11. The Publisher may disclose the personal data to business partners involved in the shipping of the copy of this journal. The data processing rights are held by Editrice Kurtis, via Arluno 4, 20010 Casorezzo (MI), Italy, to whom the readers may request at any time to update, integrate, cancel their data or perform any other operation foreseen by Italian law 196/03, Art. 7.

A norma dell'art. 74 lettera c del DPR 26 Ottobre 1972 e del DM 28 Dicembre 1972, il pagamento dell'IVA, assolto dall'Editore, è compreso nel prezzo dell'abbonamento o dei fascicoli separati. Non verrà rilasciata fattura se non su specifica richiesta.

Pubblicazione mensile edita a cura di Editrice Kurtis s.r.l. - Sede Legale, Amministrativa, Redazione e Stampa: Via Arluno, 4 - 20010 Casorezzo (MI) - Italy - Tel. +39 02.48202740 - Fax +39 02.48201219 - e-mail: jei@kurtis.it - Sede Commerciale: Via Luigi Zoja, 30 - 20153 Milano - Tel. +39 02.48202740 - Fax +39 02.48201219 - e-mail: info@kurtis.it - Direttore Responsabile: Canzio Fusé - Autorizzazione n. 370 del Tribunale di Milano del 11.11.77 - ROC (Registro Operatori Comunicazione) no. 6133.

Finito di stampare nel mese di maggio 2013

Associato all'USPI
Unione Stampa Periodica Italiana

A.N.E.S. Associato all'ANES
Associazione Nazionale Editoria Specializzata

CONFINDUSTRIA

The **Journal of Endocrinological Investigation** publishes original studies on clinical and experimental research in endocrinology and related fields. Details on the types of manuscripts that will be considered for publication are given in the Instructions to Authors. Full version of the Instructions to Authors is available at www.jendocrinolinvest.it

All editorial correspondence should be addressed to the Editorial Office:

Editor-in-Chief
Luigi Bartalena MD
Endocrinology Unit
Ospedale di Circolo
Viale Borri, 17
21100 Varese, Italy

Editorial Office
Elisabetta Bersezio
JEI Editorial Office
c/o Department of Internal Medicine
C.so Dogliotti, 14
10126 Turin, Italy

Tel. +39 011.6334317
Fax +39 011.6647421
E-mail: elisabetta.bersezio@unito.it

The **Journal of Endocrinological Investigation** is published monthly by Editrice Kurtis s.r.l., and is cited in Current Contents/Index Medicus, Excerpta Medica, Biosis, Chemical Abstracts, SIIC (Sociedad Iberoamericana de Información Cientifica).

Journal recognized as having "high cultural value"
by the Ministry of Cultural Heritage

Impact Factor: 1.566

XXXVI NATIONAL CONGRESS OF THE ITALIAN SOCIETY OF ENDOCRINOLOGY

Padova, PadovaFiere
June 5-8, 2013

ORGANIZING SECRETARIAT

FASI S.r.l. - Via R. Venuti, 73 - 00162 Roma
Tel. 0697605610 - Fax 0697605650 - info@fasiweb.com - www.fasiweb.com

OVERVIEW OF THE MEETING PROGRAM

Wednesday June 5

13.00 - 15.30	Parallel Symposia
15.30 - 15.45	Opening Ceremony
15.45 - 17.00	SIE-ESE Symposium
17.00 - 17.45	Symposium
17.45 - 18.30	Plenary Lecture

Thursday June 6

08.15 - 10.15	Parallel Symposia
10.15 - 12.00	Parallel Oral Top Sessions
12.00 - 13.00	Parallel Clinical Cases and Meet the Expert
13.00 - 15.30	Parallel Symposia
15.30 - 16.30	Plenary Lecture
16.30 - 18.05	Pharmaceutical Industries Symposia
18.05 - 19.30	Members Assembly

Friday June 7

08.15 - 10.15	Parallel Symposia
10.15 - 12.00	Parallel Oral Sessions
12.00 - 13.00	Parallel Clinical Cases and Meet the Expert
13.15 - 14.45	Pharmaceutical Industries Symposia
14.45 - 16.00	Round Table
16.00 - 17.45	SIE Award Lecture
17.45 - 19.30	Parallel Symposia

Saturday June 8

08.15 - 10.00	Parallel Oral Sessions
10.00 - 11.00	Lecture
11.00 - 12.45	Parallel Symposia
12.45 - 13.00	Closing Ceremony

JOURNAL OF ENDOCRINOLOGICAL INVESTIGATION

Volume 36 Supplement to no. 5 2013

XXXVI NATIONAL CONGRESS OF THE ITALIAN SOCIETY OF ENDOCRINOLOGY

Padova, PadovaFiere
June 5-8, 2013

CONTENTS

TOP ORAL SESSION

The abstract number is the same reported in the program.

OT01

RISK-STRATIFIED EVALUATION OF OVER 16,000 THYROID MICROCARCINOMAS: AT PRESENTATION MORE THAN ONE THIRD HAVE TWO OR MORE RISK FACTORS FOR RECURRENCE

P. Malandrino[1], G. Pellegriti[1], M. Russo[1], I. Marturano[1], G. Sapuppo[1], E. Messina[1], C. Scollo[1], C. Regalbuto[1], R. Vigneri[1]

[1]Endocrinology, Dept. of Clinical and Molecular Biomed., Univ. of Catania - Catania

Introd.: Most of papillary thyroid microcarcinomas (PTMC, ≤10mm size) are very low risk cancers. A certain number of PTMCs, however, may cause persistent/recurrent disease and even distant metastases and death. Risk factors at presentation have been established to identify PTMCs that may have unfavorable outcome and require more aggressive treatment and follow-up. Aim: To analyse the prevalence of host (age, gender) and tumor (size, multifocality, extrathyroid extension and nodal metastases) risk factors in PTMC at presentation. Patients & Methods: Retrospective study of all PTMCs incident cases recorded in a five-year period in the Sicilian Regional Registry for the Thyroid Cancer (SRRTC, 2002-2006) and the Surveillance and Epidemiology End Results (SEER, 2004-2008). Age at diagnosis (≤45 vs. >45), gender, tumor size (1-6 vs. 7-10 mm), multifocality, extrathyroid extension and nodal metastases were evaluated. Results: PTMC incidence was much higher in Sicily (1,777 PTMCs, ASRw =5.8/10^5) than in the US (14,423 PTMC, ASRw =2.9; p<0.01). Within Sicily, the incidence was much higher in the Catania province (volcanic area): ASRw =10.4 vs. 4.6 in the rest of Sicily (p<0.01). In both registries, a significant inverse correlation was observed between age and tumor size (r=-0.86; p<0.0001). Among the 6,093 cases who, in both cohorts underwent nodal excision, at multivariate analysis, the following factors were independently associated to the presence of nodal metastases: age ≤45 (O.R.=1.6; 95% C.I.=1.4-1.8); male gender (O.R.=3.3; 95% C.I.=2.8-3.8); tumor size 7-10 mm (O.R.=1.6; 95% C.I.=1.4-1.8); multifocality (O.R.=2.1; 95% C.I.=1.8-2.3); extrathyroid extension (O.R.=4.4; 95% C.I.=3.7-5.3). The presence of 2 or more than 2 of the above mentioned risk factors occurred in 648 (36.5%) and 5,803 (40.2%) patients in the SRRTC and SEER cohorts, respectively. Patients having both age ≤45 y and tumor size 7-10 mm were at higher risk for extrathyroid extension (O.R.=1.8; 95% C.I.=1.6-2.1), multifocality (O.R.=1.5; 95% C.I.=1.3-1.6) and nodal metastases (O.R.=1.9; 95% C.I.=1.6-2.1). Concl.: Conservative therapeutic strategies are appropriate for most patients with PTMC. Nearly 40% of them, however, have two or more risk factors for unfavorable outcome. At preoperative evaluation, size 7-10 mm, young age (≤45 y) and male gender should suggest PTMC treatment like that of thyroid macrocarcinomas, in accordance to the current guidelines.

OT02

EPIDEMIOLOGICAL FEATURES OF CUSHING'S SYNDROME FROM COMPLICATION TO MORTALITY: NOVEL INSIGHT FROM MISSION STUDY ON NEARLY 5000 PATIENTS

P. Vitale[1], A. Cozzolino[1], C. Simeoli[1], M. De Leo[1], D. Iacuaniello[1], G. Trifirò[2], A. Colao[1], R. Pivonello[1]

[1]Clinical Medicine and Surgery - Section of Endocrinology, University Federico II - Napoli, [2]Clinical and Experimental Medicine and Pharmacology, University of Messina - Messina

Background: MISSION study is a retrospective observational study assessing the epidemiological characteristics and the mortality rate in a very large population of patients with all type of CS.

Aim: The objective of the present study consists in the calculation of the risk factors and complications related mortality rate and survival curve of the patients included in MISSION Study.

Patients and method: 4956 worldwide patients entered the study, 1029 males (20,7%) and 3927 females (79,3%). There was the absolute etiologic prevalence of pituitary adenoma (68,2%) distantly followed by adrenal disease (22,3%) and by ectopic disease (3,1%). Occult and unknown origins amounted to 6,1%. Moreover, 21,3% of patients showed active disease, 31,6% were in short term remission, 40,1% were cured while 6,7% were under pharmacological control.

Results: The cumulative crude mortality rate was 8,89 per 1000 person-years; all the crude mortality rate ratio and survival curve trends (3,14 - p= 0,000 for hypertension, 2,72 - p= 0,000 for alteration of glucose metabolism, 1,34 - p= 0,01 for dyslipidemia, 2.17 - p= 0,000 for coagulopathy, 3,45 - p= 0,000 for cardiopathy, 2,07 - p= 0,000 for brain diseases, 1,57 - p= 0,000 for psychiatric diseases and 4,02 - p= 0,000 for infections) revealed an higher rate of death for patients presenting with the analyzed risk factors or complications. Moreover, the association of different risk factors or complications seemed to increase the mortality rate and worsen the survival curve compared with each one alone (p=000 for hypertension and alteration of glucose metabolism). Furthermore, patients with pituitary adenoma and with active disease had a worse survival curve compared with patients with adrenal adenoma (p= 0,02) and with other disease status (p= 0,000).

Conclusion: This preliminary data analysis underlines that mortality is higher in patients presenting all types of complications, and that the combination of some of them has an enhanced effect on mortality. Moreover, the pituitary origin (compared to adrenal adenoma) and active disease seems to give an additional death risk.

OT03

COMPARISON BETWEEN EFFECTIVENESS OF INOSITOL/ALPHA LIPOIC ACID AND METFORMIN IN IMPROVING METABOLIC PARAMETERS IN WOMEN WITH POLYCITIC OVARY SYNDROME

L. Granieri[1], R. S. Auriemma[1], A. Cozzolino[1], L. F. S. Grasso[1], M. Galdiero[1], P. Vitale[1], F. Lo Calzo[1], C. Simeoli[1], Y. Perone[1], F. Orio[2], A. Colao[1], R. Pivonello[1]

[1]Dip. di Medicina Clinica e Chirurgia, Sezione di Endocrinologia, Università "Federico II" - Napoli, [2]Dip. di Endocrinologia, Università "S.Giovanni di Dio e Ruggi d'Aragona" - Salerno

Background: Given the role of the impairment of insulin sensitivity in the pathogenesis of Polycystic Ovary Syndrome (PCOS), insulin-sensitizing compounds, such as metformin, have been proposed to solve the hyperinsulinemia-induced dysfunctions. Furthermore, some abnormal actions of insulin might be dependent from a deficiency of inositol phosphoglican mediators so that the administration of inositol also could improve metabolic profile of these patients (pt). The aim of the study was to compare the effects of treatment with inositol-apha lipoic acid and metformin on metabolic parameters in PCOS pt.

Patients and Methods: Fifty-two PCOS pt (aged 24.67±8.35) entered the study. Treatment with metfomin (500-2000 mg, 26 patients; aged 22.6±9.8) or inositol with alpha lipoic acid (Inosidex® 2gr, 26 patients; aged 25.9 ±6.5) was randomly assigned. All pt were daily administered for 6 months. At baseline and after therapy, hormonal (LH, FSH, androstenedion, progesterone, estradiol, total testosterone) and metabolic parameters (total and HDL- cholesterol (C), triglycerides (Tg), fasting glucose (G) and insulin (I), 120-min G and I after glucose load, homa-IR, homa beta, Quicki, G/I and BMI) were evaluated.

Results: Compared to baseline, inositol-alpha lipoic acid treatment induced a significant decrease in homa-IR (p=0.007), I (p=0.002), G (p=0.006), G/I (p=0.03) and Quicki (p=0.015), as well as in body weight (p=0.002) and BMI (p=0.005) after 6 months. Similarly, metformin induced a significant decrease in homa-IR (p=0.000), homa beta (p=0,000), I (p=0,003), G (p=0.001), Quicki (p=0,001), 120-min G (p=0.005) and I (p=0.005), also improving lipid profile: total-C (p=0,005), HDL-C (p=0.033) and Tg (p=0.008), with no significant impact on body weight and BMI after 6 months. In pt treated with inositol-alpha lipoic acid, 120-min I was significantly lower (p=0.005) than in those receiving metformin.

Conclusion: In PCOS pt, inositol appears as effective as metformin in improving metabolic parameters and insulin sensitivity and even more effective than metformin in reducing post-prandial insulin levels.

.

OT04

MORTALITY IN PATIENTS WITH ADRENAL INCIDENTALOMAS ASSOCIATED WITH PROGRESSIVELY INCREASED PATTERNS OF SUBCLINICAL CORTISOL HYPERSECRETION

G. Di Dalmazi[1], V. Vicennati[1], S. Garelli[1], E. Casadio[1], E. Rinaldi[1], A. Paccapelo[1], U. Pagotto[1], R. Pasquali[1]

[1]Scienze mediche e chirurgiche - Università Alma Mater - Bologna

Background. Subclinical Cushing's syndrome (SCS) is defined as alterations in hypothalamic-pituitary-adrenal axis without classic signs or symptoms of glucocorticoid excess. This condition has been associated to increased risk of adverse metabolic and cardiovascular outcomes, independently of other potential risk factors. However, is still not known if this condition could lead to a higher mortality respect to non-secreting adrenal masses (NSA).

Aim. To evaluate the overall mortality of long-term follow-up patients with NSA, intermediate phenotypes of subclinical hypercortisolism (IP), and SCS.

Methods. For this study were enrolled 222 subjects. None of the patients underwent to surgical treatment of the adrenal mass. The 1-mg dexamethasone suppression test (DST) was used as primary diagnostic tool, and patients were classified as below: 155 were defined NSA and 7 SCS, using the most stringent cut-off values (<50 nmol/L and >138 nmol/L, respectively). The 60 patients with cortisol post-DST between 50 and 138 nmol/L were defined IP. Mean duration of follow-up was 6,8 years. Patients were classified at the last follow-up using the same diagnostic criteria. Patients were then divided into 2 groups: "stable NSA" and "stable subclinical hypercortisolism (SH)" if they did not change the secreting pattern during the follow-up (NSA and IP/SCS, respectively).

Results. Patients age was different in the 2 groups (P=0,004). Mortality analysis was then performed by Cox regression, using age as covariate. The overall unadjusted hazard ratio was 3.31 in stable SH (P<0.001) respect to stable NSA. The hazard ratio adjusted for age was 2.99 in stable SH (P=0.001) when compared to stable NSA.

Conclusion. Increasing patterns of subclinical hypercortisolism are associated with increased overall mortality, independently of the effect of age. We are increasing the study population and we are performing further analysis on causes of mortality of these patients.

OT05

AUTOIMMUNE POLYGLANDULAR SYNDROME TYPE 1 IN ITALY: A SURVEY ON 114 PATIENTS*

S. Garelli[1], S. Masiero[1], S. Barollo[1], A. Fierabracci[2], A. Crinò[2], R. Perniola[3], F. De Luca[4], G. Weber[4], G. Picca[7], G. Radetti[4], S. Chen[8], J. Furmaniak[8], B. Rees Smith[8], C. Betterle[1]

[1]Dipt. di Medicina - Padova, [2]O. Bambin Gesù - Roma, [3]O.C. Fazzi - Lecce, [4]U.O. Pediatria - Bolzano, [5]U.O. Pediatria - Messina, [6]U.O. Pediatria - Milano, [7]U.O. Endocrinologia - Foggia, [8]FIRS Lab. - Cardiff, UK

Objectives: Autoimmune Polyglandular Syndrome type 1 (APS-1) is a rare recessive inherited disease, caused by the mutation of AIRE gene on chromosome 21. Various groups of patients have been published worldwide, describing overall 450 patients, with more than 80 different mutations. APS-1 is classically characterized by the presence of two out of three main diseases: chronic mucocutaneous candidiasis (CC), chronic hypoparathyroidism (CH) and Addison's disease (AD); most of the patients have also other endocrine and non-endocrine autoimmune manifestations.

Patients: we studied a group of 114 patients, coming from different Italian Regions, analyzing clinical diseases, organ- and non organ-specific autoantibodies (to interferon omega (IFNAbs), tryptophan hydroxylase, L-aminoacid decarboxylase, adrenal cortex, 21-hydroxylase, steroid-producing cells, 17-alfa-hydroxylase, side-chain cleavage, parietal cells, intrinsic factor, islet cell, glutamic-acid decarboxylase, melanocytes) and AIRE gene mutations.

Results: 81 were females and 33 males (F/M ratio 2.5); 31 had at least one sibling affected (15 families). The mean actual age was 32 years (range 3-84), with a mean follow-up of 14.5 years. IFNAbs were positive in 92% of patients, confirming to be the best marker of APS-1. CC was present in 76%, CH in 90% and AD in 81% of patients with a mean age at onset of 9, 9 and 15 years, respectively. As regards other autoimmune diseases, 35% were affected by alopecia, 33% by thyroid diseases, 29% by chronic gastritis, 25% by vitiligo, 22% by hepatitis and 47% of females by POF. Two AIRE mutations were found in 85% of cases, only one mutation in 5% and no mutations in 10%. There was a great variability of mutations, but in many cases they were peculiar of the geographical origin. The most frequent mutation was R257X followed by W78R, R139X, R203X and del13. We registered 12 deaths at a mean age of 33 years (range 12-61).

Conclusions: this study describes the largest group of patients with APS-1 in a Nation.

*The study was funded from the EU 7th Framework Programme,Grant No. 2008-201167.

OT06

METFORMIN INHIBITS ANDROGEN-DEPENDENT INSULIN-LIKE GROWTH FACTOR-I RECEPTOR UP-REGULATION IN PROSTATE CANCER CELLS

R. MALAGUARNERA[1], A. SACCO[1], C. VOCI[1], R. VIGNERI[2], A. BELFIORE[1]

[1]Depatment of Health Science - Catanzaro, [2]Department of Clinical and Molecular Biomedicine - Catania

Introduction: Insulin-like growth factor-I receptor (IGF-IR) plays an important role in prostate cancer progression. We have shown that, in prostate cancer cells, androgens upregulate IGF-IR by inducing membrane initiated events involving AMP-response element-binding protein (CREB) activation and CREB-dependent IGF-IR gene transcription. Anti-androgen therapy is ineffective in modifying these events. Metformin exerts pleiotropic anti-tumoral effects in several cancer models.

Aim: We aimed at evaluating whether metformin may affect androgen-dependent IGF-IR upregulation.

Methods and results: in androgen receptor (AR) positive prostate cancer cells (LNCaP), we found that metformin specifically inhibits androgen-dependent activation of CRE activity by inducing the nuclear exclusion and degradation of the CREB cofactor TORC2. This event disrupts the integrity of the CREB-CBP-TORC2 complex and inhibits CRE activity. Accordingly, TORC2 specific silencing, partially counteracted androgen mediated IGF-IR overexpression, as well as metformin actions. Metformin also blocked androgen-induced mTOR/p70S6K activation by inhibiting AR phosphorylation and its association with Src. This effect also contributes to block IGF-IR upregulation in response to androgens.

Conclusion: In prostate cancer cells metformin abrogates membrane initiated androgen effects responsible for IGF-IR upregulation through different mechanisms: a) by inhibiting CREB-CBP-TORC2 complex transcriptional activity; b) by disrupting AR/Src association and inhibiting mTOR/p70S6K pathway. Thus, metformin could represent a novel promising approach to prostate cancer therapy.

OT07

FUNCTIONAL CHARACTERIZATION OF NOVEL TSH RECEPTOR PATHOGENETIC VARIANTS ASSOCIATED WITH NON-AUTOIMMUNE HYPERTHYROTROPINEMIA

G. Gelmini[1], M. Bonomi[1], F. Guizzardi[1], L. Persani[1]

[1]Scienze Cliniche e di Comunità - Milano

Non **autoimmune hyperthyrotropinemia** (NAHT) is an inheritable thyroid disorder associated with loss-of-function TSH receptor (TSHR) variants that determine the condition known as "TSH resistance". In this study we evaluated the presence of variants in TSHR gene in a cohort (n = 131) of pediatric patients with non-autoimmune hyperthyrotropinemia (NAHT). We identified 16 variants, 4 of which are never been described before (L57P, P162S, P668S, S745C), in approximately 12% of cases. The new missense variants have been functionally studied in vitro after transient transfection in COS-7 cells. The results obtained show that the variants L57P and P668L cause an almost complete disruption of both Gs and Gq mediated pathways through different molecular mechanisms, as the interaction with the two G protein is probably affected in the case of P668L whereas the membrane expression is severely compromised in L57P. The P162S variant is associated with a small reduction of the membrane expression , a dose-response curve, representative of the accumulation of intracellular cAMP post stimulus, characterized by reduced Emax and a reduced ability to stimulate the intracellular IP3 and calcium accumulations. The combination of these with previously published data shows that the P162 residue is often subject to loss-of-function variations and therefore represent a hot spot for receptor inactivation. Finally, the S745C change is the first variant affecting the intracellular tail of the receptor, a domain with so far unknown functional activity. This variant presents a membrane expression and a Gs-stimulating activity comparable to the WT receptor, but has a significant impact on the ability to stimulate the Gq-dependent pathway.

In conclusion, this study shows that the causative variants on TSHR gene are associated with a frequency of 12% in isolated pediatric NAHT. The results obtained show that disruption of the Gq mediated signal may frequently contribute to the pathogenesis of TSH resistance highlighting the relevance of this pathway for the activation of the enzymes involved in iodide organification. These results contribute to the understanding of the receptor domains involved in the pleiotropic interaction with the different G proteins.

OT08

THE FXR AGONIST OBETICHOLIC ACID NORMALIZES LIPID DROPLET HANDLING AND INSULIN SENSITIVITY IN PREADIPOCYTES FROM A RABBIT MODEL OF METS

E. Maneschi[1], L. Vignozzi[1], A. Morelli[1], T. Mello[1], S. Filippi[1], I. Cellai[1], P. Comeglio[1], E. Sarchielli[1], A. Calcagno[1], B. Mazzanti[1], R. Vettor[1], G. B. Vannelli[1], L. Adorini[1], M. Maggi[1]

[1]Experimental and Clinical Biomedical Sciences - Florence

Adipose tissue (AT) dysfunction is characterized by ectopic fat deposition in the abdominal viscera and liver, inflammatory and adipokine dysregulation, and insulin resistance and may be a more important mediator than total fat mass of type 2 diabetes, hypertension and dyslipidaemia development, all these features clustering in the metabolic syndrome (MetS). We recently demonstrated that the selective FXR agonist obeticholic acid (OCA, INT-747) ameliorates the metabolic profile and reduces visceral AT (VAT) in a high-fat diet (HFD)-induced rabbit model of MetS. We now report the effects of in vivo OCA dosing (10 mg/kg, daily, oral gavage, 12 weeks) on the adipogenic capacity of isolated VAT preadipocytes (rPAD) from MetS rabbits, as compared to regular diet. VAT and liver were studied by immunohistochemistry, Western blot, and RT-PCR. rPAD were exposed to a differentiating mixture (DIM) to evaluate adipogenesis. Adipocyte size, hypoxyprobe staining, expression of perilipin-1 (anti-lipolytic protein) and cytosolic GLUT-4 were all significantly increased in VAT of HFD and normalized by OCA treatment. TNFα expression, along with other steatosis (PPARγ and adiponectin) and inflammation (IL-6 and IL-10) markers, were also increased in HFD liver and normalized by OCA. rPAD from HFD-rabbits were less sensitive to DIM, and in particular to insulin, as demonstrated by decreased triglyceride synthesis and glucose uptake, impaired lipid droplets fusion, as well as by the reduced adipogenesis-specific genes. OCA treatment preserved all the DIM-induced adipocyte functions, normalizing the markedly increased lipid droplet size in HFD-derived adipocytes and the increased major lipid-fusion complex SNARE. In conclusion, OCA dosing in a MetS rabbit model ameliorates liver and VAT functions, increasing their efficiency in triglyceride and lipid droplet handling. This could reflect the ability of OCA to restore insulin sensitivity in committed adipose tissue unable to finalize its storage function, thus counteracting MetS-induced metabolic alterations and pathological fatty tissue deposition.

OT09

SIMULTANEOUS DETERMINATION OF SALIVARY TESTOSTERONE AND ANDROSTENEDIONE BY ISOTOPIC DILUTION LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY (LC-MS/MS)

M. Mezzullo[1], F. Fanelli[1], E. Dalla Benetta[1], A. Gambineri[1], V. Vicennati[1], R. Pasquali[1], U. Pagotto[1]

[1]Scienze Mediche e Chirurgiche-Università di Bologna - BOLOGNA

The assessment of hormones in saliva is emerging as new valuable tool for clinical and research purposes. Salivary levels are supposed to reflect the free fraction of circulating hormones, thus representing a more informative indicator of their activity. Moreover, saliva collection is not stressful and doesn't require medical assistance and allows multiple sampling in a day. LC-MS/MS technology offers high sensitivity and specificity to detect small concentration found in saliva, and allows high throughput multi-analyte quantitation. In this study we proposed a LC-MS/MS method for the simultaneous measurement of testosterone (T) and androstenedione (A). After addition of the internal standard d5-T, 0.3ml of saliva were purified with 3ml of hexane-ethyl acetate (8/2, v/v). The extracts were separated by an HPLC system (Serie200, PerkinElmer) on a C18 3.0x100mm, 3.5µm column. Analytes were revealed by electrospray ionization and multiple reactions monitoring by the 4000Q-Trap MS (AB-Sciex). Total runtime was 10.5min. Calibration curves linearity ranged between 1.95-2000pg/ml for T and 3.91-2000pg/ml for A. Limit of detection (LOD), the smallest analyte quantity yelding a signal-to-noise ratio (S/N) >3, was 439 and 880fg for T and A, respectively. Limit of quantitation (LOQ), the smallest concentration measurable with acceptable accuracy and imprecision yelding a S/N>10, was 1.95 and 3.91pg/ml, while sensitivity in saliva matrix was 7.8 and 15.6pg/ml for T and A. Intra-assay imprecision at the low (7.8pg/ml), medium (31.2pg/ml) and high (500pg/ml) level was 5.0, 7.2 and 2.5% for T and 10.0, 9.1 and 3.1% for A, while inter-assay imprecision was 12.2, 7.1 and 5.7% for T and 16.7, 15.1 and 14.9% for A. Accuracy at the low, medium and high level was 103.0, 94.2 and 92.6% for T and 98.3, 94.6 and 88.9% for A. Preliminary data on healthy subjects revealed early morning levels ranging between 20-50 and 60-110pg/ml, and levels <30 and <50pg/ml in the late afternoon for T and A, respectively. Our assay displayed the sensitivity, precision and rapidity needed for a proper measurement of low salivary T and A concentrations and for application over epidemiological studies or in routine settings. Moreover, the multi-analytical profile allows a more effective analysis of androgen status, and will be used to define the circadian rhythm in healthy subjects as well as in hypogonadic patients. This research was supported by the European Union grant (NEUROFAST FPVII-KBBE-2009-3-245009).

OT10

MINERALOCORTICOID RECEPTOR ANTAGONISM PROMOTES BROWNING OF ADIPOSE TISSUE AND PROTECTS FROM ADVERSE METABOLIC CONSEQUENCES OF DIET-INDUCED OBESITY

V. Marzolla[1], A. Armani[1], F. Cinti[2], A. Antelmi[1], G. Carpinelli[3], R. Canese[3], U. Pagotto[4], C. Quarta[4], G. Rosano[1], S. Cinti[2], M. J. Young[1], A. Fabbri[5], M. Caprio[1]

[1]IRCCS San Raffaele Pisana - Roma, [2]Università di Ancona - Ancona, [3]Istituto Superiore di Sanità - Roma, [4]Università di Bologna - Bologna, [5]Università di Roma Tor Vergata - Roma

It is known that mineralocorticoid receptor (MR) blockade has a potent antiadipogenic activity on murine and human preadipocytes. In this study the effects of MR antagonists were investigated in a model of diet-induced obesity. Female 10-week-old C57bl6 mice were fed with normal chow or a high fat (HF) diet for 12 weeks. Mice fed HF were concomitantly treated for 12 weeks with drospirenone (DRSP, 6 mg/Kg/day), a potent MR antagonist with progestative properties, or spironolactone (SPIRO, 20 mg/kg/day). Mice fed HF diet showed a significant increase in total body weight, fat mass, mean adipocyte size, expression of WAT markers and showed impaired glucose tolerance after intraperitoneal plasma glucose tolerance test. MR antagonism prevented weight gain and white fat mass expansion induced by HF in parametrial, perivescical, and inguinal depots without affecting interscapular fat pad weight. Magnetic Resonance Imaging confirmed that MR antagonists counteracted the HF-driven expansion of abdomino-pelvic fat volume. Importantly, both DRSP and SPIRO prevented the impaired glucose tolerance in mice fed HF, countered HF-induced up-regulation of WAT markers transcripts and reduced mean adipocyte size. Indeed, MR antagonists markedly increased UCP1-positive cells interspersed in WAT depots analysed, suggesting that MR antagonism promotes browning of adipose tissue. Accordingly, Magnetic Resonance Spectroscopy in vivo analysis performed in inguinal fat showed a significant increase in the percentage of water in mice treated with MR antagonists, reflecting an increase in number of brown-like adipocytes, known to be richer in water. In addition, PET/CT analysis showed increased glucose uptake in visceral abdomino-pelvic and interscapular fat in MR antagonists-treated mice, confirming a notable rise in activity of metabolically active brown fat. Finally, 2 days treatment of murine primary preadipocytes extracted from inguinal fat with MR antagonists up-regulated PRDM-16, a determinant of brown fat-like gene program, whereas 6 days treatment increased UCP-1 expression, suggesting a specific involvement of adipocyte MR in browning of adipose tissue observed in vivo.

OT11

PITUITARY TUMORS: MEN1 AND CDKN1B V109G POLYMORPHISM

G. Sciortino[1], G. Manfredi[1], G. Vitale[1], U. Verga[2], M. Filopanti[2], S. Massironi[2], A. G. Lania[3], F. Guizzardi[1], L. Persani[1]

[1]Dep. of Clinical Sciences and Community Health & Lab.of Endocrine and Metabolic Research Istituto Auxologico Italiano IRCCS University of Milan - Milano, [2]Fondazione IRCCS Ca' Granda Osp.Maggiore Policlinico - Milano, [3]Dep. Biometra IRCCS Istitituto Clinico Humanitas - Milano

Introduction: The prevalence of pituitary tumors in MEN1 ranges from 10 to 60%. Such tumors may occur as the first clinical manifestation of MEN1 in less than 10% of familial and in 25% of sporadic cases. In about 10-20% of patients with MEN1-like features MEN1 gene mutations were not identified. Germline mutations in the CDKN1B gene had been potentially associated with the development of a MEN1-like phenotype but the role of CDKN1B variations in MEN1-like patients is not well established. **Design**: We screened germline DNA from 114 patients affected by MEN1 apparently sporadic (index cases) for MEN1 gene. We evaluated the prevalence of pituitary adenoma in these patients and germline screening for CDKN1B in those affected by MEN1-like syndrome but negative for MEN1 gene mutations. **Results**: MEN1 gene mutation was detected in 28% (32 patients) of index cases, 75% of whom were familial MEN1. Among the index cases with an indication for MEN1 analysis, we selected the patients affected with pituitary adenoma (54 patients, ie 47,3%). Of these, 13 patients (24%) were carriers of mutation, 9 of whom were classified as cases of familial MEN1 (69.2%) and 4 (30.7%) as sporadic MEN1 (negative family history). In the familial MEN1 group, pituitary adenoma was the first clinical manifestation in 55.5%, compared to 25% of the sporadic group. Among the index cases who were wild type (wt) for MEN1, we selected 48 patients with MEN1-like clinical features, whom were screened for germline mutations of CDKN1B gene. No gene mutation was found in this group but in 47.9% a single nucleotide polymorphism of the CDKN1B gene, leading to a nonsynonymous change (Val109Gly), was found. This variant has been evaluated in several malignancies, but not in MEN1 patients, with the aim of establishing a potential predisposing role. Among the 48 patients analyzed for the CDKN1B 45.8% (22/48) had a pituitary involvement and 54.5% (12/22) of these were carriers of the polymorphism p.V109G. **Discussion**: In conclusion, in our study the prevalence of pituitary adenoma as the first clinical manifestation of familial MEN1 is higher than that described in the literature (55.5% vs 10%). In addition, our results suggest that the prevalence of the polymorphism p.V109G of CDKN1B may be higher in patients with MEN1-like phenotype (47.9%) either than in the general population (23%) or the population affected by other types of malignancies (6-40%).

OT12

AUTOIMMUNE HYPOGLYCEMIA INDUCED BY A-LIPOIC ACID IS MORE COMMON THAN PREVIOUSLY THOUGHT AND MOSTLY ASSOCIATED WITH HLA-DRB1*0403 ALLELE

A. Tumminia[1], G. Sortino[2], D. Consoli[1], M. Russo[1], G. Parrinello[1], S. Tumino[3], F. Di Raimondo[2], S. Squatrito[1], R. Vigneri[1], D. Gullo[1]

[1]Endocrinology, Garibaldi-Nesima Hospital - Catania, [2]Hematology, Ferrarotto Hospital - Catania, [3]Department of Medicine and Pediatric Sciences - Catania

Introduction. *Insulin Autoimmune Syndrome* (IAS, Hirata disease), a rare cause of hypoglycemia characterized by extremely high insulin levels and autoantibodies against endogenous insulin, has been mostly reported in Japan and associated with the use of sulfhydryl compounds, including *α-lipoic acid* (ALA). Among Japanese, HLA DRB1*0406 and DRB1*0403 (at a lower level) are the alleles with the highest risk for susceptibility to IAS. We report a series of 6 cases observed in Sicily in a short period of time and all associated with ALA use, in which HLA-DRB1* allelic association was evaluated.**Methods and Results.** From March 2011 to December 2012 six patients (M=2 F=4), median age 70 yrs (IQR 43-75), were admitted for severe hypoglycemia. No one had been previously treated with insulin. During hypoglycemic episodes median insulin and C-peptide serum levels were 4,065 µU/ml (IQR 1,783-19,225) and 5.8 ng/ml (IQR 4.6-6.9), respectively. All patients had positive insulin autoantibodies by PEG precipitation. HLA analysis showed DRB1*0403 in 5 patients and DRB1*0406 in 1 patient. Hypoglycemic symptoms appeared between 30 and 120 days after taking ALA 600 mg/d. Discontinuation of the drug resulted in a reduction of hypoglycemic episodes. All patients were treated with prednisone 12.5-25 mg/d, gradually tapering dosage. Insulin values returned to normal within 3 months with total remission of hypoglycemic symptoms. **Conclusions.** The high prevalence of HLA DRB1*0406 in Japan (allele frequency: 0.031) is considered a genetic risk factor for the development of IAS. Nonetheless, the rarity of this disease in Caucasians has been explained by the low frequency of DRB1*0406 (0.0025 in Italy). Most of our patients (5 out of 6) have DRB1*0403, an allele common among Caucasians and widely distributed across various populations. The wider presence of DRB1*0403 in our population, combined with the increasing use of ALA, a common nutritional supplement that does not require medical prescription, may explain why the IAS cases are rising.The use of ALA can cause severe hypoglycemia in genetically predisposed individuals, also outside Japan, and must be considered in the differential diagnosis of hypoglycemic syndromes.

OT13

ALTERATIONS IN ISLET MORPHOLOGY AS A FUNCTION OF INSULIN SENSITIVITY IN HUMANS

T. Mezza[1], G. Muscogiuri[1], G. P. Sorice[1], J. Hu[2], G. Clemente[3], R. N. Kulkarni[2], A. Pontecorvi[1], A. Giaccaro[1]

[1]Endocrinologia e Malattie del Metabolismo - Roma, [2]Islet cells & Rigenerative Medicine - Boston (MA), [3]Chirurgia Epatobiliare - Roma

Type 2 diabetes develops when insulin secretion fails to cope with worsening insulin resistance. It is becoming evident that insulin resistance can also impact non-classical tissues including the pancreatic β-cells. For example, obese insulin resistant patients exhibit an enhanced β-cell mass as an adaptive response although the underlying mechanism(s) is not fully understood. To explore the alterations that occur in islet morphology, as part of the adaptive mechanism, we performed hyperinsulinemic euglycemic clamps in 18 Caucasian non-diabetic patients, dividing them into insulin resistant (IR, n=9) or insulin sensitive (IS, n=9) (10 F/8 M, 51±15 yrs., BMI 27.9±5.3 kg/m2) groups according to glucose uptake; incretins were evaluated during a Mixed Meal Test. Subsequently, all patients underwent duodeno-pancreatectomy, and pancreases were collected for immunohistochemistry for glucagon, insulin and somatostatin+ cells to assess islet morphology. Apoptosis was evaluated by TUNEL, proliferation by Ki67, and ductal cells by CK19 immunostaining. Assessment of the entire group revealed a direct correlation between GU and islet size (r= -0.74; p<0.001), and between GU and % glucagon area expressed as a fraction of the total pancreas (%GLUCA, r=-0.65; p=0.003). IR group displayed a significant reduction in β/α ratio and increased islet size (2456±332.2 vs 5156±944.8 μm2, p<0.01). While no differences were evident in proliferation, apoptosis or β cell area, the IR group displayed increased insulin+CK19+ cells (p<0.001), scattered islets (<8 cells) (p=0.04) and a larger β-cell nuclear area (p=0.03).

Further, GLP1 levels correlated with %GLUA (r=0.63, p=0.04) and we detected GLP-1 immunoreactivity in α-cells. To our knowledge this is the first report correlating in vivo insulin sensitivity indices and incretin parameters with islet morphology. Our data suggest that impaired insulin sensitivity and an increase in GLP-1 in α-cells contribute to enhance β-cell mass.

OT14

CIRCULATING TUMOR CELLS IN ADRENOCORTICAL CARCINOMA: A MONOCENTRIC PRELIMINARY STUDY

G. Poli[1], P. Pinzani[1], C. Scatena[2], F. Salvianti[1], E. Corsini[1], L. Canu[1], V. Piccini[1], G. Nesi[2], M. Mannelli[1], M. Luconi[1]

[1]Department of Clinical and Experimental Biomedical Sciences, University of Florence - Florence, [2]Department of Surgery and Translational Medicine, University of Florence - Florence

OBJECTIVE: Adrenocortical carcinoma (ACC) is a rare malignancy, with prognosis mainly dependent on stage at diagnosis. The identification of disease-associated markers for early diagnosis and drug monitoring is mandatory to improve survival rate and life quality. Circulating tumor cells (CTC) originate from primary tumor or metastases. The tumor-induced angiogenesis and invasion allow aggressive tumors to release CTC into the blood stream before any detectable metastases are established. Therefore, CTC detection may have enormous potential for diagnosis, prognosis and monitoring the disease. The presence of CTC in ACC patients have never been investigated so far.

DESIGN: CTC analysis was performed in 14 ACC and 10 adrenocortical adenoma (ACA) patients. Blood samples obtained before (n=3 pts) and after (n=10 pts) surgery were filtered on Screencell devices (Screencell®) which isolate CTC on size-base.

RESULTS: CTC were isolated in all ACC but not in ACA samples. Immunocytochemistry on CTC, compared to the primary tumors, revealed positivity for adrenocortical markers, confirming the adrenocortical origin. When ACC patients were stratified in two classes according to the cut-off of the median value of the clinical parameters (tumor diameter, Ki67, Weiss) or to the presence/absence of metastasis, a statistical significant difference was found in the number of CTC post-surgery only when diameter (CTC/ml mean±SD: 2.70±3.70 vs 0.59±0.67, P=0.028 for diameter>/=8 and<8cm, respectively) and metastatic stage (CTC/ml mean±SD: 3.91±4.83 vs 0.70±0.70, P=0.031, for stage=4 versus the others, respectively) were considered.

CONCLUSIONS: Our findings provide the first evidence that CTC may represent a valid marker to support diagnosis in adrenocortical tumor pathologies. Moreover, CTC seem to correlate with some clinical parameters. Although very preliminary, these results suggest a potential use of this so-called "liquid biopsy" for prognosis and non-invasive monitoring of progression and response to treatments.

FIRB fund of the Italian Minister of University, Research and Instruction to MM (prot number: RBAP1153LS).

OT15

MOLECULAR SCREENING FOR PERSONALIZED TREATMENT APPROACH IN ADVANCED ADRENOCORTICAL CANCER

M. C. De Martino[1], A. Al Ghuzlan[2], C. Do Cao[3], G. Assié[4], J. Scoazec[5], S. Leboulleux[1], S. Aubert[3], R. Libé[4], C. Nozières[5], F. Pattou[3], F. Borson-Chazot[5], R. Pivonello[6], C. Mazoyer[7], J. Bertherat[4], M. Schlumberger[1], L. Lacroix[7], E. Baudin[1]

[1]Nuclear Medicine and Endocrine Oncology, Institut Gustave Roussy - Villejuif (France), [2]Pathology, Institut Gustave Roussy - Villejuif , [3]Hôpital C.-Huriez, CHRU de Lille - Lille, [4]Endocrinology, Assistance Publique Hôpitaux de Paris, Hôpital Cochin - Paris, [5]Hôpital Edouard Herriot - Lyon, [6]Endocrinology & Oncology, "Federico II" University - Naples, [7]Translational Research Laboratory, Institut Gustave Roussy - Villejuif

Propose: to screen for the presence of putative targets for new treatments in a large cohort of advanced adrenocortical cancer (ACC)

Experimental design: in 40 adult stage III-IV ACC primary samples, we used comparative genomic hybridization (CGH) and hotspot gene sequencing (with Ion Torrent) to describe the presence of copy number abnormalities and mutations in more than 40 genes involved in cancer development and putative drug sensitivity (HER2; EGFR; BRAF; KRAS; PIK3CA etc.).

Results: the most frequent copy number alteration observed was the deletion of the tumor suppressor genes CDKN2A (4 cases of 28 which generated informative profiles; 14.3%) and CDKN2B (3/28 cases 10.7%) both located in the region Chr.9p21. Lower level loss of the region Chr.9p21 were also frequently observed (7/28 cases 25%). The most frequent mutations were in the genes of TP53, ATM and CTNNB1 (6, 5 and 4 cases 15%, 12.5%, 10%). Amplifications of FGFR1, FGF9 and FRS2 have been seen in 3 different subjects 7.5%. Other abnormalities were detected in single patients (BRCA1, PSME3; RPTOR; MYC; ABL1; PTK2; FLT3, MDM2; ERBB4, SMO; STK11 and GNAS). Same recurrent associations of abnormalities were: deletion of CDKN2A and ATM mutation; TP53 and CTNNB1mutation.

Conclusions: Drugs targeting cell cycle could represent nowadays the most relevant new therapeutic approach for patients with advanced ACC. FGFs pathway could be a potential target for treatment in a subset of ACC patients, while treatment with other targeted therapies could have a rational, based on the genomic alterations, only in selected patients.

ORAL COMMUNICATIONS

The abstract number is the same reported in the program.

OC01

CONSTITUTIVE ONCOGENIC ACTIVATION OF THE ERK PATHWAY CAUSES THYROID CANCER CELLS RESISTANCE TO GEFITINIB

M. L. Nicolosi[1], F. Giani[1], V. Vella[1], F. Geraci[1], R. Vigneri[1], S. Squatrito[1], F. Frasca[1]

[1]Department of Clinical and Molecular Biomedicine, University of Catania - Catania

Background. Poorly differentiated thyroid carcinomas are refractory to conventional treatments and they lack effective treatment. The EGF receptor (EGFR) tyrosine kinase is overexpressed in these tumors. Therefore, inhibition of the EGFR signaling pathway is an attractive candidate for anticancer therapy. However, EGFR inhibitors have provided poor results in thyroid carcinomas.

Objective. Aim of our study is to evaluate signaling pathways involved in the resistance to the EGFR tyrosine kinase inhibitor Gefitinib in different thyroid cancer cells.

Results. Western blot analysis indicated that EGF-R was overexpressed in thyroid cancer cells and Gefitinib effectively inhibited EGFR phosphorylation. In contrast, it was poorly effective in reducing cell viability in 7 out 9 cell lines and it has a limited effect on ERK phosphorylation, with the exception of the WRO and HTH-74 cells. Hence, cell sensitivity to Gefitinib was related to the inihbition of ERK. These results suggested that the activation of ERK pathway may be responsible for thyroid cancer cell resistance to Gefitinib. Since BRAF(V600E) mutation is the most common genetic alteration in thyroid cancers leading to a constitutive activation of the ERK pathway. We evaluated whether BRAF(V600E) is predictor of Gefitinib sensitivity. BRAF(V600E)-positive thyroid cancer cells were incubated with the specific BRAF(V600E) inhibitor PLX4032. As expected, sensitivity to Gefitinib was restored, as well as the effect of Gefitinib on ERK phosphorylation. This addictive/synergistic effect was not observed in thyroid cancer cell lines expressing wild type BRAF. In concordance with these experiments, transiently knockdown of BRAF by specific small interfering restored the cytotoxic effect of Gefitinib in BRAF(V600E) positive thyroid cancer cells (Gefitinib alone: IC50=20±3,3μM; combination therapy: IC50=12±2,8μM), while it did not significantly affected BRAF negative cells (Gefitinib alone: IC50=11±2,2μM; combination: IC50=10±3,7μM). Similar results were obtained with specific MEK inhibitor PD98059.

Conclusions. These results indicate that BRAF(V600E) by activating the ERK pathway may play a major role in determining Gefitinib resistance in thyroid cancer cells harbouring this mutation.

OC02

RADIOIODINE ABLATION BY RHTSH STIMULATION OF POST-TX THYROID REMNANTS IN PATIENTS WITH MODERATELY SEVERE AND SEVERE GRAVES' ORBITHOPATHY

M. Moleti[1], M. A. Violi[1], D. Montanini[3], B. Di Bella[1], G. Sturniolo[1], S. Presti[1], A. Alibrandi[4], A. Campenni[5], S. Baldari[5], F. Trimarchi[1], F. Vermiglio[1]

[1]Medicina Clinica e Sperimentale - Messina, [2]Medicina Clinica e Sperimentale - Messsina, [3]Chirurgia Specialistica - Messina, [4]Scienze Economiche Finanziarie e Sociali - Messina, [5]Scienze Radiologiche - Messina

Context. Recent evidence suggests thyroidectomy (Tx) followed by radioiodine remnant ablation to be beneficial to Graves' Ophthalmopathy (GO) patients. However, prolonged hypothyroidism prior to treatment risks causing GO to worsen.

Objective. To evaluate the effect of total thyroid ablation following recombinant human TSH (rhTSH) stimulation of 131I uptake in patients with moderately severe and severe GO.

Design/Setting. The study, conducted at a university hospital thyroid-eye clinic, was prospective, randomized, and single-blind.

Patients/Interventions. Forty consecutive patients with moderately severe and severe GO were randomized into: 1) Tx-RAI group, including 20 subjects who underwent total Tx and radioiodine ablation of post-surgical thyroid remnants after rhTSH stimulation; 2) Tx group, including 20 subjects who underwent total Tx alone.

Outcome measures. The overall GO outcome 12 months after thyroidectomy/radioiodine ablation was the main measure. The effects of acute rhTSH and 131I administration on GO were also evaluated.

Results. GO outcome was significantly better in Tx-RAI than in Tx patients at 6 and 12 months (p 0.027 and p 0.007, respectively), and inactive in a significantly higher percentage of Tx-RAI than of Tx patients at 6 ($\chi 2$ 4.91, p 0.026) and 12 ($\chi 2$ 6.65, p 0.0099) months. No patient complained of clinically significant ocular effects following rhTSH administration, except for a transient increase in retrobulbar pain in one patient.

Conclusions. Our results indicate that combined thyroidectomy-radioiodine ablation is more effective than thyroidectomy alone in achieving early and steady GO improvement lasting at least 12 months.

OC03

SORAFENIB TREATMENT IN PATIENTS WITH RADIOIODINE REFRACTORY ADVANCED THYROID CANCER

F. Michelon[1], F. Felicetti[1], A. Piovesan[1], M. Gallo[1], G. Boccuzzi[2], E. Arvat[1]

[1]ENDOCRINOLOGIA ONCOLOGICA UNIVERSITA DI TORINO - TORINO, [2]DIPARTIMENTO DI SCIENZE MEDICHE UNIVERSITA DI TORINO - TORINO

Introduction: Differentiated thyroid cancer (DTC) has generally an excellent prognosis: surgery and radioactive iodine (RAI) warrant the control of DTC in the majority of patients. Treatment options for recurrent or metastatic DTC refractory to RAI, however are scanty. Conventional chemotherapy is not recommended for its inadequate efficacy and high toxicity. Recently, multitargeted kinase inhibitors (MKI) have showed promising results in the treatment of RAI refractory DTC in phase II and III studies. We report our experience on the use of Sorafenib (SOR), a MKI, in an off-label open protocol for patients with metastatic progressive RAI refractory DTC. Aim of the study was to evaluate tolerability and clinical response to SOR in this cohort of patients. **Methods** From march 2011 12 patients [3M 9F, median age 70 (52-81) years] with RAI refractory metastatic DTC (4 PTC, 6 FTC, 2 PDTC) were enrolled. All patients received initially SOR 400 mg twice daily; the dose was tapered in case of relevant side effects. SOR was stopped if radiological progressive disease (according to RECIST criteria) or severe toxicity (grade 3 or 4) occurred. Clinical, cardiovascular and biochemical evaluations were performed at baseline and at least monthly. CT scans were performed every 8-12 weeks.

Results One patient stopped SOR after 30 days for severe gastrointestinal toxicity and was excluded from analysis. Median progression free survival was 235 days. Three patients (27%) had partial response (PR), achieved in all cases within 3 months, whereas 7 (63%) had stable disease (SD). One patient had progressive disease. Commonest adverse events, included hand-foot syndrome (6), other skin (10) and gastrointestinal (4) toxicities, alopecia (4). One patient stopped SOR for a severe adverse event (myocardial infarction). By the time the follow-up of the study ended (December 2012) 4 patients were still on SOR. **Conclusions** In our cohort of patients with progressive, metastatic or locally advanced RAI refractory DTC, SOR confirmed relevant antitumor activity. SOR led to SD or PR in the majority of these patients despite frequent adverse events.

OC04

THE PREVALENCE, THE TUMORIGENIC ROLE AND THE FUNCTIONAL IMPLICATIONS OF RARE BRAF ALTERATIONS IN A COHORT OF ITALIAN PATIENTS WITH THYROID CARCINOMAS.

S. Barollo[1], R. Pezzani[1], A. Cristiani[2], M. Redaelli[3], L. Zambonin[1], B. Rubin[1], L. Bertazza[1], M. Zane[4], C. Mucignat-Caretta[3], A. Bulfone[2], G. Pennelli[1], M. R. Pelizzo[4], F. Mantero[1], S. Moro[5], C. Mian[1]

[1]Department of Medicine - Padua, [2]Biomedicine Sector, Center for Advanced Studies, Research and Development in Sardinia - Pula (CA), [3]Department of Molecular Medicine - Padua, [4]Department of Surgery, Oncology and Gastroenterology - Padua, [5]Department of Pharmaceutical Sciences - Padua

Background: Papillary thyroid carcinoma (PTC) is the most common malignant tumor of the thyroid gland, accounting for 74-80% of all thyroid cancers. The T1799T>A transversion mutation is an activating mutation of the *BRAF* oncogene that is common in conventional PTC and specific to it.

Aims: To study the prevalence, tumorigenic role and biomolecular implications of rare genetic variants in a large cohort of patients.

Study design: One thousand six hundred and forty-one fine-needle aspiration biopsy (FNAB) samples were collected and subjected to *BRAF* mutation analysis: 494 were PTC.

Results: *BRAF* mutations were found in 271 (54.8%) of those. The classical (c.1799T>A) mutation was found in 97% of the PTC samples while only 3% (8/271) presented rare genetic variants. A total of 9 infrequent alterations were detected: c.1795_1797dupACA (p.T599dup) found in 2 patients (one with the follicular variant and the other with classical PTC); c.1801A>G (p.K601E) found in 3 patients (1 with poorly differentiated follicular carcinoma and 2 with the follicular variant of PTC); c.1799_1801delTGA (p.V600_K601>E) found in 3 (1 with hobnail, 1 with tall cell variant and the last not yet operated): and c.1799_1814>A (p.V600_S605>D) in 1 patient (the classical PTC variant). The rare genetic variants were also analyzed by Western blot to investigate their susceptibility in modulating fundamental signaling pathways, by immunofluorescence and by means of *in silico* analysis to evaluate their molecular role in large-scale exploration of conformational spaces.

Conclusions: This study delineated the prevalence, tumorigenic role and functional implications of rare *BRAF* alterations of thyroid carcinoma.

OC05

RAS MUTATIONS AND MEDULLARY THYROID CANCER: PREVALENCE AND RELATIONSHIP WITH CLINICAL-PATHOLOGICAL FEATURES AND ANALYSIS OF ANGIOGENESIS GENES

C. Durante[1], A. Verrienti[1], M. Spenziello[1], M. Dima[1], G. Tamburrano[1], A. F. Campese[2], G. Tallini[3], D. Meringolo[4], F. Monzani[5], M. Ferdeghini[6], C. Colato[7], R. Bruno[8], F. Nardi[9], D. Russo[10]

[1]Dip. Med. Interna e Specialità Mediche - Roma, [2]Dip. Med. Molecolare - Roma, [3]Dip. Ematologia e Scienze Oncologiche - Bologna, [4]Unità Operativa Semplice Dipartimentale di Endocrinologia - Bologna, [5]Dip. di Med. Clinica e Sperimentale - Pisa, [6]Dip. di Scienze Morfologico-Biomediche - Verona, [7]Dip. di Patologia e Diagnostica - Verona, [8]Unità di Endocrinologia - Matera, [9]Dip. di Radiologia, Oncologia ed Anatomia Patologica - Roma, [10]Dip. di Scienze della Salute - Catanzaro

RAS point mutations have recently been described in sporadic medullary thyroid carcinomas (MTC) negative for mutations in RET oncogene. Aim of this study was to investigate the somatic mutational status of the three RAS proto-oncogenes in a large series of patients with MTC and correlate the genotypic characteristics with the clinical-pathological features of MTC patients. A subgroup of tumors was also investigated for the expression of a series of transcripts involved in the tumor-related angiogenesis (VEGF, PDGF and the correspondent receptors), as well as the calcitonin gene. DNA extracted from 123 MTC sporadic samples was directly sequenced to search for alteration in KRAS, HRAS, and NRAS genes (exons 2, 3, and 4). When available (21 samples), RNA was used to analyze by real time quantitative RT-PCR the expression levels of VEGF-A, VEGF-C, VEGFR1, 2 and 3, PDGF-A, PDGF-B, PDGFR-alfa, PDGFR-beta, angiopoietin 1 and 2 and calcitonin. In this series of MTC, the prevalence of RAS mutations resulted 20.7% of total. They involved HRAS at codons Q61 (ex. 3) and G13 (ex. 2) and KRAS at codons Q 61 (ex. 3), G12 (ex. 3) and K17 (ex. 4), the latter not a classical mutational hot spot of RAS gene. All the mutations detected were somatic. No relationship was found between the occurrence of RAS mutations and the clinical-pathological features of the patients, including pTNM parameters and clinical outcome (cure or persistent disease). Expression analysis of genes involved in angiogenesis revealed absence of association between a particular gene and the RAS status of the tumor. In contrast the expression of calcitonin resulted significantly increased in the RAS mutated tumors vs. RAS wild type ones (2.36 fold, p=0.039). In conclusion, we found a low prevalence of RAS mutation in this large series of MTC and absence of relationship with the clinical-pathological characteristics of the patients, except for higher levels of calcitonin gene, suggesting a more differentiated phenotype related with RAS alterations of the tumors.

OC06

PAPILLARY THYROID CANCER: A NEW CASE-CONTROL STUDY INVOLVING PRE-MIR-146A AND PTTG1 GENES.

M. Marino[1], V. Cirello[2], V. Gnarini[1], E. Pignatti[1], L. Casarini[1], C. Diazzi[1], V. Rochira[1], K. Cioni[1], B. Madeo[1], M. Simoni[1], L. Fugazzola[2]

[1]Università di Modena e Reggio Emilia, Dip. Scienze Biomediche, Metabolismo e Neuroscienze. - Modena, [2]Università di Milano, Dip. Scienze Biomediche, Unità di Endocrinologia, Padiglione Granelli, IRCCS Cà Granda - Milano

Background

Papillary thyroid carcinoma (PTC) is the most common endocrine malignancy. Two studies revealed the predisposition to PTC by the heterozygous state of rs2910164 within the *pre-miR-146a*. Interestingly, on the chromosome 5, almost 40Kb separate the *pre-miR-146a* from the pituitary tumour transforming gene (*PTTG1*), also involved in thyroid carcinomas. Furthermore, an association of the genomic region encompassing these two genes with another pathology was described. In this study, we analyzed, with a case-control design, the genetic association between PTC and *pre-miR-146a* rs2910164 as well as *PTTG1* (rs1862391 and rs2910202).

Methods

307 PTC patients and 206 controls, matched for age and gender, were enrolled. The diagnosis of PTC was done at histology, and the possible presence of thyroid nodules in the controls was excluded by ultrasounds. SNP genotyping of all SNPs was performed by Sanger sequencing and High Resolution Melting (HRM). Statistics was performed with Haplowiev 4.2 and GraphPad Prism5 software.

Results and conclusions

Pre-miR-146a rs2910164 genotypic and allelic frequencies are not statistically different in patients and controls and the SNP is not in LD with the investigated *PTTG1* SNPs. We do not confirm the reported association of the heterozygous GC genotype with PTC, in 2/3 populations of Caucasian origin. On the contrary, in our patients, the GG genotype is prevalent (58.6%). The *PTTG1* SNPs (rs1862391 and rs2910202), in perfect LD, have the same allelic frequency in patients and controls and are not associated with PTC. In conclusion, the study show that in our well-selected Italian population, either *pre-miR-146a* rs2910164 or *PTTG1* rs1862391 and rs2910202 are not associated with PTC, consistent with the well known difficulty in the identification of common genetic markers for all populations of Caucasian origin.

OC07

PROPHYLACTIC CENTRAL COMPARTMENT LYMPH NODE DISSECTION DOES NOT IMPROVE THE OUTCOME OF PATIENTS WITH A LOW RISK DTC IN A SHORT TERM FOLLOW UP

L. Valerio[1], D. Viola[1], G. Materazzi[2], P. Miccoli[2], F. Basolo[2], E. Sensi[2], P. Faviana[2], E. Molinaro[1], L. Agate[1], R. Elisei[1]

[1]Department of Endocrinology and Metabolism - Pisa, [2]Department of Surgery - Pisa

The clinical benefit of the prophylactic central compartment lymph node dissection (CCL) in differentiated thyroid cancer (DTC) is still controversial. This treatment seems to reduce DTC recurrence rates and the need of performing a reoperation. The risk of a higher rate of surgical complications represents the major concern. The possibility to find preoperative prognostic factors for the presence of central compartment lymph node metastasis (CCLM) is a need.

The aim of this prospective study was to evaluate the pros and cons of CCL and the outcome of DTC patients treated with either total thyroidectomy (TTx) or TTx+CCL and to evaluate if we could find any factor able to predict the presence of CCLM.

A total of 169 DTC patients with no preoperative evidence of lymphnode metastases (N1) were randomly assigned to TTx, (Group-A, n=84) or TTx+CCL, (Group-B, n=85).

The two groups did not differ for their epidemiological and clinical features. As expected the Group-B showed a higher prevalence of N1 (47.1% vs 7.1%) while no differences were observed for other pathological features (i.e multifocality, histological variants, BRAF mutation, etc). After a mean follow-up of 3.5 years, no difference was observed in the outcome of the 2 groups. At the present, reoperation was needed in one patient of Group-A. However, a statistically significant higher number of ^{131}I courses was administered to Group-A (p=0.0017). Conversely, a statistically significant higher prevalence of permanent hypoparathyroidism was observed in Group-B (p=0.04). We did not find preoperative prognostic factors for the presence of CCLM. Tumoral capsule infiltration, extrathyroid extension and advanced stage are postsurgical factors able to predict the presence of CCLM.

In conclusion, this study showed that the short term outcome of DTC patients treated with either TTx or TTx+CCL was very similar. However, the patients treated with TTx have to undergo a higher number of 131I courses than those treated with TTx+CCL. Conversely, those treated with TTx+CCL have a higher number of permanent hypoparathyroidism. No presurgical factors able to predict the presence of CCLM were found. A longer follow-up is needed to verify the recurrences in the 2 groups.

OC08

HBA1C FOR THE DIAGNOSIS OF DIABETES AND PRE-DIABETES: DIAGNOSTIC IMPACT AND PHENOTYPIC CHARACTERIZATION

L. Perra[1], M. Incani[1], F. Sentinelli[1], M. G. Pani[1], A. Boi[1], A. Olla[1], G. Frau[1], A. Lenzi[2], F. Leonetti[2], E. Cossu[1], M. G. Baroni[2]

[1]Department of Medical Sciences, University of Cagliari - Cagliari, [2]Department of Experimental Medicine, Sapienza University of Rome - Roma

HbA1c is the gold standard for monitoring glycaemic control in patients with diabetes mellitus. ADA and other Societies proposed diagnostic criteria based on HbA1c levels for diabetes (HbA1c≥6.5%) and pre-diabetes (HbA1c 5.7–6.4%). Epidemiological studies have shown significant discordance between HbA1c and glucose-based tests for defining diabetes and prediabetes. Aim of our study was to evaluate the impact of HbA1c criteria to diagnose diabetes and prediabetes in a cohort of subjects undergoing OGTT.We also looked at the phenotypic characteristics of those subject that had a diagnosis of prediabetes with the OGTT but had a normal HbA1c.795 subjects were selected. 640 with a BMI>30 and 155 normal weight (BMI 25.1). All patients underwent OGTT and HbA1c measurement. 65% of the subjects diagnosed as diabetic by OGTT had an HbA1c≥6.5%, but merely 41,61% of the prediabetic subjects by OGTT had an HbA1c between 5.7% and 6.49%.The normoglycemic subjects were well identified by HbA1c, with 74.3% of NGT subjects having an HbA1c<5.7%. HbA1c≥6.5% for diabetes showed a moderate sensitivity of 65.2%, with high specificity 93.5%. HbA1c between 5.7% and 6.49% did not identify well prediabetic subjects. ROC analysis showed that an HbA1c cutpoint of >6.0% for diabetes and >5.5% for prediabetes yielded the highest accuracy. We observed in our population a high level of discordancy between HbA1c and OGTT results.When analyzing data in the prediabetc range,129 (41,6%) of the prediabetic subjects by OGTT had a concordant HbA1c between 5.7% and 6.5%.149 subjects had a positive OGTT for prediabetes, but had an HbA1c within the normal range. 217 (74,3%) of the subjects had both HbA1c and OGTT in the normal range. We therefore looked at the clinical and biochemical characteristics of these latter two groups, aiming to identify possible factors that might help to select those at higher risk for prediabetes, that should be therefore analyzed primarly by OGTT. These subjects with a normal HbA1c but a prediabetic OGTT were significantly different in age, sex, lipid profile and insulinemia. Conclusion: HbA1c shows good sensitivity and specificity for diabetes when matched to OGTT. However, HbA1c 5.7-6.49% performed poorly when compared to OGTT for the diagnosis of prediabetes. In older male subjects OGTT should be preferred because HbA1c fails in a large number of them to identify their prediabetic status

OC09

P2X7 RECEPTOR DEFICIENCY ATTENUATES NON-ALCOHOLIC STEATOHEPATITIS (NASH) INDUCED BY HIGH-FAT DIET: POSSIBLE ROLE OF THE NLRP3 INFLAMMASOME

C. Blasetti Fantauzzi[1], A. Solini[2], S. Menini[1], C. Rossi[2], C. Ricci[1], E. Santini[2], C. Iacobini[1], G. Pugliese[1]

[1]Dipartimento di Medicina Clinica e Molecolare, Università La Sapienza - Roma, [2]Dipartimento di Medicina Interna, Università di Pisa - Pisa

Visceral obesity is associated with inflammatory, metabolic and vascular abnormalities including insulin-resistance, atherosclerosis and non-alcoholic steatohepatitis (NASH). The purinergic receptor P2X7 (P2X7R) might contribute to progression from steatosis to NASH, since it is modulated by NEFA and, in turn, mediates ATP-induced activation of the NLRP3 inflammasome. This study was aimed at investigating the role of P2X7R through activation of the NLRP3 inflammasome in NASH induced by a high fat diet (HFD), an established experimental model of the metabolic syndrome. To this end, P2X7R knockout (KO) mice and coeval wild type (WT) controls were fed a HFD (60% saturated fat) or a normal-fat diet (NFD, 10% saturated fat) for 4 months. Body weights were significantly higher in KO vs. WT mice and increased in both genotypes upon HFD. HFD-induced hepatic lesions were attenuated in KO vs. WT mice. This was confirmed by morphometric analysis, showing mixed, macro- and micro-vesicular, steatosis in both genotypes on a HFD, though it was less severe and predominantly of the micro-vesicular type in the KO as compared with the WT mice. The majority of WT animals (4 out of 7) met the American Association for the Study of Liver Disease criteria for diagnosis of moderate-to-severe NASH, i.e. lobular inflammation, ballooning, Mallory's bodies and fibrosis. In contrast, only 1 out of 7 KO mice showed signs of NASH of mild degree, with the remaining 6 animals showing simple steatosis, predominantly micro-vesicular, with little or no inflammation. The expression of pro-fibrotic (fibronectin, collagen I and TGF-β), pro-inflammatory (MCP1, CXCR3 and TNF-alfa), and lipid metabolism (FAS, SREBP1c, CPT1 and LXR-alfa) genes increased to a significantly lesser extent in KO vs. WT mice on a HFD. Immunohistochemical analysis showed a strong positivity for P2X7R and NLRP3 (in sinusoids, bile ducts and infiltrating inflammatory cells) in the WT mice, whereas staining was absent for P2X7R and very low for NLRP3. These data were confirmed by RT-PCR analysis of gene expression. Preliminary experiments in liver sinusoidal endothelial cells showed that P2X7R and the NLRP3 inflammasome components (NLRP3, caspase-1 and IL-1beta) are expressed under basal conditions and are upregulated in response to the pro-inflammatory cytokine TNF-alfa. These data show that P2X7R ablation protects mice from HFD-induced NASH, possibly through a blunted activation of NLRP3 inflammasome.

OC10

HIGH MOLECULAR WEIGHT ADIPONECTIN AND CARDIOVASCULAR-DEATH IN PATIENTS WITH TYPE 2 DIABETES.

C. Menzaghi[1], L. Salvemini[1], G. Palladino[1], C. De Bonis[1], S. Bacci[2], V. Trischitta[1]

[1]IRCCS CSS Research Unit of Diabetes and Endocrine Diseases - San Giovanni Rotondo, [2]IRCCS CSS Unit of Endocrinology - San Giovanni Rotondo

Cardiovascular disease is the first-cause of death in type 2 diabetes (T2D). Novel markers are needed in order to predict and prevent such event. Recently, high adiponectin has been surprisingly associated with increased mortality in elderly people with T2D; whether a similar counter-intuitive association is observed also with cardiovascular (CV)-death in T2D is not known. Our aims were to investigate the association between serum High Molecular Weight Adiponectin (HMW-A) and CV-death in T2D and whether such association is a causal one.

We studied 359 patients with T2D and coronary artery disease from the Gargano Heart Study and performed a "mendelian randomization analysis" testing the association between rs822354 (a SNP in the adiponectin locus strongly associated with HMW-A in GWAS) and CV-death in the same cohort.

During a 7 years follow-up, 58 CV-death/1,934 person-year occurred. HMW-A predicted CV-death in a model comprising age, sex, smoking habit, BMI, HbA1c, insulin therapy, hypertension, total cholesterol, HDL cholesterol and triglycerides levels: HR per SD increment of 1.38 (1.10-1.74). This association was strongly significant among males (n=242, HR=1.39, 95%CI 1.16-1.66), but not females (n=117, HR=0.91, 95%CI 0.55-1.47), thus pointing to a sex specific effect of HMW-A on CV-death (p value for HMW-A-by-sex interaction=0.045).

Notably, rs822354 (similarly associated with HMW-A levels in both males and females: per allele adjusted β±SE=0.22±0.09 μg/ml, p=0.01 and 0.26±0.15 μg/ml, p=0.08, respectively), was associated with CV-death among males (HR=1.80, 95%CI 1.16-2.80), but not females (HR=0.85, 95%CI 0.42-1.71; p for SNP-by-sex interaction=0.050), thus mirroring the sex specific association observed between HMW-A levels and CV-death and strongly supporting a cause-effect relationship underlying the association in males.

In conclusion, in patients with T2D high serum HMW-A is an independent risk factor for CV-death among males, but not females. Further studies are needed to confirm this finding.

OC11

PROLONGED EXPOSURE OF PANCREATIC BETA-CELLS TO PALMITATE RESULTS IN REDUCED INCRETIN ACTION AND SIGNALING VIA CREB AND AKT

G. Biondi[1], A. Natalicchio[1], N. Marrano[1], F. Tortosa[1], R. Labarbuta[1], S. Perrini[1], L. Laviola[1], F. Giorgino[1]

[1]Dipartimento dell'Emergenza e dei Trapianti di Organi-DETO - Bari

The incretin effect is attenuated or markedly diminished in type 2 diabetic and obese subjects. The aim of this study was to investigate the mechanisms of incretin resistance induced by prolonged exposure to high FFA concentrations in pancreatic beta-cells. Action and signaling of the GLP-1 analog exendin-4 were investigated in rat INS-1E cells treated with 0.25 mM palmitate for 24 h and in islets obtained from mice fed a high fat diet for 3 weeks. Prolonged exposure of INS-1E cells to palmitate reduced the ability of exendin-4 to augment insulin mRNA levels and to induce insulin release by 50% and 60%, respectively ($p<0.05$). In addition, palmitate blocked exendin-4-stimulated CREB and Akt phosphorylation, whereas phosphorylation of MEK and Erk-1/2 was not altered. Moreover, palmitate did not interfere with the ability of IGF-I to activate CREB and Akt, indicating that the inhibitory effects of palmitate are specific for exendin-4 signaling. Both islets from mice fed a high fat diet and INS-1E cells exposed to palmitate showed reduced PDX-1 mRNA levels (by 40-50%; $p<0.05$) and GLP-1 receptor mRNA and protein levels (by ~30% and 50%, respectively; $p<0.05$). Furthermore, RNAi-mediated suppression of GLP-1 receptor expression prevented exendin-4-induced CREB and Akt phosphorylation ($p<0.05$), but did not impair the ability of exendin-4 to stimulate MEK and Erk-1/2. In contrast to palmitate, 24 h exposure of INS-1E cells to oleate led to increased PDX-1 and GLP-1 receptor mRNA and protein levels, and enhanced the ability of exendin-4 to stimulate CREB and Akt phosphorylation ($p<0.05$). In conclusion, prolonged exposure of pancreatic beta-cells to excess palmitate results in reduced exendin-4 action. The mechanism of FFA-induced incretin resistance appears to involve diminished GLP-1 receptor expression and reduced activation of the CREB/Akt pathways, which differently than the MEK/Erk-1/2 pathway, are critically controlled by the cellular levels of GLP-1 receptor.

OC12

INHIBITION OF RETINAL ANGIOGENESIS BY A LEPTIN RECEPTOR ANTAGONIST PEPTIDE

C. Parrino[1], L. Scolaro[2], L. Otvos[3], E. Surmacz[2]

[1]Dipartimento di Biomedicina Clinica e Molecolare Università di Catania/ Sbarro Institute for Cancer Reaserch and Molecular Medicine Temple University - Catania/Philadelphia, [2]Sbarro Institute for Cancer Reaserch and Molecular Medicine Temple University - Philadelphia, [3]Biology Department Temple University - Philadelphia

Ocular neovascularization is a pathological hallmark of prominent forms of blindness including diabetic retinopathy. Emerging studies suggest that angiogenic and pro-inflammatory cytokine leptin might be implicated in the pathogenesis of diabetic eye disease. However, the potential ofinhibiting leptin function in diabetic eye models has never been explored. We investigated mitogenic, angiogenic, signaling activities of leptin in a relevant ophthalmic cell model, and explored the potential of our leptin receptor (ObR) antagonist peptide (Allo-aca) to inhibit these functions. Leptin at 50-250 ng/mL stimulated the growth of monkey retinal (RF/6A) endothelial cells in a dose-dependent manner. The maximal mitogenic response (35±7 %) was noted at 24 h of 250 ng/mL leptin treatment. Leptin-dependent proliferation was reduced to base levels with 10 and 100 nM Allo-aca. Leptin induced angiogenic response, i.e., formation of tube-like structures. The maximal increase of angiogenesis (163±10%) was observed with a 250 ng/mL leptin treatment for 4 h, and this effect was totally blocked in the presence of 100 nM Allo-aca. In addition, 250 ng/mL leptin modulated several intracellular pathways controlling cell growth, inflammatory activity and angiogenesis, including the phosphorylation of STAT3, Akt, and ERK1/2 at 15 min, and expression of COX2 and NF-kB at 12 h. The above leptin responses were significantly inhibited with Allo-aca at 100 nM concentration. Notably, leptin treatment of RF6/A cells for 6-24 h increased by ~4-fold leptin mRNA expression, and this autocrine loop was downregulated in the presence of 100-250 nM Allo-aca. An unrelated control peptide did not demonstrate any antagonistic activity in all the above assays. At present, anti-VEGF based therapeutics represent the only biologics approved for treatment of diabetic eye disease, however targeting other angiogenic molecules may represent an alternative or complementary strategy to inhibit intraocular neovascularization. Our data provide new insights into the role of leptin in retinal endothelial cells and offer basis for development of ObR antagonists as therapeutics for diabetic microvascular complications affecting the eye.

Supported by NovoNordisk Diabetes Innovation Award

OC13

THE HMGA1-IGF-I/IGFBP SYSTEM: A NOVEL BIOCHEMICAL PATHWAY FOR MODULATING GLUCOSE HOMEOSTASIS

B. Arcidiacono[1], S. Iiritano[1], V. Ventura[1], M. T. Nevolo[1], E. Chiefari[1], D. Foti[1], A. Brunetti[1]

[1]Dipartimeto di Scienze della Salute, Università di Catanzaro - Catanzaro - Italy

Insulin hypersensitivity, despite of impaired insulin action, supports the existence of molecular adaptation mechanisms whose functional activation may promote tissue glucose uptake and utilization by insulin-independent mechanisms. This possibility is documented by a wide variety of observation in vivo, in animal models of diabetes. We have previously reported that in Hmga1-knockout mice, peripheral insulin hypersensitivity paradoxically coexisted with a condition of impaired glucose tolerance and overt diabetes. This study aims to provide evidence for the existence of molecular adaptation mechanisms in vivo linked to the HMGA1-IGF-I/IGFBP system, that plays a role in the recruitment of Glut4 to muscle plasma membrane and glucose uptake under adverse circumstances in which insulin action is precluded.

Using chromatin immunoprecipitation studies we showed that HMGA1 is required for gene activation of the IGF-binding proteins 1 (IGFBP1) and 3 (IGFBP3), two major members of the IGF-binding protein superfamily. Furthermore, by using positron emission tomography (PET) with 18F-labeled 2-fluoro-2-deoxy-D-glucose, in combination with euglycemic clamp with IGF-I, we demonstrated that IGF-I's bioactivity was increased in Hmga1-knockout mice, in which both skeletal muscle Glut4 protein expression and glucose uptake were enhanced compared with control mice.

In conclusion, our findings propose that the increased responsiveness to IGF-I represents the primary mechanism promoting glucose disposal in Hmga1 deficient mice with defective insulin receptor and insulin action. Finally, HMGA1, by affecting the expression of both IGFBP protein species, can serve as a modulator of IGF-I activity, therefore representing an important novel mediator for glucose disposal.

OC14

LIPOCALIN-2 IS A TARGET OF ALDOSTERONE ACTION IN ADIPOSE TISSUE: CLINICAL AND EXPERIMENTAL STUDIES

R. Urbanet[1], A. Nguyen Dinh Cat[2], N. Lòpez Andrés[3], P. Rossignol[4], R. Vettor[1], F. Jaisser[5], F. Fallo[1]

[1]Dipartimento di medicina - Padova, [2]Cardiovascular Research and Medical Sciences Institute - Glasgow, [3]INSERM U961 - Nancy, [4]INSER U961 - Nancy, [5]INSERM U872 - Paris

Introduction and Objective. Animal and human studies evidenced a strong correlation between levels of aldosterone (aldo), lipocalin-2 (NGAL) or the complex NGAL-matrix metalloproteinase-9 (MMP9), and the prevalence of obesity, hyperglycemia and insulin resistance. Our laboratory demonstrated that NGAL is a target of aldosterone action in cardiovascular systhem. Our hypothesis is that NGAL may also be a target of aldosterone in adipose tissue.

Design. We used multiple approaches: 1) analysis of aldo/NGAL/NGAL-MMP9 serum concentrations in 134 patients with or without abdominal obesity; 2) study of the effect of treatment with mineralocorticoid receptor antagonist (MRA) on NGAL serum concentration in obese db/db mice; 3) exploration of the mechanism linking aldosterone action and expression/secretion of NGAL in adipose tissue in adipo-MR mice, a transgenic model that conditionally overexpresses aldosterone receptor, i.e., mineralocorticoid receptor (MR), in adipocytes.

Results. In patients, Aldo and NGAL-MMP9 were positively correlated each other ($rs=0.30$, $p<0.0004$) and independently correlated with BMI (respectively, $rs=0.35$, $p<0.0001$ and $rs=0.47$, $p<0.0001$) and HOMA index (respectively, $rs=0.33$, $p=0.0001$; $rs=0.24$, $p=0.007$). Db/db mice treated 8 weeks with MRA showed reduced NGAL circulating levels (3 fold reduction, $p<0.01$) and decreased NGAL mRNA levels in visceral fat (3 fold reduction, $p<0.001$). In adipo-MR mice, MR was specifically overexpressed in adipose tissues (10 fold increase, $p<0.05$), and this was coupled with a significantly rise in NGAL mRNA levels (2-8 fold increase, $p<0.05$).

Conclusion. We confirmed a strong correlation between high levels of aldosterone, NGAL, overall fat mass and prevalence of metabolic diseases. We demonstrated a relationship between MR activation in adipose tissue and NGAL expression. Further analyses are required to explain the molecular mechanism leading aldosterone to enhance NGAL expression and secretion in/by adipose tissue.

OC15

EFFECTS OF MINERALOCORTICOID AGONISTS AND ANTAGONISTS ON SURVIVAL AND PROLIFERATION OF ADULT RAT HIPPOCAMPAL PROGENITOR CELLS

I. Gesmundo[1], F. Settanni[1], E. Ghigo[1], E. Arvat[1], R. Granata[1]

[1]Department of Medical Science - Division of Endocrinology, Diabetology and Metabolism - School of Medicine - University of Turin - Turin

Hippocampus is a key area in the brain and influences the neuroendocrine functions, especially the hypothalamo-pituitary-adrenal (HPA) axis that is mainly regulated by corticotrophin-releasing hormone (CRH), vasopressin (ADH) and glucocorticoid (GC). This feed-back action is mediated by both glucocorticoid (GRs) and mineralocorticoid (MRs) receptors. GRs are distributed throughout the brain, but mostly in hypothalamic neurons, while the MRs highest expression has been detected in the hippocampus. Recent reports suggest that an impairment of hippocampal MRs more than GRs, might have clinical relevance in some pathological conditions such as depression or neurodegenerative diseases. Aim of the study was to clarify the role of fludrocortisone (MRs agonist) and spironolactone (MRs antagonist) on cell proliferation, differentiation and survival in adult rat hippocampal progenitor (AHP) cells, a totipotent cell line that can differentiate in neurons and glial cells. Furthermore we investigated the effect of fludrocortisone on AHP cell survival after treatment with amyloid ß-protein (fragment 1-42). AHP cells were cultured with Neural Stemline Medium enriched with FGF-basic. The presence of GRs and MRs was evaluated by RT-PCR. Cell survival was measured by MTT assay and cell proliferation by BrdU incorporation. Apoptosis was studied by Caspase-3 activity analysis. Activation of survival signalling pathways was determined by Western blotting, i.e. phosphorylation of phosphatidylinositol 3-kinase/Akt (PI3K/Akt), glycogen synthase kinease-3 (GSK-3ß) and of cAMPresponse element binding (CREB). Fludrocortisone, but not spironolactone, stimulates proliferation, and protects against growth factor deprivation-induced apoptosis. Fludrocortisone activates the GSK-3ß, the PI3K/Akt pathway and the cAMP/PKA/CREB pathways. Moreover it counteracts the effect of the amyloid ß-protein (1-42) on cell death and inhibition of cell proliferation. The survival and proliferative effect of fludrocortisone in neuronal precursors candidate MRs agonists as potential molecules in the treatment of neurodegenerative conditions, such as Alzheimer`s disease

OC16

HIGH GLUCOSE INDUCES HYPOGONADOTROPIC HYPOGONADISM BY INTERFERING WITH GPR54 SIGNALING IN THE PREOPTIC AREA OF THE HYPOTHALAMUS.

A. Morelli[1], L. Vignozzi[1], E. Sarchielli[1], P. Comeglio[1], S. Filippi[1], G. Rastrelli[1], E. Maneschi[1], I. Cellai[1], G. B. Vannelli[1], M. Maggi[1]

[1]Experimental and Clinical Biomedical Sciences - Florence

The metabolic syndrome (MetS) is a clustering of metabolic and cardiovascular risk factors, having in insulin resistance the key element. In males, MetS is also associated to hypogonadism. We recently found that rabbits with high fat diet (HFD)-induced MetS also developed hypogonadotropic hypogonadism (HH), showing a reduced immunopositivity for GnRH in the hypothalamus. We evaluated the pathogenetic link between MetS components and HH in HFD rabbits compared to regular diet (RD) group. Correlation analysis showed that testosterone and gonadotropins levels decreased as a function of the number of MetS components. Elevated glucose and cholesterol levels resulted the major determinants. Accordingly, gonadotropins negatively correlated with glucose intolerance, as evaluated by oral glucose tolerance test, and with hypothalamic gene expression of glucose transporters (GLUT1, GLUT3 and GLUT4). GLUT1 mRNA was also negatively associated with GnRH expression. In a multivariate analysis with all GLUT isoforms as covariates, only the insulin-sensitive GLUT4 retained the negative association with gonadotropins. GLUT4 positively correlated with increasing number of MetS components, severity of glucose intolerance and mRNA expression of inflammation markers (COX2, IL-6).Similarly, IL-6 mRNA positively correlated with increasing number of MetS components, while was negatively associated with gonadotropin levels. HFD significantly induced both mRNA expression and immunopositivity of GLUT4 and IL6 in the hypothalamus, as compared to RD group. In contrast, GPR54 immunopositivity was reduced by HFD. Consistent with this observation GPR54 mRNA negatively correlated with glucose intolerance. KiSS1/GPR54 system is a central regulator of GnRH neurons. We therefore studied the effects of high glucose in human fetus-derived GnRH-secreting neuroblasts, FNCB4 cells. High glucose exposure significantly reduced GnRH, KiSS1, GPR54 and leptin receptor expression in FNCB4. A subset of HFD rabbits was treated with INT-747, able to ameliorate glucose metabolism in HFD rabbits. This treatment significantly increased GnRH mRNA while reduced GLUT4 and IL-6 immunopositivity, without preventing HFD-related HH. In conclusion, our results suggest that negative effects of hyperglycemia on hypothalamic function may contribute to testosterone deficiency in MetS.

OC17

IRON OVERLOAD IMPAIRS THE MIGRATORY PROPERTIES OF IMMATURE GNRH NEURONS IN VITRO

L. Steffani[1], F. Di Nitto[1], C. Macchi[1], P. Dongiovanni[2], L. Valenti[2], S. Fargion[2], M. Ruscica[1], P. Magni[1]

[1]Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano - Milano, [2]Dipartimento di Fisiopatologia e dei Trapianti, Università degli Studi di Milano, Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico - Milano

Iron is an essential micronutrient required for fundamental biochemical activities and for proper brain development in the fetal and early neonatal period. However, cellular iron overload produces toxic build-up in many organs, including brain. In thalassaemic and juvenile hemochromatosis patients, excess iron is the most important factor afflicting the hypothalamic-pituitary axis in a dose dependent fashion leading to hypogonadotropic hypogonadism. Aim of this study was to investigate the mechanisms of iron toxicity in vitro in GN-11 cells, a model of immature and migratory GnRH neurons. A 24-h treatment with 150 μM Ferric Ammonium Citrate (FAC, source of ferric iron) was not toxic for GN-11 cells as displayed by the unchanged cell morphology and by a cell viability assay (ATPlite). Gene expression analysis by semi-quantitative PCR showed that GN-11 cells express the iron-related proteins ferritin, transferrin receptor and hepcidin. Exposure of GN-11 cells to 150 μM FAC resulted in the inhibition (-35%,***$p<0.001$ vs Control) of fetal bovine serum (FBS)-induced chemo-migration, as assessed by Boyden chamber assay. Pre-treatment with 100 μM deferoxamine (DFO), a specific iron chelator, reverted the above reported effect (###$p<0.001$ vs FAC). Time-course experiments showed that 150 μM FAC was associated with induction of phosphorylation of chemomigration-related cell signalling extracellular signal-regulated kinase1/2 (ERK1/2) and 5' adenosine monophosphate-activated protein kinase (AMPK) proteins after 10 min treatment (*$p<0,05$ vs no-treated cells), as evaluated by Western blotting. Specific ERK and AMPK inhibitors, U0126 and Compound C (both 10 μM), respectively, abolished FAC-mediated signalling. Moreover, U0126 and Compound C counteracted FAC-driven phosphorylation of ACC, an AMPK downstream protein. The present data, though preliminary, show that acute iron treatment negatively affects the migration of GN-11 neuronal cells in vitro and it is associated with the activation of ERK and AMPK signalling pathways. We hypothesize that iron overload may impair migration of GnRH neurons from the olfactory placode into forebrain and hypothalamus, where they promote reproductive competence.

OC18

OBESTATIN PROMOTES ADULT RAT HIPPOCAMPAL PROGENITOR CELL PROLIFERATION AND SURVIVAL THROUGH ACTIVATION OF PI3K/AKT, GSK-3ß/ß-CATENIN AND AC/CAMP/PKA/CREB SIGNALING

E. Gargantini[1], A. Baragli[1], F. Settanni[1], M. Taliano[1], E. Ghigo[1], R. Granata[1]

[1]Divisione di Endocrinologia, Diabetologia e Metabolismo, Dipartimento di Scienze Mediche, Scuola di Medicina, Università degli Studi di Torino - Torino

Obestatin is a recently discovered peptide encoded by the ghrelin gene. Obestatin was initially claimed to bind to the orphan receptor GPR39; however, this finding has been question and at present, obestatin receptor is still unknown. We have previously shown that obestatin exerts antiapoptotic effects in pancreatic β-cells and human pancreatic islets, through activation of survival pathways and interaction with the glucagon-like peptide 1 receptor (GLP-1R). Besides peripheral actions, obestatin displays central effects, such as regulation of sleep and mnemonic functions. Hippocampal neurogenesis, which is essential for mnemonic functions and learning, consists of progenitor cell proliferation and differentiation. Interestingly, both ghrelin and the synthetic peptidyl growth hormone secretagogue hexarelin have been previously shown to stimulate proliferation of adult rat hippocampal progenitor cells (AHPs). Here, we investigated obestatin effects on proliferation and apoptosis of AHPs and the underlying signalling pathways. Cell survival was assessed by MTT assay, cell proliferation by 5-bromo-2-deoxyuridine (BrdU) incorporation, and apoptosis through caspase-3 activity and Bcl-2 expression. Obestatin increased cell proliferation and survival and reduced apoptosis of AHPs that were cultured in growth factor-deprived medium. These effects involved increased activity of phosphatidylinositol 3-kinase (PI3K)/Akt, GSK-3β/β-catenin and AC/cAMP/PKA/CREB pathway, as demonstrated by immunofluorescence, Western blot analysis and use of specific inhibitors. Furthermore, the specific antagonist of GLP-1R exendin-(9-39) abolished obestatin-induced proliferation and survival. Finally, the agonist of GLP-1R exendin-4, exendin-(9-39) and unlabelled obestatin displaced binding of fluorescent-obestatin in AHPs. These data suggest an involvement of GLP-1R in obestatin effects. In conclusion, obestatin promotes survival and proliferation and inhibits apoptosis of AHPs through activation of pathways which play a key role in neuroprotection. In addition, these results suggest a possible role of obestatin in neuronal precursor cell protection and candidate this peptide as potential therapeutic molecule in conditions such as hippocampal damage.

OC19

MIR-26A AND CELL CYCLE CONTROL IN ACTH SECRETING PITUITARY ADENOMA

E. Gentilin[1], F. Tagliati[1], C. Filieri[1], D. Molè[1], M. Minoia[1], M. R. Ambrosio[1], E. degli Uberti[1], M. C. Zatelli[1]

[1]Scienze Mediche - Ferrara

MicroRNAs (miRNAs) have several physiological functions, but have been also implicated in human neoplastic initiation and progression. We previously demonstrated that 30 miRNAs are differentially expressed in normal human pituitary as compared to pituitary adenomas. However, the most of miRNAs target genes remain unknown, hindering the understanding of miRNAs contribution to pituitary tumorigenesis. In order to clarify this issue, we investigated the expression of 10 miRNAs among those that were found as mostly dysregulated in human pituitary adenoma tissues in the settings of a murine ACTH-secreting pituitary adenoma cell line, AtT20/D16v-F2 cells. Among these, we selected the most dysregulated mouse miRNA and searched for miRNA targets and their biological function. We found that AtT20/D16v-F2 cells have a specific miRNAs expression profile and that miR-26a is the most dysregulated miRNA. The latter is overexpressed in human pituitary adenomas and can control cell viability in the "in vitro" model without involving caspase 3/7 mediated apoptosis. We here demonstrate that protein kinase C delta (PRKCD) is a direct target of miR-26a and that miR26a inhibition delays cell cycle in G1 phase. This effect involves downregulation of cyclin E and cyclin A expression via PRKCD modulation. In conclusion, miR-26a and related pathways, such as PRKCD, play an important role in cell cycle control of ACTH pituitary cells, opening new therapeutic possibilities for the treatment of persistent/recurrent Cushing's disease.

OC20

ISOLATION AND CHARACTERIZATION OF MESENCHYMAL CELLS FROM PITUITARY TUMOURS

M. Orciani[1], G. Appolloni[1], G. Arnaldi[1], L. Trementino[1], R. A. Ricciuti[2], A. Rychlicki[2], M. Boscaro[1], R. Di Primio[1]

[1]Scienze Cliniche e Molecolari - Ancona, [2]Neurochirurgia - Ancona

Pituitary adenomas are an important and frequently occurring form of intracranial tumor. Although, different molecular mechanisms are involved in pituitary tumorigenesis, the pathogenesis of these tumors is not fully characterized. In the past few years the introduction of the cancer stem cells (CSCs) notion opened new perspectives for the diagnosis and cure of solid tumors. The concept that a specific subpopulation of tumor cells possesses distinct stem cell properties implies that cancer stem cells arise as an intrinsic property of tumor biology and development. Such cells are proposed to persist in tumors as a distinct population and cause relapse and metastasis by giving rise to new tumors.

Therefore, development of specific therapies targeted at CSCs holds hope for improvement of survival and quality of life of cancer patients. Actually, no informations are available about stem cells and cancer stem cells on pituitary tumours.

This work depicts some essential features of stem cells isolated from human pituitary adenomas (2 non secreting pituitary macroadenomas and 1 GH-secreting pituitary macroadenoma). Three tumour samples were collected and cultured with a specific culture medium for mesenchymal cells isolation. Cells began to appear near to the explants after 7 days; adherent cells started to divide rapidly, reaching confluence within 7 more days, with a fibroblastoid morphology. For immunophenotyping, cells were stained with fluorescin isothiocyanate (FITC)-antibodies against selected markers. As expected, cells appeared to be strongly positive for CD73, CD90 and CD105 and negative for HLA-DR, CD14, CD19, CD34 and CD45, indicating a stem-like immunophenotype. In addition, the expression of Oct-4, Sox-2, Nanog and Klf-4 was tested by PCR. The densitometric analysis revealed a detectable expression of the selected genes, with no significant differences among the donors. Even if further studies are needed for the fully comprehension of the specific nature of these cells and on their role on tumour onset and maintenance, this study opens to the possibility of isolation of stem cells from pituitary tumour, allowing a molecular targeting of it.

OC21

CHARACTERIZATION OF A STABLE CELL LINE OF MOUSE FETAL HYPOTHALAMIC NEURAL STEM CELLS

A. Cariboni[1], L. Conti[1], V. Andrè[1], D. Aprile[1], J. Zasso[1], R. Maggi[1]

[1]Scienze Farmacologiche e Biomolecolari, CIRMAR, Università degli Studi di Milano - Milano

The mammalian hypothalamus is involved in regulating several physiological functions; many of these functions are exerted by the neuroendocrine system. The neuroendocrine hypothalamus contains two distinct subsystems, the parvicellular and magnocellular neuronal systems, however, the molecular pathways that mediate the development of such neurons are largely unknown. The study of neural stem cells (NSC) offers a useful model to investigate such mechanisms. Neurospheres of NSC from both fetal and adult rat hypothalami, able to differentiate into glia and neurons, have been recently isolated but the proportion of stem cells in neurosphere is low and they cannot directly observed and studied.

The present communication describes the setup and the characterization of a pure stable cell line of NSC from E12 fetal mouse hypothalamus. The cell line (named AC1) grows as a monolayer in continuous expansion, by symmetrical division, in a defined medium enriched in FGF-2 and EGF. AC1 cells express stemness (nestin, Sox-2 and Pax-6), neuronal, but not astrocytic, markers; moreover, the expression of hypothalamic patterning genes (Sim1, Sim2, Arnt2, Brn2) has been also confirmed. After prolonged expansion, they remain able to differentiate efficiently into neurons and astrocytes in vitro. In normal culture conditions, AC1 were found to express POMC and CRH; however, detectable transcripts for TRH, GHRH and somatostatin were evident after short-term induction of neuronal differentiation. The ability of AC1 cells to develop neuroendocrine lineages in vitro will help to elucidate the mechanisms involved in the specific differentiation of neurohormonal hypothalamic neurons as well as other physiological hypothalamic developmental processes. In perspective, AC1 cells, would offer a new valuable tool to develop future cellular approaches to neuroendocrine disorders (i.e., diabetes insipidus, obesity, Prader-Willi syndrome, etc.). *(granted by MIUR)*

OC22

EXPERIMENTAL MODELS OF PROSTATE AND BLADDER INFLAMMATION: GPR30/GPER1 AS A PARADIGM FOR TISSUE-SPECIFIC SEX STEROIDS TARGETING

P. Comeglio[1], L. Vignozzi[1], A. Morelli[1], I. Cellai[1], S. Filippi[1], E. Maneschi[1], F. Corcetto[1], C. Corno[1], M. Maggi[1]

[1]Experimental and Clinical Biomedical Sciences - Florence

In males, benign prostatic hyperplasia (BPH)/lower urinary tract symptoms (LUTS) has been associated to obesity, hypogonadism and metabolic syndrome (MetS). We previously described, in MetS animals, specific prostate and bladder alterations, including inflammation and tissue remodeling. These alterations were positively related to a low-testosterone and high-estrogen milieu. In addition to ERα and ERβ, also GPR30/GPER1 is able to mediate several estrogen non-genomic actions. Supplementing a subgroup of MetS rabbits with tamoxifen, a classical ERs antagonist and GPR30 agonist, we analyzed its effect on MetS-induced prostate and bladder alterations, and, using human BPH (hBPH) cells, we studied the effect of selective ERs compounds and GPER1 silencing on prostate inflammation. Sex steroid receptors were expressed in rabbit prostate and bladder, with ERα expression higher in the latter. MetS was associated with an increased ERα expression in both tissues, and specifically associated with GPER1 in the prostate. ERα, ERβ and PR receptors were upregulated in MetS rabbits bladder, where tamoxifen decreased ERα and PR expression, while further stimulating ERβ. Tamoxifen dosing decreased the MetS-induced overexpression of inflammatory- and tissue remodeling genes. In MetS rabbit prostate, sex steroid receptors, pro-inflammatory and pro-fibrotic genes were upregulated. However, tamoxifen did not affect them. In hBPH cells, we found a sex-steroid receptors distribution that recapitulates the one observed in rabbit prostate. 17β-estradiol significantly increased IL8 secretion, an effect blunted by co-treatment with the GPER1 antagonist G15. The GPER1 agonist G1 also induced a rise in IL8 secretion and the specific antagonist for the genomic estrogen receptors ICI even increased the stimulation of IL8 release. In vitro siRNA analysis of GPER1 effects on hBPH cells demonstrated that the stimulatory effect of the various compounds was reverted by the GPER1 silencing. In conclusion, GPER1 should be considered as the main mediator of estrogen action in prostate, whereas in bladder the mechanism appears to rely on ERα, as indicated by in vivo experiments with tamoxifen dosing in rabbits. The crucial function of GPER1 in prostate has been confirmed by in vitro experiments. Limiting the effects of the MetS-induced estrogen action via GPR30 could offer new perspectives in the management of BPH/LUTS, counteracting MetS-associated prostatic alterations.

OC23

HIGH FREQUENCY OF E2F1 COPY NUMBER VARIATION IN SEVERE OLIGOZOOSPERMIA AND NON-OBSTRUCTIVE AZOOSPERMIA

D. Zuccarello[1], S. Ferrari[1], M. Menegazzo[1], A. Garolla[1], A. Ferlin[1], C. Foresta[1]

[1]University of Padova, Department of Molecular Medicine & Centre for Human Reproduction Pathology - Padova

Mice lacking or overexpressing E2F1 (E2 Transcription Factor 1) have testicular atrophy with severe loss of spermatogonia, probably due to a disruption of the inter-Sertoli tight junctions (TJs) barrier. Here we tested the hypothesis that alterations of E2F1 gene could be involved the pathogenesis of male infertility. Since the changes of expression of E2F1 seems to be important for the pathogenesis of spermatogenic impairment in mice, we analyzed the number of the copies (Copy Number Variations, CNV) of E2F1 in 43 men affected by idiopathic severe oligozoospermia (SO, sperm count < 5 million/mL), 104 subject affected by Sertoli cell only syndrome (SCOS), and 107 fertile normozoospermic men. CNVs has been analysed by quantitative real-time PCR using the TaqMan Copy Number Assay (Life Technologies). We found a frequency of CNV of 30% in SO (13/43) and of 24% in SCOS (25/104) patients compared to 10% of controls (11/107) (p=0.003, OR 3.782, 95% CI 1.408-10.245; p=0.008, OR 2.762, 95% CI 1.208-6.411, respectively). In particular, only duplication or triplication-CNV (3 or 4 copies of the gene) was statistically more frequent in cases than in controls (30% in SO, 20% in SCOS, 5% in controls). By real-time PCR and western blotting we showed that deletion or duplication CNVs influenced also the total amount of RNA and correspondent produced protein. Moreover, by immunofluorescence we demonstrated the presence of E2F1 protein in all the cellular steps of spermatogenesis except in mature spermatozoa. Our results showed for the first time a high prevalence of alterations in E2F1 gene in SO and SCOS, suggesting an important role of this gene in normal spermatogenesis. In conclusion, E2F1 overexpression due to CNV seems to have a key role in the pathogenesis of SO and SCOS, acting both on mechanical and functional interaction of Sertoli with the cells of spermatogenesis, and on apoptotic mechanisms, finally leading to complete clear of these cells in the testis.

OC24

COMPLETE AROMATASE DEFICIENCY IN FOUR ADULT MEN: DETECTION OF A NOVEL MUTATION AND TWO KNOWN MUTATIONS IN THE CYP19A1 GENE

E. Pignatti[1], K. Ajlouni[2], N. Khawaja[2], K. Unluhizarci[3], E. Kartal[4], C. Carani[1], M. Marino[1], M. Simoni[1], E. Vighi[1], V. Rochira[1]

[1]Dipartimento di Scienze Biomediche, Metaboliche e Neuroscienze, Unimore - Modena, [2]National Center for Diabetes, Endocrinology and Genetics - Amman, Giordania, [3]Department of Endocrinology - Kayseri, Turchia, [4]Department of Endocrinology and Metabolisim Disease - Ýzmir, Turchia

INTRODUCTION. At present, only eight men with loss-of-function mutations in the *CYP19A1* gene have been described. We report the genetic study of four adult men with undetectable serum estrogens, unfused epiphyses, eunuchoid skeletal proportions, continuing linear growth, tall stature, *genu valgum*, osteoporosis, obesity and *achantosis nigricans*. Patient 1 (26-yrs/182cm) and 2 (28-y/187cm) are from Turkey, Patient 3 (44-yrs/185cm) and 4 (29-yrs/197cm) are two brothers from Jordan. All patients had a history of consanguinity. **METHODS.** All coding exons with their flanking intronic sequences of *CYP19A1* gene, amplified by PCR, were sequenced and compared with known human *CYP19A1* gene sequences. **RESULTS.** Patient 1 was homozygous for a point mutation in the first nucleotide of intron 3 (IVS3+1G>T); Patient 2 was homozygous for a point mutation (c.1124 G>A) in exon IX resulting in protein missense mutation p.R375H. The two brothers (Patients 3 and 4) had a homozygous point mutation in exon IV (c.434 G>A) leading to Arg to Gln substitution (p.R115Q). All patients had impaired glucose tolerance, Patient 3 was diabetic, Patient 2 had a history of three forearm bone fractures after minimal trauma, Patient 1, 3, and 4 had impaired liver function. Patient 1 had documented GH-deficiency, all other patients had no evidence of GH hypersecretion. **CONCLUSIONS.** The description of these new four aromatase-deficient men confirms the detrimental effects of congenital estrogen deficiency on glucose, liver and bone metabolism. The p.R115Q mutation (Patients 3 and 4) is new: it probably leads to protein structure distortion. The other two known mutations are found in homozygosis for the first time. Clinical evidence of osteoporotic fractures is described for the first time and further emphasizes estrogen role on bone health in men and the need for fracture prevention in these patients. Finally, tall stature with concomitant GH-deficiency or with low to normal GH secretion depends on unfused epiphyses that allows bone elongation resulting in eunuchoid skeletal proportions.

OC25

RECURRENT X CHROMOSOME-LINKED DELETIONS: DISCOVERY OF NEW GENETIC FACTORS IN MALE INFERTILITY

C. Krausz[1], D. Lo Giacco[2], C. Chianese[1], E. Ars[2], E. Ruiz-Castané[3], G. Forti[4]

[1]Andrology Unit, Department of Biomedical, Experimental and Clinical Sciences, University of Florence - Florence, [2]Molecular Biology Laboratory, Fundació Puigvert - Barcelona, [3]Andrology Service, Fundació Puigvert - Barcelona, [4]Endocrine Unit, Department of Biomedical, Experimental and Clinical Sciences, University of Florence - Florence

Introduction Up to now, only Y-linked CNVs have been demonstrated to contribute to spermatogenic impairment in humans, whereas whether the X chromosome contains *AZF*-like regions remains unknown. Through our first X-chromosome array-CGH screening we identified: i) a significantly higher number of deletions ("deletion-burden") in patients in respect to controls; ii) 3 recurrent deletions (frequency >1%) with potential clinical sgnificance. Here we aim to explore the role of such recurrent deletions in oligo/azoospermia. **Material and methods:** 3 deletions of interest, named CNVX1, CNVX2, and CNVX3, mapping to the Xq, were studied by a multi-step PCR method. The study populations included Italian and Spanish subjects and we analyzed: i) 494 cases and 492 controls (CNVX1); ii) 591 cases and 591 controls (CNVX2); iii) 359 cases and 391 controls (CNVX3). **Results:** All 3 deletions were found with a significantly higher frequency in patients than controls. CNVX1: 6,7% in patients and 4,0% in controls (p<0,05; OR:1,643, 95%CI:0,956-2,823); CNVX3: 4,2% of patients and 1,5% of controls (p<0,05; OP: 2,723; 95%CI:1,068-6,942); CNVX2: exclusively found in patients (1,2%). The flanking regions does not contain Segmental Duplications, therefore NHEJ is the most likely mechanism for the formation of these deletions. The deletions do not remove directly coding genes, with the exception of CNVX2 which could affect the expression of one gene belonging to the cancer testis antigen family. **Conclusions:** Based on 1182 subjects, we discovered an AZF-like region on the X-chromosome (CNVX2), specific to men with impaired sperm production. The associated phenotype ranges from azoospermia to severe oligozoosperrmia and similarly to Y–linked deletions it may become a new diagnostic test.

OC26

RESULTS OF 2-YEARS ANDROLOGICAL SCREENING OF YOUNG MEN IN CAMPANIA

F. Lo Calzo[1], P. Vitale[1], M. Galdiero[1], R. S. Auriemma[1], C. Salzano[1], L. Granieri[1], D. Iacuaniello[1], A. Cozzolino[1], A. M. Isidori[2], A. Lenzi[2], A. Colao[1], R. Pivonello[1]

[1]Medicina Clinica e Chirurgia, Sezione di Endocrinologia, Università "Federico II" - Napoli, [2]DFM - Fisiopatologia Medica, Università "La Sapienza" - Roma

Background: In Italy since the abrogation of military service and its related medical examination, andrological screening is no longer conducted on a large scale. Therefore, a national campaign designed by the Sapienza University of Rome and conducted in collaboration with the Italian Society of Andrology and Sexuality Medicine (SIAMS) and the Ministry of Health has been started in order to perform andrological examination in young adult men. The screening was directed to male students aged 18 and over in their final year of secondary education. We report the main results of andrological screening of two years period (2010-2012) in a population of 18 years old young students of Campania.
Methods: The campaign was conducted in 12 schools of Campania. An anonymous 60-item questionnaire was completed by 755 students, among which 269 accepted to be subjected to an andrological medical examination on school.
Results: Only 134/755 (17.7%) of students had already performed an andrological examination: 330/755 (43.7%) of students didn't know the role of andrologist and only 127/755 (16.8%) of students received information about sexual and reproductive health by a physician. In 240/755 (32%) of students one or more risk factors for sexual and reproductive health (altered body weight, alcohol and substances abuse, smoking, high-risk sexual behaviour, previous cryptorchidism) was documented. Andrological disorders were found in 80/269 (29.7%) of students, and in 87.5% of them, it represented the first diagnosis. A grade II or higher varicocele was diagnosed in 112/269 (41%) of the students, being the first diagnosis in 91% of them. A phimosis was discovered in 10/269 (3.7%) of students. A low testicular volume, unilateral or bilateral, was found in 11/269 (4%) of students. Genital skin lesions were discovered in 8/269 (3%) of students.
Conclusions: The results of this study displayed a poor familiarity of the students with the figure of the andrologist and a frequent undiagnosed andrological diseases. This underlines the importance of a permanent screening in order to increase awareness and knowledge about male reproductive health and to prevent reproductive disorders.

OC27

MITOCHONDRIAL DEPOLARIZATION IS INVOLVED IN THE INHIBITION OF ANGIOGENIC CELLS FROM HEALTHY MEN BY HUMAN SERUM FROM MEN WITH ERECTILE DYSFUNCTION

S. D'Andrea[1], A. D'Angeli[1], A. Barbonetti[1], F. Francavilla[1], S. Francavilla[1]

[1]Univ. of L'Aquila, Department of Life, Health and Environment Sciences - L'Aquila

Introduction: Soluble factors in the serum of men with Erectile dysfunction (ED) and vascular risk factors (VRFs) inhibited mononuclear circulating cells (MNCs) of healthy men to differentiate circulating angiogenic cells (CACs) putatively involved in endothelial damage repair. We explored potential involved molecular mechanisms. **Material and Methods:** MNCs from healthy men were cultured in standard conditions. After 4 days adherent cells were maintained in culture for further 3 days with or without hrTNF-α (10 ng/ml), with or without a competitive anti-type 1 TNF-α receptor (TNFR1) mAb (100 mg/ml). Other samples were cultured with serum of 10 healthy men or with serum of 10 patients with ED and VRFs. CACs were identified by uptake of acetylated low-density lipoprotein and binding of Ulex-lectin. Mitochondrial membrane potential (ΔΨm) was assessed at flow cytometry with JC-1, which emits orange or green fluorescence in the presence of high or low DYm, respectively. Caspase activation was evaluated at flow cytometry using permeable FITC-conjugated peptides (IETD-FMK, LEHD-FMK and DEVD-FMK), which bind to the activated caspase-8, -9 and -3, respectively, in apoptotic cells. **Results:** *Effect of TNF-α on CACs from healthy men.* The mean number of CACs was significantly reduced in presence of TNF-α compared to medium (p<0.05), which significantly decreased cellular ΔΨm compared to control medium (p<0.05). ΔΨm suppression was associated to increased percentages of cells with activated caspase-8, caspase-9 and caspase-3 compared to controls (p<0.05). All effects of TNF-α were prevented by the anti-hTNFR1 mAb. *Effect of serum of men with ED on CACs from healthy men.* The mean number of CACs was significantly reduced after culturing MNCs with human serum from ED patients compared to serum from healthy men (p<0,017). The inhibition was not prevented after incubation with anti-hTNFR1 mAb, and was associated to ΔΨm suppression, compared to control medium (p<0,02). **Conclusion:** Increased circulating levels of TNF-α, reported in men with ED, could adversely impact CACs differentiation from MNCs by triggering both death-receptor (Caspase-8 activation) and mitochondrial pathway (Caspase-9 activation). TNF-α inhibition on CACs was associated to ΔΨm suppression. Mitochondrial depolarization was also involved in the inhibitory control on CACs differentiation by human serum from ED men and this effect was not reverted by Ab-antiTNFR1. This suggests that TNF-α isn't the main soluble factor involved in inhibition of CACs by serum of men with ED and vascular risk factors.

OC28

SOMATOSTATIN RECEPTOR SUB-CELLULAR LOCALIZATION AND DIMERIZATION AFFECT SOMATOSTATIN ANALOG-DRIVEN ANTIPROLIFERATIVE EFFECT IN PROSTATE CANCER CELLS

M. Arvigo[1], M. Ruscica[2], L. Steffani[2], M. Albertelli[3], F. Gatto[3], C. Macchi[2], P. Ameri[3], M. Culler[4], F. Minuto[1], P. Magni[2], D. Ferone[1]

[1]Dip. di Med. Interna e Specialità Mediche e Centro di Eccellenza per le Ricerche Biomediche, Università di Genova - Genova, [2]Dip. di Sc. Farmaceutiche e Biomolecolari, Università di Milano - Milano, [3]Dip. di Med. Interna e Specialità Mediche, Università di Genova - Genova, [4]Biomeasure Incorporated/IPSEN - Milford, MA, USA

In recent years, somatostatin (SRIF) and somatostatin receptors (ssts) have been highlighted as critical regulators involved in the progression and neuroendocrine differentiation of human prostate cancer (PCa). However, conflicting results have been reported in the literature on sst heterogeneity, specific cell localization and ligand-induced receptor activation. Clinical studies testing the currently available SSAs on advanced hormone-refractory PCa did not show convincing results probably due to the prevalent sst2 specificity of these drugs. Aim of this study was to evaluate in two androgen-independent human PCa cell lines DU-145 and PC-3 cells: a) the sst membrane and sub-cellular distribution; b) the effects of new mono- and bispecific SRIF analogs (SSAs) on cell proliferation; c) the constitutive and SSAs-driven sst dimerization; d) the correlation between SSAs-induced antiproliferative effect and membrane receptor dimerization. DU-145 and PC-3 cells express all SRIF receptors (sst1-5) on cell membrane. A sub-cellular organelle separation showed that sst1 and sst2 were mainly expressed in the microsomal fraction while sst5 in the lysosomal one. In both cell lines, treatment with BIM-23244 (sst2/5) and BIM-23926 (sst1) analogs were more effective in inhibiting cell proliferation (-30%, dose-range 10-10-10-6 M), compared to BIM-23120 (sst2), BIM-23206 (sst5) and BIM-23704 (sst1/sst2). Moreover, treatment with BIM-23704 (sst1/sst2) and BIM-23244 (sst2/ss5) increased the amount of pre-constituted sst1/sst2 and sst2/sst5 membrane dimers, respectively. In both cell lines, a significant correlation was found between BIM-23244-induced sst2/sst5 dimeration and inhibition of cell proliferation. Conversely, only in PC-3 cells, BIM-23704-induced sst1/sst2 dimerization significantly correlated with the inhibition of cell proliferation. In conclusion, our data highlight sst1 and sst2/sst5 dimer receptors as possible preferential targets for the development of new peptide-based drugs for the treatment of hormone-refractory.

OC29

SOMATIC MUTATIONS IN THE KCNJ5 GENE AFFECT CARDIAC REMODELLING AND REGRESSION OF LEFT VENTRICULAR HYPERTROPHY IN PRIMARY ALDOSTERONISM DUE TO APA

T. M. Seccia[1], M. V. Cicala[2], L. Lenzini[1], C. Maniero[1], C. Letizia[1], B. Rubin[1], R. Pezzani[2], F. Mantero[2], G. Rossi[1]

[1]dipartimento di Medicina, Clinica Medica 4 - Padova, [2]Dipartimento di Medicina, Endocrinologia - Padova

Context. Considering the detrimental cardiovascular (CV) effects of aldosterone, APA patients carrying somatic mutations in the selectivity filter of KCNJ5 K+ channel, which were found to be associated with higher plasma aldosterone secretion from aldosterone producing adenomas (APA), might develop a more prominent CV damage than those without such mutations.

Objective. To test this hypothesis we compared the echocardiographic changes between APA patients with(mutAPA) and without (wtAPA) the G151R, G151E, L168R, and T158A mutations.

Design. From a cohort of 250 consecutive PA patients, we identified 170 patients who had an unequivocal diagnosis of APA by the four corners criteria, and high-quality echocardiographic data. Of them 106 who had comprehensive clinical and KCNJ5 sequencing information and outcome data at long-term follow-up, were analyzed using the rest as controls.

Results. The KCNJ5 mutations were about two-fold more prevalent in women than in men and overall involved 18.8% of the APA. At baseline the mutAPA patients were similar to the wtAPA patients for systolic and diastolic blood pressure and need for antihypertensive medications, in spite of higher plasma aldosterone (PAC,70.0 (41.9 -98.1) ng/dl vs 44.5 (38.2 - 50.7), < 0.0001), aldosterone-renin-ratio (ARR, 513 (381 - 1571)ng/dl/ng/ml/h vs169 (72 - 266), < 0.0001), and left ventricular mass index (LVMI, 60±7.4 mg/h2.7vs 49±3.6, p=0.004).

At long-term follow-up after adrenalectomy the mutAPA showed a greater fall of LVMI than the wtAPA (14.9±3.9 mg/m2vs 6.4±1.2, p=0.007), despite a similar fall of BP and a similar normalization of PAC and ARR.

Conclusions. In APA patients the occurrence of the somatic KCNJ5 mutations implies higher PAC, ARR, and LVMI, and a greater decrease of LVMI after adrenalectomy than in the wild type APA patients. However, the presence of these mutations did not compromise the chances of being cured from the hyperaldosteronism and the high blood pressure.

OC30

A LONG-TERM FOLLOW UP IS NEEDED IN PATIENTS WITH ADRENAL INCIDENTALOMAS AND SUBCLINICAL HYPERCORTISOLISM

V. Morelli[1], G. Reimondo[2], R. Giordano[3], S. Della Casa[4], C. Policola[4], S. Palmieri[1], A. S. Salcuni[5], A. Dolci[6], M. Arosio[6], B. Ambrosi[7], A. Scillitani[5], E. Ghigo[8], P. Beck-Peccoz[1], I. Chiodini[1], M. Terzolo[2]

[1]Endocrinology and Diabetology Unit, Fondazione IRCCS Cà Granda, University of Milan - Milan, [2]Division of Internal Medicine I, San Luigi Hospital, University of Turin - Orbassano, [3]Dpt of Biological and Clinical Sciences, University of Turin - Turin, [4]Endocrinology Unit, Università Cattolica - Rome, [5]Endocrinology Unit, Casa Sollievo della Sofferenza Hospital, IRCCS - San Giovanni Rotondo, [6]Endocrine Disease and Diabetology Unit, San Giuseppe Hospital, Multimedica Group, University of Milan - Milan, [7]Endocrinology and Diabetology Unit, IRCCS Policlinico San Donato, University of Milan - San Donato Milanese, [8]Division of Endocrinology, Diabetology and Metabolism, University of Turin - Turin

The need of a long-term follow-up in adrenal incidentalomas (AI) is debated and data on cardiovascular events (CVE) are lacking. In this retrospective study all patients referred to 7 Italian Endocrine Units for AI, without signs of hypercortisolism at baseline and with a ≥5 yrs follow-up (82±32 months, range 60-286), were enrolled. In 196 patients, aged 59±10 yrs (range 25-79), we evaluated the changes in weight, glucose and lipid metabolism, blood pressure control and the occurrence of CVE. Patients were classified as affected with subclinical hypercortisolism (SH) in the presence of cortisol after 1–mg dexamethasone suppression (1-mgDST) test >5 µg/dl or ≥2 parameters out of low ACTH, increased urinary free cortisol and 1-mgDST >3 µg/dl. At baseline SH was found in the 10.7% of patients. The prevalence of obesity, diabetes mellitus, dyslipidemia and arterial hypertension (29%, 20%, 43% and 56%, respectively) was similar between patients with (SH+) and without SH (SH-). At baseline the prevalence of CVE in SH+ patients was higher than in SH- ones (24% vs 7%), regardless of age (OR 3.8, 95%CI 1.1-12.4, P<0.05). At the end of follow-up a new diagnosis of SH was made in the 7.1% of patients. The adenoma size (baseline 2.3±0.8 cm) increased >2.5 cm in the 2.0% of cases. The glucose and lipid metabolism, blood pressure and weight control worsened in the 24.5%, 16%, 33% and 15% of patients, respectively. Moreover, the SH persistence/appearance was significantly associated with the worsening of ≥2 of the metabolic parameters (P=0.005) and with the occurrence of new CVE (P=0.035). In conclusion, in AI patients a long-term follow-up is recommended for the risk of diameter increase and of SH development. SH patients are at risk of worsening of the metabolic control and, importantly, of CVE.

OC31

C-MYC MODULATION AFFECTS DRUG RESPONSIVITY IN ADRENOCORTICAL CANCER CELLS

A. Stigliano[1], L. Cerquetti[1], C. Sampaoli[1], P. Fiore[1], B. Bucci[2], P. Lardo[1], V. Toscano[1]

[1]Medicina Clinica e Molecolare - Facoltà di Medicina e Psicologia "Sapienza" Università di Roma - Roma, [2]Centro Ricerca Ospedale San Pietro Fatebenefratelli - Roma

c-myc gene amplification, maintaining the transformed state, appears to underlie c-Myc over-expression in many cases of carcinoma and is associated with more aggressive tumors therefore linked to poor prognosis.

We examined the c-Myc protein expression in adrenocortical cancer (ACC) cells to find out the possible role of this protein in the neoplasm, its response to therapy and to determine whether the protein is a prognostic factor in patient with ACC.

H295R and SW-13 ACC cell lines were treated with different drugs, taxol, mitotane and tamoxifen considered in this study. Taxol is highly efficacious in the treatment of a broad spectrum of neoplastic diseases, mitotane is an adrenal-specific agent employed in the treatment of ACC. Finally, tamoxifen is the most common adjuvant treatment for hormone receptor-positive cancer, it acts by blocking estrogen binding to its receptor, however, data on its effectiveness are limited in ACC.

c-Myc over-expressing cell clones was achieved by transfecting H295R cell line with the pcDNA3-hMyc plasmid expressing the full-lengh MYC coding sequence. Moreover SW-13 cell line were transfected with siRNA oligonucleotides for c-myc. Cell cycle analysis were evaluated by flow cytometry. c-Myc, cell cycle molecules and apoptotic markers were evaluated by Western blot. High c-Myc level was present in poor differentiated SW-13 cell line, and low level was present in well differentiated H295R cell line. Differential rate of drugs sensitivity was revealed after c-Myc modulation, in fact different drug concentrations were required to induce a significant antineoplastic effects in both cell lines. This event occurred early in the SW-13 cells compared to H295R. Interestingly, we observed in H295R cell line the overexpression of c-Myc protein rendered cells more prone to growth inhibition to taxol exposure. Moreover silencing of c-myc mRNA prevented taxol-induced apoptosis.

In conclusion c-Myc plays a central role for some treatment in proliferation control in ACC cell lines. The current study directly demonstrate that c-Myc could be considered as a marker predicting response to chemiotherapeutic agents in ACC cell lines.

OC32

RAPID CHANGE IN WEIGHT, WAIST CIRCUMFERENCE AND QUALITY OF LIFE AFTER SHORT-TERM HYDROCORTISONE MODIFIED RELEASE IN ADULTS WITH ADRENAL INSUFFICIENCY

C. Simeoli[1], A. Cozzolino[1], D. Iacuaniello[1], A. Albano[1], C. Di Somma[1], A. Colao[1], R. Pivonello[1]

[1]Department of Clinical Medicine and Surgery - Section of Endocrinology, Federico II University - Naples, Italy

Background: Patients with Adrenal Insufficiency (AI) need a life-long glucocorticoid (GC) treatment. An increased cortisol exposure, due to multiple daily doses, is a known risk factor for weight gain and abdominal obesity. Moreover, AI patients frequently experienced fatigue showing impaired Quality of life (QoL), and reported difficulties remembering to take doses, particularly the midday and afternoon doses, worsening treatment compliance.

Aim: The aim of this study was to evaluate in AI patients the impact of one-three months of hydrocortisone modified release tablets (HMRT) treatment, a new hydrocortisone formulation, given once daily in the morning, aimed at achieve a more physiological exposure-time cortisol profile.

Materials and Methods: Twelve patients (9 F, 3 M, 22-41 yrs) affected by AI, 10 (8F, 2M) with congenital adrenal hyperplasia and 2 with secondary AI (1F, 1M), 10 chronically treated with conventional hydrocortisone treatment (15-35 mg/die) and 2 with prednisone (5-10 mg/die), were switched on HMRT (Plenadren, Viropharma) and were evaluated before and after 1-3 months of HMRT treatment (15-40 mg/die). Body weight, Body Mass Index (BMI), waist circumference (WC) and 5 items of ADDIQoL questionnaire, aimed to assess general health perception, vitality, working ability, depression and body pain perception, were evaluated.

Results: After 1-3 months of HMRT treatment a significant decrease in body weigh (p= 0.021), BMI (p=0.027) and WC (p= 0.002) was observed. Six patients (50%) improved their general health perception, 3 (25%) their vitality, and 4 (33%) their working ability. Three patients (25%) decreased their depression and 3 (25%) their body pain perception. Furthermore, five patients (33%) showed a clear improvement in treatment compliance.

Conclusion: The results of this preliminary study demonstrated that the switch from standard GCs to HMRT induces a significant and very rapid change in body weight, BMI and WC as well as QoL and treatment compliance after a short period of treatment. This data support the concept that the use of a more physiological cortisol replacement is highly beneficial for patients with AI.

OC33

INVESTIGATION OF ß-CATENIN, N-CADHERIN AND E-CADHERIN EXPRESSION IN ADRENOCORTICAL TUMORS

B. Rubin[1], R. Pezzani[1], M. V. Cicala[1], M. Iacobone[2], A. Fassina[3], F. Mantero[1]

[1]Dipartimento di Medicina. U.O. di Endocrinologia. Università degli Studi di Padova - Padova, [2]Dipartimento di Scienze Chirurgiche Oncologiche e Gastroenterologiche. U.O. di Chirurgia Endocrina. Università degli Studi di Padova - Padova, [3]Dipartimento di Medicina. U.O. di Citopatologia e Patologia Chirurgica. Università degli Studi di Padova - Padova

Background: Adrenocortical tumors (ACT) are classified as adenomas (ACA) or carcinomas (ACC). β-catenin constitutive activation is a frequent alteration in benign and malignant ACT. E-cadherin was discovered as a protein associated with β-catenin which plays a crucial role in cadherin-mediated cell adhesion. N-cadherin seems to be involved in the development of malignant ACT, but information regarding expression of N-cadherin or E-cadherin in ACT is very limited.

Aim: to evaluate the expression of N-cadherin, E-cadherin and β-catenin in ACT and in ACC cell line models (H295R and SW13).

Methods: We analyzed differential expression of β-catenin, N-cadherin and E-cadherin by immunohistochemistry and by quantitative Real time-PCR in 71 sporadic ACT. This study included 8 normal adrenal cortex samples (NA), 24 ACC, 18 aldosteronomas (APA), 23 cortisol producing adenomas (CPA) and 6 non-secreting incidentalomas (NSA).

Results**:** Real-time PCR: Compared with NA, β-catenin was over-expressed in 50% of ACC (12/24) and 51% of ACA (24/47); N-cadherin was down-regulated in 75% of ACC (18/24) and in 60% of ACA (28/47).

IHC: 47% of ACC (7/15) and 33% of ACA (11/33) presented increased cytoplasmic and/or nuclear β-catenin accumulation; furthermore 100% of ACC (15/15) presented down-expression of N-cadherin and 18 of 33 ACA (55%) were down-regulated. We did not find expression of E-cadherin in any ACT. Interestingly, Spearman analysis showed correlation between β-catenin and N-cadherin expression (ACC vs ACA).

Conclusion: Our preliminary data suggest that β-catenin overexpression together with the aberrant expression of N-cadherin may participate to progression of ACT. Identification of these and other differentially expressed genes may enhance our understanding of the molecular biology of ACT development, and may contribute in creating new diagnostic and prognostic tools.

OC34

THE ADIPOSE VISCERAL DYSFUNCTION PLAYS AN IMPORTANT ROLE IN DIABETES IN CUSHING DISEASE.

V. Guarnotta[1], M. C. Amato[1], A. Ciresi[1], C. Simeoli[2], R. Pivonello[2], A. M. Colao[2], C. Giordano[1]

[1]Section of Endocrinology, Diabetology and Metabolism (Di.Bi.M.I.S) - Palermo, [2]Section of Endocrinology - Napoli

Background: Cushing disease (CD) is associated with increased morbidity and mortality caused by cardiometabolic alterations. Visceral Adiposity Index (VAI) expresses impaired adipose distribution and function which are related to the cardiometabolic risk. **Aim**: To evaluate in a cohort of CD patients the correlation between VAI and other parameters, such as gender, etiology, age, cortisol values measured in the morning (8 am) and at the midnight, urinary free cortisol (24h sample of urine, average of three samples) and glucose tolerance as normal glucose tolerance (NGT), impaired fasting glucose (IFG), impaired glucose tolerance (IGT), IFG+IGT and diabetes mellitus. **Materials and methods**: We performed a retrospective study in 140 CD patients consecutively afferent in outpatients clinic of the Universities of Naples and Palermo. Patients were divided by VAI tertiles and trend analysis was evaluated. **Results**: 27 men and 113 women, mean age of 40.98±16,61 years and BMI of 30.86 ± 6.01 Kg/m2 were studied. 62.7% of patients had a metabolic syndrome, 30.7% diabetes mellitus, 5.7% IFG, 12.1% IGT and 0.7% IFG+IGT. Among all parameters evaluated, only the midnight cortisol showed a significant increasing trend according to VAI tertiles (I tertile 172.57±77.24, II tertile 168.40±73.67, III tertile 227.06±119.42; p=0.045). Significant correlations between HOMA IR increase and VAI tertiles (I tertile 2.16±1.18, II tertile 3.13±1.49, III tertile 2.81±1.37 p=0.020), HbA1c increase and VAI tertiles (I tertile 5.87±0.84%, II tertile 6.40±1.23%, III tertile 6.60±1.19% p=0.018) were found. No significant trend for HOMA-b was observed. **Conclusions: CD w**omen have a higher cardiometabolic risk than men and this risk becomes more higher more older is the patient. Therefore these patients have a condition of visceral adiposity dysfunction that contributes to decrease of insulin sensitivity, but not at a reduction in beta-cell function, like the typical diabetes mellitus at onset. Thus, visceral fat dysfunction seems to be a key factor not only in favouring diabetes mellitus onset but also in worsening glycaemic control in Cushing diabetic patients.

OC35

HYPOTHALAMIC-PITUITARY-ADRENAL AXIS (HPA) SUPPRESSION FOLLOWING AN INTRA-BURSAL INJECTION OF TWO DIFFERENT STEROIDS

F. Guaraldi[1], M. Battaglia[2], E. Castiello[3], M. Leporati[4], D. Gori[5], S. Grottoli[1], F. Settanni[1], A. Sudanese[3], E. Ghigo[1], E. Arvat[1]

[1]Dip. Scienze Mediche - Torino, [2]U.O. Ecografia e Radiologia, IOR - Bologna, [3]U.O. Ortopedia e Traumatologia, IOR - Bologna, [4]Centro Regionale Antidoping - Orbassano (TO), [5]Dip. Igiene e Medicina Preventiva - Bologna

Intra-articular steroids are frequently injected to treat pain and inflammation associated to articular disorders and orthopedic procedures. HPA axis' suppression is a major but poorly considered side effect. Materials and methods: blind, randomized, case-control study. 40 patients with rotator cuff painful calcific tendonitis underwent percutaneous ultrasound-guided treatment. Then, they randomly received (20 pts per group) an intra-bursal (IB) injection of 40 mg metilprednisolone acetate (MA) (group A) or triamcinolone acetonide (TA) (group B). The morning before (T0) and 1 (T1), 7 (T2), 15 (T3), 30 (T4) and 45 (T5) days after treatment we evaluated in all patients plasmatic cortisol and ACTH (RIA), urinary cortisol and MA/TA (LC-MS/MC). Results: ACTH, plasmatic and urinary cortisol were in the normal range and similar in both groups at T0. A significant ($p<0.00001$) and similar decrease (mean ± SD, mean difference 95% CI) of plasmatic ACTH (Group A 9.1 ± 9.1 vs 23.77 ± 11.29; 15±7 pg/ml. Group B 5.49 ± 6.96 vs 29.2 ± 16.14; 24±8 pg/ml) and cortisol (Group A 45.94 ± 50.66 vs 182.55 ± 59.67; 136±36 ng/ml. Group B 36.97 ± 52.9 vs 179.71 ± 58.41; 143±35 ng/ml) and urinary cortisol (Group A 7.23±8.1 vs ±24±11; -16.77 ±5.57 µg/die. Group B 12.83±20.88 vs 24±9; -11.17 ±8.27 µg/die) was observed at T1 in both groups (mean under min. ref. value in >85% of patients). No significant differences were found for ACTH after T2 vs T0; cortisol was similar to T0 after T2 in group A, and after T3 in group B; urinary cortisol was still lower ($p<0.001$) up to T4 in group B (13.88±8.79; -10.12±5.79 mcg/die) and up to T5 in group A (14.01±10.15; -9.99±5.9 mcg/die). Drugs' urinary levels decreased sharply from T1 to T2 (MA: 23.15±14.51 vs 5.37±4.38 ng/ml. TA: 12.33 ± 7.53 vs 4.56 ± 3.08 ng/ml), then more gradually (detectable in ~10% of pts at T5). Conclusions: a single IB injection of 40 mg MA/TA is sufficient to severely suppress HPA axis' function up to 45 days, with no significant differences between drugs, and a trend of cortisol production converse but parallel to drug's elimination. For this reason, treated patients deserve a particular attention and replacement therapy, especially under stress conditions.

OC36

CORRELATES OF BONE STRENGTH IN DIABETES: THE STUDY ON THE ASSESSMENT OF DETERMINANTS OF MUSCLE AND BONE STRENGTH ABNORMALITIES IN DIABETES (SAMBA)

F. Conti[1], S. Balducci[1], L. Pugliese[1], L. Salvi[1], C. Maccora[1], A. Bazuro[1], V. Della Chiara[1], G. Salerno[1], A. Nicolucci[2], G. Pugliese[1]

[1]Dip. Medicina Clinica e Molecolare, Università La Sapienza - Roma, [2]Dip. Farmacologia Clinica ed Epidemiologia, Consorzio Mario Negri Sud - S. Maria Imbaro

Diabetes mellitus is associated with an increased risk of bone fractures, which seems to be dependent on mechanisms at least partly differing from those associated with senile or post-menopausal osteoporosis. Moreover, risk of fractures is higher in subjects with type 1 diabetes (T1D) than in those with type 2 diabetes (T2D), who have normal or even increased bone mineral density. This paradox might be explained by reduced bone quality despite normal/higher density due to increased mechanic load. This study was aimed at assessing bone strength correlates in subjects with T1D and T2D from the Study on the Assessment of Determinants of Muscle and Bone Strength Abnormalities in Diabetes (SAMBA), encompassing a wide-range of peripheral nerve function and various degrees of micro and macrovascular complications. Four-hundred consecutive patients were examined by quantitative ultrasound (QUS). Univariate and multivariate regression analyses were applied to identify correlates of QUS parameters. Broadband ultrasound attenuation (BUA), speed of sound (SOS) and the quantitative ultrasound index (QUI) were higher in males (M) than in females (F) and similar in subjects with T1D and T2D (BUA: T1D M 79.3±22.5, F 64.7±25.1 -T2D M 78.1±19.0, F 62.0±21.0 dB/MHz; SOS: T1D M 1,557±38, F 1,542±39 – T2D M 1,551±33, F 1,523±130 m/s; QUI: T1D M 99,7±24.3, F 87.8±26.0-T2D M 96.9±20.7, F 84.0±22.0) At univariate analysis, these parameters were strongly associated with physical activity level, cardio-respiratory fitness, upper and lower body strength, BMI, waist, uric acid, triglycerides and, inversely, age and HDL cholesterol, but not HbA1c, blood pressure and LDL cholesterol. Among surrogate measures of complications, sural sensory nerve amplitude and heart rate response to cough test and standing, but not eGFR, albuminuria, intima-media thickness, ankle-brachial index and motor nerve parameters, correlated with bone strength. Multivariate analysis revealed that BUA, SOS and QUI were independently associated with age, male gender, BMI, and some sensory and autonomic nerve function parameters. When fat mass and fat free mass were included in the model in place of BMI, only the former remained as an independent correlate of bone strength. These data confirm that bone strength is related to central obesity and associated atherogenic dyslipidemia, but also indicate that peripheral nerve dysfunction, especially sensory and autonomic, may contribute to bone quality abnormalities and increased fracture risk in diabetes.

OC37

ROLE OF BONE MINERAL DENSITY IN PREDICTING MORPHOMETRIC VERTEBRAL FRACTURES IN PATIENTS WITH HIV INFECTION

T. Porcelli[1], E. Quiros-Roldan[2], M. Gianizza[1], D. Gotti[2], F. Maffezzoni[1], I. Patelli[1], F. Castelli[2], G. Mazziotti[1], A. Giustina[1]

[1]Cattedra di Endocrinologia, Università di Brescia - Brescia, [2]Cattedra di Malattie Infettive, Università di Brescia - Brescia

Over the recent years, there has been convincing evidence that HIV infection per se and antiretroviral therapy may cause skeletal fragility with an increased risk of fractures. However, most of the previous studies investigated bone turnover and bone mineral density (BMD), whereas available data on fractures are scanty and predictors of fractures in this clinical setting are still largely unknown. Indeed, it is unclear whether BMD is a reliable marker of skeletal fragility in patients with HIV infection. In this cross-sectional study, we aimed at investigating the predictors of low BMD and radiological vertebral fractures in patients with HIV infection. One hundred and forty-nine consecutive patients with HIV infection (102M, 47F, median age 51y, range: 34-78) underwent BMD measurement by dual-energy X-ray absorptiometry (DXA) at lumbar spine (Lunar Prodigy, GE Healthcare): 33.6% of patients showed normal BMD, while 44.3% were osteopenic and 22.1% osteoporotic. Prevalence of low BMD (osteopenia and osteoporosis) was higher in females as compared to males (80.9% vs 59.8%) with no significant correlation with age and body mass index. Eighty-seven patients were studied for vertebral fractures by a radiological and morphometric approach and 29.9% of them showed vertebral fractures. Vertebral fractures occurred more frequently in patients with low BMD (T-score <-1 SD) as compared to patients with normal BMD (37.3% vs 4.3%; p<0.001), without any significant difference between osteopenia and osteoporosis (35.3% vs 40.0%; p=0.71). Moreover, vertebral fractures were significantly associated with age (odds ratio for each decade of age: 2.3, C.I.95% 1.18-4.56; p=0.01), whereas no significant associations with BMI and sex were found. This study confirms that prevalent vertebral fractures are very frequent in HIV occurring in about one-third of the patients and shows that they are associated with age and low BMD. Interestingly, however, the prevalence of vertebral fractures was comparable between osteopenic and osteoporotic patients. These results suggest a lumbar spine BMD <-1 threshold to identify patients at risk of skeletal fragility and therefore good candidates to morphometric evaluation of spine X-ray. This is in line with previous indications in other forms of secondary osteoporosis such as that induced by glucocorticoids and may depend on reduced bone quality associated with HIV infection or on the consequences of antiretroviral therapy.

OC38

ASSESSMENT OF TEN-YEAR PROBABILITY OF FRACTURE THROUGH THE FRAX ALGORITHM IN PATIENTS WITH AND WITHOUT TYPE 2 DIABETES MELLITUS.

M. Fallarino[1], V. Carnevale[2], T. Filardi[1], E. Romagnoli[1], M. A. Annese[2], A. Fontana[2], M. Rossetti[1], A. Carnovale[1], M. Copetti[2], F. Pellegrini[2], C. Eller-Vainicher[3], V. V. Zhukouskaya[3], I. Chiodini[3], G. D'Amico[2], S. Morano[1]

[1]Medicina Interna, "Sapienza" Università - Roma, [2]"Casa Sollievo della Sofferenza", IRCCS - San Giovanni Rotondo, Foggia, [3]IRCCS "Ca Granda" - Milano

Introduction:type2 diabetes mellitus and osteoporosis are common conditions, causing relevant disability, morbidity, and mortality, namely in the elderly. Moreover, patients with long-standing diabetes have an increased risk of falls and consequent susceptibility to fractures, partly because of complications. The pathogenesis of bone involvement in diabetes is still not fully elucidated, patients with type2 diabetes seem to have increased skeletal fragility, despite their preserved or high areal bone mineral density (BMD). Particular interest has been recently attributed to algorithms based on clinical risk factors, among which the Fracture Risk Assessment Tool (FRAX). The FRAX algorithm estimates the 10-year probability of major osteoporotic fractures (including hip, clinical spine, humerus, and forearm fractures) and/or of only hip fractures. Objective: to investigate fracture risk by the FRAX tool in patients with type2 diabetes mellitus (DM), compared to a concomitantly enrolled control group (CS). Research design and methods: multi-centric cross-sectional observational study, carried out in three Italian Diabetes outpatients Clinics. We assessed the FRAX scores of 974DM and 777CS. In DM several parameters and complications of the disease were faced to FRAX-estimated fracture risk. Results:patients with DM had equal or significantly lower FRAX- estimated probability of both major osteoporotic (p<0.001) and hip fractures (p=0.023), with noticeable gender difference. They also had a number of prior fractures higher than CS (p<0.001). Some clinical features of diabetes (hypoglycemia, and -only for hip fractures score- vascular disease in male DM; only for hip fracture score, HbA1c and neuropathy in female DM) were associated with the FRAX scores in multiple regression models. The FRAX - estimated probability of both major osteoporotic and hip fractures is not negligible in defined subsets of patients with DM, segregated by CART analysis. Conclusions:mean FRAX scores of DM were lower than those of CS but the number of previous fractures was higher in DM patients. A portion of patients with DM had a high FRAX-estimated fracture risk, and distinct clinical profiles of DM patients had estimated risk often exceeding the National Osteoporosis Foundation (NOF) threshold for treatment.

OC39

PRIMARY ALDOSTERONISM AND CALCIUM AND PHOSPHATE METABOLISM

V. Ronconi[1], L. Ceccoli[1], L. Giovannini[1], M. Marcheggiani[1], F. Turchi[1], M. Boscaro[1], G. Giacchetti[1]

[1]Clinica di Endocrinologia, Università Politecnica delle Marche-Ospedali Riuniti Umberto I-GM Lancisi- G Salesi - Ancona

Recent studies have shown that aldosterone induces urinary calcium excretion leading to a reduction of calcemia with consequent secondary hyperparathyroidism and bone mineral density (BMD) loss. In patients with primary aldosteronism (PA) this picture of hyperparathyroidism is significantly improved by treatment with adrenal surgery or with mineralocorticoid receptor antagonists.

On these premises, aim of the present study was to evaluate calcium and phosphate metabolism parameters in PA patients, compared with patients with essential hypertension (EH) and the effect of treatment of aldosterone excess on bone mineral desnity in PA patients.

We studied 226 patients: 116 with PA (46 with an aldosterone producing adenoma-APA and 70 with bilateral adrenal hyperplasia-IHA), and 110 patients with EH. In 40 patients with PA we evaluated biochemical parameters and bone mass, using the dual-energy x-ray absorptiometry (DXA), at basal and after a mean follow-up of 24 months since treatment.

In PA patients, compared with EH, PTH levels and urinary calcium excretion were significantly increased, while serum calcium was significantly decreased with comparable vitamin D levels. At follow-up in PA patients, PTH levels were significantly reduced compared with basal evaluation, despite similar vitamin D amounts.

At follow-up, we observed a significant improvement of the Z-score at lumbar spine, at femoral neck and at total hip.

In conclusion, our results support previous data showing secondary hyperparathyroidism in PA patients, which is reversible after treatment. Moreover, such targeted treatment appears to be able to determine a significant improvement of BMD both at spine and hip sites.

OC40

THE EMBRYONIC TRANSCRIPTION FACTOR TBX1 IS DEREGULATED IN PARATHYROID TUMORS

C. Verdelli[1], V. Vaira[2], M. Meregalli[3], V. Guarnieri[4], A. Terranegra[5], A. Scillitani[6], L. Vicentini[7], G. Steffano[8], S. Corbetta[9]

[1]Lab.Biol. Molecolare, IRCCS Pol. San Donato - S.Donato M.se (MI), [2]Div.Patologia, Fondaz.IRCCS Cà Granda - Milano , [3]Lab.Cell.Staminali, DFMCT, Univ.Milano, Fondaz.IRCCS Cà Granda - Milano , [4]Genetica Medica, IRCCS Osp. Casa Soll. Sofferenza - S.Giovanni Rot. (FG), [5]DMCO, Univ.Milano - Milano , [6]U.O.Endocrinologia, IRCCS Osp. Casa Soll. Sofferenza - S.Giovanni Rot. (FG), [7]Endocrinochirurgia, Fondaz.IRCCS Cà Granda - Milano , [8]Chirurgia I, IRCCS Pol. San Donato - S.Donato M.se (MI), [9]U.O.Endocrinologia, DSBS Univ. Milano, IRCCS Pol. San Donato - S.Donato M.se (MI)

Cancer cells and embryonic stem cells share key biological properties: transcription factors regulating self-renewal and differentiation have been found expressed in human cancer cells. We focused our attention on TBX1, the gene deleted in 22q11.2 microdeletion/DiGeorge syndrome and involved in heart, thymic and parathyroid cells differentiation. TBX1 mRNA and portein were expressed in adult normal parathyroid glands, while most typical parathyroid adenomas (PA), MEN1-related hyperparathyoid glands and few carcinomas (4 out of 17) expressed *TBX1* mRNA at higher levels (2-4 folds). TBX1 gene regulation and function in parathyroid neoplasia were further investigated. Functional studies were performed in HEK293 cells, since they expressed *TBX1* mRNA and protein. We tested the hypothesis that TBX1 expression might be regulated by embryonic signalling pathways such as bone-morphogenetic protein (BMP)/SMAD and Wnt/beta-catenin. Treatment for 3-16 hours with 20 ng/ml BMP4 increased *TBX1* mRNA levels. Interestingly, the BMP4 receptor BMPR1A was detected in PA cells. By contrast, beta-catenin accumulation induced by 8-hours treatment with 10-20 mM lithium chloride inhibited *TBX1* mRNA levels in HEK293 and PA cells. Furthermore, the activation of the calcium sensing receptor (CaSR) by stimulating for 8 hours HEK293 cells stably transfected with the human CaSR, with increasing concentrations of both calcium and the CaSR agonist R568, induced a reduction in *TBX1* mRNA levels. Silencing of TBX1 gene in both HEK293 and PA cells induced a significant reduction in TBX1 target genes such as WNT5a and BMP4-induced increase in inhibitor of differentiation-1 (*Id1*) mRNA levels. In conclusion, TBX1 might be involved in parathyroid tumorigenesis regulating genes such as WNT5a and Id1, known to promote cancer development and progression.

OC41

EFFICAY AND SAFETY OF TERIPARATIDE TREATMENT

S. Lupo[1], P. Franceschetti[1], M. Bondanelli[1], T. Tommasin[2], S. Bianchi[3], R. La Corte[4], M. C. Zatelli[1], E. C. degli Uberti[1], M. R. Ambrosio[1]

[1]Section of Endocrinology, University of Ferrara - Ferrara, [2]Section of Ortophedy, University of Ferrara - Ferrara, [3]Dept of Pharmacology, University of Ferrara - Ferrara, [4]Section of Reumatology, University of Ferrara - Ferrara

Teriparatide is the only available anabolic drug for treatment of post-menopausal and glucocorticoid-induced osteoporosis. Recent studies demonstrated the efficacy of teriparatide, but few data are available concerning treatment adherence and tolerance. We studied 165 patients [157 F, 8 M; mean age 72.06 ± 9.09 (SD)] treated with teriparatide from 2005 to 2012, with the aim to evaluate long term therapy efficacy, safety and tolerance. We analyzed the incidence of new fractures, the Visual Analogue Scale (VAS) score, serum levels of serum calcium, phosphorus, PTH, uric acid, ALP, urinary calcium and the side effects, before and after 6, 12 and 18 months of therapy. Data were available for all patients at 6 months of therapy, for 146 at 12 months and 112 at 18 months of therapy. Seven patients have not yet completed 12 months of therapy and 29 patients have not yet completed 18 months of treatment. 15 patients were lost at follow-up: 10 after 12 months and 5 after 18 months; 2 patients stopped treatment because of adverse events, probably not related to therapy (1 worsening of dyspnea; 1 liver metastasis). Therefore, adherence to treatment was of 95.54 %. Efficacy of the drug was demonstrated by the appearance of new fractures only in 4 patients (2.4 %), before 6 months of therapy in 2 cases, before 12 months in 1 case and 18 months in 1 case, respectively. A significant ($p<0.0001$) reduction of VAS score was already obtained after 6 months of therapy and maintained after 12 and 18 months (medium VAS: 6.98 ± 2.75, before therapy; 5.23 ± 2.9 at 6 months; 4.43± 2.7 at 12 months; 3.86 ±2.2 at 18 months). Biochemical parameters did not significantly changes during therapy. Only phosphorus levels significantly ($p<0.001$) increased at 12 and 18 months of treatment. No patients had serious side effects. These data demonstrate the efficacy of teriparatide in reducing back pain and preventing new fractures in a large group of patients, and indicate good tolerance and adherence to therapy.

OC42

PREVALENCE OF NORMOCALCEMIC PRIMARY HYPERPARATHYROIDISM IN A SMALL VILLAGE OF SOUTHERN ITALY

A. Meola[1], E. Vignali[1], R. Centoni[1], F. Cetani[1], S. Chiavistelli[1], F. Saponaro[1], C. Marcocci[1]

[1]Medicina Clinica e Sperimentale, Università di Pisa - Pisa

Primary hyperparathyroidism (PHPT) is characterized by the presence of hypercalcemia and high PTH levels. A variant of PHPT, the so-called normocalcemic PHPT (NPHPT), has been identified in recent years. It is characterized by normal serum calcium and high PTH levels, in the absence of other causes of secondary hyperparathyroidism. The epidemiology of NPHPT is not well understood.

In the early fall of 2010 we performed a survey in a small Southern Italian village, in which all adult residents (n=1811) were invited for an interview and eventually blood tests. One thousand fifth-six individuals accepted to participate. Daily calcium intake was also evaluated using a self-administered questionnaire. Blood samples were collected for measurement of serum calcium, albumin, creatinine, PTH, and 25OHD.

Complete biochemical and questionnaire results were available in 679 subjects (age 18-89 yr; 422 F and 257 M). Classical, hypercalcemic PHPT was diagnosed in 4 women (0.6%). Increased plasma PTH (nl: 10-65 pg/ml) and normal albumin-adjusted serum calcium (alb-Ca; nl: 8.6-10.2 mg/dl) were found in 288 individuals (42.4%). Two-hundred sixty-three of them were excluded because of serum 25OHD <30 ng/ml (n=241, 83.7%) or eGFR<60 ml/min/1.73 m^2(n=22, 7.6%). NPHPT was diagnosed in the remaining 25 subjects [11 F(mean age 47 yr, 5 postmenopausal) and 14 M(mean age 47 yr)], with an prevalence of 8.7% among individuals with increased plasma PTH and normal albumin-adjusted serum calcium, and an overall prevalence of 25/679 (3.5%). PTH, alb-Ca and 25OHD (mean ±SD)concentrations were 89.0±21.5 pg/ml, 9.0±0.3 mg/dl, and 37.6±7.2ng/ml, respectively. No relationship was found between quartiles of daily calcium intake and plasma PTH levels.

In conclusion,the finding of high plasma PTH and normal alb-Ca is rather common and, in the majority of cases, is associated with low 25OHD levels and, less frequently, renal failure. However, a definite proportion of subjects (3.5%) met the criteria of NPHPT. A long-term follow up of these subjects is needed to establish whether NPHPT represents an early stage of classical PHPT or a separate entity.

OC43

A SINGLE-CENTER, OPEN-LABEL, PHASE II, PROOF-OF-CONCEPT STUDY WITH PASIREOTIDE LAR IN PATIENTS WITH PROGRESSIVE MTC: 6-MONTH RESPONSE

A. Faggiano[1], P. Ferolla[2], G. Vitale[3], M. Del Prete[1], V. Ramundo[1], R. Esposito[1], V. Marotta[1], F. Marciello[1], A. C. Carratù[1], L. Camera[4], R. Fonti[4], A. Colao[1]

[1]Department of Clinical Medicine and Surgery, Federico II University - Napoli, [2]Department of Internal Medicine, University of Perugia - Perugia, [3]Ospedale San Luca IRCCS, Istituto Auxologico Italiano, Milan; Department of Medical Sciences, University of Milan - Milano, [4]Department of Biomorphological and Functional Sciences, Federico II University - Napoli

Introduction: Medullary thyroid cancer (MTC) is a well-differentiated neuroendocrine tumor in which somatostatin receptor (sst) expression is higher for sst1 and sst5 than for sst2. This may explain why the available sst2–selective analogues do not work in these patients and why pasireotide (SOM230), a novel, multi-receptor targeted somatostatin analogue with high-binding affinity for sst1,2,3 and sst5 could be effective.
Aim: To evaluate the effectiveness of pasireotide long-acting release (LAR) in MTC pts with progressive disease.
Study Design: Trial enrollment started in February 2012 (study registration no. NCT01625520). Twenty pts are expected to be enrolled. At now, 14 consecutive pts with progressive metastatic or persistent postoperative MTC have been enrolled and received pasireotide LAR 60 mg/m.
Results: At 1 mth evaluation, calcitonin hypersecretion was significantly decreased in 7 pts (by 32 to 73%), stable in 6 and progressive in 1 other. Among the 10 pts who were evaluable at 3 mth follow-up, calcitonin was significantly decreased in 6 pts, stable in 3 and further increased in 1. Target tumor lesions were stable in 9/10 pts and progressive in 1/10 who had a 3- and 6-mth CT scan. FDG-PET SUVmax was decreased by 36 to 50% in 2/9 pts with stable disease at CT scan.
Conclusions: This is the first experience on the use of pasireotide (SOM230) in pts with MTC. A 6-month antisecretive and antiproliferative response to pasireotide LAR has been observed.

OC44

GENOME-WIDE ANALYSIS OF ESTROGEN-DEPENDENT ENOS SIGNALING BY CHIP-SEQUENCING: NOVEL TRANSCRIPTIONAL MECHANISM IN AGGRESSIVE PROSTATE CANCER

S. Nanni[1], A. Aiello[1], A. Re[2], A. Guffanti[3], V. Benvenuti[4], C. Colussi[1], A. Felsani[4], S. Bacchetti[5], C. Gaetano[6], A. Pontecorvi[1], A. Farsetti[4]

[1]Catholic University - Rome, [2]Univ Messina - Messina, [3]Genomnia - Milan, [4]CNR - Rome, [5]Regina Elena Instit - Rome, [6]Goethe Univ - Frankfurt

Our previous works identified nuclear endothelial NOS (eNOS) as partner of both Estrogen Receptor Beta and Hypoxia Inducible Factors in Prostate Cancer (PCa). These transcriptional complexes determined localized chromatin remodeling in response to estrogen and hypoxia stimuli that in turn regulate genes associated with adverse prognosis in PCa. To explore the role of nuclear eNOS in the acquisition of aggressive phenotype in PCa, we performed ChIP-Sequencing on chromatin-associated eNOS from cells from a primary tumor associated with poor outcome and from metastatic LNCaP cells, before and after treatment with 17b-estradiol (E2). By this approach, we have documented the genome-wide existence of a considerable number of eNOS-DNA associations that define transcriptional active regions modulated by estrogen. In summary, we found that: 1. the eNOS-bound regions (peaks) are widely distributed across the genome encompassing multiple transcription factors binding sites, including Estrogen Response Elements. 2. E2 increased the number of peaks, indicating hormone-dependent eNOS re-localization. 3. Peak distribution was similar with/without E2 with ≈ 55% of them in extragenic DNA regions and an intriguing involvement of the 5' domain of several miRs deregulated in PCa. Numerous potentially novel eNOS-targeted genes have been identified suggesting that eNOS participates in the regulation of large gene sets. The parallel finding of downregulation of a cluster of miRs, including miR-34a, in PCa cells associated with poor outcome led us to unveil a molecular link between eNOS and SIRT1, an epigenetic regulator of aging and tumorigenicity, negatively regulated by miR-34a and in turn activating eNOS. In particular, we identified of novel positive feedback loop of eNOS involving transcriptional downregulation of pri-miR34a mediated by an estrogen dependent eNOS/SIRT1 complex, as consequence we observed an up-regulation of mir-34a-target SIRT1 that in turn actives eNOS itself (by a post-transduction modification). These findings reveal unprecedented functions of eNOS and of eNOS/SIRT1 interplay, fine-tuned by E2-activated ER signaling, and attributed to eNOS a critical molecular role in aggressive PCa.

OC45

HETEROGENEITY OF PROLIFERATION IN NEUROENDOCRINE TUMORS

M. Albertelli[1], F. Grillo[2], T. Borra[2], P. Calamaro[2], L. Mastracci[2], F. Annunziata[1], M. Falanga[1], F. Minuto[1], D. Ferone[1]

[1]DIMI, Endocrinologia, IRCCS AOU San Martino IST, Università di Genova - Genova, [2]DISC, Anatomia Patologica, IRCCS AOU San Martino IST, Università di Genova - Genova

Background: The neuroendocrine tumor (NET) proliferation-based grading system (ENETs) has proved reliable for prognostic stratification, however concerns exist on Ki67 heterogeneity. Our aim was to evaluate intratumor Ki67 index heterogeneity in primary and metastatic sites.

Methods: A total of 170 GEP-NETs (between 1993-2011) were identified, 50 of them with clinical follow-up (mean follow up was 59 months, range 2-168 months). Twenty-five cases had multiple paraffin blocks on which Ki67 immunohistochemistry was performed.

Results: Thirteen out of 21 (62%) primary sites presented exactly the same Ki67 percentage and therefore the same grade in each paraffin block. Six (29%) tumors presented different Ki67 indices between paraffin blocks, but with no change in grade. Two (10%) tumors showed Ki67 index discrepancy (7% vs 2% and 4% vs 2%) which was enough to change grade (G1 to G2). Out of 14 patients with primary NET and synchronous metastases, 9 (64%) presented exactly the same Ki67 index between sites while 2 (14%) showed variability in their Ki67 index, but not in grade. Three (21%) cases showed discrepancy between primary tumor and metastases. In particular two cases showed an increase in proliferation index in nodal metastases (1% vs 5% and 17% vs 31%) and one case showed increased Ki67 index in a mesenteric localization (1% vs 5%). One case with multiple hepatic metastases showed discrepancy between each metastasis (7% vs 1%). Six patients underwent surgical excision of metachronous metastases during follow up. Three (50% - 1 nodal and 2 hepatic metastases) patients showed an increase in Ki67 rate in the metastatic site and a change in grade, from G1 to G2 (1% vs 10%; 2% vs 5%; 1% vs 7%).

Conclusions: Heterogeneity in NETs is well recognized and any sampling variability may be overcome by evaluating Ki67 on multiple tissue blocks. This may however not prove necessary in primary tumors as few cases (10%) in our study showed sufficient variability to change grade. Differences in grade between primary and synchronous and metachronous metastatic site are however more important and evaluation of Ki67 at all sites may be significant for patient management. These findings will be correlated with patient outcome so as to identify whether the increase in proliferation rate in secondary sites is important in the correct determination of prognosis.

OC46

PXD101 PREVENTS THE ONSET OF CASTRATION-RESISTANT PHENOTYPE MODULATING ANDROGEN RECEPTOR, HSP90 AND CRM1 IN PRECLINICAL MODELS OF PROSTATE CANCER

G. L. Gravina[1], C. Festuccia[1], F. Marampon[1], A. Mancini[1], M. Piccolella[2], P. Negri-Cesi[2], M. Motta[2], P. Muzi[3], E. Di Cesare[1], V. Tombolini[4], A. Lenzi[5], E. A. Jannini[1]

[1]Dipartimento di Scienze cliniche applicate e biotecnologiche - L'Aquila, [2]Dipartimento di endocrinologia - Milano, [3]Dipartimento di Medicina clinica, sanità pubblica, scienze della vita e dell'ambiente - L'Aquila, [4]Divisione di radioterapia, Ospedale Policlinico Umberto I - Roma, [5]Dipartimento di Medicina Sperimentale - Roma

Aims. Aberrant activation or "reactivation" of androgen receptor during androgen-ablation therapy shows a potential cause for the development of castration-resistant prostate cancer. This study tested the hypothesis that PXD101, a potent pan histone deacetylase inhibitor, may prevent onset of castration resistant phenotype and potentiate hormonal therapy.Material and Methods. A panel of human prostate cancer cells with graded castration resistant phenotype and in vivo models were used to verify this hypothesis. Results. In this report we demonstrated that hormonal manipulation favors the onset of castration-resistant phenotype increasing HDAC expression and activity as well as modulating expression and activity of AR, EGFR, HER2 and Akt. Consistent with these observations, the functional knockdown of HDACs by PXD101 prevented the onset of castration resistant phenotype with a significant down-regulation of AR, EGFR, HER2 and Akt expression/activity. The disregulation of functional cooperation between HDAC-6 with hsp90, on one hand, and between GSK-3β with CRM1, on the other hand, may explain the biological effects of PXD101. In this regard, the HDAC-6 silencing or the functional knockdown of hsp90 by 17AAG resulted in the selective down-regulation of AR, EGFR, HER2 and Akt expression/activity, while the decreased phosphorylation of GSK-3β mediated by PXD101 increased the nuclear expression of CRM1 which in turn modified the AR and survivin recycling with increased caspase-3 activity. Conclusions. HDAC inhibitors retain the ability to prevent the onset of castration resistant phenotype and, therefore, merit clinical investigation in this setting. However, additional data are needed to develop clinical treatment strategies for this disease stage.

OC47

ENDOCRINE TOXICITIES AFTER TREATMENT WITH IPILIMUMAB.

P. Sartorato[1], V. Chiarion Sileni[2], J. Pigozzo[2], S. Faoro[3], V. Camozzi[1], G. Opocher[1], S. Zovato[1]

[1]Oncoendocrinology-IOV - Padova, [2]Medical Oncology-IOV - Padova, [3]Pharmacy-IOV - Padova

INTRODUCTION: Ipilimumab (IPL) is a MAB directed against the CTLA-4 which promotes activation of cytotoxic lymphocytes and augments an immune-mediated anti-tumour response. IPL is now licensed for the treatment of unresectable or metastatic malignant melanoma. A host of immune-related adverse events are associated with the anti CTLA-4 therapy. The endocrinologist may be involved in the management of IPL treated patients in whom may arise secondary hypophysitis with hypopituitarism in 1-6 % of patients, followed by hypo- and hyperthyroidism secondary to thyroiditis in 2,7 and 0,3 %, respectively and primary adrenal insufficiency in 2,1 %. We present our experience of a series of patients treated with IPL which came to our attention for a suspicion of hypophysitis. PATIENTS AND METHODS: We studied 8 patients (age: 51±7; M/F:6/2) with metastatic melanoma enrolles in different trials with IPL. They presented hypophysitis symptoms after a median time of 11 weeks (range 7-16).All patients presented fatigue and asthenia and 4 also severe headache. All 8 patients had biochemical evidence of secondary adrenal insufficiency (median random cortisol level 105 ±112 nmol/L with undetectable ACTH levels); four patients presented also low FT3 (mean 2,86 pmol/L) and FT4 levels (mean 11.1 pmol/L) with low TSH in 3 cases (mean 0.245 mU/L) and in normal range in one (2,59 mU/L). 3 male patients also presented with a severe deficiency of testosterone (6±3 nmol/L).Three cases had also very low values of IGF-1 (65±22ug/L). One patient had the MRI which documented diffuse enlargement of the pituitary gland. All patients, as required by trial safety procedures, started with prednison 1 to 2 mg/kg orally once per day with gradual tapering and then substituted with cortison acetate. 3 patients also started thyroxine treatment.1 patient experienced a concomitant autoimmune hyperthyroidism that still requires thyrostatic treatment. Long term follow-up of our patients (11±8 months after IPL discontinuation) showed a persistent need for glucocorticoid replacement, while 2 patients recovered the thyroid axis function after 4 months from hypothyroidism and 2 patients the gonadal axis after 2 months CONCLUSION: The prevalence of IPL induced hypopituitarism may be higher that previously thought and the effects on adrenal axis may be irreversible. This emphasizes the clinical relevance of endocrine toxicity and the importance of warning for early screening of pituitary insufficiencies in these patients.

OC48

THE PROGNOSTIC VALUE OF CHRONIC LYMPHOCYTIC THYROIDITIS AND THE ROLE OF LYMPHOCYTIC SUBTYPES IN PAPILLARY THYROID CANCER PATIENTS.

T. Pilli[1], P. Toti[2], M. G. Castagna[1], R. Occhini[2], M. Caselli[1], L. Barbagli[1], S. Memmo[1], F. Maino[1], F. Pacini[1]

[1]Sezione di Endocrinologia, Scienze Mediche, Chirurgiche e Neuroscienze - Università di Siena, [2]Sezione di Anatomia Patologica, Biotecnologie Mediche - Università di Siena

Background. A link between thyroid cancer and the chronic lymphocytic thyroiditis (CLT) has long been recognized but it is still debated.A worse prognosis has been reported but mostly either a protective or no effect of thyroid autoimmunity has been shown.Moreover background lymphocytic thyroiditis and the presence of lymphocytes within and/or surrounding tumors (TAL),that may represent tumor-specific immune response,should be assessed separately. **Aim.** The aim of this study was to evaluate the prognostic value of CTL in a retrospective cohort of PTC patients and to characterize the lymphocytic infiltration (LI) and the different lymphocytic subtypes. **Materials and Methods**. We assessed 375 PTC patients,aged 45.2±16.4 (m±SD),with a mean follow-up of 6.28±3.86 years, treated with surgery and radioiodine remnant ablation.Archived thyroid tissue sections from 60 PTC patients were independently reviewed for the presence of CTL and TAL and lymphocytic subsets were assessed by immunohistochemistry. **Results**. At histological examination 75/375 patients (20%) showed CLT while the remaining patients did not have lymphocytic infiltration.Patients with CTL were more frequently women (90.7% vs 69.7,p=<0.001) and more commonly, but with no statistical difference, classified as low risk (according to ATA criteria) (53.3% vs 33.9%), compared to those with no CTL. At the last follow-up patients with CTL showed a significantly better outcome compared to those with no CTL, even when we divided the patients in low and high risk (cure rate defined as undetectable thyroglobulin and negative imaging: 100% vs 89.8%,p=0.039 and 82.9% vs 66.3%,p=0.043, respectively). Data from LI characterization are available for 23/60 patients:4 patients did not have any lymphocytic infiltration, 6 patients had CTL and 10 patients TAL. Patients with CTL, with respect to those with TAL,tend to have tumors with not aggressive variant, intra-thyroidal extension and no lymph node metastases.Of particular interest the lowest CD8+/Foxp3+ lymphocytic ratio was observed in the intra-tumoral TAL.

Conclusions. Our data suggest that the presence of CTL is associated to a better outcome in PTC patients and that the cytotoxic to regulatory lymphocytic imbalance in the intra-tumoral TAL may attenuate the tumor-specific immune response favoring a more aggressive behavior of cancer.

OC49

NEUROENDOCRINE TUMOURS OF LUNG: NEW DATA ON ATYPICAL CARCINOID AND LARGE CELL NEUROENDOCRINE CARCINOMA FROM A ITALIAN-FRENCH MULTICENTRIC STUDY

F. Marciello[1], O. Mercier[2], P. Ferolla[3], D. Planchard[4], P. L. Filosso[5], A. Chapelier[6], F. Grimaldi[7], G. Blanco[8], B. R. De Latour[9], J. Guigay[4], P. Dartevelle[10], J. Scoazec[4], M. Papotti[5], A. Colao[1], E. Baudin[4], A. Faggiano[1]

[1]Università "Federico II" - Napoli, [2]Centre Chirurgical "Marie Lannelongue" - Le Plessis Robinson, [3]Univeristà di Perugia - Perugia, [4]Institut"Gustave Roussy" - Villejuif, [5]Univeristà di Torino - Torino, [6]Foch Hopital - Suresnes, [7]Università di Udine - Udine, [8]Istituto Oncologico del Mediterraneo - Catania, [9]CHU Rennes - Rennes, [10]Centre Chirurgical"Marie Lannelongue" - Le Plessis Robinson

Introduction - Natural history of lung neuroendocrine tumors (NET) and in particular of two histological subtypes, atypical carcinoid (AC) and large cell neuroendocrine carcinoma (LCNEC), is poorly known. Aim of the study was to determine disease free survival (DFS) and overall survival (OS) of sporadic, resectable, non-metastatic lung AC and LNEC. Patients and Methods - This retrospective study involved 116 consecutive patients surgically treated (R0) between 1998-2008 for AC and LCNEC in two Italian and French expert networks. Slides were reviewed by two expert pathologists to validate the histological definition (WHO 2004). Results - The files of 86 patients were reviewed (49 male, 37 female). Mean age at diagnosis was 58±15 years for AC and 63±15 years for LCNEC. The most frequent presenting symptom was respiratory infection (AC: 24%, LCNEC: 31%); endocrine syndrome occurred in only one AC patient (Cushing's syndrome). Mean follow-up time was 80.6 months in AC and 50.6 months in LCNEC. The most used surgery was lobectomy (AC:81%, LCNEC:59%). N-positive status was found in 29% and 57% of AC and LCNEC, respectively. The rate of recurrence was 39% among AC (15% local, 24% distant metastasis) and 41% in LCNEC (27% local, 14% distant metastasis). The mean time of recurrence from surgery was 24.6 months in AC (4-88 months) and 15 months in LCNEC (4-44 months). Median OS was 5.4 years in LCNEC (not reached in AC). Median DFS was not reached in both histotypes. Recurrence rate was higher in N+ AC patients than in N0 ones (76% vs 24%).

Conclusion - Patients with lung AC and LCNEC experience a high rate of recurrence after surgery. Beside LCNEC patients, N-positive AC patients should be considered for adjuvant therapy and be subjected to an intensive monitoring on. These findings underline that lung NETs could not be considered tumors with benign behaviour and require a careful follow-up for long-time after surgery.

OC50

IN PATIENTS WITH NEWLY-DIAGNOSED TYPE 2 DIABETES BETA CELL FUNCTION IS AN INDEPENDENT PREDICTOR OF GLUCOSE CONTROL EVOLUTION OVER 18 MONTHS

M. Trombetta[1], G. Fainelli[2], L. Boselli[2], S. Bonetti[2], M. Dauriz[1], C. Negri[1], V. Stoico[1], E. Bonora[1], R. Bonadonna[1]

[1]Dipartimento di Medicina - Università degli studi di Verona e AOUI - Verona, [2]Dipartimento di Medicina - Università degli studi di Verona - Verona

We asked the question whether the metabolic phenotype at baseline and/or a number of type 2 diabetes mellitus (T2DM) risk genes may predict the evolution of glucose control (GC) within the first 18 months after diagnosis of the disease. 593 GAD-antibodies negative patients with newly diagnosed T2DM (age:59±0.4 yrs; BMI: 30±0.2 kg/m^2) were studied with: 1. Prolonged (5-hours) frequently sampled OGTT to assess beta cell function (BCF) by state of art mathematical modelling of glucose and C-peptide; 2. Standard euglycemic insulin clamp to assess insulin sensitivity (SI); 3. Genotyping the common T2DM risk variants of the following genes: ADAMTS9, CDKAL1, FTO, G6PC2, GCK, GCKR, GNPDA2, HHEX, HNF1B, IGF2BP2, IRS1, JAZF1, KCNJ11, MTNR1B, NOTCH2, PPARG, SLC30A8, TCF7L2, THADA, TMEM18, TSPAN and WFS1. GC evolution was defined as the difference between HbA1c at diagnosis (7.0±0.1%) and HbA1c at 18 months (6.5±0.1%). 141 patients were lost to follow-up, thereby leaving 452 patients for evaluation. In all multivariate regression models, basal HbA1c (standardized beta [stBETA]:0.92, $p<0.0001$) was the strongest positive predictor of favourable GC evolution (i.e. the higher HbA1c at diagnosis, the greater its fall within 18 months). No role for T2DM risk gene variants, either as single SNP or as a genetic score derived from all SNPs, could be detected. BCF (stBETA:0.08), but not age nor BMI nor SI nor pharmacological theraphy, were positive independent predictors of favourable GC evolution ($p<0.001$ e $p<0.01$, respectively). Thus, better BCF at diagnosis, but not SI not the T2DM genotype assessed in this study, is an independent predictor and a putative determinant of more desirable short-term (18 months) GC evolution.

OC51

IDENTIFICATION OF HMGA1 AS A NOVEL DOWNSTREAM TARGET OF THE INSULIN-NUTRIENT SIGNALING SYSTEM IN HUMANS AND MICE

E. Chiefari[1], B. Arcidiacono[1], M. T. Nevolo[1], S. Iiritano[1], V. Ventura[1], C. Palmieri[1], D. Foti[1], A. Brunetti[1]

[1]Dipartimento di Scienze della Salute, Università di Catanzaro - Catanzaro (Italy)

The insulin signaling pathway is fundamental in keeping euglycemia in response to physiological changes. Binding of insulin to the insulin receptor (INSR) activates a variety of cytoplasmatic and nuclear proteins that leads to the regulation of gene expression. We previously demonstrated that the high-mobility-group A1 (HMGA1) protein is a key regulator of the INSR gene and that HMGA1 gene defects cause insulin resistance and T2D in humans and mice. Here we investigated whether activation of the INSR by insulin affected the transcriptional activity of HMGA1.

In cultured cells (HepG2 and HEK293), and in in vivo normal mice, in fed and fasting states, we performed functional experiments to evaluate HMGA1-DNA dynamic interaction and its modulation following pharmacological and physiological stimuli.

As shown with chromatin immunoprecipitation, binding of HMGA1 to the INSR gene promoter and other glucose-related genes (IGFBP1, PEPCK, G6Pase) occurred in serum-starved/fasted conditions, whereas binding of HMGA1 was severely attenuated after insulin treatment. In Fluorescence Recovery After Photobleaching (FRAP) analysis, insulin treatment caused a marked redistribution of HMGA1 from the nuclear interior site (transcriptionally active DNA) to the peripheral region of the nucleus (inactive DNA). As measured by real-time PCR, a significant reduction of INSR, IGFBP1, PEPCK, and G6Pase gene expression, occurred after insulin-mediated detachment of HMGA1 from DNA. This reduction was abolished in HepG2 cells markedly depleted of HMGA1, as well as in cells treated with distamycin A, an inhibitor of HMGA1 protein binding to DNA. Similarly, following insulin treatment, mRNA levels of IGFBP1, PEPCK, and G6Pase decreased by 50% in primary hepatocytes from normal mice, whereas no changes were detected in cells from Hmga1-null animals, indicating that HMGA1 plays a role in the regulation of these genes by insulin.

Thus, these data indicate that HMGA1 is a novel downstream nuclear target of the INSR signaling pathway, and a critical mediator of the nutrient response and glucose homeostasis.

OC52

RESISTANT HYPERTENSION IN SUBJECTS WITH TYPE 2 DIABETES: CLINICAL CORRELATES AND ASSOCIATION WITH RENAL, RETINAL AND CARDIOVASCULAR COMPLICATIONS

A. Solini[1], G. Penno[1], E. Bonora[2], E. Orsi[3], G. Zerbini[4], C. Fondelli[5], R. Trevisan[6], M. Vedovato[7], M. Chirio[8], R. Buzzetti[9], M. G. Baroni[9], G. Pugliese[9]

[1]Università di Pisa - Pisa, [2]Università di Verona - Verona, [3]Fondazione IRCCS Cà Granda – Ospedale Maggiore Policlinico - Milano, [4]Istituto San Raffaele - Milano, [5]Università di Siena - Siena, [6]Ospedali Riuniti - Bergamo, [7]Università di Padova - Padova, [8]Università di Torino - Torino, [9]Università La Sapienza - Roma

The phenotype of resistant hypertension (RH) has not been characterized in subjects with type 2 diabetes. This analysis was aimed at assessing the independent correlates of RH and the association of this condition with renal, retinal and cardiovascular complications in subjects with type 2 diabetes. We used the baseline data from the Renal Insufficiency And Cardiovascular Events (RIACE) Italian Multicenter Study, including 15,773 patients consecutively visiting 19 Diabetes Clinics throughout Italy in years 2007-2008. RH was defined as systolic and/or diastolic blood pressure values not on-target (i.e.>130 and 80 mmHg, respectively) in subjects on ≥3 anti-hypertensive agents or on-target but using >4 drugs (n=2,363; 15% of the whole RIACE cohort). Patients on-target with 2 drugs (Ctr1; n=1,369) and 3 drugs (Ctr2, n=803) served as control groups. No clinically significant differences among groups were observed for age, diabetes duration, HbA1c, BMI, waist and lipid profile. Subjects with RH had higher albuminuria, lower eGFR and, hence, higher prevalence of chronic kidney disease (CKD) than control groups (56.3% vs 41.9% in Ctr1, and 46.1% in Ctr2; $p<0.0001$). Likewise, rate of advanced retinopathy, but not of cardiovascular disease (CVD), was higher in individuals with RH. Logistic regression analysis with backward variable selection showed that RH was independently associated with age (1.020 [1.012-1.027]), waist (1.026 [1.020-1.032]), micro (1.379 [1.201-1.583]) and macroalbuminuria (2.131 [1.644-2.762]), eGFR 30-59 ml/min/1.73m2 (1.269 [1.053-1.529]) and <30 ml/min/1.73m2 (1.527 [1.007-2.318]), and advanced retinopathy (1.271 [1.048-1.542]). Moreover, RH was associated with a higher risk of Stages 1-2 CKD (1.510 [1.282-1.778]), Stages 3-5 nonalbuminuric CKD (1.366 [1.135-1.645]), and particularly Stages 3-5 albuminuric CKD (1.954 [1.594-2.395]), after adjusting for several confounders. No independent correlation was found between RH and CVD, either total or by vascular bed (coronary, cerebrovascular and peripheral). These data show that, in subjects with type 2 diabetes, RH is associated with age, waist, and microangiopathy, especially albuminuric CKD, but not with macroangiopathy, thus suggesting a strict bi-directional relationship between RH and renal damage.

OC53

ROLE OF INTERACTION BETWEEN SIRTUIN 1 AND HUR PROTEIN ON E-SELECTIN RELEASE IN METABOLIC SYNDROME

S. Vigili de Kreutzenberg[1], A. Martellini[1], G. Ceolotto[1], A. Cattelan[1], A. Fabricio[2], E. Squarcina[2], M. Gion[2], A. Semplicini[1], A. Avogaro[1]

[1]Department of Medicine - University of Padova - Padova, [2]Department of Internal Medicine - SS Giovanni e Paolo Hospital - Venezia

Background. Endothelial dysfunction and inflammation play a pivotal role in the pathogenesis of vascular complications associated with metabolic diseases. Cytokines, oxidative stress and age-dependent parameters trigger endothelial activation and adhesion molecule release. We investigated the expression of SIRT-1, an ageing-associated protein, in peripheral mononuclear cells (PBMNC) and circulating biomarkers of endothelial activation obtained from subjects with insulin resistance and metabolic syndrome (MS). Furthermore, we assessed the potential role of SIRT-1 on the mechanisms underlying the pro-inflammatory pathways of endothelial activation.

Materials and Methods. Twenty-four subjects with MS, defined by ATPIII criteria, and 61 without (non-MS) participated in this study. Circulating TNF-a, I-CAM, V-CAM and E-Selectin were determined, along with insulin sensitivity (Si), by minimal model analysis, and gene and protein expression of SIRT-1, in PBMNC of the subjects. In HUVECs, exposed to TNF-a and high glucose, we studied: E-Selectin release; NF-kB acetylation; NFkB interaction with E-Selectin promoter; interaction between SIRT1 mRNA and ELAV-like protein 1 (HuR protein), a post-transcriptional regulator of SIRT1. The same experiments were performed overexpressing or silencing SIRT1 and HuR.

Results. E-Selectin (32±3.2 vs. 23±1.1; $p<0.01$) and TNF-a (1.49±0.1 vs. 1.03±0.05; $p<0.01$) were significantly higher, while SIRT1 gene expression was significantly lower (0.74±0.04 vs. 1.10±0.05; $p<0.01$) in MS subjects compared to non-MS; I-CAM and V-CAM were similar. E-Selectin negatively associated with Si ($r=-0.317$; $p= 0.007$) and SIRT1 gene expression ($r=-0.528$; $p=0.008$), only in MS. In HUVEC: SIRT1 overexpression reduced the release of TNF-α-induced E-Selectin production, through both the inhibition of p65 NF-kB acetylation, and the attenuation of NF-kB binding to the E-Selectin promoter. Molecular mechanisms involved in high glucose-mediated attenuation of SIRT1 expression implicate the reduced interaction between HuR and SIRT1 mRNA ribonucleoprotein.

Conclusions. We demonstrate that an abnormal regulation of SIRT1 by HuR leads to elevated circulating E-Selectin, in presence of MS: the longevity-associated proteins may exert a beneficial action through the inhibition of endothelial activation.

OC54

THE ANTI-APOPTOTIC EFFECT OF GLP-1 IS BLUNTED IN PANCREATIC BETA CELL CARRYING THE Q121 VARIANT OF ENPP1 GENE

N. Caporarello[1], C. Parrino[1], M. R. Stancampiano[1], R. Vigneri[1], L. Frittitta[1]

[1]Department of Clinical and Molecular Biomedicine, University of Catania - Catania

Ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1), a negative modulator of insulin receptor activation, plays a relevant role on insulin secretion, insulin signaling and glucose metabolism. Using the rat beta cell line INS-1E transfected with either the K121 (K) or the Q121 (Q) variant of ENPP1 or only the neomicine-resistance gene (NEO) as a control, we investigated the effect of 100 nmol/l glucagon-like peptide-1 (GLP-1) on staurosporine induced apoptosis (caspase-3/7 activity assay and flow citometry) and on the pathways involved in apoptosis activation (phosphorylation of Akt-Ser473 and ERK1/2-Thr202/Tyr204 and the Bax/Bcl-2 ratio by western blot analysis).

The amount of caspase 3/7 was significantly reduced in the presence of GLP-1 both in in NEO (-54±9%) and in K cells (-56±16%) ($p<0.05$ for both) but not in Q cells (-29±5%, p=ns). Similar results were obtained with flow citometry: -57+10% ($p<0.05$) in NEO cells; -38±16% ($p<0.05$) in K and -18±7% (p=ns) in Q cells. Furthermore, GLP-1 increased Akt and ERK 1/2 phosphorylation in NEO (Akt = +66±43% and ERK 1/2 = +133±45%, $p<0.05$ in respect to staurosporine incubated cells) and also in K cells (Akt = +28±16%, $p<0.05$ and ERK 1/2 = 121±29%, $p<0.05$) while it had no effect in Q cells (18±4% and 21±24%, p=ns). Finally, GLP-1 decreased Bax/Bcl-2 ratio in NEO (-62±20%, $p<0.05$) and in K cells (-53±28%, $p<0.05$) but not in Q cells (-0%±0.1, p=ns). In conclusion, our findings indicate that ENPP1, more marked the Q121 variant (known to cause insulin resistance), reduces the anti-apoptotic effects of GLP-1 on beta cells. It may play, therefore, a pathogenic role for defective insulin secretion in patients harbouring this variant.

OC55

ROLE OF GALNT2 IN THE MODULATION OF ENPP1 EXPRESSION, INSULIN SIGNALING AND INSULIN RESISTANCE TRAITS

A. Marucci[1], F. Cozzolino[2], C. Dimatteo[1], M. Monti[2], L. Di Mauro[3], C. Menzaghi[1], S. Prudente[4], D. Mangiacotti[1], G. Fini[1], G. Lotti[3], P. Pucci[2], V. Trischitta[1], R. Di Paola[1]

[1]Research Unit of Diabetes and Endocrine Diseases, IRCCS "CSS" - S. Giovanni Rotondo, [2]CEINGE Biotecnologie Avanzate, Naples and Dipartimento di Scienze Chimiche, Università Federico II - Naples, [3]Blood Bank IRCCS "CSS" - S. Giovanni Rotondo, [4]CSS-Mendel - Rome

Aims/hypothesis. Ectonucleotide pyrophosphatase phosphodiesterase 1 (ENPP1) inhibits insulin signaling and action. Understanding the mechanisms underlying ENPP1 expression may help unravel molecular mechanisms of insulin resistance. Recent data suggest a role of ENPP1-3'untraslated region (UTR), in controlling ENPP1 expression. We sought to identify trans-acting ENPP1-3'UTR binding proteins, and investigate their role on insulin signaling.

Methods. By RNA pull-down, 49 proteins bound to ENPP1-3'UTR RNA were identified by mass spectrometry (MS). Among these, in silico analysis of genome wide association studies and expression profile datasets pointed to GALNT2 for subsequent investigation. Gene expression levels were evaluated by RT-PCR. Protein ENPP1 content and insulin receptor (IR) and Akt phosphorylation were evaluated by Western blot.

Results. In HepG2 cells, GALNT2 and ENPP1 mRNA levels were inversely correlated. GALNT2 down-regulation increased ENPP1 mRNA and protein content and reduced insulin effect on IR and Akt phosphorylation and on PEPCK expression. GALNT2 over-expression reduced ENPP1 mRNA levels but neither ENPP1 protein nor IR phosphorylation. In peripheral white blood cells (PWBC), GALNT2 and ENPP1 mRNA levels were directly correlated in control individuals and diabetic patients. In the latter group, both GALNT2 and ENPP1 mRNA levels were reduced.

Conclusions. Our functional data point to GALNT2 as a novel factor involved in the modulation of ENPP1 expression and insulin signaling in human liver cells. Additional experiments in other cells and tissues relevant to insulin action are needed to address the exact role of GALNT2 on insulin action as well as on human insulin resistance.

OC56

EARLY RETINAL NEUROPROTECTION IN EXPERIMENTAL DIABETES: A ROLE FOR GLIAL CELLS

M. Villa[1], A. Matteucci[2], L. Gaddini[2], A. M. M. Di Stasi[2], C. Mallozzi[2], M. Varano[1], F. Malchiodi Albedi[2], F. Pricci[2]

[1]Retina Medica-IRCCS GB Bietti - Roma, [2]Dip. Biologia Cellulare e Neuroscienze-Istituto Superiore di Sanità - Roma

BACKGROUND. Diabetic Retinopathy represents the primary cause of vision loss or blindness in the working age people. Recently beyond the vascular lesions hallmarking the disease, neurodegeneration and gliosis have been described in the diabetic retina before the clinical vasculopathy. Retinal glial activation seems to be a constant feature in early diabetic condition in contrast to neuronal damage whose evidence are more discrepant. Excess glucose disposal could unbalance the neuroglial homeostasis probably through the alterations of retinal mediators. AIMS. Aim of this study is to analyze the effect of diabetic milieu on retinal neuroglial unit and on the separate neuronal and glial cells in order to verify the resultant effect and the time course on neuroglial remodeling. METHODS. For in vitro studies, rat retinal tissue, primary cell cultures, either mixed (neuronal and glial) or pure (neuronal or glial), were exposed to normal glucose (NG:5.5mM) and high glucose (HG:30mM) for 48 hours. For in vivo studies, Sprague-Dawley rats were rendered diabetic with streptozotocin and studied at different times of disease (1-12 weeks). At the end of the experimental procedures, functional and structural markers of neuroglial compartment (glial activation, apoptosis) and retinal signaling (CREB phosphorylation) and mediators (Vascular Endothelial Growth Factor, VEGF) were evaluated by WB analysis and IHC. RESULTS. In retinal tissues and mixed primary retinal cell cultures, HG precociously activated Müller glial cells (as GFAP levels), without affecting neuronal population in terms of apoptotic cells, and induced neuronal pCREB nuclear translocation. In pure glial primary retinal cell cultures, HG activated Müller cells, whereas in pure neuronal cultures HG induced apoptosis of neuronal cells. VEGF, usually considered only a vascular marker, was increased in HG-treated retinal tissue (380,2vs239,4pg/ml/mg) and unchanged in mixed and pure cultures. In STZ-diabetic rats, retinal Müller glia is precociously activated, already after 1 week of diabetes, neurons maintained their functional and structural characteristics throughout all the experimental periods. CONCLUSION. These data suggest that in diabetic conditions, HG directly affects the retinal neuroglial compartment, inducing precocious glial activation. This event could regulate pro-survival transcription factor, such as CREB, and neuroprotective mediators, as VEGF, thus resulting in an initial attempt of neuroprotection.

OC57

ROLE OF THYROID HORMONES AND EPIGENETIC DRUGS IN CARDIAC DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS

A. Re[1], S. Granata[2], S. Nanni[2], A. Aiello[2], A. Rossini[3], A. Barbuti[3], C. D'Angelo[4], A. Biroccio[4], S. Mattiussi[5], S. Bacchetti[4], C. Gaetano[6], F. Trimarchi[1], A. Pontecorvi[2], A. Farsetti[5]

[1]University of Messina - Messina, [2]Catholic Univ - Rome, [3]Milan Univ - Milan, [4]Inst.Regina Elena - Rome, [5]CNR - Rome, [6]Goethe Univ - Frankfurt

Heart disease often leads to cardiomyocyte death and pathologic remodelling. Heart transplantation is the usual solution, although limited by donors number and restrictive inclusion criteria. These limits prompted research into stem cell–based alternatives, restricted however by a scarce source of adult stem cells and their relatively inefficient contribution to heart regeneration. Embryonic stem cells (ESCs) retain great promise as unlimited source of pluripotent progenitors for myocardial regeneration, but their therapeutics use is still impaired by ethical concerns and incomplete understanding of factors governing cardiomyocytes differentiation. We aimed at creating ESC-derived cardiomyocytes for experimental cell transplantation therapies in mice. We treated murine ESCs (mESCs) with Triiodothyronine (T3) and/or anacardic acid (AA), a natural epigenetic drug inhibiting histone acetylases, and investigated whether and how efficiently cardiac cell differentiation occurred. For easier identification of differentiated cells, engineered mESCs expressing red fluorescent protein (RFP) under the NCX1 gene promoter, an early cardiac differentiation marker, were used. The hanging-drop embryoid body (EB) technique was adopted to reproduce in vitro an embryo-like architecture. mESC-derived RFP-positive cardiomyocytes were collected and analysed by RT-PCR, western blot, electrophysiology and FACS. Both AA and T3 promoted cardiac differentiation, anticipating EBs beating and increasing beating areas but with effects apparently mediated by non-overlapping pathways. While T3 did not affect acetylation, AA decreased lysine acetylation of histonic and non histonic proteins. RT-PCR showed decreased stemness genes expression in EBs and only AA increased expression of Nkx2.5, a cardiac differentiation key gene. Gene profiling showed that AA upregulated only genes for early cardiomyocites differentiation, generating immature cardiac cells, whereas T3 induced genes for terminal cardiac differentiation, facilitating formation of mature cardiomyocytes. These results were confirmed by electrophysiology showing increased spontaneous firing, in support of T3 inducing peacemaker-cell-like differentiation. These findings reveal a master role of multiple epigenetically controlled signals mediated by T3 and AA, and provide an alternative for potentiating production of mature and functional cardiac cells for therapeutic intervention.

OC58

IODINE USE INDUCES INCREASE OF SERUM THYROID AUTOANTIBODIES AND SPREADING OF ABTG EPITOPE ONLY IN SUBJECTS WITH LYMPHOCYTIC THYROIDITIS

M. A. Provenzale[1], F. Latrofa[1], E. Fiore[1], T. Rago[1], L. Antonangeli[1], L. Montanelli[1], D. Ricci[1], M. Scutari[1], M. Frigeri[1], F. Aghini-Lombardi[1], M. Tonacchera[1], P. Vitti[1]

[1]Clican and Experimental Medicine - Section of Endocrinology, Univeristy of Pisa - Pisa

Iodine prophylaxis has been associated with a higher frequency of thyroid autoimmunity. We correlated thyroid autoimmunity and iodine use in 1295 subjects (502 M and 793 F, 45.7±19.2 yrs old), living in Pescopagano, an Italian village. 906 subjects (70.0%) declared to routinely use iodized salt (Iodized Salt Users: IS) and 389 (30.0%) not to use iodized salt (non-Iodized Salt Users: non-IS). Urinary iodine excretion (UIE), thyroglobulin (TgAb) and thyroperoxidase (TPOAb) autoantibodies were measured in all subjects; high levels of TgAb and TPOAb were considered expression of clinical Hashimoto's Thyroiditis (HT). According to thyroid ultrasound, the subjects were divided into two groups: HT pattern (HT-US; n. 87) and non HT pattern (non HT-US; n=1208). TgAb epitopes of 16 IS and 17 non-IS HT patients were evaluated by inhibition of TgAb binding to Tg in ELISA using 4 human monoclonal TgAb-Fab. Results. UIE was significantly higher in IS than in non-IS (112.0 µg/L vs 86.5 µg/L). The frequency of positive TgAb was significantly higher in IS than in non-IS (18.9% vs 13.6%) while for TPOAb this difference was only close to significance (16.9% vs 13.1%; p=0.09). In the HT-US group the frequency of positive TgAb was significantly higher in IS than in non-IS (58.5% vs 31.8%) while for TPOAb this difference was only close to significance (69.2% vs 45.5%;p = 0.05). In the HT-US group the percent of high levels of both TgAb and TPOAb were significantly higher in IS than in non-IS (50.8% vs 13.6% and 61.5% vs 31.8%, respectively). In the non HT-US group the percent of positive and high levels of both TgAb and TPOAb were similar in IS and non-IS. The levels of inhibition by region B TgAb-Fab were significantly higher in IS than non-IS (27.5% vs 3.0%; p=0.047); inhibitions by the other three TgAb-Fab were similar in IS and non-IS. In summary: i) serum thyroid autoantibodies are more common in IS than in non-IS; ii) the effect of iodine is evident in subjects with HT-US; iii) iodine is associated with serum autoimmunity mainly to thyroglobulin; iv) thyroid autoimmunity associated with the use of iodine is correlated with the expression of different TgAb epitopes. In conclusion, the use of iodized salt is associated with the onset of serum thyroid autoimmunity, but this effect is observed only in subjects with a hypoechoic pattern at thyroid ultrasound, expression of a lymphocytic infiltration of the thyroid.

OC59

L-T4 MONOTHERAPY IS NOT ADEQUATE FOR NORMALIZING SERUM FT3 IN MOST PATIENTS WITH CENTRAL HYPOTHYROIDISM

D. Gullo[1], A. Latina[1], I. Marturano[1], A. Caff[1], P. Tita[1], G. Parrinello[1], S. Squatrito[1], R. Vigneri[1]

[1]Endocrinology, Department of Clinical and Molecular Biomedicine, Garibaldi-Nesima Hospital - Catania

Context. In *central hypothyroidism* (CH) the treatment of choice, as in primary hypothyroidism, is L-T4 because peripheral conversion to T3 is believed to account for the overall tissue requirement for thyroid hormones. In these patients TSH cannot be used to evaluate euthyroidism and FT4 is currently the most used measurement to adjust the replacement dose. In a large series of CH patients we evaluated whether L-T4 monotherapy can normalize serum thyroid hormones, and more specifically, the most active hormone FT3. **Methods.** In a cross-sectional retrospective study we measured FT3, FT4 and FT3/FT4 ratio in 76 CH patients, median age 58 yrs (IQR 37-69), M=33 F=43, all having normal FT4 serum levels under L-T4 monotherapy (reference range 9.0-20.6 pmol/L). Data were compared to those observed in a cohort of euthyroid controls (n=3,875). **Results.** In L-T4-treated CH patients median FT4 levels (14.0 pmol/L) was not different from those of euthyroid controls (13.2 pmol/L; P=0.60) whereas median FT3 was significantly lower (3.11 pmol/L *vs* 4.47; P< 0.001). In L-T4-treated CH patients, 97.4% had FT3 levels below the median FT3 of controls and 35.5% had FT3 lower than the reference range (2.93-6.01 pmol/L). The FT3/FT4 ratio, an index of peripheral deiodination, was 0.23 *vs* 0.32 (P< 0.001). FT3 and FT4 levels were influenced by gender, age and the treatment with other pituitary hormones. In 26 CH patients having serum FT4 in the highest tertile, suggested as the target value for optimal L-T4 therapy, FT3 levels were below the median FT3 of reference range in all cases and below 2.5 percentile of the normal range in 8/26 cases (30.8%). **Conclusions.** CH patients on L-T4 monotherapy have FT3 levels significantly lower than euthyroid controls, despite normalization of FT4. Most important, in one third of cases, even when FT4 is in the higher range, FT3 is often lower than the reference range, indicating that these patients' ability to convert T4 to T3 is insufficient. The accompanying pituitary and peripheral hormone deficiencies may play a role in the reduced FT4 to FT3 conversion. These data indicate that FT4 alone is not sufficient for assessing TH status in CH patients. L-T4 monotherapy may not adequately guarantee euthyroidism in a significant subgroup of CH patients that may require a more physiological treatment with combined L-T4 and L-T3 administration.

OC60

INNOVATIVE METHODS OF IODINE PROPHYLAXIS: FROM IODINE FORTIFIED VEGETABLES TO HUMANS

F. Niccolai[1], A. Molinaro[1], A. Dimida[1], M. Frigeri[1], M. De Servi[1], P. Agretti[1], D. M. Giuseppina[1], E. Ferranini[1], P. Piaggi[2], G. Lucia[1], F. Aghini Lombardi[1], P. Vitti[1], M. Tonacchera[1]

[1]Endocrinology and Metabolism - Pisa, [2]Energy and Systems Engineering - Pisa

Introduction. Iodine deficiency (ID) is the result of insufficient dietary iodine intake and has multiple adverse effects due to inadequate thyroid hormones production. The most effective way to control iodine deficiency is through the universal salt iodisation (USI). USI alone may not be sufficient to assure adequate iodine nutrition.

Objectives. Biofortification of vegetables with iodine offers an excellent opportunity to increase iodine intake. In this study we tested the intake of vegetables (potatoes, tomatoes, carrots and green salad) fortified with iodine in 50 healthy volunteers to assess the efficiency of this model of iodine prophylaxis.

Methods. Each portion of vegetables (200 g of potatoes, 100 g of carrots 100 gof tomatoes and 70 g of salad) contains about 45 ug of iodine (30% of RDA).The volunteers consumed a portion of vegetables, 5 times/week for 1 week. To assess the iodine intake we measured iodine excretion in urine. The first urine samples were obtained before starting the study, successively, we measured the urinary iodine 7 days following the intake of the vegetables in the diet and then 7 days after the end of vegetables intake.

Results. The median urinary iodine before the treatment was 98,3 mcg/L, during the treatment was 117,5 mcg/L and after the treatment was 85 mcg/L. Our results indicate that the median urinary iodine has increased by 19% and return to the basal values after the discontinuation of the intake.

Conclusions. Biofortification of vegetables with iodine provide a mild increase in UI concentration that, together with the abitual use of iodised salt, contributes to improve the iodine nutritional status of the population without risk of iodine excess.

OC61

IMPAIRED OUTCOME OF CONTROLLED OVARIAN HYPER-STIMULATION IN WOMEN WITH THYROID AUTOIMMUNE DISEASE: THYROID AUTOIMMUNITY, THYROID FAILURE OR BOTH?

V. Capelli[1], F. Magri[1], M. Gaiti[1], E. Brambilla[2], F. Zerbini[1], M. Rotondi[1], R. E. Nappi[2], L. Chiovato[1]

[1]Medicina Interna ed Endocrinologia, Fondazione Salvatore Maugeri - Pavia, [2]Centro per la procreazione medicalmente assistita, IRCCS Policlinico San Matteo - Pavia

Introduction: Controlled ovarian hyper-stimulation (COH) is a crucial step of assisted reproduction technologies (ART). Thyroid dysfunctions and autoimmune thyroid disease (ATD) may negatively affect the outcome of ART, but the underlying mechanisms are still poorly understood.

Aim: To evaluate the respective role of ATD and thyroid function, as assessed by serum TSH, on the early outcome of COH. We decided to focus on this short-term outcome in order to selectively study the influence of thyroid diseases on the ovarian sensitivity to gonadotropin stimulation. Using this design we avoided the confounding effect of the many other factors, which contribute to the final outcome of ART (endometrial microenvironment, male factor, anatomical abnormalities).

Subjects and methods: 262 (202 ATD-negative and 60 ATD-positive) euthyroid sub-fertile women underwent ART. Prior to COH, serum FSH, LH, estradiol (E2) were measured at cycle day 3, and progesterone at cycle day 21. At oocytes pick-up and at embryo-transfer we evaluated the performance of recombinant-FSH (r-FSH), as assessed by serum E2 concentration/total administered r-FSH units (E2/r-FSH) ratio and by oocytes quality.

Results: at both oocytes pickup and embryos transfer, the performance of r-FSH was significantly poorer in ATD-positive than in ATD-negative women. In the ATD-positive group, women with a TSH < 2.5 mIU/L displayed a higher serum E2 concentration at oocytes pickup, a higher E2/r-FSH ratio, and a greater number of mature metaphase II (M II) oocytes than women with a TSH > 2.5 mIU/L. When ATD-positive women were divided in quartiles according to their serum TSH level, both the ovarian response to r-FSH and the number of M II oocytes significantly decreased from the lowest to the highest quartiles of serum TSH concentration.

Conclusions: ATD has a negative effect on the early outcome of COH, but this negative influence may be avoided with adequate L-thyroxine therapy aimed at keeping TSH < 2.5 mU/L. Thyroid antibodies and serum TSH should be checked in any woman undergoing ART.

OC62

IODINE PROPHYLAXIS RATHER THAN L-T4 TREATMENT IS EFFECTIVE IN IMPROVING NEUROINTELLECTUAL PERFORMANCES IN CHILDREN BORN TO IODINE DEFICIENT MOTHERS

M. Moleti[1], G. Ilardo[2], M. Boncoddo[2], A. Candia Longo[2], B. Di Bella[1], S. Presti[1], G. Sturniolo[1], G. Tortorella[2], F. Trimarchi[1], F. Vermiglio[1]

[1]Medicina Clinica e Sperimentale - Messina, [2]Neuropsichiatria Infantile - Messina

Background. Adequate iodine intake during pregnancy is essential to maintain maternal and fetal euthyroidism and to guarantee normal brain development in the fetus. Objective. To verify the effects of iodine prophylaxis by iodized salt on neuro-intellectual outcome of children born to mothers residing in a moderately iodine deficient area. Subjects. Sixty children aged ≥6 and ≤12 years, born to mothers who had referred to our antenatal thyroid screening clinic between 2000 and 2006, divided into the following groups: 1) iodized salt (Is) group, n=15 children born to mothers who had been regularly using iodized salt for at least two years prior to becoming pregnant; 2) iodized salt + L-Thyroxine (Is-LT4+) group, n=15 children born to mothers regularly using iodized salt for at least two years prior to becoming pregnant and who were on L-T4 treatment (suppressive or substitutive doses) prior to pregnancy; 3) no iodized salt (no-Is) group, n=15 children born to mothers who never used iodized salt; 4) no iodized salt but L-T4 treatment (no-Is-LT4+) group, n= 15 children born to unsupplemented mothers who were on L-T4 treatment (suppressive or substitutive doses) prior to pregnancy. Methods. Wechsler Scale of Intelligence Scale for Children 4th ed. (WISC-IV), 13 subscales. Results. Children born to iodine supplemented mothers had a similar total Intelligence Quotient (tIQ) (Is group tIQ 93.7±12.2 and Is-LT4+ tIQ 95.8±18.2, p NS), which was significantly higher of those recorded in childre born to unsupplemented mothers (no-Is group tIQ 82.3±15.1 and no-Is-LT4+ group tIQ 84.1±14.7, p NS). This finding was observed irrespectively of maternal serum FT4 levels that were, as expected, consistently higher at any point in time during pregnancy in the mothers on LT4 therapy. The proportion of children with cognitive deficit (tIQ<85 points) was about threefold lower among children born to iodine supplemented mothers (Is and Is-LT4+ groups 23% vs no-Is and no-Is-LT4+ groups 63.3%, $\chi 2$ 8.21 p 0.004). Conclusions. Our data seem to indicate that neuro-intellectual outcome is affected by maternal iodine status rather than by maternal thyroxine levels, likely because of a more adequate fetal thyroid function. Nonetheless, the high rate of cognitive deficit also observed in children born to mothers who regularly used iodized salt indicates this mean of iodine prophylaxis to be insufficient to face the increased needs for iodine during pregnancy.

OC63

STEADY STATE SERUM T3 CONCENTRATIONS FOR 48 HOURS FOLLOWING THE ORAL ADMINISTRATION OF A SINGLE DOSE OF T3 SULFATE

F. Santini[1], M. Giannetti[1], I. Ricco[1], G. Querci[1], G. Saponati[2], D. Bokor[3], G. Rivolta[3], L. E. Braverman[4], P. Vitti[1], A. Pinchera[1]

[1]UO Endocrinologia I-Ospedale di Cisanello-Azienda Ospedaliero-Universitaria Pisana - Pisa, [2]Ispharm CRO - Lucca, [3]Bracco S.P.A - Milano, [4]Section of Endocrinology, Diabetes, and Nutrition, Boston University School of Medicine - Boston

Sulfate conjugation of thyroid hormones is an alternate metabolic pathway that facilitates the biliary and urinary excretion of iodothyronines and enhances inner ring deiodination, leading to the generation of inactive metabolites. A de-sulfating pathway reverses this process and thyromimetic effects have been observed following the parental administration of 3,5,3'-triiodothyronine sulfate (T3S) in rats. The present study investigated whether T3S is absorbed after oral administration in humans and if it represents a source of 3,5,3'-triiodothyronine (T3). Twenty-eight hypothyroid patients (7 men and 21 women, mean age 44 ± 11 years [SD]) who had a thyroidectomy for thyroid carcinoma were enrolled. Replacement thyroid hormone therapy was withdrawn (40 days for thyroxine, 14 days for T3) prior to radioiodine remnant ablation. A single oral dose of 20, 40, 80 (4 patients/group) or 160 mcg (16 patients/group) T3S was administered. Blood samples for serum T3S and total T3 (TT3) concentrations were obtained at various times up to 48 hours after T3S administration. At all T3S doses, serum T3S concentrations increased reaching a peak at 2-4 hours and progressively returned to basal levels 8 hours later. The T3S Cmax and area under the curve (AUC 0-48h) were directly and significantly related to the administered dose (expressed as mcg T3S/kg BW). An increase in serum TT3 concentration levels was observed, significant after 1 hour, further increased at 2 and 4 hours, and then remained steady up to 48 hours after T3S administration. In the 160 mcg group, the mean serum TT3 increase (ng/dl, minus baseline) was: 10.5(1h), 17.7(2h), 23.6(4h), 17.7(8h), 19.7(12h), 28.2(24h), 22.7(48h). There was a significant direct correlation between the TT3 AUC 0-48h and the administered dose of T3S. No changes in serum free thyroxine concentrations during the entire study period were observed, while serum TSH levels increased slightly at 48h not related to the dose of administered T3S. No adverse events were reported. In conclusion: 1)T3S is absorbed following oral administration in humans; 2)the oral administration of a single dose of T3S is converted to T3 in a dose-dependent manner and results in steady state serum T3 concentrations for 48 hours; 3)T3S may represent a new agent in combination with thyroxine in the therapy of hypothyroidism.

OC64

SILDENAFIL TARGETS IN VITRO AND IN VIVO CXCL10 RELEASE: A NOVEL PHARMACOLOGICAL TOOL FOR CARDIAC DISEASES

M. Colleti[1], A. Isidori[2], A. Lenzi[2], L. Di Luigi, C. Crescioli

[1]Dep Mov Human & Health Sc, Foro Italico - Rome, [2]Dep of Exp Med, Sapienza - Rome

Phosphodiesterase type 5 inhibitors (PDE5i), used to treat erectile dysfunction, emerged to improve cardiac kinetics in diabetic subjects with dilatative cardiomyopathy. CXCL10, a potent Th1 cell chemoattractant, seems deeply engaged in cardiac inflammation and dysfunctions, i.e., heart rejection, vasculopathy development after transplantation or cardiopulmonary bypass. CXCL10 likely drives a self-inflammatory loop in damaged heart: human cardiomyocytes under inflammatory stimuli secrete CXCL10, which, in turn, perpetuates Th1-driven inflammatory cascade, leading to cardiac damage. Furthermore, CXCL10 is involved in diabetes as well. We aimed 1. to investigate the effect of the PDE5i Sildenafil onto CXCL10 secretion induced by inflammatory stimuli in human cardiomyocytes in comparison with cyclosporin A (CsA) and methylprednisolone (MeP), both drugs with immunomodulatory properties. IL-8 and IL-6, related to cardiac inflammatory status, were also tested. The same assays were performed on human PBMCs to investigate the effect of Sildenafil onto circulating immune cells; 2. to measure CXCL10 in sera from 30 diabetic patients with dilatative cardiomyophaty before and after chronic treatment with Sildenafil (100mg/day), vs. placebo and in comparison with other inflammatory cytokines (IL-8, IL-6, MIP1 β , MCP-1). ELISA and Bio-Plex array system were used to analyze cell supernatants and sera. We found that Sildenafil: 1. *in vitro*, decreased CXCL10 secretion by cardiomyocytes, similarly to CsA and differently from MeP; IL-8 and IL-6 secretion did not change; in PBMCs it did not affect CXCL10 secretion; IL-8 and IL-6 decreased with each treatment, except for IL-8 with CsA; 2. *in vivo*, decreased serum CXCL10 only in those patients with higher basal levels, thus categorizing, responder vs. non responder groups; placebo intake exerted no effect. Interestingly, a correlation between circulating CXCL10 and markers related to cardiac function, such as BNP and NT-proBNP were observed; no correlation was found with diabetes related parameters; IL-8, IL-6, MIP1-β, MCP-1 were significantly reduced. In conclusion, our *in vitro* and *in vivo* data, while confirm the relevance of CXCL10 in heart dysfunction, suggest Sildenafil as a novel pharmacological tool for cardiac diseases, likely depending on disease-related inflammatory status.

OC65

RECURRENT PREGNANCY LOSS AND GENOMIC INSTABILITY OF THE MALE GAMETE

C. Krausz[1], S. Vinci[1], A. Costantino[2], E. Guarducci[1], I. Natali[3], M. C. Meriggiola[2], G. Forti[4]

[1]Andrology Unit, Department of Biomedical, Experimental and Clinical Sciences, University of Florence - Florence, [2]Department of Obstetrics and Gynecology - Bologna, [3]Sterility Center, S.S. Cosma and Damiano Hospital - Pescia, [4]Endocrine Unit, Department of Biomedical, Experimental and Clinical Sciences, University of Florence - Florence

Recurrent pregnancy loss (RPL) is a multifactorial disease and in about 50% of cases the etiology is not defined. Previous studies have focused on abnormal karyotype, sperm aneuploidy rate and complete AZF microdeletions on the Y chromosome. We investigated on genetic factors in the male partner of RPL couples: i) complete AZF deletions ii) partial AZFc deletions and duplications iii) instability of microsatellites (MSI) comparing DNA from spermatozoa versus blood cells. For the first two objectives, we screened 139 patients by STS +/-, AZFc gene dosage (Genescan software) and compared the data to 637 normozoospermic controls. Complete AZF deletions were not found in any RPL patient, whereas we observed a significantly higher frequency of partial AZFc deletion subtype, called gr/gr, in patients versus controls (2.9% versus 0.47%, p=0.022; OR=6.125 CI,95%=1.387-27.047). The frequency of AZFc partial duplications showed a higher incidence in patients than in controls (4.31% versus 2.67%), without reaching statistical significance. For the third objective, 75 patients from RPL couple and 63 fertile normozoospermic controls were studied. We analyzed 7 microsatellites (mono-, di- and trinucleotide) in DNA extracted from both lymphocytes and spermatozoa (Genescan software). The length of microsatellites was compared between the two cell types and when a discrepancy was found the carrier was referred to as "unstable". The subjects with highly unstable condition (>40% of markers discordant) were present exclusively in the patient's group (6/75; 8% versus 1/63; 1.6% in controls). In conclusion, we found a significant association between gr/gr deletion and RPL. The enlargement of the study population will allow to validate the potential association between RPL and the two other genetic alterations (partial AZFc duplications and microsatellite instability).

OC66

LABEL-FREE BOTTOM-UP PROTEOMIC APPROACH IN SEMINAL PLASMA TO IDENTIFY ANDROGEN TARGETS IN MALE HYPOGONADISM.

D. Milardi[1], G. Grande[2], F. Vincenzoni[3], A. Giampietro[2], I. Messana[4], M. Castagnola[3], R. Marana[1], L. De Marinis[2], A. Pontecorvi[2]

[1]Istituto Scientifico Internazionale "Paolo VI" - Università Cattolica del S. Cuore - Roma, [2]Dipartimento di Endocrinologia - Università Cattolica del S. Cuore - Roma, [3]Istituto di Biochimica e Biochimica Clinica - Università Cattolica del S. Cuore - Roma, [4]Dipartimento di Scienze della Vita e dell'Ambiente - Università di Cagliari - Cagliari

Seminal plasma (SP) contains proteins secreted by testis, epididymis and male accessory glands, involved in the successful fertilization of the oocyte. The function of epididymis, prostate and seminal vesicles are dependent upon the presence of androgenic stimuli.

To investigate the role of testosterone in the modulation of the proteomic pattern in SP, we analyzed human SP proteome comparing seminal proteome by five normogonadal males with the proteome by five patients with severe hypogonadism. H

ormonal assays and standard semen analysis were performed. Label-free proteomic analysis was performed after trypsin digestion by an Ultimate 3000 Nano/Micro-HPLC apparatus equipped with an FLM-3000-Flow manager module and coupled with an LTQ Orbitrap XL hybrid mass spectrometer. Among the list of proteins identified in control samples we evaluated the proteins which were absent in all samples by hypogonadic patients.

Protein identification criteria resulted in the identification of a significative lower number of proteins in hypogonadic patients compared with proteins identified in controls. Among the 60 proteins identified in fertile normogonadal men, 50 proteins were absent in hypogonadic patients, including protein S-100, WD repeat-containing protein, semaphorin, TNF receptor-associated factor, lactotransferrin, prolactin-inducible protein, prostatic acid phosphatase, cystatin, carboxypeptidase E, thyroglobulin.

The 50 missing proteins might represent indicators of androgen-mediated protein synthesis at cellular level. Furhermore, the absence of seminal proteins involved in fertility in hypogonadal patients may explain the association between male hypogonadism and infertility.

OC67

R31C GNRH1 MUTATION CAUSES AN AUTOSOMAL DOMINANT FORM OF NORMOSMIC CONGENITAL HYPOGONADOTROPIC HYPOGONADISM

L. Maione[1], J. Young[2], R. P. Millar[3], M. Lombès[2], A. Guiochon-Mantel[2], A. Colao[1], J. Bouligand[2], R. Pivonello[1]

[1]Dipartimento di Medicina Clinica e Chirurgia, Sezione di Endocrinologia, Università Federico II - Napoli, [2]Université Paris Sud - Kremlin-Bicetre, [3]University of Pretoria - Pretoria

Normosmic congenital hypogonadotrophic hypogonadism (nCHH) is a rare disease of the hypothalamo-pituitary-gonad axis leading to lack of puberty and infertility. Loss-of-function mutations of *GNRH1* gene (encoding GnRH decapeptide) are a very rare cause of autosomal recessive nCHH. R31C *GNRH1* is the only missense mutation described to date affecting the GnRH decapeptide. The heterozygous condition is in striking contrast with the mode of inheritance traditionally described for frameshift and non-sense mutations. We report two additional nCHH families with heterozygous R31C *GNRH1* mutation. We postulate a mutational «hot spot» since the nucleotide base change is located on a CpG islet in four unrelated nCHH families. We first excluded a second genetic event at *GNRH1* locus by sequencing regulatory regions (upstream and downstream promoters, and intron 1). Then we excluded oligogenism by sequencing coding regions of other nCHH-associated genes. Finally we conducted a comprehensive functional characterization of mutant GnRH decapeptide on three different cell models to unravel disease mechanisms. We clearly demonstrate a dramatic reduction of the mutant decapeptide activity. R31C decapeptide is less able to activate ERK phosphorylation, to stimulate serum-response-element coupled to luciferase, to trigger intracellular calcium mobilization and inositol phosphate accumulation when compared to wild-type. Additionally, R31C peptide is unable to induce *lh beta* transcription and LH secretion in LbetaT2 murine gonadotrope cells. This loss of function is explained by a drastic reduction in the affinity to GnRH receptor. We did not find any negative dominance of the mutant peptide over the wild type *in vitro*. This offers a unique opportunity to discuss the complex *in vivo* patho-physiology of this apparently dominant form of nCHH.

OC68

MUTATION ANALYSIS OF NR5A1 GENE ENCODING STEROIDOGENIC FACTOR 1 (SF-1) IN CRYPTORCHIDISM AND MALE INFERTILITY

M. S. Rocca[1], C. Vinanzi[1], M. Ghezzi[1], R. Selice[1], C. Foresta[1], A. Ferlin[1]

[1]Dipartimento di Medicina Molecolare - Padova

The gene *NR5A1*, encoding Steroidogenic Factor 1 (SF1), is a member of the nuclear receptor superfamily and is a key transcriptional regulator of genes involved in male sex determination and differentiation, testicular descent and reproduction. Initially, mutations in this gene has been identified in 46,XY patients affected by primary adrenal insufficiency (AI) and/or DSD (disorders of sexual development) with various degree of gonadal dysgenesis.

Individuals with heterozygous *NR5A1* mutations present a broad range of clinical phenotypes in agree with preliminary studies suggesting that unexplained severe spermatogenetic failure and cryptorchidism might be linked to mutations in *NR5A1*.

In order to better understand the role of NR5A1 in male infertility and cryptorchidism, we performed a mutation analysis of the coding region of NR5A1 gene in 673 subjects (275 newborns with cryptorchidism, 112 men with history of orchidopexy, 286 idiopathic infertile men with azoo-oligozoospermia) and 286 normozoospermic men (controls).

The molecular analysis of the *NR5A1* gene revealed eight new and one previously described heterozygous mutations in the 673 cases studied. All mutations identified in the *NR5A1* gene were heterozygous missense mutations in exon 4 and were located in the hinge region and proximal Ligand Binding Domain (LBD). Furthermore, three of the new identified mutations have been predict to be probably damaging. No variations in *NR5A1* were found in subjects with normal semen parameters.

In addition, in our group of patients we have found the previously described polymorphism (Gly146Ala) that was also associated in patients with cryptorchidism and micropenis. This study strongly suggests that variations in *NR5A1* gene might be associated also with "mild" phenotypes represented by spermatogenic impairment and especially cryptorchidism and it would be necessary demonstrate this data with molecular modeling and in vitro studies.

OC69

AUTOANTIBODIES TO STEROIDOGENIC ENZYMES IN MALES WITH AUTOIMMUNE ADDISON'S DISEASE: MARKERS OF AUTOIMMUNE ORCHITIS?

M. Dalla Costa[1], G. Bonanni[1], S. Masiero[1], S. Chen[2], J. Furmaniak[2], B. Rees Smith[2], R. Perniola[3], G. Radetti[4], S. Garelli[1], S. Chiarelli[5], D. Faggian[1], M. Plebani[1], C. Betterle[1]

[1]Dipt. di Medicina - Padova, [2]FIRS Lab. - Cardiff, [3]O.C. V. Fazzi - Lecce, [4]O.Civile - Bolzano, [5]Dipt. di Scienze Medico Diagnostiche e Terapie Speciali - Padova

Objective and Design: In females with autoimmune Addison's disease(AAD) steroidogenic enzymes autoantibodies (SEA) are markers of autoimmune premature menopause. In contrast the prevalence of SEA in males with AAD has not yet been studied in detail. SEA including autoantibodies to:a)steroid-producing-cells (StCA),b) 17α-hydroxylase (17α-OHAbs), c)side chain cleavage (SCCAbs) were studied in a large cohort of men with AAD. In a group of SEA(+) and SEA(-) AAD males the gonadal function was evaluated by testing levels of FSH, LH, testosterone, sex hormone binding globulin (SHGB), anti-Müllerian Hormone (AMH) and inhibin-B (I-B) compared to a group of SEA(-). A group of SEA(+) and SEA (-) males were followed-up to assess predictive value of SEA in relation to testicular dysfunction. **Patients and Methods**: The study included 147 males with AAD (mean age 34yrs; range 2-84); 27 with APS-1, 76 with APS-2, 11 with APS-4, 33 with isolated AAD. StCA were measured by indirect IF on human testis, ovary and adrenal, 17α-OHAbs and SCCAbs by IPA using ^{35}S-labelled recombinant antigens. The levels of LH, FSH, testosterone, SHGB, AMH and I-B were determined using electrochemiluminescence methods. **Results**: SEA were found in 25% of males. Specifically, in 66% with APS-1; in 22% with APS-2, in 19% with APS-4; and in 3% with isolated AAD. StCA correlated with 17α-OHAbs and/or SCCAbs in 88.4%. LH, FSH, testosterone, SHBG, AHM and I-B levels were tested in 25 SEA(+) and 58 SEA(-) patients. The levels of hormones were in the normal range for the different age groups and there was no significant difference between the group of SEA(+) and SEA(-) males ($p>0.05$). 10 SEA(+) and 14 SEA(-) males were followed-up for a mean period of 4.3yrs and all maintained a normal testicular function. **Conclusion**: SEA were found in males with AAD in particular the patients with APS-1. However SEA do not appear to be markers of clinical, subclinical or potential autoimmune testicular failure. The different value of these Abs in females and males with AAD suggests that the testis could be considered a privileged site protected from autoimmune destruction. "The study was fund from the EU 7th Framework Programme,Grant No. 2008-201167.

OC70

LIPOPOLYSACCHARIDE INHIBITS MITOCHONDRIAL MEMBRANE POTENTIAL IN HUMAN SPERMATOZOA THROUGH THE ACTIVATION OF CB-1 BY SPERM-GENERATED ANANDAMIDE

M. R. C. Vassallo[1], A. Barbonetti[1], A. Sperandio[1], S. Filipponi[1], S. Francavilla[1], F. Francavilla[1]

[1]Andrology Unit, Department of Life, Health and Environment Sciences, University of L'Aquila - L'Aquila

Introduction. Gram-negative bacteria, frequently involved in urogenital tract infections, release the endotoxin lipopolysaccharide (LPS), and its receptor, Toll-like receptor-4 (TLR4), has been recently identified in human spermatozoa. It has been recently reported that sperm exposure to LPS affects sperm motility and activates sperm apoptotic processes, although the underlying signal transduction remains to be clarified. In macrophages, LPS induces the generation of the endocannabinoid Anandamide (AEA) from its membrane phospholipid precursors, through the activity of the calcium-dependent NAPE-hydrolyzing phospholipase D (NAPE-PLD). In human spermatozoa, which exhibit a completely functional AEA-related endocannabinoid system (including NAPE-PLD), the activation of cannabinoid receptor-1 (CB1) inhibited sperm mitochondrial membrane potential (MMP). In this study we tested the hypothesis of a contribution of CB1 activation by sperm-generated AEA in the adverse effects exerted by LPS on human spermatozoa. **Material and methods.** Sperm motility was evaluated with CASA and spermatozoa exhibiting an average pathway velocity >5mcm/sec were categorized by the software as motile sperms. Sperm MMP was assessed at flow cytometry with JC-1, which emits red or green fluorescence in the presence of high or low MMP, respectively. **Results.** The exposure of motile sperm suspensions for 6 h to LPS (100 ng/ml) produced a significant decrement in sperm MMP, as indicated by the lower percentage of spermatozoa emitting red JC-1 fluorescence with respect to untreated samples (43.1±9.4% vs 65.0±9.1%, p=.007); this effect was not associated to decreased sperm motility (motile sperms: 55.8±18.9% vs 55.5±21.3%). The LPS-induced inhibition of MMP was prevented by the selective CB1 cannabinoid receptor antagonist, SR141716 (0.1 mcM), which preserved high percentages of spermatozoa with red JC-1 fluorescence (60.6±13.0%). **Conclusions.** These data suggest that the activation of CB1 by AEA generated by calcium-dependent NAPE-PLD activation could account for the adverse effect of LPS on sperm MMP. However, it is not here confirmed that LPS affect sperm motility. Actually, MMP depression cannot induce sperm immobilization in a standard medium containing glycolysable sugars, as glycolysis actively compensates for any lack of ATP production by mitochondria in maintaining sperm motility.

OC71

METABOLIC CHARACTERIZATION OF ALMS1(GT/GT)EI , A MOUSE MODEL FOR OBESITY AND INSULIN RESISTANCE.

F. Favaretto[1], G. Milan[1], G. Collin[2], J. Marshall[2], P. Maffei[1], J. Naggert[2], R. Vettor[1]

[1]Department of Medicine-UNIPD - Padova, [2]The Jackson Laboratory - Bar Harbor

Introduction and aim: The Alström syndrome (ALMS) is a rare recessive disease caused by mutations in ALMS1 characterized by a wide spectrum of clinical manifestations including obesity and insulin resistance. ALMS1 is ubiquitous expressed and localizes on centrosomes, basal bodies and recycling endosomes, suggesting an involvement in the cilia function and/or intracellular transport, including ALMS in the ciliopathy disorders. The protein function is still unknown and its relationship with metabolic complications is not yet clarify. Our study focused on the characterization of the adipose tissue (AT) in a mouse model for ALMS.

Methods: We characterized a gene-trap insertion in the *Alms1* gene in the C57BL6/Ei mouse strain, (*Alms1*$^{(GT/GT)Ei}$) evaluating the growth curve on standard diet, by measuring the AT depots weight, the diameter of adipocytes, gene expression (by qPCR) and protein (by WB) in subcutaneous (SAT) and visceral (VAT) AT of animals before the development of obesity (6 weeks). We evaluated the proliferation, differentiation, insulin-stimulated glucose uptake and GLUT4 distribution in adipocytes in vitro differentiated .

Results: In *Alms1*$^{(GT/GT)Ei}$ hyperinsulinemia appears before the increase in weight and glucose levels. The weight of SAT and VAT and the diameter of the adipocytes are larger in *Alms1*$^{(GT/GT)Ei}$ than in controls and are associated with an increased expression of lipogenetic enzymes. In culture, the *Alms1*$^{(GT/GT)Ei}$ preadipocytes do not proliferate nor differentiate more of the controls, but, after adipogenic differentiation, show a reduced uptake of glucose, a lower content and translocation of GLUT4.

Conclusion: Adipose tissue GLUT4 defects are the early signs of metabolic dysfunctions in *Alms1*$^{(GT/GT)Ei}$ providing a possible explanation for the tissue insulin resistance and the compensatory hyperinsulinemia. The conserved ability for lipogenesis, in association with the increased insulin levels may contribute to the rapid enlargement of fat mass that results in obesity. Our findings suggest a role of ALMS1 in GLUT4 localization and intracellular trafficking and make *Alms1*$^{(GT/GT)Ei}$ a model to study pathological pathways driving obesity starting from AT defects.

OC72

AN OBESITY PREVENTION INTERVENTION IN TEENAGERS: THE EAT PROJECT. EFFECTIVENESS OF A SCHOOL-BASED PROGRAM ON MEASURES OF FATNESS AND BEHAVIOR

A. E. Malavazos[1], S. Briganti[1], F. Ermetici[1], R. Zalaschi[1], C. Oggioni[1], S. Goggi[1], G. Pelissero[2], L. Morricone[1]

[1]IRCCS Policlinico San Donato. U.O. Diabetologia e Malattie Metaboliche - San Donato MIlanese (MI), [2]IRCCS Policlinico San Donato. Direzione Scientifica - San Donato MIlanese (MI)

The **E.A.T**. (educazione alimentare teenagers) project was designed as a controlled study performed in a period of five years (2009 – 2014). The aim of this study was to determine whether a multicomponent health promotion intervention for adolescents (aged between 11 and 15 years) could be successful in influencing measures of fatness, dietary and physical activity behavior over 2 school years. Preliminary results of the first two years of the intervention are here reported. A total of 870 adolescents (mean age, 13.2 years) were enrolled. **Intervention**: An interdisciplinary program including classroom nutrition lessons, physical education by the use of pedometer and environmental change options (restructured vending-machine filled up with fresh and healthy choice). Moreover, in a subgroup of subjects, short educational messages by mobile (SMS) were employed to enhance the effectiveness of intervention. **Main Outcome Measures**: Height, body weight and waist circumference were directly measured while dietary behavior and physical activity were detected by a specific questionnaire at the beginning and at the end of each school year. **Results**: At the end of the second school year of our program, in the intervention group, on one hand we observed a significant increase in the prevalence of normal-weight adolescents (+10.3%, $p<0.01$), on the other a reduction in the prevalence of overweight and obese adolescents (-7.9%, $p<0.05$ and -2.4%, p=ns, respectively), as well as a significant reduction in mean waist circumference (-2 cm, $p<0.05$). Moreover, we observed an increase in the frequencies of consumption of vegetables and fruits and a reduction in the intake of sugar-sweetened beverages, as well as a significant enhancement in daily walking, especially in the subgroup of subjects who received SMS. No significant change in measures of fatness and in dietary or physical activity-related habits, was observed in the control group. **Conclusion**: The EAT project, after 2 school years, resulted beneficial to improve measures of fatness and behavior, to increase the frequencies of consumption of vegetables and fruits and to reduce the intake of sugar-sweetened-beverages.

OC73

EPICARDIAL ADIPOSE TISSUE INDUCES MYOCARDIAL FIBROSIS

V. Guglielmi[1], N. Venteclef[2], E. Balse[3], F. Atassi[3], J. Amour[4], P. Leprince[4], A. Dutour[5], K. Clement[2], S. Hatem[3], D. Lauro[1], P. Sbraccia[1]

[1]Dip.to Medicina dei Sistemi, Univ. degli Studi di Roma "Tor Vergata" - Roma, [2]INSERM U872, Cordeliers Research Center - Paris, [3]INSERM U956 - Paris, [4]Heart and Metabolism Division, Pitié-Salpêtrière Hospital - Paris, [5]INSERM U626 - Paris

Background: The epicardial adipose tissue (EAT) is a metabolically active fat depot, source of many adipo-cytokines and growth factors which, for the absence of fascial structures, can diffuse and have paracrine effects on the underlying myocardium. Recent studies have reported a relation between EAT thickness and the incidence and severity of atrial fibrillation (AF). However, the underlying mechanisms are unknown. Aim of this study was to test the hypothesis that EAT could promote atrial myocardial remodelling, an important determinant of the substrate of AF. **Methods:** We developed an organo-culture model of rat atrium which allowed to investigate the effects of epicardial and subcutaneous adipose tissue (SAT) conditioned medium on atrial myocardium, maintaining its histological and cellular integrity. Paired EAT and SAT biopsies were obtained from 39 patients during coronary artery bypass graft surgery. After 1 week incubation, tissue remodelling was determined by histological assays and Second Harmonic Generation Imaging, quantified using a histomorphometry software and characterized by immunofluorescence analysis. **Results:** Interestingly, we observed that the EAT secretome induced marked remodelling of rat atria in organo-culture conditions (EAT: 33.78%±1.33 vs SAT: 15.33%±1.6; P=0.001). In addition, immunofluorescence revealed the increased deposition of collagen types I, III and VI at the periphery of trabeculae and in the interstitium. Finally, in primary cultures of rat atrial fibroblasts, the EAT conditioned medium promoted the expression of Collagen type I at both transcriptional and protein levels and their differentiation into myofibroblasts, the principal cellular effectors of fibrotic processes. **Conclusion:** This study constitutes the first evidence that the secretome of human EAT can induce fibrosis of the atrial myocardium. Given the role of fibrosis in the substrate of AF, this work could provide a mechanism that may explain the relationship between EAT thickness and the risk of arrhythmia.

OC74

VISCERAL ADIPOSITY INDEX (VAI) AS A SIMPLE INDICATOR OF "ADIPOSE TISSUE DYSFUNCTION" IN PATIENTS WITH TYPE 2 DIABETES.

M. C. Amato[1], V. Torregrossa[1], S. Milano[2], G. Misiano[2], S. Vitabile[3], M. Midiri[3], G. Pizzolanti[1], C. Giordano[1]

[1]Section of Endocrinology, Diabetology and Metabolism (Di.Bi.M.I.S) - Palermo, [2]Clinical Pathology (DIBIMEF) - Palermo, [3]Section of Radiology (DIBIMEF) - Palermo

ScrAlthough still there is no a clear definition of "adipose tissue dysfunction", the identification of clinical and biological markers of altered fat distribution and function may provide the needed tools to early identify a condition of cardiometabolic risk. Visceral Adiposity Index (VAI) is a mathematical gender-specific index estimated with the use of simple anthropometric [(BMI and Waist circumference (WC)] and biochemical parameters [HDL cholesterol (HDL) and Triglycerides (Tg)], that in recent studies has shown to reflect accurately the degree of visceral adiposity and insulin resistance. However, although VAI has been indirectly shown to be a marker of impaired fat distribution and function, until to date there are only few studies in which it was correlated with the main adipokines (leptin and adiponectin). Our aim was to evaluate the correlations among various measures of fat distribution [VAI, BMI, WC, WHR, visceral adipose tissue volume (VAT) and subcutaneous adipose tissue volume (SAT) measured by MR between L2 and L4] and a complete panel of adipocytokines [Visfatin, Resistin, Leptin, Soluble leptin receptors (sOB-R), Leptin/sOb-R ratio, Adiponectin, Ghrelin, Adipsin, PAI-1, vascular endothelial growth factor (VEGF), TNF-alpha,??hs-CRP, IL-6, IL-18, Hepatocyte growth factor (HGF)]?in patients with type 2 diabetes (DM2). Ninety-one DM2 patients (age: 65.25±6.38 years; duration of disease: 9.73±5.99 years; 42 men and 49 women) in stable treatment for the last two months with metformin in monotherapy at 2 g/day were screened for the study. In all patients the serum levels of adipocytokines were assayed by Luminex-based kits. In a subset of 13 patients an assessment of VAT and SAT volume was performed through a MR. At the Pearson's correlation, among all the investigated measures of fat distribution, only VAI showed a significant correlation with almost all analyzed cytokines [visfatin (r=0.331, p=0.001), resistin (r=0.354, p=0.001), leptin (r=0.285, p=0.005), sOb-R (r=-0.214, p=0.041), ghrelin (r=-0.225, p=0.032), adiponectin (r=-0.394, p<0.001), VEGF (r=0.247, p=0.018), TNF-alpha (??r=0.333, ??p=0.001), HGF (r=0.245, p=0.019), IL-18 (r=0.317, p=0.002)]; no significant correlation with PAI-1 and adipsin was found. Our study suggests VAI, among the most common indexes of adiposity assessment, is a very useful tool well mirroring "adipose tissue dysfunction" in type 2 diabetes.

OC75

INVOLVEMENT OF CANNABINOID RECEPTOR TYPE 1 IN BRITE ADIPOSE TISSUE RECRUITMENT

R. Mazza[1], C. Quarta[1], R. Pasquali[1], U. Pagotto[1]

[1]Scienze Mediche e Chirurgiche - Bologna

Recent data showed the existence of a specific adipocyte population composed of "brite" cells in white adipose tissue (WAT), characterized by a peculiar gene expression pattern including PRDM16. Despite showing a low basal UCP1 expression, these cells display higher UCP1 expression when stimulated. In view of the involvement of the endocannabinoid system in energy homeostasis, the aim of our work is to establish whether the CB1 receptor could be a target for brown phenotype induction in vivo.

We analyzed UCP1 and PRDM16 mRNA expression by QT-RTPCR in brown adipose tissue (BAT), white subcutaneous inguinal (scWAT) and visceral epididymal (eWAT) adipose tissues, obtained from CB1KO (CBN) and Rimonabant-treated (10 mg/Kg/day i.p., 4 weeks) mice vs respective controls (CT), undergoing standard diet (SD) or high-fat diet (HFD) from the 8th to 20th week of age. In CT mice, HFD significantly stimulates UCP1 mRNA in all adipose depots (eWAT $p<0.001$; scWAT and BAT $p<0.01$) when compared to SD mice, whereas PRDM16 expression is increased only in eWAT depot ($p<0.05$), suggesting a tissue-specific gene expression response induced by HFD. We observed constant and similar gene expression modulation in CBN and Rimonabant treated animals. Comparing CT and CBN/Rimonabant mice, relevant changes in gene expression were found in scWAT where CB1 signaling inhibition induced a strong increase in UCP1 expression ($p<0.01$) regardless of diet type, whereas CB1 blockade induced PRDM16 increase ($p<0.05$) only in HFD condition. These data, together with the finding of the sole increase of CB1 expression in HFD vs SD in scWAT ($p<0.01$), strengthen the hypothesis that CB1 receptor antagonization may modulate brown-cell recruitment mechanisms on specific WAT depots. Further studies are underway to complete the characterization of the gene expression pattern of these putative "brite" cells.

OC76

PEDIATRIC OBESITY AND VITAMIN D DEFICIENCY: A PROTEOMIC APPROACH IDENTIFIES MULTIMERIC ADIPONECTIN AS A KEY LINK BETWEEN THESE CONDITIONS

G. E. Walker[1], F. Prodam[2], R. Ricotti[2], M. Roccio[1], S. Moia[1], S. Bellone[2], G. Bona[2]

[1]Department of Health Sciences - Novara, [2]Division of Pediatrics - Novara

Key circulating molecules that link vitamin D (VD) to pediatric obesity and its co-morbidities remain unclear. Using a proteomic approach, our objective was to identify key molecules in obese children dichotomized according to 25OH-vitamin D (25OHD) levels.

A total of 42 obese children (M/F=18/24) were divided according to their 25OHD3 levels into 25OHD3 deficient (VDD; n=18; 25OHD<15 ng/ml) or normal subjects (NVD; n=24; >30 ng/ml). Plasma proteomic analyses by two dimensional (2D)-electrophoresis were performed at baseline in all subjects. VDD subjects underwent a 12mo treatment with 3000 IU vitamin D3 once a week to confirm the proteomic analyses.

The proteomic analyses identified 53 "spots" that differed between VDD and NVD ($p<0.05$), amongst which adiponectin was identified. Adiponectin was selected for confirmational studies due to its tight association with obesity and diabetes mellitus. Western Immunoblot (WIB) analyses of 2D-gels demonstrated a downregulation of adiponectin in VDD subjects, which was confirmed in the plasma from VDD with respect to NVD subjects ($p<0.035$) and increased following 12mo vitamin D3 supplementation in VDD subjects ($p<0.02$). High molecular weight (HMW) adiponectin, a surrogate indicator of insulin sensitivity, was significantly lower in VDD subjects ($p<0.02$) and improved with vitamin D3 supplementation ($p<0.042$). A direct effect *in vitro* of 1α,25-(OH)2D3 on adipocyte adiponectin synthesis was demonstrated, with adiponectin and its multimeric forms upregulated, even at low pharmacological doses (10^{-9}M) of 1α,25-(OH)2D3. This upregulation was paralleled by the adiponectin interactive protein, DsbA-L, suggesting that the VD regulation of adiponectin involves post-transciptional events.

Using a proteomic approach, multimeric adiponectin has been identified as a key plasma protein that links VDD to pediatric obesity.

OC77

PRELIMINARY DATA FROM THE CORE DATASET OF THE EURO-WABB REGISTRY

P. Maffei[1], V. Bettini[1], A. Farmer[2], S. McCafferty[3], S. Aymé[4], W. Mlynarski[5], V. Nunes[6], V. Paquis[7], K. Parkinson[8], V. Tillmann[9], G. Milan[1], F. Favaretto[1], R. Vettor[1], T. Barrett[10]

[1]DIMED - Padova, [2]Birmingham Children's Hospital - Birmingham, [3]NeSC - Glasgow, [4]INSERM - Paris, [5]MUL - Lodz, [6]IDIBELL - Bellvitge, [7]CNRS - Paris, [8]ASUK - Paignton, [9]TU - Tartu, [10]UB - Birmingham

Objectives: We aimed to develop a Registry for the rare genetic diseases Wolfram (WS), Alström (AS), Bardet-Biedl (BBS) and other diabetes syndromes, containing clinical, genetic diagnostic and outcome data (www.euro-wabb.org). The purpose is to: establish the natural history of these diseases, assess clinical management, characterize cohorts for future clinical trials, establish genotype phenotype relations. This abstract describe the first 111 patients recruited. **Methods:** Patients with a confirmed diagnosis (clinical or genetic) were recruited from both within and beyond Europe by their physicians. Anonymised data was entered into a secure web-based registry (https://registry.euro-wabb.org/) at the recruiting site. Data includes 42 'core' fields agreed by consensus, permitting the differentiation of syndromes and we have analysed prevalence of core clinical symptoms. Genetic information was added in the website using the Leiden Open Variation Database software (LOVD). **Results:** 111 participants were recruited from 7 EU countries (45 males). The age range was 2 to 54 yrs, median age 15. There were 50 WS patients (median age 19 yrs, range 4-45), 29 AS (17, range 3-54), 29 BBS (9, median 2-20) and also 3 patients diagnosed with Wolcott-Rallison and with vision and hearing impairment were included. The prevalence of diabetes and median ages of onset were: WS (1/50; 8 yrs); AS (9/29; 15 yrs); BBS (2/29; 13 yrs). The prevalence of obesity and median ages of onset were: WS (1/50; 8 yrs); AS (21/29; 1); BBS (25/29; 1). The prevalence of vision and hearing impairment in WS, AS and BBS were respectively 103/108 (48/50, 29/29, 25/29) and 55/108 (28/50, 21/29, 5/29). 22/29 BBS patients presented with polydactyly. We have compiled a comprehensive mutation database listing 22 genes associated with WABB and including over 723 unique DNA variants. All these data are now freely available at: https://lovd.euro-wabb.org. **Conclusions:** This Registry allowed us to capture sufficient data to differentiate these rare genetic diseases. Increasing the number of patients and recruitment centres will allow us to accurately characterize the phenotype and establish genotype phenotype correlations. These goals will be also achieved through the new consensus extended dataset of 400 fields.

POSTERS

The abstract number is the same reported in the program.

PP001

BODY WEIGHT LOSS REVERTS OBESITY-ASSOCIATED HYPOGONADOTROPIC HYPOGONADISM: A META-ANALYTIC STUDY

G. Corona[1], G. Rastrelli[2], M. Monami[3], F. Saad[4], M. Luconi[2], M. Lucchese[5], A. Sforza[1], G. Forti[2], E. Mannucci[3], M. Maggi[6]

[1]U.O. Endocrinologia, Ospedale Maggiore - Bologna, [2]U.O. Endocrinologia, Università di Firenze - Firenze, [3]U.O. Geriatria, sez Diabetologia, Università di Firenze - Firenze, [4]Gulf Medical University - Ajman, United Arab Emirates, [5]Unità di chirurgia bariatrica, Università di Firenze - Firenze, [6]U.O. Medicina della sessualità e Andrologia, Università di Firenze - Firenze

Introduction. Few randomized clinical studies have evaluated the impact of diet and physical activity on testosterone (T) levels in obese men with conflicting results. Conversely, studies on bariatric surgery in men generally showed an increase in T levels. The aim of the present study is to perform a systematic review and meta-analysis of available trials on the effect of body weight loss on sex hormones levels.
Methods. An extensive Medline search was performed including the following words "testosterone", "diet", "weight loss" and "bariatric surgery" and "males". The search was restricted to data from January 1, 1969 up to August 31, 2012.
Results. Out of 266 retrieved articles, 24 were included in the study. Of the latter, 22 evaluated the effect of diet or of bariatric surgery, whereas 2 compared diet and bariatric surgery. Overall both low calorie diet and bariatric surgery are associated with a significant ($p<0.0001$) increase plasma sex hormone binding globulin bound and unbound T levels (TT), bariatric surgery being more effective in comparison with low calorie diet (TT increase = 8.73[6.51-10.95] vs 2.87[1.68-4.07] for bariatric surgery and low calorie diet, respectively; both $p<0.0001$ vs baseline). Androgen rise is greater in those patients that lose more weight as well as in younger, non-diabetic subjects with a greater degree of obesity. Body weight loss is also associated with and decrease in estradiol and increase in gonadotropins levels. Multiple regression analysis shows that the degree of body weight loss is the best determinant of TT rise ($B=2.50\pm0.98$; $p=0.029$).
Conclusions. Present data show that weight loss is associated with an increase of both bound and unbound T levels. The normalization of sex hormones induced by body weight loss is a possible mechanism contributing to the beneficial effects of surgery in morbid obesity.

PP002

DNA FRAGMENTATION AND POLIPLOIDY IN MACROCEPHALIC SPERM HEADS: A CASE REPORT

M. Mirabelli[1], E. Molinari[2], F. Rumbolo[1], A. Bertagna[1], A. M. Barberis[1], C. Dibisceglie[1], A. Revelli[2], C. Manieri[1]

[1]Università degli Studi di Torino, Dipartimento di Scienze Mediche - Torino, [2]Università degli Studi di Torino, Dipartimento di Discipline Ginecologiche e Ostetriche - Torino

Macrocephalic-sperm head syndrome is associated with large-headed, multi-flagellated spermatozoa and a poor outcome after ART. Here we show the situation of a patient with macrocephalic sperm heads, absence of flagellar abnormalities and a history of repeated abortions after in vitro fertilization. Seminal parameters of a 46-years old patient were analyzed according to WHO guidelines (2010). We evaluated, also, sperm DNA fragmentation with a modified S.C.S.A. and A.O.T., apoptosis rate with Annexin VFITC/P.I. Assay and aneuploidies with FISH for chromosomes 18, X, Y, 13, 21. Sperm concentration was 95 mln/ml, total number was 380 mln/ml, progressive motility was 73% and we observed a 100% teratozoospermia with increased head size. Second level analysis showed 65,5% (S.C.S.A.) and 85,2% (A.O.T.) cells without DNA fragmentation and 3,7% cells AnnVFITC-/PI-. FISH analysis pointed out a 98% of diploid cells. Sperm macrocephaly is commonly associated with oligozoospermia and characterized by polyploid large-headed and multi-tailed spermatozoa, which show increased chromatin decondensation. In this case, the analysis revealed normal concentration and motility, and the absence of any other significant structural aberration (i.e. multiple flagelli). Considering the observed nucleus abnormality, we expected a high proportion of DNA fragmentation as a consequence of altered sperm size and decreased vitality. Apoptotic markers within the sample, showed an extremely abnormal rate of late cellular death, confirmed by eosin vitality test subsequently performed (not shown). To explain this observation we could hypotize the presence of factors such as ROS in seminal plasma that could affect the cells viability. It would be important to better investigate the level of oxidant factors, to evaluate the mithocondrial membrane potential and to use a direct method, such as TUNEL Assay, to assess the DNA fragmentation rate.

PP003

NITROBLUE TETRAZOLIUM TEST FOR SEMEN OXIDATIVE STRESS EVALUATION

F. Rumbolo[1], M. Mirabelli[1], C. Buzzoni[1], A. Bertagna[1], C. Di Bisceglie[1], A. M. Barberis[1], C. Manieri[1]

[1]Università degli Studi di Torino, Dipartimento di Scienze Mediche - Torino

Oxidative stress in spermatozoa is a very important physiological mechanism for its maturation and proper spermatogenesis. It is confirmed, however, that the prolonged state of oxidation and the decrease of the ratio between oxygen free radicals (ROS) and antioxidant activity causes membrane permeability alterations, morphological abnormalities and decreased sperm motility. Oxidation effects can be associated with severe alterations in sperm parameters, such as severe oligozoospermia and azoospermia, and a drastic fertility decline. Nowadays scientists know that seminal standard parameters, by themselves, are not a good indicator of sperm quality and that it is necessary to find second level markers, for example the study of ROS. The purpose of this work was to quantify the seminal fluid oxidation state through the nitroblue Tetrazolium test (NBT test) and look for correlations between the data obtained and standard semen parameters of the same samples analyzed. The data obtained indicate that samples with less than 14% normal sperm morphology (under 50th percentile) show a significant increase of ROS; in consideration of these results, we suggest to consider the use of 50th percentile as an important cut-off in the overall assessment of fertility status. We also found a highly significant correlation between poor sperm motility and an increased level of ROS, but it remains to be investigated whether the increased of ROS levels is the cause or the consequence of poor motility. These results suggest the importance of conducting a refined search of the seminal values that go beyond the simple standard semen analysis. The quantification of the oxidative status can be a significant value for the diagnosis of male infertility and it is also necessary to enlighten the mechanisms underlying the excessive production of ROS, as for example an absence of antioxidant agents. In particular, the NBT test performed in our laboratory, has proved to be a rapid, economical, reproducible and easy to introduce also in daily routine laboratories.

PP004

TOTAL, FREE AND BIOAVAILABLE TESTOSTERONE ACUTELY INCREASE AFTER BOTH SHORT-TERM SUB-MAXIMAL AND MAXIMAL ENDURANCE EXERCISE IN HEALTHY MALES

P. Sgrò[1], C. Baldari[1], F. Romanelli[2], A. Lenzi[2], L. Guidetti[1], L. Di Luigi[1]

[1]Scienze Motorie, Umane e della Salute - Università di Roma "Foro Italico", [2]Medicina Sperimentale - Università di Roma Sapienza

The acute hormones responses to exercise are necessary to guarantee a physiological adaptation and to protect health. Few and conflicting data exist when the role of male acute testosterone adaptation to endurance exercise is concerned. The aim of this study is to investigate on the acute hypothalamus-pituitary-testicular responses to endurance exercise in a laboratory setting. Twelve healthy males volunteers performed a thirty minutes sub-maximal exercise at individual anaerobic threshold and a maximal exercise until exhaustion on cycle-ergometer. Blood samples were collected before exercise [30, 15 min and immediately before (0-pre)], immediately after (0-post), and at different time points during recovery (+15, +30 and +60 min) for hormones assays. Serum PRL, GH, ACTH, LH, cortisol, DHEAS, testosterone [total (TT), calculated free (cFT) and bioavailable (cBioT)], SHBG and respective ratios before and after exercise were evaluated. Together with well known hormones increases (ACTH, GH, C, DHEAS), we observed that TT, cFT and cBioT acutely increased after both exercises in all volunteers. TT concentration increased immediately after both sub-maximal and maximal exercise and remained significantly higher than pre-exercise values during recovery (at +15, +30, +60 min) in both trials. Compared to maximal exercise, TT was higher at + 60 min of recovery after sub-maximal exercise. Mean TT-post.exAUCs were significantly higher than their respective pre.exAUCs after both sub-maximal and maximal exercise (+ 20.05% and +20.89% respectively). cFT and cBioT increased immediately after both sub-maximal and maximal exercise and remained significantly higher than pre-exercise values until + 60 min and + 30 min of recovery, respectively. Compared to maximal exercise, cFT and cBioT were higher at + 60 min of recovery after sub-maximal exercise. cFT- and cBioT-post.exAUC were significantly higher than their respective pre.exAUC after both sub-maximal and maximal exercise. Differently from other hormones, TT, cFT and cBioT maximal increases after exercise were not correlated to exercise-intensity related parameters. The anabolic/catabolic steroids ratios were higher after sub-maximal exercise with respect to maximal exercise. Low intensity and maximal endurance exercises increase testosterone in males. Due to its rapid adaptation and effects, endogenous testosterone should be also considered a dynamic hormone. Considering the adaptive role of testosterone, new concerns arise when diagnosis and management of hypogonadism in male athletes are concerned.

PP005

VISCERAL OBESITY INFLUENCES THE PROSTATE GROWTH IN BASAL CONDITION AND AFTER TESTOSTERONE SUBSTITUTION THERAPY IN KLINEFELTER SYNDROME

R. Selice[1], N. Caretta[2], A. Di Mambro[2], A. Ferlin[2], M. Torino[2], P. Palego[2], C. Foresta[2]

[1]Dipartimento di Medicina Molecolare - Padova, [2]Medicina Molecolare - Padova

Context: Klinefelter syndrome (KS) is a chromosomal alteration characterized by increased incidence of metabolic syndrome, mainly caused by visceral obesity. In the last years, obesity has been studied as potential risk factor for prostate disease and recently a link has been demonstrated between insulin resistance and visceral adiposity with prostate volume.

Objective: To find a possible relation between obesity and prostate volume and to evaluate obesity as a predictive factor of major prostate growth in subjects affected by KS after testosterone therapy.

Subjects and Methods: A total of naïve 121 patients having non-mosaic KS were evaluated with reproductive hormones, metabolic parameters, anthropometric measures, PSA and prostate volume. Out of 121 patients 56 hypogonadic subjects were treated with testosterone-gel 2%. Serum levels of total testosterone, estradiol, LH, FSH, PSA, hemochrome and prostate volume were measured in all subjects at baseline and after 18 months of testosterone-treatment.

Results: In all patients prostate volume positively related with waist circumference (WC). The group with WC ≥ 94 cm showed significantly higher prostate volume, BMI, insulin plasma levels, HOMA index, total cholesterol, triglycerides and glycemia and significantly lower HDL levels with respect to the group with WC < 94 cm. No relation was found between prostate volume and testosterone, estradiol plasma levels, E/T or LH/T ratio. After treatment only the hypogonadic WC ≥ 94 cm group showed a statistically significant increase of prostate volume.

Conclusions: Visceral obesity, insulin-resistance, lipidic and glycidic metabolism alterations seem to be a risk factor for a major prostate growth in KS, despite androgen or estrogen levels. Furthermore the findings about the major prostate volume increase in hypogonadic obese KS subjects, after testosterone-therapy, may provide the basis for individualized T treatment or detect subjects to closer surveillance.

PP006

RELATIONSHIP BETWEEN BIOCHEMICAL AND CLINICAL HYPOGONADISM IN SPINAL CORD INJURED MEN

A. Barbonetti[1], F. Cavallo[2], G. Felzani[2], S. Francavilla[1], F. Francavilla[1]

[1]Andrology Unit, Department of Life, Health and Environment Sciences, University of L'Aquila - L'Aquila, [2]San Raffaele Sulmona - Sulmona

Introduction. A high prevalence of low testosterone (T) levels has been reported in spinal cord injured (SCI) men, but whether they are reflected in clinical hypogonadism is difficult to ascertain, as several clinical features of hypogonadism may be related to other pathophysiological determinants in these patients. The aim of this study was to analyze clinical correlates of T levels in SCI men.

Patients and methods. Twenty patients, aged 45.4±16.8 years, admitted to a rehabilitation program at the Centre for Clinical Research San Raffaele of Sulmona because of traumatic spinal cord injury were included in the study. All patients had a documented history of neurologically stable spinal cord injury, with a mean time post-injury of 9.5±8.6 years. All patients underwent blood and anthropometric measurements. As Body Mass Index (BMI) may underestimate body fat in men with SCI, an estimate of per cent body fat (%fat) was performed using a regression equation to predict %fat [%fat = 3.929+1.246 (BMI)]. Free T was calculated using the Vermeulen formula. Insulin sensitivity was assessed by HOMA index and QUICKI. All participants were asked to complete the AMS (Aging Male Symptom) questionnaire.

Results. The total T values were 439±267 ng/dL (mean±SD). Forthy per cent of patients exhibited T levels < 300 ng/dL. Significant correlates of total T levels were: age (r=-0.63; p=0.05); % fat (r=-0.65; p=0.04); HOMA (r=-0.72; p=0.02); QUICKI (r=0.7.2; p=0.02) and sexual subscale of AMS (r=-0.67; p=0.03);. Free T also correlated with [haemoglobin] (r=-0.68; p=0.03) and somatic subscale of AMS (r=-0.68; p=0.03).

Vitamin D levels were 16.0±5.9 ng/mL. Seventy per cent of patients exhibited levels <20 ng/mL. No correlation was found with total or free T.

Conclusions. This study confirms a high prevalence of low T in SCI patients. The significant correlation between low T levels and several clinical features of hypogonadism, notwithstanding the small population analyzed in this preliminary study, indicates, as a novel datum, a relationship between biochemical and clinical hypogonadism in these patients. An interplay between hypogonadism and insulin resistance appears to be stronger in this than in other clinical settings, suggesting that SCI men are at high risk of hypogonadism as a consequence of metabolic abnormalities as well as of metabolic consequences of hypogonadism.

PP007

INVOLVEMENT OF MITOCHONDRIAL DYSFUNCTION IN THE ADVERSE EFFECT EXERTED BY SEMINAL PLASMA FROM MEN WITH SPINAL CORD-INJURY ON SPERM MOTILITY

A. Barbonetti[1], M. R. C. Vassallo[1], S. Filipponi[1], A. Sperandio[1], A. Di Gregorio[1], S. Francavilla[1], F. Francavilla[1]

[1]Andrology Unit, Department of Life, Health and Environment Sciences, University of L'Aquila - L'Aquila

Introduction. The multi-factorial etiology of severe asthenozoospermia, usually occurring in men with spinal cord injury (SCI) includes an adverse impact of seminal plasma (SP) on sperm motility. In this study we investigated the effect exerted by SP from men with SCI on donor sperm mitochondrial activity and its reflection on motility. **Material and methods.** 22 SP were recovered by centrifugation of ejaculates obtained from SCI men by penile vibratory stimulation. Seminal fructose levels were determined by spectrophotometry. SP from SCI men as well as from healthy donors was tested on donor motile sperm suspensions for their effect on sperm motility, vitality, mitochondrial membrane potential (MMP) assessed at flow cytometry (FC) with JC-1, mitochondrial generation of ROS, evaluated at FC with MitoSOX Red (MSR), lipid peroxidation, evaluated at FC with BODIPY and caspase activation, evaluated at FC using permeable FITC-conjugated peptides (LEHD-FMK and DEVD-FMK), which irreversibly bind to the activated caspase-9 and -3, respectively. Sperm motility was evaluated with CASA. **Results.** Only SP exhibiting both low fructose levels and inhibitory effect on MMP (N=13), affected donor sperm motility when evaluated 1h after coincubation (motile sperms: 20.5±12.7% vs 72.9±7.3% in control SP, p<.001). This effect was reverted by washing in medium containing glucose in spite of persistently depressed MMP, as indicated by the lower percentage of spermatozoa emitting red JC-1 fluorescence (38.9±5.0%) with respect to washed controls (79.4±7.2%, p<.001). In the same samples, sperm motility dramatically decreased with respect to controls when evaluated 6 hours after washing and re-suspension in the glucose-containing medium and the loss of motility was associated to a decrease in the % of viable spermatozoa. SP which induced a disruption of MMP, also enhanced a mitochondrial ROS generation. The enhanced mitochondrial ROS generation was associated with a late induction of lipid peroxidation, when evaluated at 6h, but not at 1h, after washing from SP. Furthermore, activation of caspase-9 and caspase-3 accompanied the loss of MMP. **Conclusions.** A double energetic blockage (glycolysis and mitochondrial respiration) represents a metabolic determinant of the early adverse effect exerted by SP from men with SCI on sperm motility. Mitochondrial dysfunction-related apoptotic/oxidative mechanisms might account for later consequences on sperm motility/vitality.

PP008

EFFECTS OF THE PDE5 INHIBITOR TADALAFIL ON PLASMA ANTIOXIDANT STATUS AND MARKERS OF EXERCISE-INDUCED MUSCLE DAMAGE

P. Sgrò[1], R. Ceci[1], G. Duranti[1], C. Baldari[1], L. Guidetti[1], F. Romanelli[2], A. Lenzi[2], S. Sabatini[1], L. Di Luigi[1]

[1]Scienze Motorie, Umane e della Salute - Università di Roma "Foro Italico", [2]Medicina Sperimentale - Sapienza Università di Roma

INTRODUCTION. A recent clinical study evidenced an increase of serum total antioxidant status (TAS) and a decrease in total oxidant status in men affected by erectile dysfunction after the administration of the PDE5 inhibitor tadalafil. It is well known that intense bouts of exercise induce oxidative stress that can contribute to skeletal muscle damage. When muscle is damaged there is a disruption to the cell membrane inducing an increase of serum concentration of creatine kinase (CK) and lactate dehydrogenase (LDH). Therefore, they are commonly used as a blood markers of muscle damage. AIMS. The present study has been designed to investigate the effects of tadalafil on plasma TAS and on exercise–induced skeletal muscle damage. SUBJECTS AND METHODS. A cross-over double blind, placebo-controlled study was conducted in well-trained subjects. Each volunteer randomly received two tablets of placebo (control group) or tadalafil 20 mg/die with 36 hrs of interval (TD group) before a maximal exercise was performed. After a two-week washout, the volunteers were crossed over. Blood samples were collected immediately before and immediately after exercise in both group to evaluate TAS (sum of endogenous and food-derived antioxidants that represents the total antioxidant activity of the extracellular fluid), CK and LDH. RESULTS. Preliminary data showed no differences in TAS values between TD and control group before exercise. Interestingly, tadalafil administration induced a greater increase of antioxidant capacity after exercise compared to placebo group. Moreover, TD group had significantly lower plasma levels of CK and LDH after exercise when compared with the control group. CONCLUSION. These results suggest that tadalafil influences plasma antioxidant status during exercise and might attenuated exercise related muscle damage. Further investigation on the anti-oxidative effects of tadalafil are warranted.

PP009

SEMINAL, ULTRASOUND AND PSYCHOBIOLOGICAL PARAMETERS CORRELATE WITH METABOLIC SYNDROME IN MALE MEMBERS OF INFERTILE COUPLES

F. Lotti[1], G. Corona[1], S. Degli Innocenti[1], E. Filimberti[1], V. Scognamiglio[1], L. Vignozzi[1], G. Forti[1], M. Maggi[1]

[1]Università di Firenze - Firenze

Introduction: Metabolic syndrome (MetS) impact on male fertility has been poorly studied. This study is aimed at systematically evaluate possible associations between MetS and clinical characteristics in men with couple infertility.

Methods: Out of 367 consecutive subjects, 351 men without genetic abnormalities (36.0±8.0 years) were studied. MetS was defined according to the IDF&AHA/NHLBI classification. All men underwent physical, hormonal, seminal and scrotal ultrasound evaluation. Erectile and ejaculatory functions were assessed by International Index of Erectile Function-15 erectile function domain (IIEF-15-EFD) and Premature Ejaculation Diagnostic Tool (PEDT), respectively, while psychological symptoms by Middlesex Hospital Questionnaire.

Results: Out of 351 patients, 27 (7.7%) fulfilled MetS criteria. In an age-adjusted model, MetS was associated with a stepwise decline in total testosterone (TT) (B=-1.25±0.33,p<0.0001), without a concomitant rise in gonadotropins, suggesting an hypogonadotropic hypogonadism in relatively young subjects. Among ultrasound features, in an age-adjusted logistic model, only testis inhomogeneity was significantly associated with increasing MetS factors (HR=1.36 [1.09-1.70],p<0.01). At univariate analysis, progressive motility and normal morphology were negatively related to the number of MetS components (both p<0.0001), but when age and TT were introduced in a multivariate model, only sperm morphology retained a significant association (B=-1.418±0.42;p=0.001). The risk of ED (IIEF-15-EFD score<26) increased as a function of the number of MetS factors, even after adjusting for age and TT (HR=1.45[1.08-1.95],p<0.02). No association between PEDT score and MetS was observed. Finally, after adjusting for age and TT, somatization and depressive symptoms were associated with increasing MetS components (B=0.66±0.03,p<0.05; B=0.69±0.03,p<0.02; respectively).

Conclusions: In men with couple infertility, MetS is associated with hypogonadotropic hypogonadism, poor sperm morphology, testis ultrasound inhomogeneity, ED, somatization and depression. Recognizing MetS could help patients to improve not only fertility but also sexual and overall health.

PP010

METABOLIC SYNDROME CORRELATES WITH SEMINAL INTERLEUKIN 8, PROSTATE VOLUME AND PROSTATE ULTRASOUND ABNORMALITIES IN MALE SUBJECTS OF INFERTILE COUPLES.

F. Lotti[1], G. Corona[1], L. Vignozzi[1], M. Rossi[1], E. Maseroli[1], M. Gacci[1], G. Forti[1], M. Maggi[1]

[1]University of Florence - Florence

Introduction: The impact of metabolic syndrome (MetS) on male infertility has been poorly studied. We recently reported that MetS is associated with hypogonadism, poor sperm morphology and testis ultrasound inhomogeneity. However, the possible associations between MetS and prostate-related symptoms and signs are still lacking.

Methods: Out of 187 consecutive subjects, 171 men (36.5±8.3 years) without genetic abnormalities were studied. MetS was defined according to NCEP-ATPIII classification. All men underwent hormonal (including total testosterone [TT] and insulin), seminal, including interleukin 8 (IL-8), scrotal and transrectal ultrasound evaluation. Prostate-related symptoms were assessed by National Institutes of Health-Chronic Prostatitis Symptom Index (NIH-CPSI) and International Prostate Symptom Score (IPSS).

Results: Out of 171 patients, 22 (13%) fulfilled MetS criteria. In an age-adjusted regression model, insulin levels increased as a function of MetS components (β=0.134, p<0.0001) and showed an inverse correlation with TT (adj.r =-0.359, p<0.0001). In an age,-TT,-insulin-ordinal logistic regression model, normal morphology was negatively related to the number of MetS components (β=-0.116,p<0.002). No association among MetS, NIH-CPSI and IPSS score was observed. Using an age,-TT,-insulin-ordinal logistic adjusted model, a positive correlation between the number of MetS components and sIL-8 levels was observed (β=0.760,p<0.05). When transrectal ultrasound features were evaluated, using an ordinal logistic regression model adjusted for the aforementioned confounders, the number of MetS components was positively related to prostate volume (β=0.086,p<0.0001), arterial prostatic peak systolic velocity (β=0.148,p=0.02), the prevalence of prostate inhomogeneity (β=0.521,p<0.01) and prostate calcification size (β=0.087,p<0.02). No association between MetS and seminal vesicles features was found.

Conclusions: In men with couple infertility, MetS is positively associated with a prostate enlargement, biochemical (sIL8) and ultrasound signs of prostate inflammation, but not with prostate-related symptoms.

PP011

THE IDENTIFICATION OF PREDIABETES CONDITION WITH ARIC ALGORITHM PREDICTS LONG-TERM CARDIOVASCULAR EVENTS IN PATIENTS WITH ERECTILE DYSFUNCTION.

G. A. Silverii[1], G. Corona[2], G. Rastrelli[1], M. Monami[3], A. Sforza[2], G. Forti[1], E. Mannucci[3], M. Maggi[1]

[1]Dip.to di Fisiopatologia clinica, SODC medicina della sessualità e andrologia - Firenze, [2]Dip.to di medicina, sezione endocrinologia, ASL - Bologna, [3]Dip.to di area critica medico chirurgica, unità geriatrica, sezione diabetologia - Firenze

Introduction: the Atherosclerosis Risk in Communities (ARIC) algorithm is one of the most efficient instruments for the prediction of incident type 2 diabetes. Recently, it has been shown to predict another relevant cardiovascular (CV) risk factor, such as chronic kidney disease.

Aim: to verify whether, in patients with erectile dysfunction (ED), the use of ARIC diabetes risk score might improve the efficacy in predicting major CV events of other CV risk algorithms specifically developed for the assessment of CV risk.

Methods: a consecutive series of 2,437 men (mean age 52.5 ± 12.9 years) attending our outpatient clinic for sexual dysfunction was retrospectively studied. A subset of this sample (N = 1,687) was enrolled in a longitudinal study (mean follow-up of 4.3 ± 2.6 years).

Main outcome measures: the assessment of metabolic risk was evaluated with the ARIC algorithm. The assessment of CV risk was evaluated using the Progetto Cuore risk engine.

Results: in the cross-sectional study, ARIC score was inversely related with testosterone levels, sexual functioning, and penile blood flow. When longitudinal sample was analyzed, higher baseline ARIC score significantly predicted major adverse cardiovascular event (MACE) even when subjects with diabetes mellitus at baseline were excluded from the analysis (hazard ratio = 1.522 [1.086-2.135]; P = 0.015 for trend). In addition, among subjects classified as "low risk" (CV risk <20% at 10 years corresponding to <9% at 4.3 years) by Progetto Cuore, a receiving operating curve (ROC) analysis for ARIC (vs. MACE) allowed the identification of a threshold of 0.22, which had a positive predictive value for 4.3-year MACE of 9%. Applying the ARIC score (with a threshold of 0.22) to Progetto Cuore "low-risk" subjects, we could classify as "at high risk" 89.8% of subjects with incident MACE vs. 79.6% with Progetto Cuore only. Conclusions: in patients with ED, identifying prediabetes, even with algorithms, predicts long-term CV events.

PP012

HAVE ANDROGEN RECEPTOR GENE CAG AND GGC REPEAT POLYMORPHISMS AN EFFECT IN INFERTILE MEN ON SPERM MOTILITY?

N. delli Muti[1], A. Agarwal[2], E. Buldreghini[1], A. Gioia[1], A. Lenzi[3], M. Boscaro[1], G. Balercia[1]

[1]Andrology Unit, Endocrinology, Department of Clinical and Molecular Sciences - Polytechnic University of Marche, Ancona, [2]Center for Reproductive Medicine, Glickman Urological and Kidney Institute - Cleveland, Ohio, [3]Andrology, Pathophysiology of Reproduction and Endocrine Diagnosis Unit - University of Rome ''La Sapienza'', Rome

Androgens and a normal androgen receptor are required for normal spermatogenesis. We investigated polyglutamine (CAG) and a polyglycine (GGC) tract in Italian men with defective spermatogenesis. We studied a group of 40 infertile men with spermatogenesis failure without Y-chromosome microdeletions compared with 60 normozoospermic ones. The distributions of both polymorphisms, within the normal range of Caucasian populations, were similar among infertile men and controls. Nonetheless, we observed that the frequency comparison of each CAG allele showed a statistical difference in the allele CAG 22; GGC 17 was the more predominant allele in infertile men than in controls. Moreover, in order to investigate the hypothesis that semen characteristics are perturbed by androgen receptor allele variants, we tried to detect a link between triplets and sperm motility in all subjects (cases plus controls). Subjects were subdivided into three groups, based on calculated allele frequencies. A significantly decreased motility, related to a longer CAG and GGC tracts, and marked differences between the groups exist for both polymorphisms. Our data highlights a probable relationship between the allele CAG 22/ GGC 17 and a defective spermatogenesis in infertile men, suggesting that these polymorphisms might have an important effect on AR function.

PP013

METABOLIC AND CARDIOVASCULAR OUTCOMES OF FATHERHOOD: RESULTS FROM A COHORT OF STUDY IN SUBJECTS WITH SEXUAL DYSFUNCTION.

A. D. Fisher[1], G. Rastrelli[1], E. Bandini[1], G. Corona[1], M. Monami[2], E. Mannucci[2], M. Maggi[1]

[1]Fisiopatologia Clinica SODC Medicina della Sessualità e Andrologia - Firenze, [2]Diabetes Section, Geriatric Unit, Department of Critical Care University of Florence - Firenze

INTRODUCTION: Previous cross-sectional and longitudinal studies reported a negative correlation between fatherhood and testosterone (T) levels, likely due to a centrally mediated downregulation of the hypothalamic-pituitary-gonadal axis. Moreover, epidemiological data indicate that fatherhood might affect metabolic and cardiovascular outcomes, although different results have been reported. Up to now, no studies have evaluated these associations in a population of men seeking treatment for sexual dysfunction (SD).**AIM:** To explore biological and clinical correlates of number of children (NoC) and its possible associations with forthcoming major cardiovascular events (MACE) in a sample of men with SD.**METHODS:** A consecutive series of 4,045 subjects (mean age 52 ± 13.1 years old) attending the Outpatient Clinic for SD was retrospectively studied. A subset of the previous sample (N = 1,687) was enrolled in a longitudinal study.Information on MACE was obtained through the City of Florence Registry Office.**RESULTS:** Among patients studied, 31.6% had no children, while 26.3% reported having one child, 33.4% two, and 8.8% three or more children. Although fatherhood was negatively related with follicle-stimulating hormone levels and positively with testis volume, we found a NoC-dependent, stepwise decrease in T plasma levels, not compensated by a concomitant increase in luteinizing hormone. NoC was associated with a worse metabolic and cardiovascular profile, as well as worse penile blood flows and a higher prevalence of metabolic syndrome (MetS). In the longitudinal study, after adjusting for confounders,NoC was independently associated with a higher incidence of MACE. However, when the presence of MetS was introduced as a further covariate, the association was no longer significant.**CONCLUSIONS:** This study supports the hypothesis that bond maintenance contexts and fatherhood are associated with an adaptive downregulation of the gonadotropin-gonadal axis, even in a sample of men with SD. Moreover, our data suggest that NoC predicts MACE, most likely because of an unfavorable, lifestyle-dependent, parenthood-associated behavior.

PP014

GENDER IDENTITY DISORDER AND EATING DISORDERS: SIMILARITIES AND DIFFERENCES IN TERMS OF BODY UNEASINESS

A. D. Fisher[1], E. Bandini[1], G. Castellini[2], M. C. Meriggiola[3], H. Casale[1], L. Benni[2], N. Ferruccio[2], C. Faravelli[2], D. Dettore[4], M. Maggi[1], V. Ricca[2]

[1]Andrology Unit, Department of Clinical Physiopathology - Firenze, [2]Psychiatric Unit, Department of Neuropsychiatric Sciences, Florence University School of Medicine - Firenze, [3]Center for Reproductive Health, - Bologna, [4]Andrology Unit, Department of Clinical Physiopathology, Florence University School of Medicine - Firenze

INTRODUCTION:Subjects with gender identity disorder (GID) have been reported to be highly dissatisfied with their body, and it has been suggested that the body is their primary source of suffering. AIMS:To evaluate quality and intensity of body uneasiness in GID subjects, comparing them with a sample of eating disorder patients and a control group.To detect similarities and differences between subgroups of GID subjects, on the basis of genotypic sex and transitional stage. METHODS:Fifty male-to-female (MtF) GID (25 without and 25 with genital reassignment surgery performed), 50 female-to-male (FtM) GID (28 without and 22 with genital reassignment surgery performed), 88 eating disorder subjects (26 anorexia nervosa, 26 bulimia nervosa, and 36 binge eating disorder),and 107 healthy subjects were evaluated. Subjects were studied by means of the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, the Symptom Checklist (SCL-90), and the Body Uneasiness Test (BUT). RESULTS:GID and controls reported lower psychiatric comorbidity and lower SCL-90 General Severity Index (GSI) scores than eating disorder subjects. GID MtF without genital reassignment surgery showed the highest BUT values, whereas GID FtM without genital reassignment surgery and eating disorder subjects showed higher values compared with both GID MtF and FtM who underwent genital reassignment surgery and controls. Considering BUT subscales, a different pattern of body uneasiness was observed in GID and eating disorder subjects. GID MtF and FtM without genital reassignment surgery showed the highest BUT GSI/SCL-90 GSI ratio compared with all the eating disorder groups.CONCLUSIONS:GID and eating disorders are characterized by a severe body uneasiness, which represents the core of distress in both conditions. Different dimensions of body uneasiness seem to be involved in GID subsamples, depending on reassignment stage and genotypic sex. In eating disorder subjects body uneasiness is primarily linked to general psychopathology, whereas in GID such a relationship is lacking.

PP015

INVOLVEMENT OF SPERM PLASMA MEMBRANE IN HUMAN MALE INFERTILITY

G. Balercia[1], A. Vignini[2], E. Salvolini[1], E. Buldreghini[1], R. Di Primio[1], L. Mazzanti[2]

[1]Dipartimento di Scienze Cliniche e Molecolari - Ancona, [2]Dipartimento di Scienze Cliniche Specialistiche ed Odontostomatologiche - Ancona

A growing body of evidence supports the role of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in the pathogenesis of sperm cell dysfunction among men with infertility. These free radicals can react with the fatty acids of plasma membrane phospholipids, thus affecting the physico-chemical features of the bilayer, which in turn may greatly influence the structural and functional properties of membrane-bound/embedded proteins, and cause a functional impairment of spermatozoa themselves. In fact, the plasma membrane structure has a great importance for successful fertilization, given that capacitation, acrosome reaction (AR) and sperm-egg fusion are membrane-associated events.

Furthermore, modifications of sperm membrane fluidity may alter the activity of enzymes involved in ionic homeostasis, such as Na+/K+-ATPase and Ca2+-ATPase. On the other hand, both ATPases are known to be suphydryl (SH)-containing enzymes and their thiol groups could be the target for both nitric oxide (NO) and peronynitrite (ONOO-).

Our aim was to isolate human spermatozoa from normospermic fertile donors and infertile subjects affected by idiopathic asthenozoospermia. In these cells we assessed the physico-chemical characteristics of plasma membrane by means of fluorescence spectroscopy. In addition we determined Na+/K+-ATPase and Ca2+-ATPase activities, free SH content and intracellular Ca2+ concentration.

Spermatozoa from the asthenozoospermic group exhibited a reduced fluidity at the lipid-water interface level, an increased fluidity of the deeper portion of the bilayer, and a lower plasma membrane hydration than normospermic cells. Moreover control spermatozoa showed Na+/K+-ATPase and Ca2+-ATPase activities, free SH content and intracellular Ca2+ concentration higher than those of asthenozoospermic samples.

PP016

TESTICULAR DAMAGE BY CHRONIC GONADOTROPINS SUPPRESSION IN UNRECOGNIZED PATIENT WITH CONGENITAL ADRENAL HYPERPLASIA: OUTCOME OF GLUCOCORTICOID THERAPY

S. C. Ingrillì[1], G. Borzì[1], P. Tita[1], D. Leonardi[1], G. Padova[1]

[1]Endocrinology, Garibaldi Nesima Hospital, Department of Clinical and Molecular Biomedicine, University of Catania - Catania

Excessive adrenal steroid production inhibits the hypothalamic-pituitary axis with failure of normal testicular maturation, if this occurs early in file. We describe the case of a 30-yrs-old man with a 9-yrs primary infertility.He reported pubarche when he was 3-yrs-old, greater stature than his peers up to 10 yrs and subsequent slowdown of the growth rate and completion of secondary sex characteristic. Despite his personal and family history (a sister already treated with dexamethasone for classic virilising congenital adrenal hyperplasia, CAH), he didn't receive appropriate diagnosis and therapy. At the physical examination, height 156 cm, weight 74 kg, BMI 30.4 kg/m², normal virilization, normal blood pressure, hypotrophic testicles (bilateral Prader 6-8). At laboratory tests: azoospermia, FSH <0.1 mU/ml, LH 0.1 mU/ml, Testosterone 6.7 ng/ml, PRL 20 ng/ml, 17-OH Progesterone (17OHP) 135 ng/ml, D4-A 25.7 ng/ml, DHEAS 3.6 mcg/ml, cortisol 15 mcg/ml, E2 23 pg/ml, ACTH 1378 pg/ml. The low values of gonadotropins were therefore consequent to the negative feedback caused by high adrenal androgens levels due to CAH.Testis ultrasound revealed subcentimetric hypoechoic and disomogeneous lesions with irregular and hyperechoic rib. He started treatment with dexamethasone and cortisone acetate, with dose adjustments on the basis of periodic laboratory tests. The correction of adrenal steroidogenesis with glucocorticoid therapy led to normalization of ACTH, 17OHP and D4A values, but testosterone values below the normal range (1.4 ng/dl). A gradual increase of gonadotropins levels was detected, highlighting the testicular damage. Therefore, the correction of adrenal steroidogenesis with glucocorticoids unmasked the preexistent hypogonadism which gradually led to subjective simptoms such as fatigue, erectile dysfunction and loss of libido, making necessary the testosterone replacement therapy.Conclusions:late diagnosis of CAH and adrenal androgen excess due to 21-hydroxylase deficiency caused the suppression of the hypothalamic-pituitary-gonad axis, leading in testicular maturation failure with fibrosis and atrophy and, finally, in a hypergonadotropic hypogonadism. The diagnosis of CAH in man, both in the classic form and late-onset is underestimated; in the evaluation of the infertile patient, especially in presence of low gonadotropins levels and normal testosterone, is essential to include the 17OHP assay.

PP017

IMPAIRED MASTURBATION INDUCED ERECTIONS: A NEW CARDIOVASCULAR RISK FACTOR FOR MALE SUBJECTS WITH SEXUAL DYSFUNCTION

V. Boddi[1], G. Rastrelli[1], G. Corona[2], G. Forti[1], M. Maggi[1]

[1]Fisiopatologia clinica- Sezione Andrologia - Firenze, [2]Endocrinologia - bologna

Introduction: Erectile dysfunction (ED) is considered an early surrogate marker of silent, or even overt, cardiovascular diseases (CVD). However, epidemiological studies take into account only sexual intercourse-related erections. Although autoeroticism is a very common practice, data on masturbation-induced erections as a possible predictor of major adverse cardiovascular events (MACE) are lacking.

Aim: To evaluate the clinical correlates of impaired masturbation-induced erections and to verify the importance of this sexual aspect in predicting MACE.

Methods: A consecutive series of 4,031 male patients attending the Outpatient Clinic for sexual dysfunction for the first time was retrospectively studied. Among these subjects, 64% reported autoeroticism during the last three months and only this subset was considered in the following analyses. In the longitudinal study, 862 subjects reporting autoeroticism were enrolled.

Main outcome measures: Several clinical, biochemical and instrumental (PGE1 test and penile color Doppler ultrasound) parameters were studied.

Results: Subjects with an impaired erection during masturbation (46% of those reporting autoeroticism) had more often a positive personal or family history of CVD, a higher risk of reduced intercourse- and sleep-related erections, hypoactive sexual desire and perceived reduced ejaculate volume, and impaired PGE1 test response. Prolactin levels were lower in those having impaired erection during masturbation. In the longitudinal study, unadjusted incidence of MACE was significantly associated with impaired masturbation-induced erections. When dividing the population according to the median age and diagnosis of diabetes, the association between impaired masturbation-induced erections and incidence of MACE was maintained only in the youngest (<55 year-old) and in non-diabetic subjects, even after adjusting for confounders (HR=3.348[1.085-10.335], p=0.032 and HR=2.108 [1.002-4.433], p=0.049; respectively).

Conclusions: This study indicates that, in subjects with male sexual dysfunction, evaluating an often neglected sexual parameter, such as masturbation-induced erections, can provide further insights on forthcoming MACE in particular in "low risk" subjects.

PP018

"IT TAKES TWO TO TANGO": THE RELATIONAL DOMAIN IN A COHORT OF SUBJECTS WITH ERECTILE DYSFUNCTION (ED).

V. boddi[1], G. Rastrelli[1], G. Corona[2], A. D. Fisher[1], G. Forti[1], M. Maggi[1]

[1]Fisiopatologia Clinica-Sez Andrologia - Firenze, [2]Endocrinologia - Bologna

Introduction. The relational domain of erectile dysfunction (ED) is difficult to investigate in a clinical setting. We developed and validated SIEDY, a 13-item structured interview, which assesses, beside the organic (Scale1) and intra-psychic (Scale 3) domains, also the relation one (Scale 2). We previously established a pathological threshold for SIEDY Scale 1 and 3.

Aim. To identify a pathological threshold of SIEDY Scale 2.

Method. A non-selected, consecutive series of 2992 subject with ED was retrospectively evaluated. In a first consecutive series of 844 patients (Sample A, studied without systematically applying a psychometric test: Middlesex Hospital Questionnaire, MHQ) a pathological threshold of SIEDY Scale 2 score was identified through receiver operating characteristic, using, as surrogate marker of impaired couple relationship, at least a positive answer to two standard questions on conflict within the couple and on the presence of extramarital affairs.

Main outcome measure. Sensitivity and specificity, along with possible associations with biological and psychological correlates were verified in a further sample of 2148 patients (Sample B).

Results. In sample A, a threshold of Scale 2 score ≥ 2 predicts couple impairment with a sensitivity of 53% and specificity of 66%, and an overall accuracy of 62.0±2.2% ($p<0.0001$). When this threshold was verified in sample B, Scale 2 score ≥ 2 was associated with a higher risk of anxiety and depressive symptoms, higher prevalence of psychopathology, and higher Scale 3 scoring, even after adjusting for confounders. In the same sample, a Scale 2 score ≥ 2 was associated with a reduced intimacy during sexual intercourse and overall worse sexual functioning.

PP019

METABOLIC SYNDROME AND BPH/LUTS: THE ROLE OF INFLAMMATION.

L. Vignozzi[1], M. Gacci[1], I. Cellai[1], R. Santi[1], G. Corona[1], A. Morelli[1], G. Rastrelli[1], P. Comeglio[1], A. Sebastanelli[1], E. Maneschi[1], G. Nesi[1], C. De Nunzio[1], A. Tubaro[1], E. Mannucci[1], M. Carini[1], M. Maggi[1]

[1]Experimental and Clinical Biomedical Sciences - Florence

BACKGROUND. Metabolic syndrome (MetS) and benign prostate hyperplasia (BPH) are often comorbid. Chronic inflammation, a determinant pathogenic factor for BPH, is a putative link between the two conditions. METHODS. In a multi-center cohort of BPH patients (n = 244) who underwent prostatectomy, we evaluated whether MetS is associated with prostatic inflammation in BPH specimens. In addition, we investigated the in vitro inflammatory effects of metabolic insults on human prostatic myofibroblastic cells (hBPH). RESULTS. Inflammatory infiltrates score (IS) in prostatectomy specimens showed a stepwise association with the number of MetS factors present (P = 0.001). After adjusting for age, reduced HDL cholesterol, and elevated triglycerides were the only factors significantly associated with IS. Prostatic volume and anterior-posterior (AP) diameter were positively associated to the number of MetS components. Among MetS determinants, only dyslipidaemia (increased serum triglycerides and reduced serum high-density lipoprotein) was associated with an increased risk of having a prostatic volume >60 cm(3) (hazard ratio (HR)=3.268, P<0.001). Increased IS was also significantly associated with hypogonadism. In an age- and testosterone (T)-adjusted model, dyslipidemia was still associated with IS. To investigate whether metabolic factors could directly trigger prostate inflammation, we performed preliminary studies in myofibroblastic hBPH. Among the different factors, oxidized low-density lipoprotein (oxLDL) showed the highest secretion of IL-8 (>10-fold)—a surrogate marker of prostate inflammation—as well as IL-6, and bFGF. Co-treatment with DHT significantly inhibited oxLDL-induced secretion of IL-8, whilst an AR-antagonist, bicalutamide, reversed DHT effects. DHT suppresses oxLDL receptor (LOX-1) expression. CONCLUSIONS. Our data suggest that fats and insulin could have a detrimental effect on prostate health, boosting inflammation, a key pathogenic factor in BPH. Conversely, beneficial effects of DHT in counteracting lipid- and insulin-induced prostatic alterations, suggest that T—via its conversion into DHT—may have unexpected beneficial effects on prostate health.

PP020

HIGH CIRCULATING LEVELS OF CCL2 IN PATIENTS WITH KLINEFELTER'S SYNDROME.

M. Rotondi[1], F. Coperchini[1], A. Renzullo[2], G. Accardo[2], G. Groppelli[1], F. Magri[1], D. Esposito[2], A. M. Isidori[3], F. Barbato[2], A. Cittadini[4], L. Chiovato[1], D. Pasquali[2], F. Barbato[2]

[1]Unit of Internal Medicine and Endocrinology, Fond. S. Maugeri, Laboratory for Endocrine Disruptors and Chair of Endocrinology, University of Pavia - Pavia, [2]Unit of Endocrinology, Department of Cardiothoracic and Respiratory Sciences, Second University of Naples - Napoli, [3]Department of Experimental Medicine, Sapienza University of Rome - Roma, [4]Department of Internal Medicine, Cardiovascular Sciences and Clinical Immunology, University Federico II, Naples, Italy - Napoli

Klinefelter's Syndrome (KS) is associated with an elevated prevalence of metabolic syndrome (MetS). While an elevation of circulating biomarkers of inflammation, in particular CCL2 was extensively reported in MetS, no data are available about the circulating profile of these pro-inflammatory molecules in KS. Aim of the current study was to measure the circulating levels of CCL2, CXCL10 and adiponectin in patients with KS in order to assess their potential role in the development of MetS in these patients.

Patients and methods: Twentysix KS patients with normal circulating testosterone level and thirty age-matched healthy male adults, who served as controls, entered the study. CXCL10, CCL2 and adiponectin were measured in all patients and controls. Serum testosterone, FSH, LH, SHBG, IGF-1, PRL, thyroid hormones, TSH, insulin and routine blood tests were also measured.

Results: A significant increase in CCL2 but not in CXCL10 and adiponectin serum levels was observed in patients with KS as compared with healthy controls. In KS a significant correlation was found between circulating serum testosterone levels and CCL2 levels. Subgroup analysis according to the presence or absence of Mets, showed similar hormones concentrations in KS patients with (KS-Mets+) or without metabolic impairment (KS-Mets-). Furthermore, KS-Mets+ and KS-Mets- did not differ for the serum concentrations of adiponectin, CCL2 and CXCL10.

Conclusions: The present study shows a significant increase in serum CCL2 levels in patients with KS with or without MetS as opposed to healthy controls. A positive correlation was observed between CCL2 and testosterone levels. These findings could suggest that, in addition to hormonal factors, a genetic interaction, possibly mediated through macrophagic infiltration of adipose tissue, is involved in development of MetS in patients with KS.

PP021

VDR AND RXR LOCALIZATION IN NORMAL AND NEOPLASTIC HUMAN MALE REPRODUCTIVE TISSUES

F. Cariati[1], V. Gigantino[2], G. Coppola[1], C. Pivonello[1], M. Galdiero[1], G. Botti[2], L. Gandini[3], A. Lenzi[3], R. Franco[2], A. Colao[1], R. Pivonello[1]

[1]Medicina Clinica e Chirurgia, Università "Federico II" - Napoli, [2]Unità di Patologia, Istituto Nazionale dei Tumori, Fondazione Pascale - Napoli, [3]Medicina Sperimentale, Università "La Sapienza" - Roma

Vitamin D is an important modulator of cell growth and differentiation, and has been introduced as one of the main regulators of spermatogenesis. Vitamin D receptor (VDR), expressed in normal and malignant human tissues, heterodimerizes with nuclear retinoid X-receptor alpha (RXRα).

Aim of this study was to investigate the localization of VDR and RXRα in normal and neoplastic tissues from male reproductive apparatus.

VDR and RXRα was detected by both immunohistochemistry and immunofluorescence. Normal specimens from epididymes, seminal vesicles, testes and prostate were included. Additionally, two tissue Micro Array platforms were built using the most representative areas from different testicular germ cell cancer (TGCTs) and prostate cancer (PC) samples.

VDR and RXRα were expressed in normal epididymes, seminal vesicles and prostate. VDR in these tissues showed both membrane and cytoplasmic immunoreactivity, while RXRα was only cytoplasmic. In normal testes, VDR was expressed in Sertoli cells and in rete testis, while RXRα was found in Leydig and Sertoli cells, and in spermatogonia.

VDR immunostaining was stronger in differentiated TGCTs than in undifferentiated. Conversely, RXRα expression was greater in undifferentiated TGCTs.

VDR expression was higher in normal prostate than in PC tissues. More specifically, VDR expression was the lowest in tumours with the highest Gleason Score (GS). Interestingly, in PC with higher Gleason Score VDR was almost exclusively localized on cell membranes.

We show a comprehensive characterization of VDR and RXRα localization in human male reproductive system. High expression of both receptors was found in all samples, but according to a tissue- and disease-specific distribution. Furthermore localization within cell structures revealed a specific compartimentalization.

The localization of VDR on cell membranes, especially in cancerous cells, might suggest that vitamin D may have non-genomic effects in these tissues, signalling through non-classical pathways.

The different distribution of these receptors in TGCTs histotypes could be used as an additional tool in differential diagnosis.

PP022

PSA AS A MARKER OF TESTOSTERONE BIOLOGICAL ACTIVITY

G. Rastrelli[1], G. Corona[2], E. Maseroli[1], A. Silverii[1], M. Monami[3], E. Mannucci[3], G. Forti[1], M. Maggi[1]

[1]Sexual Medicine and Andrology Unit, Department of Clinical Physiopathology - University of Florence - Florence, [2]Endocrinology Unit, Maggiore-Bellaria Hospital - Bologna, [3]Diabetes Section, Geriatric Unit, Department of Critical Care, University of Florence - Florence

Objective: To verify whether, in a large sample of male subjects seeking medical care for sexual dysfunction (SD), prostate-specific antigen (PSA) might represent a reliable marker of testosterone (T) bioactivity.

Design: Cross-sectional study

Methods: A consecutive series of 3156 patients attending our Unit for SD studied. Among them, only subjects without history of prostate disease and with PSA levels <4 ng/mL (n=2967) were analyzed. Several clinical and biochemical parameters were studied.

Results: Receiver operating characteristic curve analysis for predicting severe hypogonadism (T<8 nmol/L) showed an accuracy of PSA=0.612±0.022 (p<0.0001), with the best sensitivity and specificity at PSA< 0.65 ng/mL (65.2% and 55.5%, respectively). After adjusting for age, low PSA was associated with higher prevalence of hypogonadism-related clinical features, (i.e. delayed puberty, lower testis volume), and associated conditions, as metabolic syndrome (HR=1.506 [1.241-1.827]; p<0.0001), type 2 diabetes (HR=2.044 [1.675-2.494]; p<0.0001) and cardiovascular diseases (HR=1.275 [1.006-1.617]; p=0.045). Furthermore, low PSA was more frequently associated with sexual symptoms, such as impaired sex- and sleep-related erections. The association between low PSA and hypogonadal symptoms and signs as well as with metabolic syndrome was retained even after adjusting for T levels.

Conclusions: PSA is a reliable marker of T biological activity and it may represent a new tool in detecting clinically relevant hypogonadism. The single determination of PSA levels might give insights not only on the circulating levels of total T, but also on its active fractions and, most importantly, on its biological activity.

PP023

BONE MINERAL DENSITY IN YOUNG ADULT UNTREATED HYPOGONADAL MEN, TREATED ONES AND CONTROL SUBJECTS

A. Gioia[1], L. Ceccoli[1], M. Boscaro[1], V. Ronconi[1], G. Giacchetti[1], G. Balercia[1]

[1]CLINICA DI ENDOCRINOLOGIA-UNIVERSITA' POLITECNICA DELLE MARCHE - ANCONA

A major cause of osteoporosis in men is hypogonadism. Androgens and estrogens have in vitro and in vivo trophic effects on skeletal development and are essential for maintaining bone mass. Aim of this study was to evaluate bone mineral density (BMD) in untreated hypogonadal males versus hypogonadal men in testosterone replacement treatment (6 with testosterone gel 2% and 11 with Injectable Testosterone Undecanoate), and control healthy subjects. We studied 21 untreated men,17 hypogonadal men treated for at least 5 years and 18 age- and BMI-matched controls (median age 46,5 yrs). Among the untreated group, the clinical onset of hypogonadism was on average 24 months before; 13 patients were affected by primary hypogonadism and 8 by secondary hypogonadism. None of the patients had history of diseases known to affect bone metabolism or taking drugs influencing bone. In all patients BMD evaluation at L1-L4, femoral neck and total hip was performed by dual-energy X-ray absorptiometry. Total, free and bioavailable testosterone, estradiol were significantly decreased in untreated patients versus treated ones and controls. Only the 5% of patients had Z-score and T-score below -2,5 DS, no differences in the prevalence of osteoporosis were found between the three groups. BMD, T score and Z score at all skeletal sites were not statistically different. Despite similar testosterone, estradiol levels and duration of disease, patients with untreated secondary hypogonadism displayed significantly lower T score and Z score at femoral neck, as well as significantly decreased LH levels compared with untreated patients with primary hypogonadism. Similar differences in densitometric parameters were found also between untreated patients with secondary hypogonadism vs treated patients with secondary hypogonadism and controls. Among the treated group, DXA parameters were similar independently of formulation of testosterone replacement therapy. In conclusion, a recent history of secondary hypogonadism appears to adversely affect the bone health, independently of testosterone and estradiol levels and duration of disease. This could be due to potential role of LH in Vitamin D 25- hydroxylation in testis.

PP024

EIACULATIO PRAECOX (PE) WORSENS THE SEXUAL DISTRESS PERCEIVED BY FEMALE PARTNER: STANDARDIZATION AND VALIDATION OF FSDS-R-PE

E. Limoncin[1], M. Tomassetti[1], G. L. Gravina[1], G. Ciocca[1], S. Di Sante[1], E. carosa[1], A. Lenzi[2], E. A. Jannini[1]

[1]Dipartimento di Scienze cliniche applicate e biotecnologiche - L'Aquila, [2]Dipartimento di Medicina sperimentale - Roma

Aim: to measure female sexual distress related to premature ejaculation (PE) with a new diagnostic tool, the Female-Sexual-Distress-Scale-Revised-PE (FSDS-R-PE). Methods: a total of 2109 women (1361 of “PE group” and 748 of “Control group”) were selected among women who visited the “eiaculazioneprecocestop” website, a thematic area of SIAMS homepage. All females completed the online version of FSDS-R-PE and the Female Sexual Function Index (FSFI) sent to them via e-mail with the International Index of Erectile Dysfunction (IIEF) for their partners. Content and discriminant validity, internal consistency and test-retest reliability of FSDS-R-PE were determined. Propensity analysis was applied to balance the impact of demographics on sexual distress perception. Results and conclusions: internal consistency of FSDS-R-PE was >0.90 and 0.84 for PE and control groups, respectively. Test-retest reliability was 0.82 (95%CI 0.72-0.87) and 0.85 (95%CI 0.79-0.92) for PE and control group. FSDS-R-PE had a high AUC (0.90; 95%CI 0.89-0.91) and the new cut-off score (>12) had Se, Spe, PPV and NPV of 79.1% (95%CI 73.8-82.5), 99.5%; (95%CI 98.0-100.0), 99.3% (95%CI 98.7-100.0) and 67.9% (95%CI 64.2-73.2), respectively. Median FSDS-R-PE scores were significantly higher in PE group (20; 95%CI 19-21) than in controls (6; 95%CI 6-7) (p<0.0001). Logistic regression, weighted for demographics, indicated that females of PE group experienced sexual distress 9.83 (OR=9.83; 95%CI 7.94 to 12.15) times higher than controls. Hence, the FSDS-R-PE was found to fulfill psychometric requirements in measuring sexual distress related to a partner’s sexual dysfunction.

PP025

PREVALENCE OF SEXUAL DYSFUNCTIONS BETWEEN INFERTILE COUPLES

E. Limoncin[1], V. Minnucci[1], G. L. Gravina[1], G. Ciocca[1], E. Carosa[1], S. Di Tommaso[1], G. Balercia[2], A. Lenzi[3], E. A. Jannini[1]

[1]Dipartimento di Scienze cliniche applicate e biotecnologiche - L'Aquila, [2]Dipartimento di Medicina interna e Biotecnologie applicate - Ancona, [3]Dipartimento di medicina sperimentale - Roma

Aim: to investigate the prevalence of sexual dysfunctions between infertile couples and infertile couples attempting to the adoption path. Materials and methods: sexual function of 61 infertile couples (clinical group) and 35 infertile couples attempting to the adoption path (control group) was measured with the International Index of Erectile Dysfunction (IIEF) and the Female Sexual Function Index (FSFI). The association level between the presence of sexual dysfunction and the variables “infertility/infertility with adoption” was measured with the Relative Risk (RR). The differences in continuous and dichotomous variables were measured with T Student test for unpaired data and with Chi-Square/Fisher test, respectively. P values <0.05 were considered statistically significant. Results: the prevalence of female sexual dysfunctions was statistically different between women of clinical [49.2% (30/61)] and control group [17.1% (6/35)] (p=0.004). The prevalence of moderate (p=0.065) and severe (p=0.249) erectile dysfunction (ED) was not statistically significant: 18% (11/61) of men in the clinical group were affected by moderate ED and 21.3% (13/61) by severe ED. In the control group 3% (1/35) were affected by moderate ED and 34.3% (12/35) by severe ED. Females of clinical group had nearly three-time higher probability (RR=2.87; 95%CI 1.32 to 6.20; p=0.0074) to develop a clinically significant sexual dysfunction respect to females of control group. No association was found in males between moderate/severe ED and infertility. Conclusions: The higher risk to develop a significant sexual dysfunction between infertile females may be partially due to the presence of depressive symptoms that can also worsen sexual desire.

PP026

PATIENTS WITH LOW TESTICULAR VOLUME HAVE WORSE BIOFUNCTIONAL SPERM PARAMETERS

R. A. Condorelli[1], A. E. Calogero[1], Y. Duca[1], S. La Vignera[1]

[1]Dip. Scienze Mediche e Pediatriche - Università di Catania - Catania

Background/Aim: Lower testicular volume (TV) (<12 cm3) is associated with lower testicular function. Several studies have examined the conventional sperm parameters (concentration, motility, and morphology) and the endocrine function (gonadotropins and testosterone serum levels) in the patients with reduction of TV. No other parameters have been examined. The aim of this study was to evaluate some biofunctional sperm parameters by flow cytometry of men with reduced TV compared with that of subjects with normal TV.

Subjects and Methods: 78 patients without primary scrotal disease were submitted to ultrasound evaluation of the testis. They were divided into two groups according to testicular volume, calculated by the ellipsoid formula (length x width x thickness x 0.52): Forty patients had normal TV (>12 cm3), whereas 38 patients had reduced TV (≤12 cm3). All patients underwent blood sampling for hormone measurements and evaluation of conventional and biofunctional (by flow cytometry) sperm parameters evaluation.

Results: As expected, TV correlated positively with the following conventional sperm parameters: semen volume, sperm concentration, progressive motility, and normal forms (p<0.001) and negatively with the percentage of spermatids (p<0.001). No statistically significant correlation was found with pH. LH, FSH, and E2 serum concentration correlated negatively with testicular volume, while no statistically significant correlation was found with T and prolactin. The following biofunctional sperm parameters mitochondrial membrane potential, phosphatidylserine externalization, chromatin compactness, and DNA fragmentation) correlated strongly negatively with TV (p<0.0001).

Conclusions: This study confirmed that conventional sperm parameters and serum hormone are worst in patients with low TV, whereas it, for the first time, showed that patients with low TV have impaired sperm mithocondrial function, early apoptosis, and abnormal chromatin/DNA integrity.

PP027

EFFECT OF THE ADDITION OF DDP-4 INHIBITOR SITAGLIPTIN TO METFORMIN TREATMENT ON SEXUAL FUNCTION IN MALE PATIENTS WITH DIABETES MELLITUS

M. Galdiero[1], P. Vitale[1], F. Lo Calzo[1], C. Simeoli[1], F. Cariati[1], C. Salzano[1], A. Colao[1], R. Pivonello[1]

[1]Dipartimento di Medicina Clinica e Chirurgia, Sezione di Endocrinologia, Università di Napoli "Federico II" - Napoli

Introduction: Diabetes mellitus is associated with an impairment of sexual function due to cardiovascular, neurological, metabolic and hormonal factors. A DDP-4 inhibitor, sitagliptin, has been reported to ameliorate glycaemic control, metabolic profile and cardiovascular damage when added to metformin to target normal HBA1c levels. Furthermore sitagliptin seems to improve endothelial function by both GLP-1 mediated and not mediated mechanisms and independently from glycaemic control. This study aimed to investigate the effects of six months treatment with sitagliptin plus metformin on sexual function of male patients with diabetes.

Patients and Methods: Twenty-six patients (51.4±9.4 yrs) with uncontrolled diabetes in treatment with maximal dose of metformin entered the study. Total testosterone (T), BMI, waist circumference, lipid and glucose profile, HBA1c and International index of erectile function score (IIEF-5) were assessed before and 6 months after the addition of sitagliptin (100 mg/day) to previous metformin therapy.

Results: At baseline, the prevalence of hypogonadism was 20%, while erectile dysfunction(ED) was diagnosed in 60% of patients. Hypertension and dyslipidemia affected 77% and 73% of patients, respectively. IIEF score negatively correlated with age(p=0.009, r=-0.49). Sitagliptin induced HbA1c normalization in 60% of patients. Serum triglycerides(p=0.03), weight(p=0.04), waist circum.(p=0.01), BMI (0.03) and systolic blood pressure(p=0.04) were reduced after 6 months treatment whereas an increase of IIEF score(p=0.04) was registered. The prevalence of hypogonadism remained unchanged while ED prevalence was reduced to 38% (p=NS). Furthermore, an amelioration of the severity of DE was registered in 10% of patients. IIEF score changes(%), after sitagliptin therapy, correlated positively with T(p=0.04, r=0.04) changes and negatively with BMI(p=0.001, r=-0.7) and waist (p=0.007, r=-0.5) changes. BMI changes resulted to a multivariate analysis the best predictors of IIEF amelioration.

Conclusions: Sexual function resulted ameliorated by adding sitagliptin to metformin in diabetics with suboptimal HBA1c levels. This improvement seems to depend more on the change in BMI that on the change in glycaemic control, but direct beneficial effects of drug itself on endothelial and erectly function cannot be ruled out.

PP028

TESTOSTERONE REPLACEMENT THERAPY REDUCES HEPCIDIN LEVELS IN YOUNG HYPOGONADAL MEN

D. Visconti[1], V. Palumbo[1], D. Esposito[1], A. Topa[1], A. La Rezza[1], A. De Bellis[1], A. A. Sinisi[1]

[1]Edocrinologia, Dpt Scienze Cardiotoraciche e Polmonari, Seconda Università di Napoli - Napoli

Erythrocytosis is a frequent adverse event associated with testosterone replacement therapy (TRT) in aged hypogonadal men, but the mechanisms involved remain poorly understood. T administration to aged men reduced hepcidin (Hpc) levels, a cytokine regulating iron availability, suggesting a potential role in deregulated erythropoiesis. Aim of present study was to evaluate the effects of T replacement therapy on Hpc levels in young hypogonadal men. Methods: 58 subjects (18-36 yrs.) with hypogonadism due to Kallmann syndrome (16), idiopathic hypogonadism (12), multiple pituitary deficiency (7), Klinefelter syndrome (18), and anorchidism (5) were studied. Blood samples were obtained basally (pts. never treated or after 3-months suspension) and after 6-12 months of T substitution therapy with 1g of T undecaonate (TU) im every 10-12 weeks (30 cases), or 250 mg of T enanthate (TE) im every 2-3 weeks (20 c), or 50-100 mg T gel/d (6 c). In all samples haematocrit (Ht), Hpc (by Elisa) and T (by RIA) were determined. T therapy increased T and Ht, and decreased Hpc levels (p<000.1). Hpc levels were negatively related to T and Ht. Fifteen subjects (27%) developed polycythaemia (Ht>50); 10 out 15 (67%) received TE. Conclusion: TRT induced erythrocytosis in young males with hypogonadism. TE administration seems favour polycythaemia respect to other formulations. T-induced suppression of Hpc may contribute mechanistically to stimulate erythropoiesis.

PP029

INVESTIGATION ON PSYCHOLOGICAL SYMPTOMS IMPROVES ANDROTEST ACCURACY IN PREDICTING HYPOGONADISM IN SUBJECTS WITH SEXUAL DYSFUNCTION

G. Rastrelli[1], G. Corona[2], E. Bandini[1], C. Strada[3], E. Maseroli[1], V. Ricca[4], C. Faravelli[3], E. Mannucci[5], M. Maggi[1]

[1]Sexual Medicine and Andrology Unit, Department of Clinical Physiopathology University of Florence - Florence, [2]Sexual Medicine and Andrology Unit, Department of Clinical Physiopathology; Endocrinology Unit, Maggiore-Bellaria Hospital - Bologna, [3]Department of Psychology, University of Florence - Florence, [4]Psychiatric Unit, Department of Neuropsychiatric Sciences, University of Florence - Florence, [5]Diabetes Section, Geriatric Unit, Department of Critical Care, University of Florence - Florence

The role of psychological symptoms in recognizing late-onset hypogonadism (LOH) is still controversial. The aim of the study is to evaluate the association between LOH and specific psychological symptoms, and to verify whether investigating intra-psychic domain improves the accuracy of a validated case-history tool (ANDROTEST) in detecting LOH. A consecutive series of 1009 subjects (mean age 49.23±13.34) consulting for sexual dysfunction was studied. Intra-psychic symptoms were investigated by Middlesex Hospital Questionnaire (MHQ), a self-reported questionnaire for screening of mental disorders.

A minimum set of two MHQ items was identified through iterative receiver-operating characteristic analysis, with assessment of sensitivity and specificity for hypogonadism (calculated free testosterone <0.225 nmol l(-1)) in an exploratory sample of 462 patients. Sensitivity and specificity were verified in a validation sample of 547 subjects, in which the final two-item version showed an accuracy of 58.4±3.2% in detecting hypogonadism. The combination of the two-item score with ANDROTEST increased the accuracy in predicting hypogonadism (0.741±0.029; P<0.0001) when compared with ANDROTEST (0.696±0.018; P<0.0001) and the two-item score (P<0.05) alone. Hence, combining these two psychological symptoms with a physical scoring system improves its ability in detecting hypogonadism. The combination of the scores should be tested in other studies.

PP030

HIGH TESTIS SIZE IS UNEXPECTEDLY ASSOCIATED WITH MACE: BIOLOGICAL DETERMINANTS IN SUBJECT WITH SD

G. Rastrelli[1], G. Corona[1], F. Lotti[1], V. Boddi[1], E. Mannucci[1], M. Maggi[1]

[1]Scienze Biomediche, Sperimentali e Cliniche - Università di Firenze - Firenze

Introduction: Measurement of testis volume (TV) in subjects with sexual dysfunction (SD) is an informative clinical procedure that can predict reproductive fitness. However, the role of TV in overall and cardiovascular (CV) fitness has never been studied. **Aim:** To analyse the clinical correlates of testis voleme in patients with SD and to verify the value of this parameter and its determinants (i.e. LH levels) in predicting major adverse cardiovascular events (MACE). **Methods:** A consecutive series of 2809 subjects without testiculopathy (mean age 51.2±13.1 years) consulting for SD was studied. A subset of this sample (n=1567) was enrolled in a longitudinal study. **Results:** After adjusting for age and testosterone, TV was negatively associated with both LH (Adj. r = -0.234; p<0.0001) and FSH (Adj. r = -0.326; p<0.0001) levels. In addition, overweight/obesity (BMI>25), smoking and alcohol abuse increased as a function of testis volume (HR=1.041[1.021-1.061], p<0.0001; 1.024 [1.005-1.044], p=0.012; 1.063 [1.015-1.112], p=0.009; respectively, for each incremental mL of testis volume). Furthermore, systolic, diastolic, mean and pulse pressure were all positively related to increased TV (Adj. r= 0.151; 0.136; 0.157 and 0.089; respectively, all p<0.0001). The effect of these lifestyle factors on TV were only partially related to changes in gonadotropins levels. In fact, in an age-adjusted model smoking was associated with an higher LH, but not FSH, whereas alcohol abuse showed no relationship with both, BMI was positively associated with FSH and negatively with LH, whereas mean blood pressure was negatively associated only with LH. In the longitudinal analysis, after adjusting for confounders, TV was independently associated with a higher incidence of MACE (HR=1.066[1.013-1.122]; p=0.014), but the stepwise introduction in the Cox model of lifestyle factors, mean blood pressure and BMI progressively smoothed the association, which was no longer statistically significant in the fully adjusted model. Conversely, the association of higher LH levels with a increased incidence of MACE was not attenuated by the progressive introduction of smoking, alcohol intake, mean blood pressure and BMI in the Cox model. **Conclusions:** Our data show that in SD subjects, TV is unexpectedly associated with an adverse CV risk profile, leading to a higher incidence of MACE. High LH level, that can be considered an early marker of testicular dysfunction, is also an independent marker of CV risk. Further studies are needed for better understanding which determinants of testis enlargement, beyond gonadotropins, could possibly mediate the increased incidence of MACE.

PP031

RISK FACTORS ASSOCIATED WITH PRIMARY AND SECONDARY REDUCED LIBIDO IN MALE PATIENTS WITH SEXUAL DYSFUNCTION

E. A. Jannini[1], G. Corona[2], G. Rastrelli[3], L. Vignozzi[3], A. Sforza[2], E. Mannucci[4], G. Forti[5], M. Maggi[3]

[1]Scuola di Sessuologia - Università dell'Aquila, [2]Unità di Endocrinologia, Ospedale Maggiore - Bologna, [3]Unità di Medicina della Sessualità e Andrologia - Università di Firenze, [4]Unità di Geriatria, sez di Diabetologia - Università di Firenze, [5]Unità di Endocrinologia - Università di Firenze

Introduction. Hypoactive sexual desire is defined as a persistent or recurrent deficient or absent sexual fantasies or desire for sexual activity which should not be comorbid with other medical conditions or with the use of psychoactive medications. Reduced libido is a symptom referring to a reduction in sexual drive for sexual activity. To investigate the risk factors of primary reduced libido (i.e not associated with conditions causing loss of libido such as hypogonadism, hyperprolactinemia, psychopathology and/or psychoactive medications) or secondary reduced libido (i.e with aforementioned conditions) in male patients with sexual dysfunction. **Methods.** A consecutive series of 3714 men (mean age 53.2±12.5 years) was retrospectively studied. Patient's reduced libido was evaluated using question #14 of SIEDY ("Did you have more or less desire to make love in the last three months?"). **Results.** Reduced libido was comorbid with erectile dysfunction, premature ejaculation and delayed ejaculation in 38%, 28.2% and 50%, respectively, whereas it was isolated in 5.1%. Reduced libido prevalence was increased by hypogonadism, almost doubled by psychopathology and universally present in subjects with hyperprolactinemia. Subjects with primary reduced libido are characterized by higher post-school qualification, more disturbances in domestic and dyadic relationships, and an overall healthy body (lower glycaemia and triglyceride levels). Accordingly, in patients with primary reduced libido the risk of major cardiovascular events as calculated with the Progetto Cuore algorithm was lower than in the rest of the sample. Features of hypogonadism- or psychopathology-associated reduced libido essentially reflect their underlying conditions. Comorbidity with other sexual dysfunctions did not modify the aforementioned results. **Conclusions.** Primary and secondary reduced libido have different risk factors and clinical characteristics. Recognizing primary or secondary reduced libido will help clinicians to identify comorbidities and to tailor appropriate treatments.

PP032

INHIBITION OF HUMAN CORPORA CAVERNOSA SMOOTH MUSCLE CELLS PROLIFERATION BY RAPAMYCIN AND METFORMIN

E. Carosa[1], C. Forcella[1], A. Castri[1], G. L. Gravina[1], P. Ronchi[2], A. Lenzi[3], E. A. Jannini[1]

[1]Department of Biotechnological and Applied Clinical Science, University of L'Aquila - L'Aquila, [2]Urology Functional Unit, "Villa Igea" Clinic - Ancona, [3]Department of Medical Pathophysiology, 1st University of Rome "La Sapienza" - Roma

Cavernous smooth muscle cells (SMCs), which relaxation is on the basis of erection mechanism, can switch their phenotype from a contractile differentiated state to a proliferative and dedifferentiated state in response to the change of local environmental stimuli. Phenotypic modulation from contractile to proliferative state of SMCs has critical role in the pathogenesis of a variety cardiovascular diseases and could have a key role in the pathogenesis of diabetic erectile dysfunction (ED), in fact the penis is considered as an extension of the vascular system (Wei A.Y. et al. Inter. J. Impot. Res. 2012, 24:196). The mammalian target of rapamycin (mTOR) is a serine/threonine kinase that positively regulates cell growth, proliferation and survival and rapamycin, the inhibitor of mTOR was implied in regulation of proliferation of carotid vascular SMCs (Wang Y et al. Br J Pharmacol 2012, 165:2378). Also, the antidiabetic agent metformin has been recently reported as direct mTOR inhibitor (Kalander A et al. 2010 Cell. Metab. 11, 390.). We are prompted to explore proliferation modulation of SMCs under rapamyicin and metformin treatment, in absence or presence of Platelet Derived Growth Factor (PDGF) that increase during vascular damage.

SMCs proliferation rate was analyzed in human corpora cavernosa cells (hCC) by the XTT dye conversion assay. Expression of gene of SMCs differentiate phenotype was monitored using semiquantitative PCR assay. We found that rapamyicin treatment of hCC significantly blocks cell proliferation, this effect became significant after 48 hours (Bas 1,879±0,202 a.u. vs. Rapamycin 20 nM 1,285±0,232 p<0,01). The rapamycin block of proliferation for 24 hours is not associated with expression of SMCs differentiate marker genes lake SM22 or SMCs α-actin. Metformin treatment also blocks hCC proliferation in dose dependent way (Bas 0,61± 0,01 a.u. vs metformin 20 mM 0,35±0,05 p<0,05) and antagonize the effect of PDGF (PDGF 20 µg/ml 0,42±0,04 a.u. vs PDGF + Metformina 20 mM 0,236±0,03; p< 0,02) on cells proliferation.

Rapamyicin and metformin inhibit the proliferation of SMCs both in absence or in presence of PDGF. We hypothesize that the benefic effect of metformin treatment observed in the reduction of ED in the diabetic rats may be mediated by its direct effect on SMCs and not only by endothelium cells.

PP033

EFFECTS OF LONG-TERM TREATMENT WITH TESTOSTERONE IN PATIENTS WITH THALASSEMIA MAJOR

P. V. Pileri[1], R. Marras[1], F. Badessi[1], I. Rum[1], P. Ferrandu[1], E. Carta[1], A. Barra[1], A. Masala[1]

[1]Medicina Interna 1 - Sassari

Hypogonadotropic hypogonadism is a frequent finding in patients with homozygous β-thalassemia. We studied the effects of long term testosterone administration in male patients affected by thalassemia.One hundred and forty male patients entered this study.All were on iron chelation therapy;all had some degree of liver impairment due to iron deposition and chronic HCV infection. Their average age at the beginning of the study was 19 ±2 years.All had low serum testosterone levels (2.15±0.89 ng/ml;mean ±SEM) and,all were started on testosterone (a commercial blend of enanthate and propionate esthers),250 mg given intramuscularly every 20 -30 days for an average observation period of 25 years (range:15 – 32 years).All of the patients were assessed every 6 months for sexual activity,liver function tests and serum testosterone levels.Therapy was able to warrant a normal sexual function in all of them ,an amelioration in muscular strength and trophism and a better quality of life.Liver function tests (ALT,AST,alkaline phosphatase,γGT) were not significantly influenced by the drug throughout the period of observation.Twelve months after starting therapy,their serum testosterone levels,measured 15 days after the administration of the drug,were in the normal range (7.23±1.12 ng/ml) and remained in the normal range throughout the period of observation.Gynaecomastia was a common side effect,being observed in the 25 per cent of the patients.No other relevant side effect was observed..Only one patient developed hepatocarcinoma at the age of 45 years.Long term replacement therapy with testosterone in patients with β-thalassemia is safe and devoid of relevant side effects.

PP034

HYPOTHETICAL ROLE OF GNRH ON LEYDIG AND SERTOLI CELLS FUNCTION IN INFANTS WITH NON-MOSAIC KLINEFELTER SYNDROME

S. Granato[1], C. Mattiucci[2], G. Ruga[1], M. Spaziani[1], N. Tahani[1], F. Impronta[1], N. Liberati[2], A. Lenzi[1], L. Tarani[2], A. Radicioni[1]

[1]Center for Rare diseases, Department of Experimental Medicine, Section of Medical Pathophysiology, Food Science and Endocrinology, Sapienza - Rome, [2]Department of Pediatrics , Sapienza – University of Rome - Rome

Context. Klinefelter syndrome (KS) is characterized by a variety of subtle, age-related clinical signs, that evolve into hypergonadotropic hypogonadism. The hypothalamic–pituitary–gonadal (HPG) axis is transiently activated in infancy; it is still unknown when testicular damage occurs.

Objective. To evaluate the HPG axis and the hormonal and clinical pattern of KS boys, comparing the results against a control population.

Methods. 46 KS boys aged 0.3 to 8.9 years and 138 controls aged 0.2 to 9.9 years were recruited. Serum FSH, LH, testosterone (T), estradiol (E2), inhibin B (INHB), sex hormone binding globulin (SHBG) and anti-Müllerian hormone (AMH) were determined. Height, weight, testicular volume and penile length were assessed.

Results. FSH was higher in KS boys than in controls under 0.75 years; 30.4% of KS boys had FSH above the 90th percentile. INHB in KS boys under 0.75 years was significantly higher than in the controls ($p<0.001$). AMH in KS boys was within the normal range and was significantly higher than in controls aged 3-6 years. T was higher in KS boys over 0.75 years, but the difference was not statistically significant. KS boys were taller and heavier than controls. No differences in LH, E2, SHBG, penile length and testicular volume were found.

Conclusion. No tubular damage was found in KS boys. We speculate that elevated FSH associated with high or normal INHB may be due to increased levels of GnRH, that probably interacts with Leydig function through normal/elevated LH values and may increase testosterone stimulation and secretion.

This study was funded by the Italian Ministry of Health and the Italian Medicines Agency (AIFA): research project MRAR08Q009 on rare diseases.

PP035

PRELIMINARY HORMONAL ASSESSMENT OF BILATERAL GYNECOMASTIA IN 75 MEN

A. Sansone[1], F. Romanelli[1], D. Lallo[1], G. Fattorini[1], V. Renzelli[1], P. Sgrò[2], L. Di Luigi[2], A. Lenzi[1]

[1]Sezione di Fisiopatologia Medica, Dip. di Medicina Sperimentale, "Sapienza" Università di Roma - Roma, [2]Unità di Endocrinologia, Università di Roma "Foro Italico" - Roma

Background Gynecomastia is a benign enlargement of male breast glandular tissue, affecting at least a third of males during their lifetime. Gynecomastia can occur in every age: transient gynecomastia is a common finding in infants and in adolescents, but in young and middle-aged men the sudden enlargement of breast glandular tissue is unusual and requires investigation. Common causes of gynecomastia include hormonal alterations, chronic liver disease and the various drugs; however, in most circumstances no definite cause might be identified.

Methods Seventy-five consecutive male patients (aged 28 to 75; mean 54+/-7.76 y.o.) who came to our practice with bilateral gynecomastia as the main presenting symptom underwent breast palpation, breast ultrasonography and anthropometric measurements; blood samples were collected in order to evaluate serum levels of hormones. Liver and kidney biochemical testing were performed.

Results Body mass index (BMI) in patients with gynecomastia was 26.2+/-2.7 kg/m2. Mean serum hormonal values were in the normal range for our laboratory (LH: 4.66+/-2.20 mUI/ml; FSH 5.20+/-3.31 mUI/ml; total testosterone 5.65+/-2.60 ng/ml; free testosterone 22.22+/-11.19 pg/ml; estradiol 80.85+/-34.2 pg/ml; SHBG 39.82+/-14.92 nmol/l; TSH 2.56+/-1.32 µUI/ml; prolactin 8.87+/-3.92 ng/ml). Nine patients were diagnosed with hypogonadism and started treatment with testosterone, with little to no improvement after 6 months of testosterone replacement therapy. Among the 9 patients with hyperprolactinemia, drug-induced gynecomastia was identified in 8 patients ; treatment suspension or change improved the symptoms of gynecomastia in all of them. Eleven patient had high estradiol levels with normal total testosterone. Imbalance between testosterone and estradiol levels might be a cause of gynecomastia both in hypogonadal patients and in patients with high estradiol and normal testosterone. Almost two thirds of the patients had idiopathic bilateral gynecomastia.

Conclusions Hormonal values in our patients showed no significant difference compared to normal, healthy people in most circumstances. In almost 25% of the 75 studied men, high E2 to T ratio was identified as a possible cause of gynecomastia.

PP036

HYPOGONADOTROPIC HYPOGONADISM IN A 23-YEAR OLD MAN WITHOUT ANOSMIA

A. Sansone[1], F. Romanelli[1], G. Fattorini[1], M. Watanabe[1], A. Lenzi[1]

[1]Sezione di Fisiopatologia Medica, Dip. di Medicina Sperimentale, "Sapienza" Università di Roma - Roma

Hypogonadotropic hypogonadism (HH) is a rare form of hypogonadism, caused by deficit in the GnRH (gonadotropin-releasing hormone)-secreting neurons of the hypothalamus. Lack or production of a defective form of GnRH results in reduced secretion of FSH and LH, which in turn lead to alterations in spermatogenesis and in sex hormone production; the lack of sex hormones leads to reduced sexual desire, osteoporosis, reduced body mass and delayed puberty. In almost half of the patients, HH is associated with anosmia – a trait which often leads to an early diagnosis; however, in the remaining 50% of the cases, sense of smell is preserved and diagnosis is usually made at a later stage. We report the case of a case of HH observed in a young man, aged 23, presenting with delayed puberty. At the time of admission the patient was underweight (BMI: 18.23 kg/m2; W: 56.5 kg, H: 176 cm) with 176 cm arm span; he described his growth rate as normal until the age of 12, with a second sudden increase in height after reaching 18 years, from 168 cm to the actual height in 2 years. The patient did not describe alterations in smell perception; scrotal and penile examination showed absence of sexual development. Total testosterone was 0.04 ng/ml; GnRH testing was then performed by administering 100 mcg of GnRH and measuring values of FSH and LH at baseline and after 30, 60 and 90 minutes. The test showed pituitary response to exogenous GnRH, with a threefold increase in FSH (from 0.86 to 2.65 mUI/ml at 90 minutes) and an even more marked increase in LH (from 0.28 to 3.32 mUI/ml at 60 minutes). Thyroid hormones and prolactin were in the laboratory ranges; vitamin D was severely deficient (10 ng/ml) and bone mineral density was severely reduced (femoral T-score: -2.5; lumbar T-score: -3.1). A RM scan of the brain showed no expansive lesions and no pituitary anomalies; an US scan of the scrotum showed small (16x12x9 mm R, 22x15x11 mm L), dysomogeneous testes. The patient is currently undergoing treatment with vitamin D and human chorionic gonadotropin: this therapy has led to improvements in quality of life, muscle mass and physical strength, and to an increase in testicular volume. Secondary sexual characteristics are starting to develop, with a marked increase in testicular volume (7-8 ml measured using an orchidometer) and growth of axillary and pubic hair.

PP037

DEHYDROEPIANDROSTERONE TREATMENT IN ELDERLY MEN: A META-ANALYSIS STUDY OF PLACEBO CONTROLLED TRIALS

G. Corona[1], G. Rastrelli[1], V. Giagulli[2], A. Sforza[3], A. Lenzi[4], G. Forti[1], E. Mannucci[1], M. Maggi[1]

[1]Scienze Biomediche, Sperimentali e Cliniche - Università di Firenze - Firenze, [2]U.O. Malattie Metaboliche ed Endocrinologia - Policlinico Conversano - Conversano (BA), [3]Unità di Endocrinologia - Ospedale Maggiore-Bellaria - Bologna, [4]Dipartimento di Fisiopatologia Medica - Università di Roma La Sapienza - Roma

Context. The age-related dehydroepiandrosterone (DHEA) deficiency has been associated with a broad range of biological abnormalities in males.
Object. To meta-analyze all double blind, placebo controlled randomized trials (RCTs) investigating the effect of oral DHEA (DHEA-RT) in comparison to placebo in elderly men.
Data source. An extensive Medline Embase and Cochrane search was performed including the following words "DHEA", "RCTs" and "males".
Study selection: Only double blind placebo controlled trials performed in elderly men were included.
Data Extraction. Data extraction were performed independently by two of the authors (A.S, V.G), and conflicts resolved by the third investigator (G.C). The quality of RCTs was assessed using the Cochrane criteria.
Results. Out of 220 retrieved articles, 26 were included in the study. The available RCTs enrolled 785 elderly men, with a mean follow-up of 39 weeks. DHEA-RT was associated with a reduction of fat mass (standardized mean differences -0.35[-0.65;-0.05]; p=0.02). However, the association with fat mas disappeared in a multivariate regression model, after adjusting for DHEA-related metabolite increase such as total testosterone and estradiol. In contrast to what observed for fat mass, no effect of DHEA-RT in comparisons to placebo was observed for different clinical parameters including lipid and glycemic metabolism, bone health, sexual function and quality of life.
Conclusions. Present meta-analysis of intervention studies does not support any role for DHEA supplementation in elderly men. DHEA and its sulphate, DHEAS, are steroids present in peripheral circulation, without a clear physiological role, apart from being precursors of bioactive androgens or estrogens.

PP038

TESTOSTERONE REPLACEMENT ITALIAN SURVEY: PRILIMINARY REPORT

A. A. Sinisi[1], L. Zanotti[2], V. Palumbo[1], M. Boschetti[3], E. Faloia[4], G. Targher[5], R. Pasquali[2]

[1]Dpt Scienze Cardiotoraciche, Seconda Università di Napoli - Napoli, [2]Endocrinologia, Università di Bologna - Bologna, [3]DISEM - Genova, [4]Endocrinologia, Università Pol. Marche - Ancona, [5]Dpt Medicina, Università di Verona - Verona

Background. Male hypogonadism (H) is a complex condition, characterized by either congenital or acquired testosterone (T) deficiency, including age related-deterioration of androgen production (Late-Onset Hypogonadism, LOH). Testosterone replacement therapy (TRT) is indicated in men with H to restore physiological T levels, reverses clinical symptoms of androgen deficiency and improves well-being and quality of life. At present several T formulations are available, including oral, transdermal and im short- (T propionate, TP, or T enanthate, TE) and long-acting (TU) preparations. **Aim**. This study was planned to assess TRT options by Italian Endocrinologists or Andrologists in their clinical practice. **Methods.** SIE and SIAMS members were invited to fill in a questionnaire form sent by mail. Here we report preliminary data of this survey. Results. Twenty-three centers (6 from north, 6 middle, 11 south/insular regions) responded and included 2000 cases. Diagnoses were primary H in 38%, secondary H in 18%, LOH in 44%. All formulations were used and in particular in 38% T esters (15% TP, 23% TE), in 34% TU, in 34% T gel; 10% received hCG. In patients >60 yr-old T gel was prescribed more frequently (54%) than T esters (46% TP or TE; 17% TU); in pts <60 yrs TU was preferred in 44% and T gel in 33%. TRT was changed or discontinued for undesired events more frequently in pts treated with older esters (24-28%) than in pts treated with T gel or im TU (7 and 9%, respectively). Therapy costs were covered by public health assistance in centers from north regions, less frequently in middle and south Italy centers. **Conclusions.** These preliminary data confirm the heterogeneous etiology of H in Italian patients, with an increasing diagnosis of LOH. Moreover, they suggest that T formulations were prescribed in all centers along with older T esters. The choice seems be influenced by age of patients, safety profile of preparations, and by potential charge of costs on Regional Health Services.
(Commissione Farmaci SIE, on the behalf of SIE and SIAMS)

PP039

A POSSIBLE ROLE OF PITUITARY AUTOIMMUNITY IN PATHOGENESIS OF HYPOGONADOTROPIC HYPOGONADISM IN TYPE 2 DIABETES

G. Bellastella[1], M. I. Maiorino[1], L. Olita[1], C. Colella[1], A. Dello Iacovo[1], A. De Bellis[2], D. Giugliano[1], K. Esposito[2]

[1]Dipartimento di Scienze Mediche, Chirurgiche, Neurologiche, Metaboliche e dell'Invecchiamento, Seconda Università degli Studi di Napoli - Napoli, [2]Dipartimento di Scienze Cardio-Toraciche e Respiratorie, Seconda Università degli Studi di Napoli - Napoli

Hypogonadotropic hypogonadism (HH) has been found to occur in about 25% of patients with type 2 diabetes. Many hypothesis have been suggested to explain the pathophysiological mechanism underlying HH in these patients such as the role of high glucose levels, impaired kisspeptin production, insulin resistance and inflammatory mediators. A role of pituitary autoimmunity has been previously demonstrated in some non diabetic patients with apparently idiopathic HH.
The aim of this study was to investigate whether pituitary autoimmunity could play a role in the HH of type 2 diabetic patients.
Forty-two type 2 diabetic males (age range 35-60 yr) have been studied and compared to 50 healthy age-matched controls. All patients were evaluated for glycemic control, morning testosterone, FSH, LH and other anterior pituitary hormones. Anti-pituitary antibodies (APA) were evaluated in patients and in controls by indirect immunofluorescence.
HH was diagnosed in 20 out of 42 (47%) patients (presence of testosterone ≤12 nmol/L). An increased prevalence of APA was found in diabetic patients (15/42, 35%) as compared with control subjects (3/50, 6%; $P < 0.001$); in particular APA were detected in 8 out of 20 with HH (40%) and in 7 out of 22 without HH (31%). While the patients without HH and controls resulted positive at low titer (≤1/8), patients with HH resulted positive at high titer (≥1/16).
The results of this preliminary study show an increased prevalence of APA in type 2 diabetic patients, with higher titer in those with HH.
An autoimmune aggression to the anterior pituitary could be responsible for some cases of HH in diabetic patients. Longitudinal studies are needed to clarify the natural history of this process and whether in these patients APA are directed against GnRH and/or gonadotropin secreting cells.

PP040

VARICOCELE AND INFERILITY: EFFECTS ON SPERMATOGENESIS

A. Palumbo[1], F. Pallotti[1], R. Conte[1], F. Lombardo[1], A. Lenzi[1], L. Gandini[1]

[1]Dipartimento Medicina Sperimentale - Università La Sapienza - Roma

Varicocele has a relevant incidence in men. Various studies have put in correlation its presence to a damage of the spermatogenesis through undefined mechanisms (increased temperature, hypoxia, catabolites). Many patients affected by varicocele don't have spermatogenesis abnormalities, in contrast with the opinion that it is frequently responsible for dispermia in infertile men. Our study evaluates the effect on spermatogenesis caused by varicocele. We have selected 2041 patients who came to our Laboratory to undergo seminal fluid and andrological examination: 1552 were affected by varicocele and 489 were not. The varicocele group (mean age 29.6±7.8) has not evidenced a significant difference compared to the control group (mean age 30.8±7.7) for ejaculate volume (3.1±1.5 ml vs 3.2±1.6 ml) and for total number of spermatozoa/ejaculate (118.7±169.8 vs 202.1±149.6). Moreover significant differences were found for motility (respectively 33.2±16.7 vs 36.0±14.7; $p<0.01$) and for atypia rate (respectively 74.8±10.9 vs 72.6±8.9; $p<0.01$). Furthermore we have divided our patients with varicocele according to age and varicocele stage: 59 patients between 14 and 17 years old, 313 patients between 18 and 27, 482 between 28 and 37 and 201 between 36 and 46. Statistical analysis has not evidenced significant differences for both volume and spermatozoa/ejaculate concentration but has revealed statistical significance ($p< 0.05$) for motility and atypia rate in patients affected by varicocele with increasing age (32.6 ± 16.2 vs 35.6 ± 16.2 vs 32.8 ± 16.8 vs 28.6 ± 17.3; 76.3 ± 9.8 vs 73.3 ± 10.2 vs 74.9 ± 11.5 vs 77.9 ±10.8). No correlation was found between seminal alterations severity and varicocele stage. Further investigations will be helpful to establish if varicocele can affect fertility.

PP041

HUMAN LEUKOCYTES IN ASTHENOZOOSPERMIC PATIENTS: ENOS EXPRESSION

E. Buldreghini[1], M. L. Macrì[2], A. Vignini[3], L. Mazzanti[3], S. Amoroso[2], M. Boscaro[1], G. Balercia[1]

[1]Dipartimento Scienze Cliniche e Molecolari - Torrette di Ancona, [2]Dipartimento Scienze Biomediche e Sanita' Pubblica - Torrette di Ancona, [3]Dipartimento di Scienze Cliniche Specialistiche ed Odontostomatologiche - Torrette di Ancona

Objective. To evaluate the pattern of mRNA-eNOS expression in human blood leukocytes isolated from normozoospermic fertile and asthenozoospermic infertile men to elucidate any pathogenic involvement in sperm cell motility.

Patients. 40 infertile men with idiopathic asthenozoospermia and **45** normozoospermic fertile donors, age-matched.

Intervention. Semen parameters were evaluated, and expression analysis of mRNA was performed in human leukocytes using Reverse Transcription - Polymerase Chain Reaction.

Main Outcome Measures. Semen analyses, to ascertain volume, sperm count, motility, and morphology. Analysis of eNOS expression and Western Blotting.

Results. A positive correlation was observed between the concentrations of NO and the percentage of immotile spermatozoa. The mRNA of eNOS was more expressed in peripheral blood leukocytes isolated from asthenozoospermic infertile men vs. those of fertile normozoospermic men (7.46 ± 0.38 vs 7.06 ± 0.56). A significant up-regulation of eNOS gene in peripheral blood leukocytes was 1.52-fold higher than that of fertile donors. .

Conclusions. eNOS expression and activity are enhanced in blood leukocytes in men with idiopathic asthenozoospermia.

PP042

MTHFR POLYMORPHISMS (677C>T E 1298A>C) AS A RISK FACTOR IN MALE INFERTILITY

M. Tenuta[1], A. Petrozzi[1], A. C. Cefaloni[1], G. Senofonte[1], F. Lombardo[1], A. Lenzi[1], L. Gandini[1]

[1]Dipartimento Medicina Sperimentale - Università La Sapienza - Roma

MTHFR (5,10-Methylenetetrahydrofolate) is a key enzyme in folate and homocysteine metabolism, involved in DNA and RNA histone methylation and ROS formation, both involved in spermatogenesis. In male mice MTHFR inactivation results in infertility and spermatogenesis arrest. Human studies have given contrasting results. Our study evaluates MTHFR 677C>T and 1298A>C polymorphism as a risk factor for male infertility. 55 Caucasian male outpatients with idiopathic infertility underwent semen and hormonal analysis, testis ultrasound and blood tests for MTHFR polymorphism, folate and B12 vitamin levels. Semen parameters (mean±SD) were: volume 3.1±1.59 mL; total sperm number 171.7±272.8; total motility 40.3±13.56%; abnormal forms 81.49±7.58%. The frequency of 677T was 50% and of 1298C was 29%. The caseload was divided into two groups: Group A: <40x106/ejaculate, consisting of 9 patients (16.36% of total); Group B: ≥40x106/ejaculate, consisting of 46 patients (83.64%). The frequencies of 677T and 1298C were 27% and 50% in Group A and 54% and 25% in Group B. The frequency of 1298C allele was statistically significant for Group A ($p<0.05$) *vs* all patients and Group B (odd ratio 2.79 and 3.00 respectively). There was no significant correlation for semen parameters, hormone values, testicular volume or MTHFR 677C>T and/or 1298A>C polymorphism. Patients with a homozygotic mutation or a double heterozygosis mutation did not have a higher frequency of oligozoospermia. Although we found no significant correlation between male infertility and MTHFR polymorphism, the results of this pilot study need to be confirmed in a greater number of patients.

PP043

CASE-CONTROL STUDY OF ANTHROPOMETRIC MEASURES AND TESTICULAR CANCER RISK

F. Giannandrea[1], G. Senofonte[1], A. C. Cefaloni[1], F. Lombardo[1], A. Lenzi[1], L. Gandini[1]

[1]Dipartimento Medicina Sperimentale - Università La Sapienza - Roma

The etiology of testicular germ cell tumors (TGCTs) is poorly understood. Recent epidemiological findings suggest that TGCT risk is determined very early in life, although the available data are still conflicting. The rapid growth of the testes during puberty may be another period of vulnerability. Body size has received increasing attention as possible risk factor for TC. To clarify the relation of body size and its anthropometric variables to TGCT risk, the authors analyzed data from 272 cases and 382 controls with regard to height (cm), weight (Kg), and body mass index (BMI; kg/m2). Overall, participants in the highest quartile of height were more likely to be diagnosed with TGCTs than participants in the lowest quartile of height, OR 2.22 (95% confidence intervals (CI): 1.25–3.93; adjusted; ptrend = 0.033). Moreover, histological seminoma subgroup was significantly associated with tallness, very tall men (>182 cm) having a seminoma TGCT risk of OR = 2.44 (95% confidence intervals (CI: 1.19–4.97; adjusted; ptrend = 0.011). There was also a significant inverse association of TGCT with increasing BMI (ptrend = 0.001; age-adjusted analysis) and this association was equally present in both histological subgroups. These preliminary results indicate that testicular cancer (TC) is inversely associated with BMI and positively associated with height, in particular with seminoma subtype. Several studies have reported similar findings on body size. As adult height is largely determined by high-calorie intake in childhood and influenced by hormonal factors at puberty, increased attention to postnatal exposures in this interval may help elucidate the etiology of TGCTs.

PP044

EFFECT OF TADALAFIL IN HUMAN SKELETAL MUSCLE CELLS

C. Antinozzi[1], M. Colletti[1], A. Lenzi[2], C. Crescioli[1], L. Di Luigi[1]

[1]Dep Mov Human and Health Sciences, Foro Italico - Rome, [2]Dep of Exp Med, Sapienza - Rome

Phospodiesterase type 5 inhibitors (PDE5i), the widely used treatment for erectile dysfunction, seem to counteract insulin resistance (IR) and improve glucose homeostasis in animals and humans. IR is primarily due to the impairment of insulin signal transduction in skeletal muscle, is a major pathogenic factor in type 2 diabetes and contributes to the morbidity of obesity. Understanding the intracellular defects responsible for IR is critical to identify pharmacological tools targeted toward the specific defects. Some effects of PDE5i have been reported onto animal muscular tissues or cells, however, whether and how PDE5i might affect metabolic processes in human skeletal muscle still remains unclear. We aimed to investigate in human fetal skeletal muscle cells (Hfsmc) whether and how the PDE5i Tadalfil affects the intracellular cascade related to insulin response, such as MAPK-, PKB/Akt-, GSK3beta-mediated signal transuction, and the transcriptional factor c-Myc, by western blot; activity of citrate synthase (CS) and succinate dehydrogenase (SDH), both enzymes of Kreb's cycle, by enzymatic assay; glucose consumption, lactate release and free fatty acid release and intracellular accumulation, by ELISA and Sudan black staining; cell proliferation and apoptosis by MTT assay and DNA fragmentation analysis.

In Hfsmc Tadalafil exerted an insulin like effect by activating the intracellular cascade related to insulin signal transduction; this effect seems to occur specifically throughout PI3K dependent activation, with no involvement of Rho-associated protein kinase (ROCK) pathway; CS activity and free fatty acid intracellular accumulation and release also significantly increased; glucose consumption did not change; apoptosis was significantly reduced. Tadalafil, like insulin, targeted part of the machinery dedicated to energy management and metabolic control, likely addressing glucose utilization towards a citrate shunt.

PP045

STUDY OF THE PITUITARY-GONADAL AXIS IN MALE PATIENTS AFFECTED BY MIOTONIC DYSTROPHY TYPE I

M. Spaziani[1], E. Bucci[2], S. Granato[1], G. Ruga[1], N. Tahani[1], A. Semeraro[1], C. Piccheri[1], A. Lenzi[1], G. Antonini[2], A. F. Radicioni[1]

[1]Center for Rare diseases, Department of Experimental Medicine, Section of Medical Pathophysiology, Food Science and Endocrinology, Sapienza - Rome, [2]Department of Neurology, II School of Medicine, Sapienza – University of Rome, c/o Ospedale Sant'Andrea - Rome

Myotonic dystrophy type 1 (DM1) is a genetic disease due to expansion of an unstable trinucleotide (CTG) repeat in the 3' untranslated region of the myotonic dystrophy protein kinase (DMPK) gene in chromosome 19q. It is the most common muscular dystrophy in adults: its worldwide prevalence has been estimated in 9,1– 96,2 x 10-6. The pleiotropic expression of DMPK gene is responsible of the premature expression of several age-related signs, symptoms and metabolic disturbances. The most important aspect of this syndrome is a progressive muscular mass decrease with reduced alertness. Other features are: presenile cataract, cardiac conduction disturbances, dyslipidemia, premature balding. Alterations of the endocrine system are often present and characterized by increased levels of plasma PTH (linked to parathyroid adenoma in most of the cases), insulin resistance with type II-diabetes and hypergonadotropic hypogonadism due to a progressive testicular atrophia. The latter, which usually leads to erectile dysfunction, is actually one of the better-described findings of DM1 and it is the consequence of both interstitial (androgenic) and tubular (spermatogenic) gonadic damage. According to this, we studied a population of 21 DM1 male patients (group 1) aged from 20 to 59 years (median age 40,5) compared to a control group (group 2) of 98 male subjects aged from 18 to 66 years (median age 36). None of DM1 patients were under replacement therapy during the study. FSH, LH, total Testosterone (T), SHBG and Inhibin b (INHB) serum concentrations were estimated for both groups. Statistical analysis showed significantly higher levels of gonadotropins ($p < 0,001$) and lower concentrations of T ($p < 0,001$) and INHB ($p < 0,001$) in the group 1 compared to group 2. On the other hand no significative differences for SHBG were found.

In conclusion, our data confirm the establishment of a gradual testicular damage that is biochemically highlighted by the progressive rise of both gonadotropins, followed by T decrease. Physicians should consider these patients as suitable candidates for T supplementation treatment, in order to improve their quality of life by avoiding metabolic syndrome, cardiovascular disease, osteoporosis and other comorbidities linked to hypogonadism.

PP046

NON-ALCOHOLIC FATTY LIVER DISEASE IS NOT ASSOCIATED WITH VITAMIN D DEFICIENCY IN ESSENTIAL HYPERTENSION

C. Catena[1], C. Cosma[2], V. Camozzi[2], M. Plebani[2], M. Ermani[3], L. A. Sechi[1], F. Fallo[2]

[1]Dpt of Experimental and Clinical Medicine - University of Udine, [2]Dpt of Medicine - University of Padova, [3]Dpt of Neurosciences - University of Padova

An independent association between non-alcoholic fatty liver disease (NAFLD), a condition characterized by insulin-resistance, and low serum 25-hydroxyvitamin D [25(OH)D] levels has been reported. 25(OH)D concentrations are directly related with insulin sensitivity, whereas low [25(OH)D] predicts development of hypertension independent of alterations in glucose homeostasis. We hypothesized that hypertensive patients with NAFLD have lower 25(OH)D than those without. We investigated in a group of essential hypertensive patients without additional cardio-metabolic risk factors the relationships between 25(OH)D levels, metabolic parameters and NAFLD. Forty-four never treated hypertensive patients (20 males/24 females, mean age 47±11 yrs.) with grade 1-2 essential hypertension were selected as having (n=23) or not having (n=21) NAFLD at ultrasonography. No patient had diabetes mellitus, obesity, hyperlipidemia, or other risk factors for liver disease. Twenty-four healthy normotensive sex-, age-, BMI-matched subjects served as controls for estimation of both NAFLD and hypovitaminosis D prevalence. The two patient subgroups were similar as to age, sex, and blood pressure levels. Body mass index, waist circumference, glucose, insulin, HOMA index and AST were higher (from $P < 0.001$ to < 0.05) and adiponectin was lower ($P < 0.05$) in patients with NAFLD than in patients without NAFLD. Prevalence of NAFLD was higher in EH patients than in controls (23/44, i.e. 52.2%, vs. 4/24, i.e.16.6%, $P < 0.001$). whereas vitamin D deficiency, as defined by 25(OH)D levels <50 nmol/L, was similarly frequent in EH patients and controls (47.7% vs. 45.8%, P NS). Prevalence of hypovitaminosis D was not different in EH patients with and without NAFLD (37.5% vs. 38.8%, P NS). No difference in serum calcium, phosphate, 25(OH)D, 1,25(OH)D and PTH levels was observed in EH patients and controls, with or without NAFLD. Conclusion: In a population of EH patients without additional cardio-metabolic risk factors, NAFLD is associated with insulin resistance but not with vitamin D deficiency.

PP047

DIRECT INHIBITION OF HEXOKINASE II BY METFORMIN IMPAIRS GLUCOSE CONSUMPTION AND SURVIVAL IN CANCER CELLS

B. Salani, C. Marini[2], A. Del Rio[3], S. Ravera[5], M. Massollo[6], A. M. Orengo[6], A. Amaro[7], M. Passalacqua[3], S. Maffioli[1], U. Pfeffer[7], G. Sambuceti[6], R. Cordera, D. Maggi

[1]DIMI - Università di Genova, [2]CNR - Sezione di Genova, [3]DIMES - Università di Bologna , [4]DIMES - Università di Genova, [5]DIFAR - Università di Genova, [6]DISSAL - Università di Genova, [7]INBB - Università di Genova

Recent evidence indicates that the widely used anti-hyperglycaemic drug metformin has important anticancer properties. This benefit at least partially reflects the drug capability to directly inhibit the proliferation of various types of cancer cells. However, the precise mechanism underlying this effect is unknown. Most solid cancers avidly use large amounts of glucose as a source for both energy production and cell building blocks. Critical to this phenotype is the production of ß-D-glucose-6-phosphate (G6P), catalysed by hexokinases (HK) I and II whose role in glucose retention and metabolism is highly advantageous for cell survival and proliferation. Here we show that metformin acts directly on the enzymatic function of HKI and II in the human non-small-cell lung cancer (NSCLC) cell line Calu-1. The inhibition is selective for these isoforms and virtually abolishes cell uptake and phosphorylation of glucose in a dose and time dependent manner, as documented by the reduced entrapment of 18F-fluorodeoxyglycose (FDG). In silico models indicate that this pharmacological action relates to metformin capability to mimic G6P features by steadily binding its pocket in HK II, thus preventing further glucose phosphorylation. The impairment of this energy source results in mitochondrial depolarization and subsequent cell death. These results demonstrate a novel action of metformin targeting the enzymatic activity of HKI and II.

PP048

STEADY STATE IS REACHED WITHIN TWO TO THREE DAYS OF ONCE-DAILY ADMINISTRATION OF ULTRA-LONG-ACTING INSULIN DEGLUDEC

S. Caputo[1], T. Heise[2], L. Nosek[2], H. Coester[2], C. Roepstorff[3], S. Segel[3], N. Lassota[3], P. Nicoziani[5], H. Haahr[3]

[1]Medicina Interna Ospedale Gemelli - Roma, [2]Profil Institut für Stoffwechselforschung - Neuss, [3]Novo Nordisk A/S - Ålborg, [4]Novo Nordisk A/S - Søborg, [5]Novo Nordisk SpA - Roma

The objective of basal insulin therapy is to ensure continuous insulin coverage throughout the 24 hrs of the day. Insulins with a duration of action of 24 hrs or less are in once-daily regimens characterized by action profiles with periods of low action rising to a peak/plateau followed by a decline. Such profiles will only provide partial basal coverage implying clinical challenges in ensuring consistent glucose control throughout 24 hrs. Insulin degludec (IDeg) has a duration of action extending beyond 42 hrs leading to a flat and stable action profile at steady state. It is important for clinical evaluation at initiation and titration of treatment to estimate the time to reach steady state with IDeg. In two randomized, double-blind trials, subjects with type 1 and 2 diabetes (T1DM/T2DM; n=66/49; age 37/59 yrs, BMI 25/30 kg/m2, A1C 8.1/7.6 %) received IDeg at 0.4, 0.6 or 0.8 U/kg for 8 (T1DM) or 6 (T2DM) days. Blood samples were taken before each dosing to determine the serum IDeg concentration on each day relative to the serum IDeg concentration before dosing on Day 7 (T1DM) or 5 (T2DM). The clinically relevant time to steady state was estimated as time from first dose until serum IDeg trough concentrations exceeded 90% of the final plateau level. For all subjects, independent of dose or type of diabetes, steady state was reached after 2–3 days of IDeg dosing. At steady state, exposure of IDeg was unchanged from day to day. In conclusion, steady-state kinetics were observed with stable serum IDeg concentrations reached within 2–3 days of once-daily dose administration with no further increase in exposure thereafter.

PP049

LESS NOCTURNAL HYPOGLYCEMIA FOR INSULIN DEGLUDEC VS. INSULIN GLARGINE IN SUBJECTS WITH T1DM AND BASELINE A1C OF 7.5–8.5%: A META-ANALYSIS

G. Grassi[1], I. Hirsch[2], L. Meneghini[3], L. Landstedt-Hallin[4], S. Rasmussen[5], N. Lassota[5], P. Nicoziani[6], J. Vora[7]

[1]Endocrinologia, Ospedale Le Molinette - Torino, [2]Med Ctr-Roosevelt - Seattle, [3]Miller School of Medicine - Miami, [4]Karolinska Institute - Stockholm, [5]Novo Nordisk A/S - Søborg, [6]Novo Nordisk SpA - Roma, [7]Royal University - Liverpool

The risk of hypoglycemia increases as A1C levels approach recommended targets. Insulin degludec (IDeg) is a new basal insulin that forms soluble multi-hexamers upon SC injection, resulting in an ultra-long action profile with a low day-to-day variability. We performed a patient level metaanalysis to investigate whether these characteristics of IDeg would allow improved glycemic control together with lower rates of hypoglycemia compared to insulin glargine (IGlar) in T1DM patients with a baseline A1C of 7.5–8.5%. Changes in A1C and fasting plasma glucose (FPG) were analyzed with a linear model and rates of hypoglycemia with a negative binomial regression model. Hypoglycemia was defined as rates of selfreported confirmed hypoglycemia (PG<56 mg/dL [3.1 mmol/L] or severe episodes requiring assistance) and nocturnal confirmed hypoglycemia (onset between 00:01 to 05:59, incl.). The analysis included all openlabeled randomized treat-to-target phase 3a trials in T1DM of 26 or 52 weeks, where IDeg (n=223) and IGlar (n=109) were dosed once daily in a basal–bolus regimen. A1C decreased from 8.0% at baseline in both groups to 7.6 vs. 7.5% at end of trial for IDeg vs. IGlar, respectively (treatment difference: 0.05 [–0.12; 0.22] 95% CI). FPG decreased from 175 to 143 mg/dL for IDeg vs. 177 to 163 mg/dL for IGlar, a treatment difference of –18.27 mg/dL [–35.05; –1.49] 95% CI. There was no difference in overall hypoglycemia (rate ratio (RR): 0.99 [0.80; 1.24] 95% CI) or severe hypoglycemia (RR: 1.05 [0.50; 2.22] 95% CI) between IDeg and IGlar. Despite the lower FPG achieved with IDeg, the rate of nocturnal hypoglycemia was lower with IDeg compared to IGlar (RR: 0.68 [0.50; 0.93] 95% CI). In conclusion, for patients with T1DM and a baseline A1C of 7.5–8.5%, treatment with IDeg results in comparable improvement in A1C with a significantly lower rate of nocturnal hypoglycemia (32%) and a greater reduction in FPG compared to IGlar.

PP050

INSULIN DEGLUDEC ALLOWS FOR FLEXIBLE DAILY DOSING IN TYPE 1 DIABETESWITH LESS NOCTURNAL HYPOGLYCAEMIA THAN INSULIN GLARGINE

A. Consoli[1], J. Cooper[2], C. Mathieu[3], P. Hollander[4], M. Bresta[5], E. Franek[6], S. Bain[7], J. Larsen[8], S. Tamer[8], P. Nicoziani[10], D. Russell-Jones[11]

[1]Diabetologia, Univ. di Chieti - Chieti, [2]Stavanger University - Stavanger, [3]UZ Gasthuisberg - Leuven, [4]Baylor Univ. - Dallas, [5]Univ. of Miami - Miami, [6]MSWiA - Varsavia, [7]Singleton Hospital - Swansea, [8]Novo Nordisk A/S - Bagsvaerd, [9]Novo Nordisk A/S - Søborg, [10]Novo Nordisk SpA - Roma, [11]County Hospital - Surrey

Current basal insulin preparations must be injected at the same time every day to ensure stable glycaemic control, particularly in patients with type 1 diabetes. Insulin degludec (IDeg) is an ultra-long-acting basal insulin that forms soluble multi-hexamers upon s.c. injection, resulting in a flat and stable glucose-lowering effect. The objective of this study was to investigate whether flexible dosing of IDeg provided comparable efficacy and safety to insulin glargine (IGlar) dosed at the same time each day. This 26 + 26-week, open-label, treat-to-target trial in patients with type 1 diabetes (n=493) compared once-daily (OD) IDeg (with evening meal) or IGlar (at same time each day) to a flexible IDeg regimen (IDeg Flex), each in combination with meal-time insulin aspart. In the first 26 weeks, IDeg Flex patients were required to alternate OD insulin administration between morning and evening, thus creating intervals of a minimum of 8 and a maximum of 40 hours between doses. For the 26-week extension, IDeg OD and IDeg Flex patients were allocated to IDeg Free Flex (IDeg FF; n=329), which allowed dosing at any time of day with dose intervals between 8 and 40 hours, and compared to IGlar (n=164), which was continued as per label.At 52 weeks, IDeg FF and IGlar reduced baseline HbA1c by 0.13 and 0.21%-points (estimated treatment difference [ETD] IDeg FF-IGlar: 0.07% [95% CI: -0.05; 0.19]). FPG reductions were significantly greater with IDeg FF versus IGlar (ETD IDeg FF-IGlar: -1.07mmol/L [95% CI: -1.82; -0.32]). Overall hypoglycaemia rates (PG <3.1mmol/L or severe hypoglycaemia requiring assistance) were similar (68.1 vs. 63.4 events/patient-year for IDeg FF and IGlar, respectively; estimated rate ratio (ERR) IDeg FF:IGlar 1.09 [95% CI: 0.91; 1.29]). Nocturnal hypoglycaemia was significantly 25% lower for IDeg FF versus IGlar (ERR: 0.75 [95% CI: 0.58; 0.97]), and severe hypoglycaemia was 26% lower (ERR: 0.74 [95% CI: 0.38; 1.42]). This study demonstrates that IDeg can be administered conveniently at any time of day with similar glycaemic control and less nocturnal hypoglycaemia than standard IGlar given OD at the same time each day over 52 weeks in patients with type 1 diabetes.

PP051

IN T2D PATIENTS WITH BASELINE A1C <8.0%, LIRAGLUTIDE ACHIEVES A1C TARGETS MORE OFTEN THAN SITAGLIPTIN OR EXENATIDE

G. Sesti[1], A. King[2], E. Montanya[3], R. Pratley[4], L. Blonde[5], C. Bette[6], C. B. Svendsen[5]

[1]Medicina Interna - Catanzaro, [2]Diabetes Center Care - Salinas (USA), [3]Università Bellvitge - Barcelona (Spagna), [4]Florida Hospital - Orlando (USA), [5]Novo Nordisk A/S - Soborg (Danimarca), [6]Novo Nordisk SPA - Roma (Italia)

Objective: Limited data are available to clinicians on the efficacy of incretin therapies in type 2 diabetes (T2D) patients who are within 1% of glycemic target (≤7.0%).
Methods: Our post-hoc analysis compared the efficacy of liraglutide 1.8 mg once-daily (OD) to exenatide 10 µg twice daily (BID) (LEAD-6) and sitagliptin 100 mg OD (LIRA-DPP-4) after 26 weeks' treatment; only patients treated as true add-on to metformin with a baseline A1c <8% were included. Patient baseline data were similar in each study (mean A1c 7.3-7.6%) except a shorter mean disease duration in LEAD-6 for exenatide vs. liraglutide (3.9 vs. 6.9 years). Change in A1c and body weight were analyzed using an analysis of covariance (ANCOVA) model based on the intention to treat (ITT) population, last observation carried forward (LOCF). Logistic regression analysis was performed on ITT population, LOCF to compare the proportion of patients achieving glycemic targets (≤6.5% and ≤7.0%).
Results: In LEAD-6, liraglutide produced a numerically greater mean A1c reduction vs. exenatide (-0.86% vs. -0.61%; estimated treatment difference (ETD) -0.27%, p=0.05), reflected in a higher proportion of patients achieving A1c ≤7.0% (84% vs. 73%; p=NS) and around twice as many reaching A1c ≤6.5% (61% vs. 37%; p<0.05). In LIRA-DPP-4, liraglutide produced a significantly greater reduction in A1c (-1.00% vs. -0.49%; ETD -0.53%, p<0.0001) and higher proportion of patients achieving both A1c ≤7.0% and A1c ≤6.5% vs. sitagliptin (82% vs. 46%; p<0.0001 and 51% vs. 20%; p<0.005). Weight loss with liraglutide was greater vs. exenatide (-3.67 kg vs. -2.63 kg; ETD -1.06 kg) but did not reach statistical significance, whereas the difference was significant vs. sitagliptin (-3.39 kg vs. -0.58 kg; ETD -2.96 kg, p<0.0001). Few patients (8-10%) experienced minor hypoglycemia with all therapies.
Discussion: In patients already close to target A1c, liraglutide 1.8 mg brings more patients to target with more weight loss than exenatide or sitagliptin.
Conclusion: In contrast to liraglutide, sitagliptin and exenatide are unlikely to reduce A1c by approximately 1% in this baseline A1c range and this should be considered when choosing an add-on to metformin in patients close to target.

PP052

LIRAGLUTIDE + METFORMIN IN TYPE 2 DIABETES: CLINICAL BENEFITS ASSOCIATED WITH SWITCH OR USE EARLY IN THE DISEASE PROCESS

A. Consoli[1], S. Bain[2], J. Seufert[3], A. B. Thomsen[4], C. Bette[5], D. D'Alessio[6]

[1]Diabetologia - Università di Chieti - Chieti (Italia), [2]Swansea University - Swansea (UK), [3]University of Freiburg - Freiburg(Germania), [4]Novo Nordisk A/S - Soborg (Danimarca), [5]Novo Nordisk SPA - Roma (Italia), [6]University of Cincinnati - Cincinnati (USA)

Metformin (Met) is generally considered the most appropriate first-line pharmacotherapy for type 2 diabetes (T2DM). When Met becomes insufficient, however, there is no general consensus on how to intensify treatment. This post-hoc analysis compared clinical benefits achieved by adding liraglutide in patients previously receiving Met only (Met-add-on) vs substituting liraglutide for sulfonylurea (SU) in subjects previously receiving Met + SU (SU-switch). Data were obtained from a large clinical trial (n=988) in which patients receiving met alone or Met + SU had their therapy changed to Met + liraglutide 1.8 mg. Baseline age (mean [SD]: 58 ([9.3] vs 56 [9.8], respectively) and A1c were similar, while duration of diabetes was significantly longer in the SU-switch subjects (9.0 [6.2] vs 6.5 [5.4]; p<0.0001). Among subjects who completed 12 weeks of treatment, the SU-switch group lost more weight, likely due to the termination of SU treatment, and subjects in the Met-add-on group had a greater reduction in A1C. These data are consistent with greater clinical efficacy of liraglutide among patients with less advanced T2DM, with ~70% of the Met add-on group reaching a target A1C of 7%. The further reduction in mean A1C among the SU switch-subjects, with ~45% reaching the glycemic goal, suggests benefits of liraglutide vs SU. These findings support the conclusions that the glycemic response to liraglutide varies across the spectrum of diabetes progression, and that changing from SU to liraglutide can bring additional benefits to some patients.

PP053

GRADUAL INTENSIFICATION OF PREMIXED INSULIN LISPRO THERAPY VS. BASAL ± MEALTIME INSULIN IN PATIENTS WITH TYPE 2 DIABETES EATING LIGHT BREAKFASTS

D. Giuliano[1], V. Woo[2], S. Shah[3], M. Tracz[4], C. Mistodie[5], A. Calle-Pascual[6], R. Duarte[7], R. Sari[8], A. Jiletcovici[9], J. Deinhard[10], S. Wille[9], J. Kiljanski[9]

[1]Second Univ. of Naples - Naples, [2]Univ. of Manitoba - Winnipeg, [3]Diabetes Action Centre - Mumbai, [4]Dept of Gastroenterology and Metabolic Diseases, Med Univ - Warsaw, [5]Spitalul Clinic de Urgenta "Sf.Apostol Andrei" - Galati, [6]Hospital Clinico San Carlos - Madrid, [7]Associacao Dos Diabeticos de Portugal - Lisboa, [8]Faculty of Medicine, Akdeniz Univ - Antalya, [9]Eli Lilly & Co - EU, [10]Accovion Gmbh - Eschborn

Randomized, open-label, 48-week, parallel arm study enrolling patients with T2DM eating light breakfasts (<15% daily calories)and not controlled with oral antihyperglycaemic medications to test if 1, 2 or 3 injections of insulin lispro mix25 and/or insulin lispro mix50 (lispro mix algorithm;LM) was non-inferior to glargine ±1, 2 or 3 injections of insulin lispro (basal ± meal-time insulin algorithm; BM) for glycaemic control, measured by HbA1c after 48 weeks, assessed using ANOVA adjusted for baseline HbA1c and with a non-inferiority margin of 0.4%.Mean number of injections/day and % of pts achieving <7.0% HbA1c without hypoglycaemia were assessed through post-hoc analyses. Pts (n=344: 176[51%] females, mean[SD] age 54.3[8.8] years, BMI 29.4[4.6] kg/m2, baseline HbA1c 9.02[0.97] %) were randomized to LM(n=171) or BM(n=173). In the per-protocol analysis (n=230) “LS means” (95%CI) endpoint HbA1c were 7.40(7.15 - 7.65) and 7.55(7.27 - 7.82) in LM and BM arms, respectively. The between-treatment difference was -0.14%(-0.42, 0.13); non-inferiority was met and confirmed in the full analysis set(n=321). Mean HbA1c changes at Week 48 were -1.68%(1.35) (LM) and -1.66%(1.31)(BM); p=0.967. Mean number of insulin injections/day at Week48 was 1.96 in the LM and 1.99 in the glargine arm. HbA1c targets of <7.0% were achieved by 48.2%(LM) vs. 36.2%(BM) and ≤6.5% were achieved by 24.8%(LM) vs. 18.5%(BM) of pts. The % of pts achieving HbA1c <7.0% without hypoglycaemia was 13.1% and 8.5%, respectively at Week 48; p=0.251. SMBG profiles, ?body weight of +2.31(3.3)kg and +2.32(3.7)kg, and total insulin doses of 46.20(28.4)IU/day and 46.45(31.4)IU/day at Week48 were similar in LM and BM, respectively. Overall(65% vs. 60%) and severe hypoglycemia rate(2%vs.4%) were similar in LM and BM groups (p=0.75); more LM pts had nocturnal symptomatic episodes (27.8%vs.17.9%; p=0.039).LM was not inferior to BM in achieving glycaemic control in this setting.

PP054

NON-ALCOHOLIC FATTY LIVER DISEASE IS ASSOCIATED WITH AN INCREASED PREVALENCE OF ATRIAL FIBRILLATION IN TYPE 2 DIABETIC PATIENTS

A. Mantovani[1], I. Pichiri[1], R. Rigolon[1], M. Dauriz[1], G. Morani[2], G. Zoppini[1], E. Bonora[1], G. Targher[1]

[1]Endocrinologia, Università di Verona - Verona, [2]Cardiologia, Università di Verona - Verona

Non-alcoholic fatty liver disease (NAFLD) and atrial fibrillation (AF) are two diseases that are highly prevalent in developed countries and share multiple cardiometabolic risk factors. Presently, the published research on the association between NAFLD (or liver function tests) and AF is sparse. The Framingham Heart Study investigators have recently reported an independent association between elevated serum transaminase levels, as proxy markers for NAFLD, and increased risk for incident AF in the community. There is currently a lack of available information on the relationship between NAFLD and AF in people with type 2 diabetes (T2DM), a group of individuals in which these two diseases are common. We studied a hospital-based sample of 702 patients with T2DM (M/F=379/323, mean age 66 years) discharged from our Division of Endocrinology during 2007-2011. NAFLD was defined by ultrasonographic detection of steatosis in the absence of other liver diseases. The diagnosis of AF was confirmed in affected participants by experienced cardiologists. Of the 702 patients included in the study, 514 (73.2%) of them had NAFLD and 85 (12.1%) had persistent AF. NAFLD was associated with an increased risk of prevalent AF (OR 3.04, 95%CI 1.5-6.0, p<0.0001). Adjustments for age, sex, BMI, systolic blood pressure, hypertension treatment, electrocardiographic left ventricular hypertrophy, CKD, pre-existing history of ischemic heart disease, heart failure, valvular heart disease, chronic obstructive pulmonary disease or hyperthyroidism did not appreciably weaken the association between NAFLD and AF (adjusted OR 4.39, 95% CI 2.0-9.3, p<0.0001). The significance of this association was consistent in all subgroups evaluated. In conclusion, our results firstly show that ultrasound-diagnosed NAFLD is strongly associated with an increased prevalence of AF in patients with T2DM, independently of several clinical AF risk factors. More research is needed to corroborate a prognostic value of NAFLD for the incidence of AF, and to further elucidate the putative underlying mechanisms that link NAFLD and AF. From the perspective of clinical practice, it is important that specialists and practicing clinicians be aware of the significant association between NAFLD and AF, especially because of the high and growing prevalence of these two pathologies.

PP055

TEMPORAL TRENDS OF HLA, CTLA-4 AND PTPN22 GENOTYPE FREQUENCIES AMONG TYPE 1 DIABETES IN CONTINENTAL ITALY

M. Spoletini[1], S. Zampetti[1], G. Campagna[1], L. Marandola[1], M. Capizzi[1], R. Buzzetti[1], .. IMDIAB Study Group[2]

[1]Experimental Medicine, Sapienza University - Rome, [2]Endocrinology, Campus Bio-Medico University - Rome

The incidence of type 1 diabetes has, progressively, increased worldwide over the last decades and also in Continental Italian population. Previous studies performed in northern European countries, showed, alongside a general increase in the disease incidence, a decreasing frequency of the highest-risk HLA genotype in type 1 diabetes populations, thus emphasizing the role of environmental factors. The aim of the study was to evaluate whether a decreasing trend of HLA, CTLA-4 and PTPN22 genotype frequencies would be present in type 1 diabetes subjects of Continental Italy, a country still considered at low incidence of the disease compared to northern European populations.

We included n=765 type 1 diabetes patients diagnosed from 1980 to 2012 in Lazio region.

For HLA, CTLA4 and PTPN22 temporal trend evaluation, subjects were sub-divided into groups of years according to the age at diagnosis. All subjects were typed for HLA-DRB1 and DQB1 by a reverse line blot. The CT60 polymorphism of the CTLA4 and C1858T of the PTPN22 gene were genotyped using ABI PRISM 7900HT (n=419 and n=364 respectively). HLA genotypes were divided in high, moderate and low-risk categories. The proportion of the HLA risk categories was not statistically different over the three decades in subjects with age of onset <15 years and ≥15 years. The genotype distribution of CT60 polymorphism of CTLA4 gene did not show any change in the frequencies during time. The analysis of the PTPN22 C1858T variant revealed, instead, that the frequency of CT+TT susceptibility genotypes decreased during time (23.8% vs 13.6%, p= 0.017). We can hypothesize that the pressure of the diabetogenic environment could be milder compared to northen European population and therefore not sufficient to reduce the need of a strong genetic background (HLA) “to precipitate” diabetes; the increased pressure of the environment could have, instead, some effects on minor susceptibility genes in our population.

PP056

ELEVATED SERUM URIC ACID LEVELS ARE ASSOCIATED WITH AN INCREASED INCIDENCE OF ATRIAL FIBRILLATION IN TYPE 2 DIABETIC PATIENTS

I. Pichiri[1], A. Mantovani[1], V. Cavalieri[1], F. Valbusa[2], L. Bertolini[2], L. Zenari[2], G. Zoppini[1], E. Bonora[1], G. Targher[1]

[1]Endocrinologia, Univ. di Verona - Verona, [2]Med. Generale, Osp. Sacro Cuore - Negrar

Recent studies reported a significant association between elevated serum uric acid (SUA) levels and increased prevalence of atrial fibrillation (AF). There is currently a lack of available information on the association between hyperuricemia and AF in people with type 2 diabetes, a group of individuals in which these two diseases are highly prevalent. We examined the relationship between SUA level and risk of developing AF in people with type 2 diabetes. We prospectively followed for 10 years a random sample of 400 type 2 diabetic outpatients (M/F=135/165, mean age 64 years), who attended the diabetes clinic at the “Sacro Cuore” Hospital of Negrar during 2000-2001, and who were free from AF at baseline. A standard 12-lead electrocardiogram was undertaken annually and a diagnosis of incident AF was confirmed in affected participants by a single cardiologist. Mean (±SD) SUA level was 5.2±1.4 mg/dl (range: 2.1-9.8 mg/dl) for all participants. Among these, 73 (18.3%) patients had hyperuricemia (i.e., SUA ≥7 mg/dl in men and ≥6 mg/dl in women or allopurinol use). Over 10 years, 42 patients developed incident AF (cumulative incidence of 10.5%). Elevated SUA was associated with an increased risk of incident AF (OR 2.43, 95% CI 1.8-3.4, p<0.0001 for each 1-SD increase in SUA). Adjustments for age, sex, BMI, hypertension, CKD, electrocardiographic features (left ventricular hypertrophy and PR interval) and use of diuretics and allopurinol did not attenuate the association between SUA and AF (adjusted OR 2.44, 95% CI 1.6-3.9, p<0.0001). Further adjustment for variables that were included in the 10-year Framingham Heart Study-derived AF risk score did not appreciably weaken this association. Results remained unchanged even when SUA was modelled as a categorical variable or when patients with history of previous heart failure and coronary heart disease were excluded from analysis. In conclusion, this study is the first to document that elevated SUA levels are associated with a greater incidence of AF in type 2 diabetic patients, independently of important clinical AF risk factors. Further research is required to elucidate the responsible mechanisms for this association, and to explore whether pharmacological interventions aimed at improving SUA levels may reduce the incidence of AF in type 2 diabetic patients.

PP057

WRIST CIRCUMFERENCE POSITIVELY CORRELATES WITH SYSTOLIC AND DIASTOLIC BLOOD PRESSURE IN OVERWEIGHT/OBESE CHILDREN AND ADOLESCENTS

M. Capizzi[1], G. Campagna[1], G. Leto[1], S. Zampetti[1], M. Spoletini[1], C. Venditti[1], M. Mastantuono[2], A. Vania[3], R. Buzzetti[1]

[1]Experimental Medicine, Sapienza University - Rome, [2]Radiological, Sciences, Oncology and Pathological Anatomy, Sapienza University - Rome, [3]Pediatrics and Child Psychiatry, Sapienza University - Rome

Cardiovascular diseases are the leading cause of death in Western countries. One of the most important cardiovascular risk factors according to many pathophysiological models is insulin resistance. The wrist circumference, in particular its bone component, has been recently demonstrated to be a clinical marker for insulin-resistance in overweight/obese children, configuring the possibility that this parameter could be taken into account in the measurement of cardiovascular risk .

Another risk factor for the developing of cardiovascular disease is blood pressure. Recent studies have shown an acceleration of skeletal growth in children in case of primary hypertension.

In the light of these findings , the aim of the present study was to evaluate the presence of a correlation between wrist circumference and blood pressure in overweight/obese children and adolescents.

N = 140 overweight / obese children and adolescents (mean age 9.8 years +2.8), were consecutively recruited for the evaluation of blood pressure, wrist circumference, weight and waist circumference. In all subjects blood samples were taken in order to evaluate insulin and blood glucose and to calculate the HOMA-IR (Homeostasis Model Assessment of Insulin Resistance)

The analysis was performed by General Linear Model with the SAS software 9.2.

The wrist circumference was significantly and positively associated with systolic and diastolic blood pressure ($p < 0.05$ and $p < 0.0001$ respectively), explaining in the model 10% of the systolic pressure variability and 25% of the diastolic pressure variability.

The circumference of the wrist in overweight / obese children and adolescents is correlated with systolic and diastolic blood pressure, confirming that this bone anthropometric marker could be of potential interest for the prediction of cardiovascular risk.

PP058

DPP4 INHIBITORS: ANTI-INFLAMMATORY EFFECTS ON M1 MACROPHAGE ACTIVATION

E. Derlindati[1], V. Curella[1], I. Zavaroni[1]

[1]Medicina Clinica e Sperimentale, Università di Parma - Parma

Background and aim. Dipeptidyl-peptidase-4 (DPP-4) inhibitors are a newer class of oral anti-hyperglycemic agents whose effect is to inhibit the degradation of the incretins, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP). It has been suggested a new role of DPP-4 inhibitors, related to a anti-atherogenic and anti-inflammatory effects. It is well established that macrophages are involved in inflammatory-related disease, in particular the pro-inflammatory macrophage polarization (M1 or classical activation) promote the inflammation process. The aim of this study is to evaluate the effects of a DPP-4 inhibitor on classical macrophage activation . Material and methods. Monocyte were isolated from healthy donors buffy coat. After the differentiation in macrophage, cells were stimulated with IFNγ and LPS, in order to induce M1 activation, in presence or in absence of 500nM of DPP-4 inhibitor (KR-62436) for 24 hours. The concentration of DPP-4 inhibitor was assessed by a cytotoxicity assay. Resting macrophages (RM) -no stimuli addiction- were used as a control. The expression of DPP-4 was evaluated in every macrophage conditions, both by real time pcr and cytofluorimetric assay. Principal pro-inflammatory genes were evaluated by real time pcr. Protein expression measurement and DPP-4 activity will be evaluated. Results. Cytofluorimetric analysis demonstrated an higher expression of DPP-4 in M1 macrophages compared to RM (Mean Fluorescence Intensity -MFI- : 1744 and 938 respectively). Nevertheless, the addiction of DPP-4 inhibitor reduced the expression of DDP-4 only in a minimal way (MFI: 1600), suggesting a possible change in DPP-4 activity rather than in protein expression (ongoing experiment). Moreover, the expression of pro-inflammatory genes COX2, IL-8 and TNF alpha are lower in M1 with DPP-4 inhibitor compared to M1 without DPP-4 inhibitor (Relative Fold Change respectively:- 2.75; -131.59 and -352.16). Conclusion. This study shows for the first time a link between DPP-4 and classical macrophage activation, proposing a pro-inflammatory role of DPP-4 The reduction of the expression of some inflammatory genes induced by DPP-4 inhibitor suggests a possible anti-inflammatory effect of DPP-4 inhibitors useful in the treatment of inflammatory – related disease, such diabetes, insulin resistance and atherosclerosis. These are preliminary data that need to be confirmed.

PP059

SCREENING OF GLUCOSE DISORDERS IN PATIENTS UNDERGOING BARIATRIC SURGERY. PRELIMINARY DATA.

E. Calvi[1], S. Belcastro[1], I. Olivetti[1], M. Tomelini[1], V. Ramella Gigliardi[1], E. Gramaglia[1], A. Benso[1], G. Scozzari[2], M. Toppino[2], M. Morino[2], E. Ghigo[1], F. Broglio[1]

[1]Divisione di Endocrinologia, diabetologia e metabolismo - Dipartimento di medicina interna - università di Torino - Torino, [2]Divisione di Chirurgia digestiva, colon-rettale e mininvasiva- Dipartimento di chirurgia - Università di Torino - Torino

BACKGROUND AND OBJECTIVE: Roux-en-Y gastric bypass (RYGBP) is known to impact on glucose metabolism. However at present the prevalence of bariatric surgery-induced alterations of glucose metabolism in patients without previous DM is unknown. Our preliminary data have shown that in 149 out of 312 patients who underwent RYGBP between January 2007 and December 2011, Edinburgh Hypoglycemia Scale (EHS) and the Sigstad's Score resulted positive for the presence of potentially hypoglycaemia-related subjective symptoms. Based on these data, aim of the present Study is to investigate the real prevalence of hypoglycaemic events in those symptomatic patients.

METHODS: to this aim, in 44 patients (6 M/38 F; age: 45,3 ± 1,5 years.; presurgical BMI: 47,7 ± 1,0 Kg/mq^2) who were positive to EHS and the Sigstad's Score, we performed Structured Self Monitoring of Blood Glucose (SMBG) for two weeks. This test was considered positive with the presence of symptoms and/or HGT <60 mg/dl.

RESULTS: out of 44 patients, 10 patients were positive for symptomatic hypoglycaemias (group A), 18 were positive for postprandial symptoms without apparent hypoglycaemias (group B), 15 were negative for both (group C), 1 patient was positive for asymptomatic hypoglycaemias (group D).

In 9 patients from group B, OGTT test was then performed allowing the diagnosis of Early neurovegetative Dumping Syndrome in 4 patients and of Dumping Syndrome with late hypoglycaemias in 5 patients. The subject in group D was tested with Continuous Glucose Monitoring (CGM) that confirmed the existence of asymptomatic hypoglycaemias.

CONCLUSIONS: in conclusion the results of this Study, although preliminary and in progress, strongly suggest that both symptomatic and asymptomatic hypoglycaemias are not uncommon as a late complication of bariatric surgery.

PP060

EVALUATION OF THE ABILITY OF ADIPOSE DERIVED STEM CELLS TO DIFFERENTIATE INTO ADIPOCYTES UNDER HYPERGLYCAEMIC CONDITIONS ALONE OR IN COMBINATION WITH PIOGLITAZONE

S. Fallucca[1], W. Rizza[1], V. Fedele[1], A. Palermo[1], B. Cagli[2], S. Tenna[2], P. Persichetti[2], R. Strollo[1], P. Pozzilli[1]

[1]Endocrinologia e Diabetologia Università Campus Bio-Medico - Roma, [2]Chirurgia ricostruttiva Università Campus Bio-Medico - Roma

Aim: Pioglitazone, a blood glucose lowering agent, may be involved in cell differentiation toward adipogenic lineage. In this study we tested the effect of high glucose alone or in combination with pioglitazone on adipose tissue-derived mesenchymal stem cells (hADSCs) differentiation.

Material and Methods: Cells were isolated from adipose tissue obtained by liposuction or surgery from normal subjects. After isolation, stem cells were characterized and differentiated toward mesenchymal cell types, including adipocytes, osteoblasts and chondrocytes to assess multipotency. hADSCs were then exposed for 15 days to high glucose (HG) media (30-55 mM) alone or in combination with pioglitazone 25 µM. An adipogenic medium was used as positive control. A cytochemical analysis with Oil Red O was performed to assess phenotypic changes and adipogenic differentiation with the formation of intracytoplasmatic lipid droplets.

Results: After 15 days, hADSCs treated with 30 mM HG showed the formation of some lipid droplets that increased in the combined treatment with 30 mM HG and pioglitazone 25 µM. Exposure to 55mM HG led to cell death within 48-72 h. By contrast, hADSCs treated with 55 mM HG and pioglitazone 25 µM prevented apoptosis and showed increased formation of intracytoplasmatic lipid droplets compared with combination media of 30 mM HG and pioglitazone 25 µM. Positive control showed that these cells differentiated into adipocytes under appropriate conditions of stimulation.

Conclusions: Our preliminary results indicate that hyperglycaemic environment may induce hADSCs differentiation toward an adipogenic lineage even if at lesser degree compared to the positive control. Presence of pioglitazone not only increased the amount of the committed hADSCs but may also prevent toxic effects of high glucose on hADSC. Therefore, glucose concentrations may modulate stem cells differentiation and pioglitazone, in addition to exert a synergistic role in the differentiation, may also have cytoprotective effects.

PP061

IGA ANTI-TRANSGLUTAMINASE AUTOANTIBODIES AND CELIAC-SPECIFIC IMMUNE RESPONSE IN TYPE 1 DIABETES VERSUS NONDIABETIC CELIAC PATIENTS, AT DIAGNOSIS

T. Filardi[1], C. Tiberti[2], F. Panimolle[1], M. Bonamico[3], B. Shashaj[3], F. Lucantoni[1], R. Nenna[3], F. Costantino[3], A. Lenzi[2], S. Morano[1]

[1]Dipartimento di Medicina Interna- "Sapienza" Università - Roma, [2]Dipartimento di Medicina Sperimentale- "Sapienza" Università - Roma, [3]Dipartimento di Pediatria - "Sapienza" Università - Roma

OBJECTIVE. To evaluate the celiac-associated humoral autoimmunity in child, adolescent, and adult patients at type 1 diabetes (DM1) onset and to determine whether DM1 celiac-specific humoral immunoreactivity occurs similarly to that in nondiabetic patients at celiac disease (CD) diagnosis. RESEARCH DESIGN AND METHODS. IgA anti-transglutaminase autoantibody (IgA-tTGAb) was detected in 654 new-onset DM1 sera. IgA-tTGAb(+) DM1 sera were subsequently analyzed for IgG-tTG, deamidated gliadin (DGP), and actin antibodies, and results were compared with those found in 83 screen-detected nondiabetic patients at CD diagnosis. RESULTS. A total of 12.8% DM1 sera were IgA-tTGAb(+), with a lower autoantibody frequency in adult patients aged >18 years (6.8 vs. 15.1%, aged ≤18 years; P = 0.005). IgA-tTGAb titers, IgG-tTGAb, and DGPAb frequency/titers and mean number of celiac-autoantibody positivities per patient were significantly lower in IgA-tTGAb(+) DM1 compared with nondiabetic CD patients. CONCLUSIONS. Age of diabetes onset is negatively associated with risk of CD. The celiac-specific humoral immunoreactivity at DM1 onset is significantly lower compared with that found in nondiabetic patients at CD diagnosis.

PP062

TESTOSTERONE AND VITAMIN D IN TYPE 2 DIABETIC MEN

N. Caretta[1], S. Vigili de Kreutzenberg[2], G. Guarneri[2], A. Avogaro[2], C. Foresta[1]

[1]Medicina molecolare - Padova, [2]Medicina Interna - Padova

Background: Epidemiological studies support a relationship between low testosterone plasma levels and type 2 diabetes mellitus (T2DM), in men. Other studies have shown an association between low serum 25-hydroxyvitamin D [25(OH)D] concentrations and increased risk of hyperglycaemia. Recently we showed the expression of CYP2R1, a key vitamin D-25hydroxylase, in human testis and a reduction of 25-hydroxyvitamin D levels in patients with testicular disease. This study was designed to evaluate testosterone and vitamin D concentrations, in T2DM men, and their possible correlations. Subjects and Methods: A total of 43 T2DM men and 22 non-T2DM age-matched controls (mean age 55.8±9.9 vs 51.4±14.6 years) were enrolled in this study. All subjects underwent accurate medical history, physical examination and biochemical blood tests. Results: T2DM men showed higher BMI (28±3.8 vs 24.9±2.3 Kg/m2; p<0.0004), waist circumference (99.9±11 vs 87±5.6 cm; p<0.0003), diastolic blood pressure (79.3±9.5 vs 73±8 mmHg; p<0.009), triglyceride (165.7±155 vs 138.7±47.6 mg/dL; p<0.001) and LH (6.4±3.4 vs 4.1±2.1 UI/L; p<0.02) concentrations compared to controls. As expected, HbA1c was higher in T2DM subjects (7.75±1.5 vs 5.3±0.4 %; p<0.001). We found lower testosterone (13.6±5.1 vs 17.3±3.8 nmol/L; p<0.001) and 25(OH)D (36.2±20.9 vs 57.1±11 nmol/L; p<0.0001) plasma levels in T2DM men compared to controls. Afterwards we correlated testosterone and 25(OH)D with anthropometric and metabolic parameters; a positive statistically significant linear correlation was present only between testosterone and 25(OH)D plasma levels (R: 0.36; p<0.024). We observed also a negative association between 25(OH)D and LH, and a positive one between 25(OH)D plasma levels and Hba1c, but both not statistically significant. Conclusions: In conclusion, in T2DM men testosterone and 25(OH)D plasma levels are reduced and related. We should speculate that these patients have a progressive reduction in Leydig cell function.

PP063

CARDIOVASCULAR EFFECTS OF TREATMENT WITH LIRAGLUTIDE IN A POPULATION WITH TYPE 2 DIABETES

M. Caputo[1], F. Prodam[1], M. Zavattaro[1], L. Pagano[1], S. Belcastro[1], M. C. Ponziani[2], G. Allochis[2], G. Aimaretti[1]

[1]Endocrinologia, Dipartimento di Medicina Traslazionale, Università del Piemonte Orientale "A. Avogadro" - Novara, [2]SCDO Malattie Metaboliche e Diabetologia, AOU "Maggiore della Carità" - Novara

Introduction: Liraglutide, a human GLP-1 analogue, is a new option for the treatment of type 2 diabetes (DM2). The purpose of this study was to evaluate the effects of liraglutide on cardiovascular risk factors in daily clinical practice in a heterogeneous population with DM2.

Subjects and methods: Four visits were scheduled in a 1 year study (baseline, 4, 8 and 12 months). Patients with a HbA1c not on target (>53 mmol/mol) during an oral hypoglycemic treatment, or patients intolerant to metformin were recruited. Exclusion criteria were: the presence of kidney diseases on dialysis, lack of compliance or refusal to injective therapy. Changes in systolic (SBP) and diastolic blood pressure (DBP) and cardiovascular risk scores (Framingham and UKPDS algorithms) were evaluated. 243 subjects (110 males; 133 females) were consecutively recruited (age, mean±SD 59.6 ± 10.4 yrs; disease duration 8.3 ± 7.0 yrs).

Results: Both SBP (154.7 ± 22.0 vs 143.5 ± 23.6 mmHg, p<0.0001) and DBP (89.1 ± 11.5 vs 84.4 ± 10.7 mmHg, p<0.0001) decreased respect to baseline. Both reductions were independent of changes in glucose, HbA1c and anti-hypertensive drugs. Framingham risk score decreased at 12 months respect to baseline (30.2 ± 17.9 vs 35.6 ± 20.3%, p<0.0001). At the same time a reduction in the risk of coronary events (15.7 ± 11.4% vs 20.7 ± 15.2%, p<0.0001) and of fatal coronary events (10.9 ± 9.5% vs 15.2 ± 13.8%, p<0.0001) was recorded. Finally, also the risk of stroke (8.6 ± 8.5% vs. 12.0 ± 15.3%, p<0.0001) and fatal stroke (1.4 ± 1.5% vs. 2.3 ± 3.3%, p<0.0001) at 12 months decreased respect to baseline. During the 12 months of observation no cardiovascular accidents occurred.

Conclusion: Liraglutide is effective in reducing blood pressure and cardiovascular risk in patients with DM2 in a relatively short-time treatment.

PP064

EFFICACY AND SAFETY OF 1 YEAR TREATMENT WITH LIRAGLUTIDE IN SUBJECTS WITH TYPE 2 DIABETES

M. Zavattaro[1], F. Prodam[1], M. Caputo[1], A. Busti[1], M. T. Samà[1], M. G. Mauri[2], L. Pagano[1], G. Allochis[2], G. Aimaretti[1]

[1]Endocrinologia, Dipartimento di Medicina Traslazionale, Università del Piemonte Orientale "A. Avogadro" - Novara, [2]SCDO Malattie Metaboliche e Diabetologia, AOU "Maggiore della Carità" - Novara

Introduction: Liraglutide, a GLP-1 analogue, is a new option for the treatment of type 2 diabetes (DM2). The purpose of this study was to evaluate the efficacy and safety of liraglutide in daily clinical practice in a heterogeneous population with DM2.

Subjects and methods: Four visits were scheduled in a 1 year study (baseline, 4, 8 and 12 months). All patients with a HbA1c not on target (>7%) during an oral hypoglycemic treatment, or patients intolerant to metformin were recruited. Exclusion criteria were: the presence of kidney diseases on dialysis, lack of compliance or refusal to injective therapy. Changes in glucose, HbA1c, body weight, BMI and lipid profile were primary outcomes. Adverse events and drop-out rate were evaluated.

Results: 243 subjects (110 males; 133 females) were recruited, with an age of (mean±SD) 59.6 ± 10.4 yrs and a disease duration of 8.3 ± 7.0 yrs. Fasting blood glucose (10.3 ± 3.1 vs 8.4 ± 2.6 mmol/l, p<0.0001) and HbA1c (8.6 ± 1.3 vs 7.4 ± 1.0%, p<0.0001) decreased at 4 months and maintained a plateau overtime. Body weight (92.8 ± 18.9 Kg vs 89.5 ± 18.2 Kg, p<0.0001) and BMI (33.8 ± 6.6 vs 32.3 ± 6.0 Kg/m2, p<0.0001) decreased at 4 months and then remained stable. Lipids slightly decreased over time. Total cholesterol (4.6 ± 0.9 vs 4.3 ± 0.9 mmol/l, p<0.01), LDL-cholesterol (2.6 ± 0.8 vs 2.4 ± 0.7 mmol/l, p<0.03) and triglycerides (1.9 ± 1.0 vs 1.7 ± 0.9 mmol/l, p<0.03) decreased respect to baseline, independently of glucose and HbA1c changes. Conversely, HDL cholesterol increased (1.16 ± 0.28 vs 1.17 ± 0.27 mmol/l, p<0.01). 35 patients left the study, 3 of them because of adverse effects.

Conclusion: Liraglutide is effective in controlling DM2 in daily clinical practice. Liraglutide could have pleiotropic actions in the control of DM2.

PP065

SERUM OSTEOCALCIN LEVELS ARE INVERSELY ASSOCIATED WITH HBA1C AND BMI IN ADULT SUBJECTS WITH TYPE 1 DIABETES

E. Maddaloni[1], A. Lauria[1], A. Maurizi[1], R. Del Toro[1], L. D'Onofrio[1], R. Strollo[1], A. Palermo[1], S. Manfrini[1], P. Pozzilli[1]

[1]Endocrinology and Diabetes, University Campus Bio-Medico - Rome

Introduction. Diabetic osteopathy is an upcoming complication of both type 1 and type 2 diabetes characterized by osteoporosis, increased risk for bone fractures and alterations in bone metabolism. Osteocalcin (OC) is a bone-specific protein product by osteoblasts that has recently been described to be involved in the regulation of glucose and energy metabolism. Several studies showed that OC serum levels are reduced in patients with type 2 diabetes and in obese patients; in type 1 diabetes osteocalcin appears to correlate positively with markers of insulin exposure, but its relationship with metabolic control has not yet clarified

Aim. To determine whether osteocalcin serum levels are correlated with metabolic control in adult subjects with type 1 diabetes.

Methods. We conducted a cross-sectional type of study in adults with type 1 diabetes referring to our outpatient clinic. A total of 93 subjects (51 males and 42 females) were enrolled in the study with age, disease duration and BMI of 39.9 ± 12.3 yrs, 17.2 ± 12.6 yrs and 24.5 ± 3.4 Kg/m2, respectively. Blood samples were drawn for measuring HbA1c, C-peptide, osteocalcin, 25-OH Vitamin D and PTHi.

Results. The following data were obtained: HbA1c 7.9 ± 1.3%; C-peptide, 0.18 ± 0.3 ng/ml; osteocalcin, 21.0 ± 13.3 ng/ml; 25-OH Vitamin D, 22.8 ± 16.5 ng/ml; PTHi, 42.1 ± 16.3 pg/ml. Significant inverse correlations were found between osteocalcin and HbA1c (r= -0.295, P= 0.004) and between osteocalcin and BMI (r= -0.218, P=0.037). A significant difference in mean HbA1c was found between the lowest and the highest osteocalcin tertile (8.5 ± 1.7% vs 7.5 ± 0.8%, P= 0.007); this significance difference was not lost after adjustment for gender, age, disease duration and BMI.

Conclusions Our data show that OC serum levels are inversely associated with BMI and HbA1c in type 1 diabetes. The mechanisms by which bone related complications develop in diabetes are not sill completely understood. Our data could support the hypothesis that a poor glycemic control could affect bone metabolism, especially osteblast function.

PP066

CONTINUOUS GLUCOSE MONITORING AND ROBOT SURGERY OF AN INSULINOMA: A CASE REPORT

E. Maddaloni[1], G. Capolupo[2], A. Palermo[1], S. Manfrini[1], F. Di Matteo[3], R. Grasso[4], R. Coppola[2], P. Pozzilli[1]

[1]Endocrinology and Diabetes, University Campus Bio-Medico - Rome, [2]General Surgery, University Campus Bio-Medico - Rome, [3]Endoscopic Ultrasound, University Campus Bio-Medico - Rome, [4]Radiology, University Campus Bio-Medico - Rome

Background: Insulinomas are rare, functioning, pancreatic neuroendocrine tumors, causing life-threatening fasting hypoglycemia. A rapid diagnosis, therapy and accurate monitoring of treatment effectiveness are important for patient's survey and quality of life. Treatment of choice for insulinoma consists in the surgical removal of the tumor, frequently associated with surgery-related complications that could be reduced by robotic-assisted laparoscopic surgery. Evidences suggest that continuous glucose monitoring could be useful to monitor the response to therapy and to confirm the complete removal of the tumor. **Case Presentation**: A Caucasian 60 year old woman referred to our clinic for evaluation of repeated episodes of symptomatic hypoglycemia. A 72 hours fasting test and a pancreatic endoscopic ultrasonography allowed a rapid diagnosis of insulinoma. The surgical enucleation of the tumor was chosen as therapy and it was performed using the daVinci Surgical System with the aid of intraoperatory ultrasonography. Before the intervention, a CGM device was applied : after the removal of the lesion an hyperglycemic rebound was recorded by the device, with a maximum glucose peak of 201mg/dl reached 4 hours after the excision of the tumor, confirming the successful outcome. The CGM has been performed even in the post-operative, showing glucose levels restored to normal values and with no hypoglycaemic events, even though the patient remained fasted for 72h post-surgery due to the onset of a grade-A pancreatic fistula. **Conclusions**: In this case the insulinoma was successfully removed using the robot surgical technique combined with the application of continuous glucose monitoring. This case report describes for the first time to our knowledge the employment of robotic surgery and intra- and post-operative continuous glucose monitoring for the treatment and follow-up of insulinoma and it supports the role of both as new and successful treatment strategies of insulinomas.

PP067

IMPACT OF SHORT-TERM TREATMENT WITH BENZODIAZEPINES AND IMIDAZOPYRIDINES ON GLUCOSE METABOLISM IN HEALTHY SUBJECTS.

I. Olivetti[1], E. Gramaglia[1], S. Belcastro[1], M. Tomelini[1], E. Calvi[1], A. Dotta[1], V. Ramella Gigliardi[1], E. Ghigo[1], A. Benso[1], F. Broglio[1]

[1]Scienze Mediche, Università di Torino - Torino

In the last years there have been a progressive reduction of the average duration of sleep and an increase in the incidence of sleep disturbances. At the same time an increase of the incidence of the metabolic syndrome has been described, partly attributable to the progressive worsening of dietary habits and the increase in sedentary lifestyle. Recent studies suggest that adequate sleep is essential to maintain good glucose metabolism and sleep disturbances may contribute to the manifestation of the metabolic syndrome. Benzodiazepines, such as brotizolam, and imidazopyridines, such as zolpidem, are frequently used as hypnotics but their potential impact on glucose metabolism has never been evaluated so far. To this aim, in 12 healthy volunteers (age [mean±SEM]: 38.3±8.1 yr.; BMI: 21.9±0.8 kg/m²) we studied glucose and insulin responses to oral glucose tolerance test (OGTT, 75 g) before and after 15-day treatment with brotizolam 0,25 mg/day or zolpidem 10 mg/day. In all the experimental conditions, OGTT induced a significant increase ($p<0.01$) of both glucose and insulin levels. Glucose and insulin responses to OGTT performed on day 0 were similar (p: n.s.) between the two sessions. 15-days treatment with brotizolam increased glucose ?AUC response to OGTT by 122% (2999.3±571.0 on day 16 vs. 1614.0±595.5 mg/dl*min on day 0; $p<0.01$) while zolpidem by 86% (3293.3±529.9 on day 16 vs. 2268.8±577.9 mg/dl*min on day 0; $p<0.01$) whereas no statistical differences were observed in terms of insulin responses both after brotizolam (4128.6±620.82 on day 16 vs. 3403.5±526.1 mU/ml*min on day 0) and after zolpidem (5762.3±1299.9 on day 16 vs. 5891.4±1309.8 mU/ml per min on day 0). In conclusion, this study suggests that benzodiazepines and imidazopyridines have a rapid glucometabolic effect that is detectable as early as after 15-days treatment.

PP068

CORRELATION BETWEEN GLYCEMIC VARIABILITY ASSESSED BY CGM AND SHORT-TERM OUTCOME IN PATIENTS UNDERGOING PERCUTANEOUS CORONARY INTERVENTION

A. Lauria[1], E. Maddaloni[1], A. Palermo[1], S. Manfrini[1], C. Proscia[2], A. Nusca[2], R. Melfi[2], I. Cavallari[2], P. Pozzilli[1]

[1]Endocrinology and Diabetes, University Campus Bio-Medico - Rome, [2]Cardiovascular science, University Campus Bio-Medico - Rome

Background. In patients undergoing percutaneous coronary intervention (PCI) dysglycemia has been correlated with peri-procedural myocardial infarction (PMI) and contrast-induced acute kidney injury. Several studies showed that daily fluctuations of blood glucose levels (BGLs) may influence PCI outcomes, but a complete assessment of glycaemic status may be not fully evaluated by fasting BGLs or glycated hemoglobin. A letter information of glycaemic excursions may be obtained by continuous glucose monitoring (CGM). **Aim**. To investigate the prognostic role of glycaemic variability assessed by using CGM, on short-term post-PCI outcomes in diabetic patients. **Methods** We prospectively enrolled 28 patients with type 2 diabetes or impaired glucose tolerance undergoing PCI. All patients were equipped with a continuous glucose recorder for 48 hours for monitoring glycaemic fluctuations in the peri-procedural (up to 24 hours after PCI) period. Glycaemic variability was expressed by the Mean Amplitude Glycaemic Excursions (MAGE - MAGEup considering nadir-to-peak fluctuations and MAGEdown peak-to-nadir excursions) and CONGA_n. Blood samples were collected at admission, 6 and 24 hours after PCI to detect blood creatinine, NGAL, CK-MB and TnI levels. **Results**. Serum creatinine and Ngal variations significantly correlated with MAGE (r=0.431, p=0.025 and r=0.399, p=0.048 respectively), MAGE up (r=0.547, p=0.003, r=0.524, p=0.007) and CONGA4 (r=0.433, p=0.024, r=0.456, p=0.022). In a multivariate model with age, baseline creatinine and left ventricle ejection fraction, CONGA4 remained an independent predictive value. Patients with PMI (11%) showed significantly higher mean values of CONGA1, CONGA2 and MAGEdown compared to patients without PMI (42.5 ± 18.5 mg/dl vs 21.5 ± 8.1 mg/dl, p= 0.001; 55.1 ± 32.1 mg/dl vs 31.0 ± 12.4 mg/dl, p= 0.014; 133.3 ± 86.2 mg/dl vs 75.1 ± 29.2 mg/dl, p= 0.016). **Conclusions**: We observed a significant correlation between glycaemic variability indexes assessed by CGM and both renal function deterioration after contrast exposure and peri-procedural myocardial damage expressed by troponin release. Our study suggest a significant impact of glycaemic variability on short-term outcome of patients undergoing coronary stenting, encouraging the use of GCM in PCI setting where an optimal glycaemic control should be achieved.

PP069

IN ADIPOCYTES BISPHENOL-A DOWN-REGULATES INSULIN SENSITIVITY AND UP-REGULATES INFLAMMATORY PATHWAYS

R. Valentino[1], V. D'Esposito[2], F. Passaretti[2], S. Cabaro[2], A. Liotti[2], F. Auriemma[2], I. Cimmino[2], S. Mabilia[2], C. Padrone[2], S. Savastano[3], P. Formisano[2]

[1]Istituto di Endocrinologia ed Oncologia Sperimentale (IEOS-CNR) - NAPOLI, [2]Dipartimento di Scienze Mediche Translazionali, Università degli Studi di Napoli "Federico II" - NAPOLI, [3]Dipartimento di Medicina Clinica e Chirurgia, Università degli Studi di Napoli "Federico II" - NAPOLI

Background: Bisphenol-A (BPA), one of the main plastic component, is a lipophilic compound with estrogen-like activities, able to alter several metabolic functions. Considering its bio-accumulation in adipose tissue, the involvement in obesity, insulin resistance and metabolic syndrome is suggestive. Aim is to investigate the effect of low and chronic BPA doses on adipocyte insulin sensitivity, inflammation and adipokine production.
Materials and methods: 3T3-L1, human preadipocytes (adipose tissue derived stromal-vascular fraction /SVF) and differentiated cells have been cultured for 24-48h in presence of 1nM BPA, and acutely stimulated (10 min) with 100 nM insulin. Next, glucose uptake was evaluated and IR, AKT, ERK, STAT and JNK activation pathways were analyzed by Western blot with phospho-specific antibodies. Moreover, a panel of adipokines were assayed in supernatants using BIOPLEX multipanel assay (BIORAD).
Results: After BPA incubation, insulin-stimulated IR, AKT and ERK phosphorylation was down-regulated with no change of protein abundance. Glucose uptake was also reduced, while pro-inflammatory adipokines (IL-6 and IFNγ) were hyper-secreted, both in human and in mouse adipocyte supernatants. In addition, STAT3 and JNK phosphorylation, markers of inflammatory pathways activation, was significantly increased in adipocytes.
Conclusion: BPA bio-accumulation in adipocytes at very low concentrations may led to low-grade inflammation, with disruption of important adipocyte functions, such as IR, AKT and ERK activities and reduction in glucose uptake, thereby causing insulin resistance. The inflammatory pathways activation is likely related to inflammatory cytokines hypersecretion and can be responsible for the main metabolic adipocyte function alterations. Further experiments are required to verify the interference of chronic BPA exposure on adipogenesis and its involvement, as contributing factor, in adult obesity and metabolic syndrome.

PP070

TURNER SYNDROME IN ADULTHOOD IS FREQUENTLY COMPLICATED BY DIABETES

D. Ibarra Gasparini[1], P. Altieri[1], E. Scarano[2], A. M. Perri[2], L. Zanotti[1], A. Gambineri[1], L. Mazzanti[2], R. Pasquali[1]

[1]Div. of Endocrinology, Dept. of Medical and Surgical Sciences, S. Orsola-Malpighi Hospital, Alma Mater University - Bologna, Italy, [2]Department of Pediatrics, S. Orsola-Malpighi Hospital, Alma Mater University - Bologna, Italy

In Turner syndrome (TS) a 3- to 11-fold increase in frequency of diabetes mellitus (DM) is reported. However, this evidence derives from cross-sectional observational studies or short-term prospective studies. This long-term prospective study was aimed at estimating the incidence and the time of appearance of DM in a cohort of Italian women with TS followed from infancy to adulthood.

Forty-nine women with TS, diagnosed by kariotyping, were followed from infancy (mean age 9, range 1-18yrs) to adulthood (mean age 37yrs, range 24-49yrs). Therefore, a mean follow-up of 26yrs (range 10-44yrs) was performed. Each subject underwent yearly a complete physical examination and familial history for DM was collected. Screening for DM was performed by the measurement of fasting glucose and HbA1c until adulthood. Afterwards, a 75-gr oral glucose tolerance test was also performed. The response of glucose at 120 minutes was used to diagnose DM according to American Diabetes Association criteria.

We observed two cases of DM before 25yrs (at 20 and at 23yrs, respectively). Other 8 subjects developed DM after 25yrs (mean age 40yrs, range 33-49yrs). Therefore, the prevalence of DM at the end of follow-up was 20.4%, significantly higher with respect to that of the general Italian female population of similar age (0.8%). The incidence rate of DM of the study population was 7 per 1000 persons-year. Age and BMI did not significantly differ between TS subjects who developed diabetes and those who did not (mean 38.5±6.1 *vs.* 36.2±6.3yrs and 25.4±4.8 *vs.* 25.3±4.6 Kg/mq, respectively). The two groups did not significantly differ also for familiarity for DM. At the diagnosis of diabetes most TS had a failure in insulin secretion.

This study demonstrates that the risk of DM is markedly elevated in middle-ages women with TS and suggests a specific pathogenetic mechanism.

PP071

CHRONIC CGMP PHOSPHODIESTERASE 5A INHIBITION IMPROVES METABOLIC CONTROL AND REDUCES ENDOTHELIAL DYSFUNCTION MARKERS IN TYPE 2 DIABETES

E. Mandosi[1], E. Giannetta[2], C. Bertolini[2], A. M. Isidori[2], S. Morano[1], A. Lenzi[2]

[1]Medicina Interna, Sapienza Università - Roma, [2]Medicina Sperimentale, Sapienza Università - Roma

Background—Endothelial dysfunction (ED) is a feature of type 2 diabetes and seems to play a key role in the pathogenesis of diabetic cardiovascular complication. Chronic hyperglycemia increases the expression of cytokines involved in inflammatory pathways of vascular damage. Inhibition of phosphodiesterase type 5 (PDE5) exerts a relaxant effect on the smooth muscle cells of the trabecular structures of the corpora cavernosa. More recently, PDE5 inhibitors have been claimed to offer cardioprotective effects. We investigated the effect of a selective phosphodiesterase type 5 inhibitor, sildenafil, in reduce endothelial dysfunction markers in type 2 diabetic patients.
Methods and Results—Fifty-nine diabetic men (60.3±7.4 years), recruited from the outpatient clinics of Policlinico Umberto I, Sapienza University Hospital of Rome, were randomized to receive sildenafil 100 mg/d (n.30, 60.7±7.6 years, BMI 28.4 ±4.9 kg/mq, HbA1c 7.8±1.3%, duration of diabetes, 6.7±5.5 years) or placebo (n.29, 60.2±8.3years, BMI 27.9±4.3 kg/mq, HbA1c 6.5±0.9%, duration of diabetes, 6.2±5.7 years) for 3 months. The following parameters were assessed at each visit: baseline and postprandial glycemia, insulin, HbA1c, HOMA index, C-peptide, lipid profile, estimated glomerular filtration rate (eGFR), 24-hour urinary microalbuminuria. Inflammatory indices and endothelial function markers, P-selectin, transforming growth factor-β[TGF-β] and Monocyte Chemotactic Protein-1 [MCP1] were evaluated before and after treatment. sP-selectin, TGF-β and MCP-1 were measured with a quantitative sandwich enzyme immunoassay (Quantikine, R&D Systems, Abingdon, UK). Results: After 3 months, sildenafil produced a significant reduction compared with placebo in post prandial glycemia (180.60±54.0 vs 155.8±51.7 mg/dl; p=0.004), HbA1c (7,8±1.2 vs 7.2±0.2 %; p=0.003), LDL cholesterol (114.3±36.8 vs 104.5±34.3 mg/dl; p=0.04). Moreover, in sildenafil-treated patients, a significant increase in HDL cholesterol (40.1±7.6 vs 43.0±8.7; p=0.025) was observed.
The levels of inflammatory markers were reduced in the sildenafil group: sP-selectin (3487.5± 596,6 vs 2719.6±416,7 ng/dl; p=0.034), MCP-1(439.7±134.5 vs 364.4±127.1 pg/ml; p=0.019), and TGF beta (34.61± 8.65 vs 30.28±8.49 ng/ml; p=0.013). Conclusions This study suggest that chronic phosphodiesterase type 5 inhibition could enhance endothelial function through the reduction of serum biomarkers of inflammatory damage and improves the glicometabolic control in patients with type 2 diabetes.

PP072

METABOLIC CONTROL AND FETAL GROWTH PARAMETERS IN WOMEN OF DIFFERENT ETHNICITY WITH GESTATIONAL DIABETES

E. Mandosi[1], M. Fallarino[1], M. E. Di Stasio[1], G. Tarquini[1], V. Adinolfi[2], I. Turinese[1], R. La Torre[3], F. Rech[3], S. Morano[1]

[1]Medicina Interna, Sapienza Università - Roma, [2]Medicina Sperimentale, Sapienza Università - Roma, [3]Ostetricia e Ginecologia, Sapienza Università - Roma

Background: Gestational diabetes mellitus (GDM) is defined as glucose intolerance with onset or first recognition during pregnancy, it is associated with multiple obstetric and neonatal complications. The incidence of GDM increased in the past decade and differs among various ethnic groups. The aim of this study was to evaluate the relationship between metabolic control and fetal anthropometric parameters in women of different ethnicity with GDM in the second and third quarter of gestation. Patients and methods: One hundred forty two pregnant women were recruited. All pregnant women without recognized pre-pregnancy diabetes (n.87) were screened at 16-18 or 24-28 weeks' gestation with 75 g glucose tolerance test as appropriate. Seventy patients resulted affected by GDM and divided in Asian (n.23, 31.6±4.6 years) and Caucasian ethnic group (n.47, 33.7±5.2 years). The following metabolic and clinical parameters were assessed at every quarter of gestation: baseline glycemia, lipid profile, HbA1c, uricemia, TSH, estimated glomerular filtration rate, microalbuminuria, and vital signs. Ultrasound measurements of the head circumference (HC), abdominal circumference (AC), femur (FL) and humerus length (HL) were performed. Results: Pre-pregnacy, second and third quarter of gestation BMI values were increased in Caucasian compared with Asian women (28.2 ±7.1 vs 22.2 ± 6.3 kg/m2 p=0.003, 31.8 ± 6.4 vs 26.6 ± 3.1 kg/m2 p=0.018 and 31.2 ± 8.6 vs 27.3 ± 3.9 kg/m2 p=0.031, respectively). In Caucasian women a significant increase in ALT (19.5 ±10.5 vs 15.7 ±2.9 UI/L, p=0.04), AST (25.1 ± 2.9 vs 14.4 ±6.7 UI/L, p= 0.035), LDL cholesterol (148.1 ± 40 vs 107.3 ± 32.2 mg/dl, p=0.003), systolic blood pressure (113.9 ± 13.2 vs 106.4 ± 10.6 mmHg, p=0.026), diastolic blood pressure (71.9 ± 8.6 vs 65 ± 7.8 mmHg, p=0.005) values was observed at third quarter. In terms of insulin therapy, Asian women had the lowest need for insulin therapy (p=0.01). Finally, an increase of estimated fetal weight (357 ± 19.2 vs 175±21.2 g, p= 0.015), HC (176 ± 9 vs 144.5 ±22.3 mm, p= 0.009), AC (150.1 ± 17.3 vs 114.6 ±17.9 mm, p= 0.004) was observed on ultrasound examination in Caucasian women in the second quarter of pregnancy, but not in the third quarter probably because of the improvement of metabolic control. Conclusion: This study shows significant differences in clinical and biochemical characteristics, and in fetal growth parameters in women with gestational diabetes among two different ethnic groups. This finding emphasizes the need to develop ethnic tailored GDM intervention.

PP073

INFLAMMATORY CONDITIONS ASSOCIATED TO TYPE 2 DIABETES INDUCE PREP1 GENE OVEREXPRESSION, BY INDUCING EPIGENETIC MODIFICATIONS.

M. Ciccarelli[1], L. Albano[1], V. Vastolo[2], S. Terreri[1], A. Desiderio[1], G. A. Raciti[1], F. Di Nardo[1], S. Ricci[1], M. R. Ambrosio[1], F. Beguinot[1], P. Ungaro[2]

[1]Dipartimento di Scienze Mediche Traslazionali, Università degli Studi di Napoli Federico II - Napoli, [2]IEOS-CNR - Napoli

Gene-environment interaction is particularly relevant to the pathogenesis of type 2 diabetes, with inflammatory conditions conferring epigenetic modifications that could lead to dysregulate gene transcription and disease progression. Prep1 is an important gene in development, that codifies an homeodomain transcription factor belonging to TALE proteins. In our lab, recent studies have identified Prep1 as a physiologic regulator of insulin sensitivity and glucose metabolism. Prep1-hypomorphic (Prep1i/i) mice - expressing about 2% of Prep1 mRNA - exhibit protection from streptozotocin-induced diabetes and increased insulin sensitivity in skeletal muscle. The aim of this study is to evaluate whether and how inflammatory diabetic conditions such as high glucose (HG), or free fatty acids (palmitate), can dysregulate Prep1 gene by inducing epigenetic modifications.

Results: L6 cells cultured in HG (25.5 mM glucose) show an increase of Prep1 mRNA levels compared to normal glucose (NG, 5.5 mM glucose) cultured cells. Time-course of HG treatment also confirmed an increase of Prep1 mRNA levels that is already present at 12 hours of incubation. The effect is dependent by glucose metabolism and is not due to osmotic stress, in fact same treatment with Xylose and 2-Deoxyglucose had no effect on Prep1 expression. Treatment of L6 cells with 0.75 mM Palmitate induced an increase in the expression of Prep1 gene at 12 hours of incubation relative to untreated cells. Up-regulation of Prep1 expression both by high glucose and palmitate is accompanied by an increase in the binding of the nuclear factor kB on Prep1 5' regulatory region. NF-kβ recruitment was associated to an increase in the dimethylation of Lysine 4 on Histone H3 and an increase in the acetylation of histone H3 and H4 - that are markers of active gene - on Prep1 promoter. This is accompanied by an increase in the binding of the histone H3-lysine 4 methyltransferase SET7/9 to the Prep-1 gene promoter.

The findings reports in the present abstract might have clinical relevance as preliminary evidence in our laboratory indicates that Prep1 gene is overexpressed in euglycemic offspring of type 2 diabetic individuals. These subjects have a very high risk of diabetes and are known to be insulin resistant, suggesting that Prep1 overexpression may provide an early contribution to diabetes progression in these individuals.

PP074

GHRH DISPLAYS PROTECTIVE EFFECTS AGAINST TUMOUR NECROSIS FACTOR-ALPHA (TNF-A)-INDUCED INFLAMMATION IN SKELETAL MUSCLE CELLS

D. Gallo[1], M. Taliano[1], M. Minetto[1], E. Ghigo[1], R. Granata[1]

[1]Department of Medical Sciences - Division of Endocrinology, Diabetology and Metabolism - School of Medicine, University of Turin - Torino

Inflammation and muscle waste are two main characteristics of idiopathic inflammatory myopathies (IIM). The immune nature of IIM is often supported by the presence of autoantibodies and the frequent association with other diseases such as diabetes, rheumatoid arthritis, AIDS and sepsis. In such terms, the immune system plays a fundamental role, in particular due to the increase in proinflammatory cytokines. Among these, one of the most important is tumour necrosis factor-alpha (TNF-α), produced by activated macrophages. TNF-α may induce muscle wasting at least via inhibition of myogenesis in myoblasts and increase of apoptosis of myoblasts and myotubes. Because of their immune nature, inflammatory myopathies are currently treated with immunosuppressive or immunomodulating drugs or intravenous immunoglobulin injections. Although many patients respond to the therapies to some degree and for a period of time, a number of patients are unresponsive or cannot tolerate these drugs. The need for more specific and safer therapies has prompted the search for newer cellular and molecular targets. GHRH (growth hormone-releasing hormone) is primarily expressed in the hypothalamus and is known for its stimulating effects on pituitary growth hormone (GH) synthesis and secretion. However, GHRH also displays effects in peripheral tissues, including stimulation of cell proliferation and survival. Notably, we and other have recently demonstrated that GHRH exerts antiapoptotic actions in cardiomyocytes and protects from ischemia/reperfusion injury and myocardial infarction in ex-vivo in vitro and in vivo animal models. Aim of this study was to investigate the effects of GHRH on proliferation, apoptosis and differentiation of skeletal muscle cells. The signaling pathways underlying GHRH effects, and fundamental for muscle functions, were also determined.

Our results show the positive effects of GHRH in TNF-α-induced inflammation in skeletal muscle cells. GHRH counteracts apoptosis and the detrimental effects of TNF-α, by activating signalling pathways that play a key role in muscle function and by inhibiting the degradation of cytoskeletal structures. In all, these results, together with our previous findings on cardiac muscle cells, suggest that GHRH may have beneficial effects in vivo in skeletal muscle disease such as inflammatory myopathies.

PP075

SEX DIFFERENCE AND ASSOCIATION OF FETUIN-A WITH ADIPOSITY-LINKED INSULIN RESISTANCE AND RESPONSE TO AN EDUCATIONAL EXCESS WEIGHT REDUCTION PROGRAM

G. Murdolo[1], C. Tortoioli[2], D. Molinari[3], F. Celi[3], V. Bini[2], F. Papi[4], A. Brozzetti[2], A. Falorni[4], A. Falorni[2]

[1]Medicina Interna - Assisi (PG), [2]Medicina Interna; sez. di Scienze Endocrine e Metaboliche - Perugia, [3]Scienze Chirurgiche; Sez. Pediatria - Perugia, [4]he; Sez. Pediatria - Perugia

Background and aims. The secreted liver protein fetuin-A emerges as a potential link between adiposity and its associated cardio-metabolic outcomes. Here, we investigated sex differences and association of fetuin-A with adiposity and insulin resistance (IR) measures, as well as fetuin-A changes after an educational-based weight excess reduction program (WERP) in prepubertal childhood.

Methods We studied 200 prepubertal children (boys/girls: 89/111; Tanner stage 1; age: 5-13 yr), included in a cohort of 44,231 adolescents who participated in an extensive Italian school-based survey. According to Cole's criteria, 100 individuals were lean (L; boys/girls: 57/43) and 100 obese (OB; boys/girls: 54/46). Additionally, 53 obese children (boys/girls: 28/25; age: 6-12 yr) were evaluated after a WERP. Serum fetuin-A, leptin, as well as a panel of inflammatory adipokines and measures of IR were determined.

Results When compared with L individuals, OB children exhibited higher ($p<0.0001$) fetuin-A, without differences between sex. Fetuin-A levels were strongly and positively correlated with adiposity, HOMA-IR, leptin, MCP-1 and ICAM-1. In a multivariate analysis, the association between fetuin-A and HOMA-IR was attenuated and became insignificant after adjustments for age, $BMI_{z\text{-}score}$, lipids and blood pressure, or when leptin and waist circumference entered the models. Notably, obese individuals who lose weight after WERP displayed a significant decrease of fetuin-A ($p<0.0001$ vs basal), which was accompanied by amelioration of HOMA-IR.

Conclusions In prepubertal children, fetuin-A emerges as a sex-independent marker of adiposity and systemic insulin resistance. The reduction of fetuin-A after WERP implicates the relevance of this liver-derived glycoprotein in mediating the beneficial effects of educational strategies to curb IR at the early stages of obesity.

PP076

TWO-FREQUENCY IMPEDANCE MEASUREMENT AS THE BASIS FOR TECHNICAL REALIZATION OF NON-INVASIVE GLUCOMETRY

I. Turkovskiy[1], V. Kharin[2], B. Paramonov[3], V. Matytsin[3]

[1]S.r.l. "Non-invasive technologies" - Saint-Petersburg, [2]Mozhaisky Military Space Academy - Saint-Petersburg, [3]Mechnikov North-West State Medical University - Saint-Petersburg

The need of technical implementation of non-invasive blood glucose level monitoring is as important as reasonably skeptical is the attitude to the possibility itself of creating such a device which would be simple, affordable, portable and meet the requirements of acceptable accuracy. Despite repeated and not quite successful attempts to use measurements of the electrical properties of tissues to evaluate glucose level in tissue fluids in vivo, impedancemetry potentialities did not reach their limits. The sequence of key physiological processes that affect the value of electrical conductivity or impedance of tissues in hyperglycemia is as follows: an increase of glucose in the extracellular fluid is accompanied by the rise of its osmolality; osmotic pressure causes excessive movement of water from the intracellular fluid into the extracellular sector. As a result, the maintenance of total water content in soft tissues in hyperglycemia is accompanied by the redistribution of fluid between intra- and extracellular compartments, leading to partial hyperhydration of the extracellular matrix and to proportional dehydration of cells. The increase of extracellular fluid volume causes pre-emptive growth of the low-frequency conductivity (decrease of low-frequency impedance) because low-frequency current runs mainly through the extracellular space. High-frequency current passes equally well through intercellular spaces due to the conductivity current and through the dielectric membranes due to the displacement currents. Therefore the high-frequency conductivity is proportional to the total water content of tissues. Thus, the different-frequency impedance measurement can provide both monitoring of the dynamics of specific volumes of liquid sectors of the body determined by blood glucose, and indirect calculation of the positive and negative increments of glucose in extracellular fluids, especially blood plasma and interstitium. Realization of the original method of glucose monitoring based on two-frequency impedance in patients with type 2 diabetes for several hours revealed a significant correlation with the level of interstitial glucose (Pearson coefficient 0.8) and blood plasma (Pearson coefficient 0.7) measured by invasive reference methods.

PP077

EXENDIN-4 COUNTERACTS PALMITATE-INDUCED APOPTOSIS BY INHIBITING DE NOVO PRODUCTION OF CERAMIDES IN HUMAN CARDIAC PROGENITOR CELLS

A. Leonardini[1], L. Laviola[1], R. D'Oria[1], M. A. Incalza[1], M. R. Orlando[1], V. Andrulli Buccheri[1], A. Natalicchio[1], S. Perrini[1], F. Giorgino[1]

[1]Endocrinologia, Dip. Emergenza e Trapianti di Organi, Università degli Studi di Bari "Aldo Moro" - Bari

Ceramides have been suggested to be important mediators of lipotoxicity-induced apoptosis in different cell types. Increased apoptosis of cardiomyocytes and cardiac progenitor cells in response to metabolic and oxidative stressors has been proposed as a mechanism of myocardial damage and dysfunction. Glucagon-like peptide-1 (GLP-1) and GLP-1 analogs exert prosurvival effects on cardiac cells. To investigate the mechanisms of fatty acid-mediated cell damage, human cardiac progenitor cells (hCPC) were isolated from right auricle biopsies and exposed to 0.1 to 0.5 mM palmitate up to 24 h. Dose- and time-dependent increase of CPC apoptosis was demonstrated by the evaluation of caspase-3 activation, caspase-3 cleavage and cytosolic release of oligosomes, respectively ($p<0.05$). In hCPC exposed to palmitate, intracellular ceramide content, evaluated by immunofluorescence, was concomitantly augmented, and palmitate also induced a significant increase in mRNA and protein levels of ceramide synthase 5, a critical enzyme in ceramide generation. Co-incubation of CPCs with fumonisin-B1, a specific ceramide synthase inhibitor, partially prevented palmitate-induced apoptosis. When cells were pretreated with the GLP-1 analog exendin-4 (20 nM for 16 h), palmitate-induced apoptosis was prevented ($p<0.05$), and the increase in ceramide levels was also reduced ($p<0.05$). Furthermore, exendin-4 prevented the increase of ceramide synthase 5 expression, both at the gene and protein levels, that occurred in response to palmitate. In conclusion, de novo ceramide accumulation contributes to palmitate-induced apoptosis of hCPC, and this is counteracted by the GLP-1 analog exendin-4. Amelioration of lipotoxicity via ceramide modulation may contribute to the cardioprotective effects of GLP-1-based therapies in subjects with type 2 diabetes.

PP078

THE DPP-IV INHIBITOR SAXAGLIPTIN INHIBITS PALMITATE-INDUCED APOPTOSIS OF HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS AND HUMAN CARDIAC PROGENITORS

M. A. Incalza[1], L. Laviola[1], M. R. Orlando[1], C. Caccioppoli[1], A. Leonardini[1], R. D'Oria[1], V. Andrulli Buccheri[1], A. Natalicchio[1], S. Perrini[1], F. Giorgino[1]

[1]Endocrinologia, Dip. Emergenza e Trapianti di Organi, Università degli Studi di Bari "Aldo Moro" - Bari

Exposure of endothelial cells and cardiomyocytes to the fatty acid palmitate results in biochemical abnormalities leading to cell dysfunction and apoptosis. DPP-IV inhibitors may exert protective actions on the cardiovascular system. The aim of this study was to investigate the protective effects of the DPP-IV inhibitor saxagliptin on palmitate-induced apoptosis of human umbilical vein endothelial cells (HUVEC) and human cardiac progenitor cells (hCPC). Expression of DPP-IV in HUVEC and hCPC was confirmed at the mRNA level by qRT-PCR, and at the protein level by immunoblotting and immunofluorescence, respectively. Exposure of HUVEC to 0.25 mM palmitate for 24 h resulted in increased expression of the adhesion molecule ICAM-1 ($p<0.05$) and of the pro-apoptotic mediator Bax ($p<0.05$), and was associated with a 5- to 7-fold increase in cellular apoptosis ($p<0.05$), evaluated by the detection of both cytoplasmic oligosomes and cleavage of caspase-3. Long-term exposure to palmitate also increased apoptosis of hCPC by 6-fold ($p<0.05$). Pretreatment with the DPP-IV inhibitor saxagliptin (0.05 mM) for short times (5 to 15 min) resulted in a dose-dependent increase in the phosphorylation of Akt, Erk-1/2 and eNOS in HUVEC, and of Akt in hCPC, respectively ($p<0.05$). Importantly, saxagliptin almost completely abrogated palmitate-induced apoptosis of both HUVEC and CPC ($p<0.05$); however, this response required preincubation of the cells for at least 2 h whereas shorter times were ineffective. Thus, saxagliptin prevents palmitate-mediated apoptosis of HUVEC and hCPC, thereby augmenting the potential for vessels and heart regeneration under conditions of lipotoxicity. Even though saxagliptin can induce pro-survival signaling via Akt within minutes, inhibition of cellular DPP-IV for at least 2 h is required to exert the anti-apoptotic effect, suggesting that this response may occur via increased bioavailability of non-GLP-1 peptides.

PP079

AUTOIMMUNITY AGAINST PANCREATIC ß CELL (GADA, IA2, IAA) IN FIRST DEGREE RELATIVES OF SARDINIAN PATIENTS WITH T1D: PREVALENCE AND TEMPORAL CHANGES

G. Frau[1], M. Incani[1], L. Perra[1], F. Scano[1], F. Sentinelli[1], V. M. Cambuli[1], A. Olla[1], M. A. Zedda[2], M. Soro[3], A. P. Frongia[4], R. Ricciardi[4], E. Cossu[1], M. G. Cavallo[5], M. G. Baroni[6]

[1]Endocrinology and Diabetes, Dep. of Med. Sciences, Univ. of Cagliari - CA, [2]I° Pediatric Clinic, AOU Cagliari - CA, [3]Div. of Pediatrics and Neonatology "S.Martino Hosp." - OR, [4]Div. of Pediatrics "Brotzu Hosp." - CA, [5]Dep. of Int. Med. Sapienza Univ. of Rome - ROME, [6]Dep of Exper. Med. Sapienza Univ. of Rome - ROME

BACKGROUND: Sardinia has one of the highest incidences of type 1 Diabetes(T1D) worldwide. The estimated incidence of T1D is more than 40 new cases/100.000/year (5-7 times higher than in the other Italian regions). In Sardinia, no studies showing the prevalence of auto-antibodies in first degree relatives of patients with T1D are available.

OBJECTIVE: to evaluate the prevalence of auto-antibodies versus pancreatic β-cells in a cohort of first degree relatives of subjects with T1D and to study the clinical and immunologic changes in time by revaluating glucose tolerance by OGTT and antibody (Ab) titers after 1 year.

METHODS: 125 families for a total of 424 subjects (125 probands, 299 first degree relatives) were studied. Each subject was sampled for GADA, IA2 and IAA auto-Ab by radioimmunoassay. Also clinical and biochemical data were recorded, including the possible presence of diabetes and other autoimmune disorders in the family.

RESULTS and CONCLUSION: 12% (36/299) of the first degree relatives were positive for at least one Ab. This prevalence is estimated 3 times higher than observed in other populations (in the DPT1 it was estimated to be 3.5%). Among these 36 cases, 25 didn't have a clinical diagnosis of diabetes (11 were already diabetic). Anti-GADA were present in 83,3% subjects, Anti-IA2 were detectable in 27,7% and Anti-IAA in 38,8%. At follow-up, of the 25 Ab+ patients without diabetes or other glucose abnormalities, 10 were revaluated after 1 year through OGTT; of these, only 1 resulted IGT, 9 resulted NGT, and the Ab titers were generally unchanged in all the 10 patients. In addition, 2 of the 25 patients developed diabetes before the follow-up visit; one had a very high titer of both GADA and IA2, the other was only IA2+. Knowing that they were Ab+, both had an early recognition of their T1D onset without acute complications. These results confirm the high susceptibility of the Sardinian population to T1D, particularly in first degree relatives, and the importance of early detection of the presence of an autoimmune process. This in order to try to initiate measures suitable to prevent acute complications at the onset of the clinical disease.

PP080

CONTINUOUS GLUCOSE MONITORING (CGMS) AND GLUCOSE VARIABILITY: EFFECTIVENESS AND PREVENTION OF HYPOGLYCEMIA.

E. Castaldo[1], D. Sabato[1], C. Tirabasso[1], A. Bellia[2], M. Cerilli[1], F. Pozzi[1], A. Galli[1], M. E. Rinaldi[1], A. Andreadi[1], M. P. Caputo[1], E. De Carli[1], M. A. Marini[2], D. Lauro[2]

[1]Centro diabete tipo 2 Università Tor Vergata - Roma, [2]Centro diabete tipo 2 Università Tor Vergata- Medicina interna e Sistemi - Roma

TITLE: Continuous glucose monitoring (CGMS) and Glucose Variability: Effectiveness and Prevention of Hypoglycemia.

BACKGROUND: latest methods developed to have a detail monitoring of glycemia is provided with continuous glycemic monitoring system (CGMS); this system evaluates the amplitude, the duration and the frequency of the glycemic's oscillations. .The system provides continuous glycemic monitoring updating values every five minutes,288/die, alerting the patient when glucose values are not in range. **Aim of the study** was to evaluate the control and prediction of hypoglycemia using CGMS in Type 1 Diabetic (T1D) patients, in treatment with a continuous system of insulin infusion (CSII). **MATERIAL AND METHODS:** we studied 7 caucasian subjects, 4M:3F, affected with T1D on continuous system of insulin infusion (CSII) treatment.Mean age was 40 years, disease duration > 5 years and mean HbA1c level ≈ 7,4%.CGMS Animas G4 Dexcom was applied in outpatient or at hospital; after short training of patient, together with routine glucose meter measurements, 2 times/24 hours. The monitoring was performed for 23 consecutive days. Patients were asked to not change the lifestyle throughout the monitoring period. **RESULTS:**during the 23 days of glucose monitoring n° 6313±524 glycemic's CGM values were obtained with intermediate value of 155 mg/dl ± 14 DS, minimum value 59 mg/dl ± 12 DS, maximum value 200 mg/dl ± 50 DS, 25% quartile 171 mg/dl, 75% quartile 92 mg/dl, no severe hypoglycemic episodes were detected. Differently, hypoglycemic's events, found with routine glucometer measurements were 2.3 vs. 4.2 observed with CGMS ($p<0.05$). Finally, we asked to the patients their opinion about CGMS monitoring system and all of them reported that they were able to prevent the most of hypoglycemic episodes, thanks to the monitoring's trend arrows.**CONCLUSIONS**:**1.** CGMS can be particularly useful in monitoring glucose profile and detecting hypoglycemia incidents, mainly nocturnal. **2.** Modification of insulin therapy on the base of CGMS helps to decrease the time of hypoglycemia and hyperglycemia, particularly during the night. **3.** Latest CGMS thanks to trend arrows can help the patient **to prevent** episodes of hypoglycemia.

PP081

PREVALENCE OF CARDIOVASCULAR DISEASE IN PATIENTS WITH TYPE 2 DIABETES ACCORDING TO STRATIFICATION BY CHRONIC KIDNEY DISEASE

G. Pugliese[1], A. Solini[2], E. Bonora[3], E. Orsi[4], G. Zerbini[5], C. Fondelli[6], R. Trevisan[7], M. Vedovato[8], G. Gruden[9], O. Lamacchia[10], L. Laviola[11], G. Penno[2]

[1]Università La Sapienza - Roma, [2]Università di Pisa - Pisa, [3]Università di Verona - Verona, [4]Fondazione IRCCS Cà Granda – Ospedale Maggiore Policlinico - Milano, [5]Istituto San Raffaele - Milano, [6]Università di Siena - Siena, [7]Ospedali Riuniti - Bergamo, [8]Università di Padova - Padova, [9]Università di Torino - Torino, [10]Università di Foggia - Foggia, [11]Università di Bari - Bari

Chronic kidney disease (CKD) is a powerful independent risk factor for cardiovascular disease (CVD). Recently, an alternate 5-risk category CKD classification, considering both estimated GFR (eGFR) and albuminuria, has been shown to be superior to the currently used NKF/KDOQI staging system, which is based primarily on eGFR, in predicting all-cause mortality and adverse renal outcomes in the general population. We verified, in subjects with type 2 diabetes from the Renal Insufficiency And Cardiovascular Events (RIACE) Italian Multicenter Study (n=15,773), whether reclassification using the alternate system provides a better definition of CVD burden (DR) associated with CKD than the NKF/KDOQI classification. Based on NKF/KDOQI classification, 62.54% of subjects had no CKD, 6.67% had stage 1, 12.03% had stage 2, 17.12% had stage 3 and 1.64% had stages 4-5 CKD, whereas 62.54%, 24.33%, 5.79%, 4.41% and 2.92% were assigned to the risk categories 0, 1, 2, 3, and 4, respectively, according to the alternate system. Prevalence of CVD, either total and by vascular bed, increased progressively both from no CKD to the advanced NKF/KDOQI stages and from risk category 0 to 4 of the alternate system. However, under the assumption that subjects classified as stages ≥2 or ≥3 (NKF/KDOQI) or category risk ≥2 or ≥3 (alternate system) would be referred for specialist care, the alternate system would led to less correct referral than the NKF/KDOQI system. Moreover, rate of CVD was quite different within each alternative system risk category. In risk category 0, prevalence was higher in subjects with an eGFR 60-89 ml/min/1.73m2 than in those with normal eGFR. In risk category 1, prevalence was higher in those with microalbuminuria and eGFR 60-89 ml/min/1.73m2 or with normoalbuminuria and eGFR 45-59 ml/min/1.73m2 than in those with microalbuminuria and normal eGFR. Similar findings were observed in risk category 3, but not in risk category 2, in which the burden of albuminuria was higher than that of reduced eGFR. These data indicate that the alternate 5-risk category classification system should be tested also for CVD and DR outcomes in large prospective studies, in order to improve risk stratification of diabetic individuals with CKD.

PP082

ASSOCIATION OF HYPERTRIGLYCERIDEMIA WITH RENAL, RETINAL AND CARDIOVASCULAR COMPLICATIONS IN SUBJECTS WITH TYPE 2 DIABETES

G. Penno[1], A. Solini[1], G. Zoppini[2], C. Fondelli[3], E. Orsi[4], R. Trevisan[5], M. Vedovato[6], F. Cavalot[7], A. Veronelli[8], M. Arosio[9], S. Morano[10], G. Pugliese[10]

[1]Università di Pisa - Pisa, [2]Università di Verona - Verona, [3]Università di Siena - Siena, [4]Fondazione IRCCS Cà Granda–Osp. Maggiore Policlinico - Milano, [5]Ospedali Riuniti - Bergamo, [6]Università di Padova - Padova, [7]Università di Torino - Torino, [8]Ospedale S.Paolo - Milano, [9]Ospedale S.Giuseppe - Milano, [10]Università La Sapienza - Roma

Atherogenic dyslipidemia, i.e. high triglyceride (TG) and low HDL cholesterol levels, contributes to the increased morbidity and mortality for cardiovascular disease (CVD) in subjects with type 2 diabetes. We assessed whether raised TG levels are associated with an increased burden from renal, retinal and CVD complications in patients with type 2 diabetes from the Renal Insufficiency And Cardiovascular Events (RIACE) Italian Multicenter Study (n=15,773). Subjects were divided in 4 groups depending on whether they had plasma TG levels below (NTG) or above (HTG) 150 mg/dl and were or not on statin therapy, which reduces TGs by up to 40%. HGT subjects, either with or without statin, had higher HbA1c, BMI, waist, non-HDL cholesterol and albuminuria, and lower HDL cholesterol and, only for subjects on statin, eGFR, as compared with NTG individuals (P<0.001). HTG patients, particularly if on statin, had higher prevalence of chronic kidney disease (CKD), especially albuminuric, than NTG subjects. In contrast, CVD and (advanced) retinopathy were more prevalent in subjects on statin than in those not, independently of TG levels. Logistic regression analysis with backward variable selection confirmed that HTG without or with statin was associated with a higher risk of CKD, Stages 1-2 (1.227 [1.081-1.393] and 1.246 [1.090-1.426], respectively), Stages 3-5 albuminuric (2.003 [1.654-2.427] and 2.667 [2.213-3.214], respectively), and Stages 3-5 nonalbuminuric (1.535 [1.294-1.820] and 1.838 [1.553-2.175], respectively), after adjusting for several confounders. NTG with statin therapy was associated only with Stages 3-5 nonalbuminuric. Conversely, statin treatment was independently associated, with no differences between NTG and HTG subjects, with a higher risk of CVD, total (2.893 [2.621-3.195] and 2.705 [2.399-3.049], respectively), coronary (4.230 [3.743-4.780] and 3.796 [3.286-4.385], respectively), cerebrovascular (2.438 [2.102-2.828] and 2.558 [2.143-3.054], respectively) and peripheral (2.128 [1.782-2.541] and 2.193 [1.779-2.704], respectively), but not of any or advanced retinopathy. In conclusion, HTG is associated with all CKD phenotypes independently of statin treatment, whereas the latter correlates with CVD independently of TG levels. These data point to a possible role of HTG in the development of CKD and to the importance of treating atherogenic dyslipidemia.

PP083

SERUM 25-HYDROXYVITAMIN D LEVEL AND INCIDENT TYPE 2 DIABETES IN OLDER MEN

N. Napoli[1], P. Pozzilli[1], D. Black[2]

[1]Area di Endocrinologia, Campus Bio-Medico - Roma, [2]University of California San Francisco - San Francisco, USA

Several studies have found an association between vitamin D levels and risk of developing diabetes. Even if the mechanism through which vitamin D regulates pancreatic function is still unclear, immunomodulation and effects of vitamin D on insulin resistance may play a main role. However, the effect of vitamin D levels on diabetes risk has not been consistent among observational studies, and also vitamin D intervention studies in diabetic patients have yielded varying results. In the present study we have investigated the effect of vitamin D on incident Type 2 diabetes in men (>65 years of age) who participated in the multisite Osteoporotic Fractures in Men (MrOS) study from March 2000 to March 2009.

After excluding 369 subjects with diabetes at baseline, 2477 subjects had baseline Vitamin D levels. . Clinical information, BMI and other factors related to type 2 diabetes were assessed at baseline visit while incident diabetes was determined at a follow-up visit 8.7 years later by self-report and medication use. Cox proportional hazards regression models were used to determine the relationship between 25(OH)D concentration and subsequent risk of a incident diabetes, with adjustment for covariates.

At baseline patients were on average 73.5 years old (±5.9), had a BMI in the overweight range (27.1±3.1) and total 25(OH)D of 24.5 ng/ml (±7.8). Incident diabetes was diagnosed in 120 subjects.

Analysis adjusted for clinic site and age (model 1) showed that vitamin d levels were associated with lower incidence of type 2 diabetes (HR=0.80 (see table)). After a further adjustment for BMI (model 2), the relationship was attenuated and no longer statistical significant. Further adjustments had little effect.

In conclusion, vitamin D levels are not associated with lower Type 2 diabetes incidence in older males. Body fat is a depot of vitamin D and it may explain the key role played by BMI.

PP084

25-OH VITAMIN D AND TYPE 2 DIABETES MELLITUS

V. Nuzzo[1], L. Tauchmanova[2], A. Gatti[3], P. Madonna[1], M. Montella[2], A. Zuccoli[2], A. Colao[4]

[1]Internal Medicine Unit “San Gennaro” Hospital - Naples , [2]Internal Medicine Unit “San Gennaro” Hospital - Naples , [3]Metabolic Unit “San Gennaro” Hospital - Naples , [4]Department of Molecular and Clinical Endocrinology and Oncology, “Federico II” University - Naples

Deficiency in 25-hydroxyvitamin D (25OHD) has been associated with insulin resistance, pancreatic beta-cell dysfunction and metabolic syndrome. More recent evidence supports an association also between 25OHD deficiency and hypertension, peripheral vascular disease, coronary artery disease, and heart failure.

In the present study was evaluated the prevalence of vitamin D deficiency and was investigated the association between vitamin D deficiency and ischemic heart disease in type 2 diabetes mellitus patients.

We assessed vitamin D status in a large cohort of outpatient type 2 diabetic patients consecutively enrolled in the period from December to March in the Units of Diabetes and Internal Medicine of San Gennaro Hospital, Naples, Italy (latitude 40 ° 49 'N, altitude 17 m) (n=698). Serum vitamin D levels were analyzed as a continuous variable and were defined as normal (≥20 ng/ml) or deficient (<20 ng/ml). In our cohort, 75% of patients were women, the mean age was 66.1 ± 9.36 years, the mean body mass index (BMI) was 31.14 ± 6.14. The mean serum levels of vitamin D were 18.23 ± 10.06 ng / ml; 234 subjects (33%) showed normal vitamin D levels while 464 (77%) were found to be in the deficient range. Moreover, we found that vitamin D deficiency was associated with coronary heart disease with an odds ratio of 1.6.

In conclusion, this study shows that low vitamin D levels has high prevalence in Southern Italian diabetic patients (67% of subjects) and is associated with an increased risk of cardiovascular disease. Unfortunately, food fortified with Vitamin D is not commonly available in Italy and supplementation should be recommended in this patient population.

PP085

INSULIN MODULATES HMGA1 DNA-BINDING ACTIVITY BY INDUCING ITS PHOSPHORYLATION

E. Chiefari[1], B. Arcidiacono[1], E. Maurizio[2], M. T. Nevolo[1], R. Sgarra[2], S. Iiritano[1], V. Ventura[1], D. Foti[1], G. Manfioletti[2], A. Brunetti[1]

[1]Dipartimento di Scienze della Salute, Università di Catanzaro - Catanzaro (Italy), [2]Dipartimento di Scienze della Vita, Università di Trieste, - Trieste (Italy)

The high-mobility-group A1 (HMGA1) protein is an architectural nuclear factor that drives gene transcription in response to multiple extracellular and intracellular signals. Such signals affects HMGA1 function by inducing post-translational modifications that influence HMGA1 ability to interact with other proteins and chromatin. For example, HMGA1 protein phosphorylation reduces its DNA-binding affinity and transcriptional activation. On the other hands, HMGA1 is a key transcriptional regulator of the insulin receptor (INSR) gene and intracellular regulatory molecules that are employed by the INSR signaling system are involved in post-translational modifications of HMGA1. Thus, we investigated whether activation of the INSR by insulin affected the phosphorylation of HMGA1 and regulated transcriptional activity of this protein.
Liquid chromatography-mass spectrometry (LC-MS) showed a significant early increase of the tri-phosphorylated HMGA1 isoform protein in HepG2 cells after insulin addition. This result was confirmed in vivo, in liver from insulin-injected mice. As determined by tryptic-peptide mapping of the phosphorylated protein and the relative extracted ion count (EIC) of the peptides, di- and tri-phosphorylation of HMGA1 occurred predominantly at the C-terminal tail (Ser98, Ser101, Ser102), a critical region for DNA binding. Single or triple mutagenesis of these serine residues prevented insulin-inhibition. Additionally, HMGA1 phosphorylation was abrogated by pretreatment with wortmannin, a potent and selective inhibitor of the PI-3K/Akt cascade. Both detachment of HMGA1 from DNA and inhibition of endogenous IGFBP-1 mRNA by insulin were prevented by inhibiting Casein Kinase 2 activity.
Collettively, our findings indicate that phosphorylation of HMGA1 represents a fundamental step in INSR signaling and that functional regulation of HMGA1 by phosphorylation/dephosphorylation may be important during acute (short-term) regulation of glucose homeostasis in response to both hormonal and nutritional changes.

PP086

POST-TRAUMATIC STRESS DISORDER AND COPING STRATEGIES IN NEW DIABETIC PATIENTS DIAGNOSED AFTER THE EARTHQUAKE IN L'AQUILA

G. Ciocca[1], E. Carosa[1], M. Stornelli[1], R. Iannarelli[2], A. Sperandio[2], E. Limoncin[1], G. L. Gravina[1], S. Di Sante[1], A. Lenzi[3], E. A. Jannini[1]

[1]Department of Biotechnological and Applied Clinical Sciences - L'Aquila, [2]Unit of Diabetology and Metabolic Dieseases, San Salvatore Hospital - L'Aquila, [3]Department of Medical Pathophysiology - Roma

Objective: Post-Traumatic Stress Disorder (PTSD) is a complex psychopathological syndrome that could occur after a traumatic event. Coping strategies can be explained as the tendency of people to react and face a possible traumatic event. The principal aim of this study is to investigate PTSD and coping strategies in new diabetic patients diagnosed after the 6 april 2009 dramatic earthquake which destroyed the medieval town of L'Aquila, Italy.
Methods: Our diabetic unit sequentially recruited 60 diabetic patients with diabetes mellitus type 2 who had diabetes before the earthquake, called pre-seism patients and 40 new diabetic patients who had diabetes diagnosis at least six months later the seism, called post-seism patients. Moreover 50 healthy people were recruited for the control group. All patients and controls lived the earthquake experience. A psychometric protocol composed by DTS for Post-traumatic Stress and Brief-COPE for coping strategy was administrated. In addition, we dosed salivary cortisol and evaluated other biological parameters as glycemia.
Results: We found a significant difference in the PTSD levels between post-seism patients (51.72 ±26.05) and pre-seism patients (31.65±22.59) ($p<0.05$), and between the post-seism patients and the control group (30.95±19.65) ($p<0.05$). The prevalence of PTSD was 67.5 % (27/40) in the post-seism group, 33.3% (20/60) in the pre-seism group and 36% (18/50) in controls. Also in this case there was a statistical difference between the post-seism group and the other two groups ($p<0.0001$). In the post-seism group, PTSD was positively linked to glycemia ($r=0.389$; $p=0.0163$) and the maladaptative coping ($r=0.667$; $p=0.0003$). Moreover, maladaptive coping was a predictive factor on PTSD, only in post-seism group (OR= 1.6826; CI-95 =1.15 to 2.45; $p= 0.006$).
Conclusions: Our results revealed greater levels of PTSD in post-seism patients compared to pre-seism patients and controls. This evidence could be considered as a risk factor for a premature development of diabetes, for an incorrect adherence to therapy and for a possible mental disease of the new patients diagnosed. In these patients, a psychological support could be important to improve the coping skills to react to PTSD and diabetes.

PP087

HYPOVITAMINOSIS D AND EARLY ATHEROSCLEROSIS IN DIABETIC DYSLIPIDEMIC PATIENTS

M. Marina[1], L. Franzini[1], D. Ardigò[1], S. Boarini[1], C. Bosi[1], S. Haddoub[1], G. Prampolini[1], A. Dei Cas[1], E. Derlindati[1], V. Spigoni[1], R. Aldigeri[1], G. Passeri[1], G. B. Vigna[1], I. Zavaroni[1]

[1]Department of Clinical and Experimental Medicine - Univeristy of Ferrara, [2]Department of Clinical and Experimental Medicine - Univeristy of Parma

BACKGROUND AND AIMS. To better understand the role of hypovitaminosis D (HD) on atherosclerotic process, we evaluated the relationship between serum concentrations of 25-hydrossi-vitamin-D (25OHvitD), the major cardiovascular (CV) risk factors and carotid intima media thickness (cIMT) as marker of early atherosclerosis, in patients with type 2 diabetes (DM2) and diabetic dyslipidemia (DD). MATERIALS AND METHODS. 138 DM2 patients of both genders (80 M and 58 F), with age 45-75 (mean 65±10) years old and DD (TG $\geq$ 150 mg/dL, HDL-C $\leq$ 40 mg/dL and LDL-C <160 mg/dL) underwent clinical examination and blood draw for determination of 25OHvitD, creatinine, oxidized LDL (oxLDL), percent of small dense LDL (sdLDL) and of large LDL (lLDL) by Lipoprint. After cIMT measurement two variables were obtained: the maximum value (IMTmax) and the average among all (IMTmean). RESULTS: High prevalence of HD was observed: only 5 individuals (3.6%) showed 25OHvitD values above 30 ng/mL. Serum levels were, as average, 14.7 ± 7.67 ng/mL. Subjects in the higher quartiles of serum 25OHvitD were on average younger (mean 66 years I vs. 61 in IV, p for trend=0.05), more frequently men (p for trend=0.025), with lower blood pressure (p for trend 0.012 and 0,010 for systolic and diastolic). Patients in the I quartile showed higher total cholesterol (p for trend=0.023), with no significant differences in HDL, LDL and TG; a higher prevalence of sdLDL (18.4% in the I quartile and 11.15% in the IV, p for trend=0.015) and a higher concentration of oxLDL (34.9 vs 30.4 U/L, p for trend=0.045). Finally, subjects in the higher quartiles showed a thinner IMTmax (IV quartile vs. I quartile 1.603 vs. 2.017, p for trend=0.036). After adjustment for major clinical variables, the circulating levels of 25OHvitD, along with age, were independently and inversely related to blood pressure (both systolic and diastolic p=0.037 and 0.045 respectively), to the % of sdLDL (p=0.035) and the concentration of oxLDL (p=0.04). Concentrations of oxLDL independently predicted, along with age and gender, a thicker cIMT ($p<0.05$). Age, gender and oxLDL explained approximately 30% of the variability of IMTmean and age, gender, and 25OHvitD about 25% of the variability of IMTmax. CONCLUSION. In patients with DM2 and DD, 25OHvitD levels are below the normal range and low 25OHvitD correlates with higher blood pressure and a more atherogenic LDL pattern, characterized by higher prevalence of sdLDL and oxLDL. OxLDL levels independently predict an increased cIMT. These results may contribute to explain the relationship between HD and CV disease.

PP088

THE ROLE OF CONTINUOUS SUBCUTANEOUS INSULIN INFUSION (CSII) ON METABOLIC CONTROL AFTER TOTAL PANCREATECTOMY IN PATIENT WITH MEN1: A CASE REPORT

E. Gamarra[1], P. Razzore[1], F. Ragazzoni[1], P. Limone[1]

[1]Div. Endocrinology AOOrdine Mauriziano - Turin

Pancreatogenic diabetes (T3cDM) is characterized by various degrees of metabolic-endocrine abnormalities ranging from mild increase in hepatic insulin resistance to complete disruption of pancreatic exocrine and endocrine activity depending on the cause of the pancreatic disease. Total pancreatectomy (TP) is characterized by complete deficiency of pancreatic enzymes and hormones, inducing extreme glycemic variability. Continuous subcutaneous insulin infusion (CSII) is being increasingly used in instable type 1 diabetes (T1DM) and pregnancy and also in T1DM children, in order to improve glycemic control by reducing hypoglycemic events and improving quality of life. Few data are available regarding the use of CSII in T3cDM, limited to anedoctal reports for total pancreatectomized patients. Here we present the case of a female patient (MF, 38 yrs old) followed from 2002 for MEN1 syndrome. She underwent lower left and lower and superior right parathyroidectomy in 2002 for multiple parathyroid adenomas; in the same year she was diagnosed with a NFPA and a non functioning pancreatic lesion (2 cm lesion in the tail of the pancreas on MRI in 2002). Besides, she also had primary autoimmune hypothyroidism on thyroxine replacement therapy. In december 2009 the patient underwent TP for volumetric progression of the lesion in the tail and evidence of multiple new lesions, maximum diameter 25 mm, in the rest of the pancreas at MRI, EUS, 68Ga-DOTATOC-PET/CT. Hystological examination revealed 6 pNET, G2 stage, maximum diameter 3 cm, 7% Ki 67. Before surgery the patient weighted 43 kg (BMI 18.6); one month after surgery, at the first diabetic evaluation, she weighted 40 kg. After surgery the patient was treated with multi insulin injections (MDI), but showed poor acceptance of disease and most of all developed important needle phobia causing difficulties in the management of her instable diabetes. The daily insulin requirement was 31 IU/day, 0.72 UI/kg/die (7 IU of basal, 24 IU bolus) and HbA1c was 7.4%. In april 2011 the patient started CSII insulin therapy (MEDTRONIC VEHO). During CSII therapy she recovered her weight loss and was able to reduce significantly her daily insulin requirement (20 IU/die, 0.45 IU/kg/die, basal 12 IU and 8 IU bolus for meals), though her diabetes control stil remains suboptimal (HbA1c 7.7%). No recurrence of neuroendocrine tumors has been reported to date and no DKA, severe hypoglycemia, local lipohypertrophy during CSII have been reported. In our patient CSII was useful in reducing her basal insulin requirements and optimizing the bolus management for meals and mostly improve her quality of life. To our knowledge, this is the first report regarding the use of CSII in T3cDM following TP in a MEN1 patient.

PP089

GESTATIONAL DIABETES MELLITUS: LIMITS OF NEW SCREENING CRITERIA

R. Vero[1], C. Capula[1], V. Ventura[2], S. Iiritano[2], V. Cristofaro[1], B. Arcidiacono[2], L. Puccio[1], V. Pullano[1], D. Foti[2], A. Brunetti[2], E. Chiefari[2]

[1]SOC-Endocrinologia-Diabetologia Az. Osp. Pugliese Ciaccio - Catanzaro (Italy), [2]Dipartimento di Scienze della Salute, Università di Catanzaro - Catanzaro (Italy)

Background – In July 2011, an expert consensus supported by the Italian Ministry of Health introduced new guidelines, for which gestational diabetes mellitus (GDM) screening must be performed in all pregnants ≥35 years old or in women <35 years old in the presence of risk factors. Our aim was to determine to what extent screening of all pregnant women would increase GDM diagnosed cases, and to examine positive testing in relation to the cut-offs established by IADPSG in 2010.

Methods – This is a retrospective cohort study of 1,232 pregnant women admitted to the Struttura Operativa Complessa Endocrinologia-Diabetologia, Ospedale Pugliese-Ciaccio, Catanzaro, and to the University of Catanzaro outpatient clinics, Italy, from May1, 2010 to July31, 2011 for GDM screening. Out of these, 487 (39.5%) pregnants were ≥35 years old and 745 (60.5%) <35 years old. In this latter group, 36.6% (273/745) showed no risk factors. GDM was diagnosed with 75g oral glucose tolerance test (OGTT) at 24-28 weeks of gestation, following the IADPSG 2010 cut-offs.

Results – Overall, 355 (28.8%) women were diagnosed with GDM. Out of these, 198 were <35 years old. In particular, GDM was diagnosed in 31.5% (86/273) of pregnants of <35 years without risk factors, who would not be tested according to the new guidelines. Interestingly, diagnosis was made at baseline (64.7%), at 1 hour (31.8%), or at 2 hours (3.5%) during OGTT.

Conclusions – Our findings, that over 30% of <35 years old pregnants without risk factors would miss diagnosis of GDM, would suggest to extent the screening to all pregnant women. To limit costs of GDM screening with a limited drop in sensitivity, OGTT could be restricted to two steps (baseline and 1 hour).

PP090

UNDERCARBOXYLATED OSTEOCALCIN, SHBG AND GPRC6A RECEPTOR IN HUMANS: NEW POSSIBLE METABOLIC IMPLICATIONS

G. Strapazzon[1], L. De Toni[1], D. Guidolin[1], S. Moro[2], V. De Filippis[2], A. Ferlin[1], C. Foresta[1]

[1]Medicina Molecolare - Padova, [2]Scienze del Farmaco - Padova

INTRODUCTION. The use of mouse genetics recently highlighted coordination by endocrine regulation between bone, energy metabolism, and endogenous sex hormones. Total serum osteocalcin concentrations in humans are inversely associated with measures of glucose metabolism and cardiovascular risk factors; however, human data are inconclusive with regard to the role of uncarboxylated osteocalcin. Intriguingly GPRC6A receptor mediates also the non-genomic effects of androgens and, although the information about SHBG-receptor structure is not conclusive, evidence seems to suggest that a G protein-coupled receptor is involved.

AIM of the STUDY. To investigate the protein-protein interaction computational predictors of osteocalcin and SHBG with GPRC6A receptor, and to validate experimentally in vitro in a two-step approach.

MATHERIAL and METHODS. 3-D protein structure alignment analysis and protein docking analysis. ClickMD-min script for computational analysis of the conformational structure of osteocalcin in presence or absence of Ca2+. Spectroscopic techniques. Gene and protein expression analysis and cell-surface receptor binding assays HEK-293T cells.

RESULTS. Our study shows for the first time the existence of the competition for a specific binding site between osteocalcin and SHBG on human cells expressing GPRC6A receptor. The results are supported by a computational prediction analysis. Our experiments describe the structure of human osteocalcin in its carboxylated and undercarboxylated forms for the first time. The influence of the binding of Ca2+ on osteocalcin structure seems to be stronger than the presence of γ-carboxylated glutamic acid residues.

CONCLUSIONS. The current two-step approach offers a target approach of investigation directly in humans, that has the potential to identify novel pathophysiological pathways as well as novel therapeutic possibilities.

PP091

IMPACT OF OBSTRUCTIVE SLEEP APNEA ON METABOLIC CHANGES FOLLOWING CALORIC RESTRICTION IN PATIENTS WITH TYPE 2 DIABETES MELLITUS AND MORBID OBESITY

L. Chioma[1], I. Giordani[1], I. Malandrucco[1], F. Picconi[1], S. Longo[1], S. Frontoni[2]

[1]Endocrinologia e Metabolismo, Ospedale San Giovanni Calibita Fatebenefratelli - Università di Roma Tor Vergata - Roma, [2]Dipartimento di Neuroscienze, Ospedale San Giovanni Calibita Fatebenefratelli - Roma

Background. Obstructive Sleep Apnea Syndrome (OSAS) is frequent in patients with type 2 diabetes mellitus (DM2) and obesity. In this population, the improvement in OSAS is proportional to weight loss. However, the presence of OSAS in DM2 patients is indipendent of obesity.

Objectives. To evaluate the effect of OSAS on the acute metabolic changes induced by caloric restriction in patients with DM2 and morbid obesity.

Patients and methods. 14 patients with DM2 and morbid obesity (BMI >40 kg/m2) were divided in 2 groups, according to the number of significant desaturations (ΔSO2>4%) per hour of sleep (ODI = oxygen desaturation index). All patients underwent an hyperglycemic clamp at baseline and after 7 days of Very Low Calorie Diet (VLCD). Before and after VLCD, insulin secretion and sensitivity, expressed as AIR (Acute Insulin Response) and M-value respectively, were investigated.

Results. At baseline OSAS and non-OSAS patients did not differ in age, sex, duration of disease, BMI, cortisol, blood pressure, fasting glucose, HbA1c, M-value and AIR. OSAS patients had a greater waist circumference (WC), although not statistically significant (p = 0.071) and, as expected, an ODI significantly higher than non-OSAS patients (25.51±5.393 vs. 2.517±0.558 events/hour, p=0.002). Despite a similar reduction of anthropometric parameters in both subgroups after VLCD, we observed a significant improvement in M-value and AIR only in non-OSAS patients (990.10 ± 170.19 vs. 1205.22 ± 145.73 µmol•min-1•m-2 BSA, p= 0.046; -1.047 ± 8.399 vs. 48.255 ± 11.899 pmol/l, p = 0.028, respectively). Moreover, in the whole sample there was a significant decrease in the average heart rate (HR) in the day and in 24 hours (p=0.041 and p=0.050, respectively).

Conclusions. The improvement in insulin sensitivity and secretion after VLCD only in non-OSAS patients, despite a similar weight loss, suggests a further negative impact of OSAS on metabolic impairments in DM2 patients. The observation of a reduction in HR could be the consequence of a modulation of sympatho-vagal balance, with a reduction in sympathetic prevalence, in patients with DM2 and morbid obesity.

PP092

CUT-OFF LEVELS OF WRIST CIRCUMFERENCE TO PREDICT INSULIN RESISTANCE IN OVERWEIGHT AND OBESE CHILDREN AND ADOLESCENTS

G. Campagna[1], M. Spoletini[1], S. Zampetti[1], M. Capizzi[1], G. Leto[1], C. Venditti[1], L. Marandola[1], A. Vania[2], R. Buzzetti[1]

[1]Experimental Medicine, Sapienza University - Rome, [2]Pediatrics and Child Psychiatry, Sapienza University - Rome

Excess fat is one of the main determinants of insulin resistance, representing the metabolic basis for developing future cardiovascular disease. One of the major priorities of clinical practice is to identify young people at increased risk for insulin resistance.

We previously demonstrated that the measurement of wrist circumference, reflecting the bone tissue area measured by RMN, was highly correlated with insulin resistance in overweight/obese children and adolescents. A recent article also demonstrated that wrist circumference is a significant predictor of diabetes in adult population.

The aim of the present study was to establish the cut off values of wrist circumference to predict insulin resistance in overweight and obese children subdivided according to pubertal status (pre-pubertal: I and II and pubertal: III, IV and V, Tanner stages).

N=637 overweight/obese children and adolescents (mean age 10.38±2.91 years) were consecutively recruited: manual measure of wrist circumference, standard deviation score body mass index, fasting biochemical parameters, pubertal stages and homeostasis model assessment of insulin resistance (HOMA-IR) were evaluated. HOMA-IR cut off values for insulin resistance in the pre-pubertal period were considered to be 2.22 in females and 2.67 in males and in the pubertal periods 3.82 in females and 5.22 in males as previously demonstrated. To determine an appropriate cut off point of wrist circumference to predict insulin resistance we performed a receiver-operator curve analysis (ROC). The cut-off of wrist circumference was defined by calculating Youden's J statistics and the maximum value of Youden's index was taken as the appropriate cut off value.

We observed a significant association between wrist circumference and HOMA-IR (b=0.37, p<0.0001). The cut offs of wrist circumference to predict insulin resistance in the pre-pubertal subjects were ≥14.8 cm in females and ≥15.4 cm in males and in the pubertal subjects were >15.8 cm in females and >17.5 cm in males.

The identification of youths with increased risk for insulin resistance in whom to perform further analyses could be now achieved by measuring the wrist circumference and referring to the specific cut-off values, thus avoiding testing the entire population of overweight/obese children.

PP093

ROLE OF PRDX6 IN THE PATHOGENESIS OF DIABETES MELLITUS

F. Pacifici[1], R. Arriga[1], G. P. Sorice[2], B. Capuani[1], D. Pastore[1], A. Coppola[1], F. Ferrelli[1], S. Caratelli[1], A. Bellia[1], G. Donadel[1], M. Federici[1], P. Sbraccia[1], G. Sconocchia[3], A. Giaccari[2], A. Orlandi[4], D. Lauro[1]

[1]Medicina dei sistemi, Università di Tor Vergata - Roma, [2]Endocrinologia e Malattie del Metabolismo, Università Cattolica del Sacro Cuore - Roma, [3]Istituto di Farmacologia Traslazionale, CNR - Roma, [4]Patologia Anatomica, Università di Tor Vergata - Roma

Introduction: Insulin resistance is a primary feature of Type 2 Diabetes and it's a metabolic disorder due to an impaired response to insulin in peripheral target tissues. Previous studies demonstrated relationship between ROS production and onset of insulin resistance, highlighting the important role of antioxidants enzymes. Peroxiredoxin 6 (PRDX6) is a peroxidase belonging to a relatively newly discovered class of antioxidant enzymes; it is capable to neutralize peroxides and phospholipid hydroperoxides. Based on these evidences, we investigated PRDX6 in the pathophysiology of Diabetes Mellitus (DM), using PRDX6 knockout (KO) and wild type (WT) mice model.

Methods and Results: IPGTT, ITT and hyperinsulinemic-euglycemic clamp showed that KO mice had an hyperglycaemic state associated with reduced glucose dependent insulin secretion measured by ELISA test. Then, WT and KO mice were stimulated with an infusion of insulin 1 U/kg into portal vein. Western Blot analysis of skeletal muscle protein, showed a significant decrease of insulin signaling activation in KO mice. The same analysis on liver protein, didn't show any significant modification in insulin signaling. Biochemical analysis revealed higher values of triglycerides (TG) and VLDL in KO mice than those observed in WT. Moreover, immunohistological analysis highlighted an important change in liver structure of KO mice. In addition, we also found an alteration in Langerhans islets number and morphology.

Discussion: These data suggested that KO mice had higher level of insulin resistance at skeletal muscle; despite we didn't observe relevant insulin signaling modification in liver. Muscle insulin resistance and altered lipid metabolism (TG and VLDL increase) might induce hepatic structural changes, as defined by NASH score. In skeletal muscle, insulin signaling analysis revealed a post-receptor defect at IRS1 level, with a significant reduction of tyrosine phosphorylation in KO compared to WT mice; this failing caused a reduction in PI3K AKT 1/2 signaling with subsequent modification in glucose uptake. Finally, we demonstrated, for the first time, the relationship between PRDX6 and DM onset, opening interesting perspectives in finding new molecular targets for DM care.

PP094

HMGB1 TRIGGERS LEPTIN PRODUCTION IN IL-2 POST ACTIVATED CD56+ PERIPHERAL BLOOD CELLS

A. Coppola[1], M. Caputo[2], B. Capuani[1], D. Pastore[1], F. Pacifici[1], R. Arriga[1], S. Caratelli[1], M. Romano[2], A. Bellia[2], F. Ferrelli[1], A. Galli[2], M. Cerilli[2], F. Pozzi[2], G. Donadel[1], M. Federici[1], P. Sbraccia[1], G. Sconocchia[3], D. Lauro[1]

[1]Dipartimento Medicina dei Sistemi,Università "Tor Vergata" - Roma, [2]Centro per il Diabete di tipo 2, Policlinico "Tor Vergata" - Roma, [3]Istituto di Farmacologia Traslazionale - Roma

Background and aims: HMGB (High-Mobility Group Box)-1 is a non histonic nuclear protein, which can be secreted and act as a proinflammatory cytokine, through the activation of TLR-2 and 4, and RAGE pathways. HMGB1 is produced by activated monocytes regulating host inflammation during metabolic diseases and pathogens invasion. Recently, investigators have shown that leptin has an immunomodulatory properties stimulating CD3+ T lymphocytes and NK cells; we tested the hypothesis that HMGB1 could modulate the expression of different cytokines in peripheral blood mononuclear cells (PBMCs) after IL-2 activation.

Material and methods:Using gradient separation we isolated PBMCs, low density PBMC (LD-P) (NK, NK-T, B cells and monocytes) and high density PBMC (HD-P) (T-Cells) and treated with IL-2 in presence or absence of HMGB1 (2ug/ml). After 48 hours culture, expression of different cytokines on supernatants were tested with protein array system. Leptin and IL-1beta levels were confirmed using ELISA assay. CD56+ and CD14+ cells were isolated by magnetic sorting utilizing anti-CD56 and anti-CD14 magnetic beads respectively. Data were analyzed utilizing t-test and a P values <0.05 was considered statistically significant.

Results and conclusions:Leptin levels were significantly increased ($p<0.05$) in IL-2 post-activated PBMCs supernatants, treated with HMGB1 compared to untreated controls. We assessed supernatants leptin levels in HD-P and LD-P and only in LD-P supernatants leptin was detected. Compared to their controls, leptin levels were significantly increased ($p<0.0005$) after IL-2/HMGB1 stimulation of NK and NK-T, and were10-15 pg/ml; This leptin amount were used to stimulate CD14+ cells and we found significant increased levels of IL-1beta in supernatants of CD14+ cells ($p<0.005$); IL-1 beta levels were similar to the one's obtained in response to LPS. We found CD56+ cells synthesize leptin that is secreted in response to IL2/HMGB1 treatment and the concentrations of secreted leptin have a biological effect. Leptin could act in paracrine way in noxae PBMCs response.

PP095

Abstract withdrawn

PP096

POSTPARTUM SCREENING IN GESTATIONAL DIABETES MELLITUS: COUNSELING EFFECTIVENESS

C. Capula[1], R. Vero[1], E. Chiefari[2], B. Arcidiacono[2], A. Vero[1], S. Iiritano[2], V. Ventura[2], L. Puccio[1], V. Pullano[1], D. Foti[2], A. Brunetti[2]

[1]SOC-Endocrinologia-Diabetologia Az. Osp. Pugliese Ciaccio - Catanzaro (Italy), [2]Dipartimento di Scienze della Salute, Università di Catanzaro - Catanzaro (Italy)

Background – Prevention and early diagnosis of type 2 diabetes mellitus (T2DM) have become public health priorities. Screening during the postpartum period offers a great opportunity for early identification of T2DM in women with a history of gestational diabetes mellitus (GDM). Neverthless, postpartum follow-up screening rates remain disappointingly low. Thus, we plan to examine the rate of postpartum glucose tolerance test for Italian women with GDM, before and after counseling, and identify demographic, clinical and/or biochemical predictors of adherence.

Methods – This is a retrospective cohort study of 1028 women with GDM enrolled to the Struttura Operativa Complessa Endocrinologia-Diabetologia, Ospedale Pugliese-Ciaccio, Catanzaro, and to the University of Catanzaro outpatient clinics, Italy, from January 2008- to June 2012. Anamnestic, clinical and biochemical parameters were assessed for all women. In the last year, verbal and written counseling on the importance of postpartum follow-up was given to pregnant women at 35-40 weeks. Women with GDM were subject to a 2-hour 75g oral glucose tolerance test (OGTT) at 6 weeks to 24 weeks postpartum, following ADA guidelines. Logistic regression analysis was used to evaluate individual effects of each patient's categorical characteristic as possible predictor of OGTT postpartum testing. Odds Ratios (OR) with 95% confidence bounds were calculated.

Results – 62.3% of women receiving counseling returned to follow-up with respect to 28.2% of women without counseling. Logistic regression analysis showed a strong association between this intervention and adherence to postpartum testing [adjusted OR 5.36 (95% CI, 3.94-7.29), $P<0.001$]. Also, women with previous diagnosis of GDM [adjusted OR 4.12 (95% CI, 2.61-6.49), $P<0.001$], with higher educational status [OR 2.80 (95% CI, 2.24-3.48) $P<0.001$] were more likely to complete the test, even after stratification for absence of counseling.

Conclusions – Counseling is an effective, inexpensive and simple tool in increasing postpartum OGTT rates in women with GDM.

PP097

TOTAL ENERGY EXPENDITURE IS INCREASED IN OBESE SUBJECTS WITH TYPE 2 DIABETES

G. Muscogiuri[1], M. Orio[2], P. Putignano[3], S. Savastano[1], A. Volpe[4], G. Lombardi[1], A. Colao[1], F. Orio[5]

[1]Dipartimento di Medicina Clinica e Chirurgia, Università "Federico II" - Napoli, [2]Centro Antidiabetico "CMSO" - Salerno, [3]Ambulatorio di Endocrinologia e Diabetologia, Presidio territoriale Ospedale San Gerardo - Monza, [4]Facoltà di medicina e Chirurgia, Università di Salerno - Salerno, [5]Endocrinologia, Dipartimento di Scienze Motorie e del Benessere, Università "Parthenope" ,SSD Tecniche di Sterilità, Endocrinologia dell'età fertile - Napoli, Salerno

The total energy expenditure (EE) of human beings is represented by basal metabolic rate (which corresponds to 60-70% of EE), dietary-induced thermogenesis (10% of EE), and the energy expended in physical activity (20-30% of EE). Obese individuals have an increased total EE compared with lean subjects; however, little is known whether alterations in carbohidrate metabolism influence energy expenditure in these patients. Thus, we aimed to investigate whether type 2 diabetes impact energy expenditure in obese subjects. Thirty-four consecutive obese subjects matched for age, body mass index (BMI) and physical activity with 32 obese with type 2 diabetes were studied. All subjects underwent to anthropometrical and metabolic evaluation and 24h-assessment of energy expenditure by SenseWear armband (SWA) for three consecutive days. During these days physical activity was similar in all subjects, in fact there was no difference in mean METs (Metabolic Equivalent).

Significant (p<0.05) differences between groups were observed in EE (2709±549 Kcal/24h *vs.*3083±739 KCal24h) for obese subjects and obese subjects with type 2 diabetes, respectively. These results demonstrate that obese subjects with alterations in glucose metabolism show an increased energy expenditure that might be likely due to increased glucagon and/or increased sympathetic activity.

PP098

HMGA1 IS A TRANSCRIPTIONAL REGULATOR OF IGFBP1 AND IGFBP3 GENES

B. Arcidiacono[1], S. Iiritano[1], V. Ventura[1], M. T. Nevolo[1], E. Chiefari[1], D. Foti[1], A. Brunetti[1]

[1]Dipartimento di Scienze della Salute, Università di Catanzaro - Catanzaro (Italy)

HMGA1 is a small basic protein that binds to AT-rich regions of DNA and functions mainly as a specific cofactor for gene activation. We previously observed that Hmga1 deficient mice showed reduced levels of insulin-like growth factor (IGF)-binding proteins 1 (IGFBP1), a circulating protein that regulates IGF-I action by modulating its bioavailability. In addition, an involvement of HMGA1 in the regulation of the IGFBP1 gene has been postulated on the basis of in vitro evidence, indicating that HMGA1 binds the insulin response element of the IGFBP1 gene promoter.

Based on this observation, we planned to verify whether HMGA1 transcriptionally regulates IGFBP1 and IGFBP3 genes, thus modulating the insulin-like metabolic effects of endogenous IGF-I on glucose metabolism.

By Chromatin Immunoprecipitation assays, we verified that endogenous nuclear factors HMGA1, C/EBPβ, and HNF-1 bound the promoters of IGFBP1 and IGFBP3 genes in HepG2 cells, in which these proteins are normally coexpressed. Perturbation of HMGA1 protein expression by siRNA significantly reduced the binding of HMGA1 to DNA and adversely affected protein-DNA interactions of C/EBPβ, HNF-1, and Sp1. The decrease in DNA-protein binding induced by anti-HMGA1 siRNA in HepG2 cells paralleled the decrease in IGFBP1 and IGFBP3 mRNA and protein expression, as revealed by real-time PCR and immunoprecipitation/Western blot analyses, respectively. Functional experiments with Luc reporter plasmids containing IGFBP1 or IGFBP3 gene promoter and various effector vectors showed that HMGA1, C/EBPβ, HNF-1, and Sp1 cooperate to activate IGFBP1 and IGFBP3 gene promoters at the transcriptional level and that this transactivation requires HMGA1.

Our findings demonstrate that HMGA1 plays an important molecular role in the transcriptional activation of the IGFBP1 and IGFBP3 genes. By affecting the expression of both IGFBP proteins species, HMGA1 can modulate the insulin-like metabolic effects of endogenous IGF-I on glucose metabolism.

...

PP099

ACUTE HYPERGLYCEMIA REDUCES CEREBRAL VASOMOTOR REACTIVITY IN SUBJECTS WITH METABOLIC SYNDROME

I. Giordani[1], F. Picconi[1], I. Malandrucco[1], L. Chioma[1], S. Longo[1], P. Palazzo[2], F. Passarelli[2], S. Frontoni[1]

[1]Endocrinologia e Metabolismo, Ospedale San Giovanni Calibita Fatebenefratelli - Università di Roma Tor Vergata - Roma, [2]Dipartimento di Neuroscienze, Ospedale San Giovanni Calibita Fatebenefratelli - Roma

Background: Cerebral Vasomotor Reactivity (CVR), a reliable method to assess cerebral hemodynamics, is reduced in type 1 and type 2 diabetic patients, thus contributing to cerebrovascular morbidity and mortality. In addition, acute glucose exposure impairs cerebrovascular reactivity in experimental animals.

Objectives: To investigate the response of cerebral reactivity to acute hyperglycemia in the presence of insulin-resistance, before the onset of diabetes.

Patients and Methods: We recruited 17 patients with metabolic syndrome (MS), but without diabetes and 4 controls (C). All subjects underwent a 2-hour hyperglycaemic clamp (HC), at a blood glucose level of 250 mg/dl (13.9 mmol/l). At baseline, 60 and 120 min of the HC, and after a 2-hour interval, CVR was evaluated. All subjects also underwent 24-h blood pressure monitoring and 24-h continuous glucose monitoring. MAGE (Mean Amplitude of Glycemic Excursions) was calculated.

Results: At baseline, CVR in MS was similar to C, but significantly higher than in a group of previously evaluated diabetic patients (p <0.001). In MS patients, but not in controls, CVR was significantly reduced after 1 hour and 2 hours of stable hyperglycemia vs. baseline (50.2% *vs.* 61.7%, p=0.002 and 51.1% *vs.* 61.7%, p=0.002 respectively). 2 hours after the end of HC, CVR value returned to baseline. Moreover, we showed a negative correlation between CVR at 120 min and mean diastolic blood pressure (in 24 hours and in the day: r=-0.408, p=0.045; r=-0.410, p=0.046, respectively) and between CVR at 120 min and MAGE (r=-0.491, p<0.05). MAGE was significantly higher in MS than in C (p=0.002).

Conclusions: The finding that acute hyperglycemia worsened CVR only in patients with MS suggests that insulin resistance per se plays a role in CVR response to acute hyperglycemia, before the onset of overt chronic hyperglycemia and that arterious hypertension and glycemic variability could influence this response.

This observation has many clinical implications, particularly on the possible negative prognostic meaning of the stress-induced hyperglycemia, in terms of cerebrovascular disease, in patients with MS, but without type 2 diabetes.

PP100

ABSENCE OF SEX DISPARITY IN THE PREVALENCE OF OBSTRUCTIVE SLEEP APNEA IN TYPE 2 DIABETES MELLITUS

G. Beccuti[1], D. Grimaldi[1], E. Tasali[1], V. Abraham[1], H. Whitmore[1], D. Ehrmann[1], E. Van Cauter[1], B. Mokhlesi[1]

[1]Department of Medicine, The University of Chicago - Chicago, IL

Obstructive sleep apnea (OSA) is one of most prevalent comorbidities in type 2 diabetes mellitus (T2DM), with a reported prevalence of up to 86% in obese patients with T2DM. In community-based studies, there is a sex disparity in OSA prevalence, where OSA is 2- to 3-fold more likely to be present in men than in women. The aim of our study was to assess the prevalence of OSA in a cohort of patients with T2DM and to examine sex differences.

Patients with T2DM were consecutively recruited from the Chicago community. All the participants underwent overnight polysomnography. The presence of OSA was defined by an apnea-hypopnea index (AHI) >5 events/hour. Hypopnea was defined as a 50% reduction of airflow for at least 10 seconds on a semiquantitative signal that was associated with either a 3% or greater oxygen desaturation or a cortical microarousal on the electroencephalography. The severity of OSA was graded according to commonly used clinical cutoffs as follows: no OSA (AHI < 5); mild OSA (5 < AHI < 15); moderate OSA (15 < AHI < 30); and severe OSA (AHI > 30).

A total of 115 subjects were studied: 50 men and 65 women. No sex difference was observed for age (mean ± SD; men: 56.2 ± 10.1 years; women: 54.3 ± 9.5 years), HbA1c levels (men: 7.3% ± 1.7; women: 7.4% ± 1.7), number of antidiabetic medications, insulin use, years of diabetes, and exercise frequency. Compared to men, women had a higher BMI (men: 33.0 ± 7.1 kg/m2; women: 35.7 ± 7.5 kg/m2; p = 0.035) and were at higher risk for T2DM based on ethnicity (men: 46%; women: 77%; p < 0.01). Out of the total of 115 patients, 98 had OSA (AHI >5), thus the overall OSA prevalence was 85%. Even after controlling for BMI and ethnicity, there was no sex difference in OSA prevalence, with 88% of men and 83% of women having OSA, respectively.

In conclusion, our study confirms that the prevalence of OSA is exceptionally high in T2DM and indicates that the sex disparity in OSA prevalence that is found in non-diabetic populations is no longer present in obese subjects with T2DM. This observation suggests that OSA may be on the causal pathway leading to T2DM development.

PP101

INFLUENCE OF DIPEPTIDYL PEPTIDASE IV INHIBITORS ON IGF-I AND ALS LEVELS

M. Boschetti[1], A. Rebora[1], M. Monachesi[2], A. Aglialoro[3], V. Albanese[1], D. Ferone[1], F. Minuto[1]

[1]DIMI Università di Genova - Genova, [2]ASL 2 Ospedale San Paolo - Savona, [3]ASL 3 Ospedale Villa Scassi - Genova

The role of glucagon-like-peptide-1 (GLP-I) in the reduction of glicemia levels through stimulation of insulin secretion and inhibition of glucagon release is well known.
Recently some new compounds acting through this mechanism such as incretin mimetics (Exenatide and Liraglutide) and dipeptidyl peptidase IV (DPP-IV) inhibitors (Sitagliptin and Vildagliptin) have been developed.
Indeed the DPP-IV inactivating enzymatic activity acts on several peptides included GHRH; the inhibition of this enzyme causes an increase of GLP-1 and GIP and contributes to the stabilization of GHRH levels. To test the hypothesis that inhibition of DPP-IV activity influence the GH/IGF-I axis we evaluated IGF-I and ALS levels (as a marker of DPP-IV action on GHRH degradation) in patients treated with Sitagliptin or Vildagliptin in comparison versus patients treated with Exenatide, Liraglutide (in addition on Metformin) or only Metformin.
Patients and methods: Three groups of patients, age-matched, were included in the study. Glucose, HBa1C, IGF-I and ALS levels were evaluated in each patient.
Group 1: 17 (8M, 9F) patients undergoing therapy with Metformin, and Exenatide or Liraglutide Group 2: 18 (9M, 9F) patients undergoing therapy with Metformin and Sitagliptin Or Vildagliptin Group 3: 25 (13M, 12F) patients undergoing therapy with Metformin. All the patients have been treated for at least 6 month.
Results: The study is on course and the enrollment of patients is still open. Preliminary data show that IGF-I (expressed as IGF-I SDS) and ALS levels are in the normal range and not influenced by therapy (no statistical differences among the 3 groups).As expected, IGF-I SDS values correlated with HbA1c ($P<0.02$) in all groups. No correlation was found between drug dosage and IGF-I and ALS levels.
Conclusion: preliminary data show that GH-IGF-I system is not influenced by DPP-IV inhibitor, confirming in humans the results observed in animal experiences.

PP102

HIGH VISCERAL ADIPOSITY INDEX (VAI) SCORE BEFORE AND AFTER TREATMENT IN YOUNGH HIV-POSITIVE PATIENTS

A. Di Sarno[1], E. Guerra[2], M. Gargiulo[1], C. Di Somma[2], A. Chirianni[1], C. Giordano[3], A. Colao[2]

[1]Osp. D. Cotugno, Az. dei Colli - Napoli, [2]Dip. di medicina clinica e chirurgia, Sez. di endocrinologia, Univ. degli Studi di Napoli Federico II - Napoli, [3]Endocrinologia e malattie del metabolismo, AOUP Paolo Giaccone Univ. degli Studi di Palermo - Palermo

The new therapies in HIV-positive patients are extremely effective in terms of immunological response. In western countries the mortality rate was reduced by 100% from 1984 to the current 8.8%. HIV people are now patients with a chronic disease characterized by also metabolic disorders. Long term virus alterations and treatment side-effects induced lipid disorders, in particular hypercholesterolemia and hypertriglyceridemia. To assess cardiovascular risk (CVR) in HIV patients. From April 2012 to January 2013 we evaluated 20 HIV-positive naive patients, no HBV-HCV co-infected (19 males, age range 21-42 years, mean 31.8 $\pm$ 4.8, BMI 23.5 $\pm$ 3.8). Before and after inhibitor proteases (IP) treatment we evaluated weight, height, waist circumference, BMI, blood pressure, triglycerides, total, HDL and LDL cholesterol and glycemia. Visceral Adiposity Index (VAI) was also evaluated in order to assess CVR. All results refer to comparison between before and after 6 mm IP-treatment. At present 10 (50%) and 8 (40%) patients completed 6 and 2 mm of follow-up, respectively; the remaining 2 (10%) were lost to the follow-up. In all 10 patients that completed 6 mm of IP-treatment, total (154.6 $\pm$ 37.2 *vs* 186.3 $\pm$ 55.7; *p=0.056*), LDL cholesterol (95.6 $\pm$ 29 *vs* 106.8 $\pm$ 33.9; *p=0.146*), and triglycerides (107.3 $\pm$ 42.6 *vs* 154.4 $\pm$ 87.2; *p=0.101*) levels were not significantly increased. On the contrary, HDL cholesterol levels (41.8 $\pm$ 8.5 *vs* 48.8 $\pm$ 12.1; *p=0.022*) were significantly increased. BMI (23.7 $\pm$ 3.6 *vs* 24.9 $\pm$ 3.4; *p=0.033*) and waist circumference (84.5 $\pm$ 5.5 *vs* 89.4 $\pm$ 7.8; *p=0.003*) were significantly increased. Before IP-treatment, VAI score, adjusted for sex and age, showed abnormal high values in 8 of 10 patients. The remaining 2 patients had normal VAI score. At 6 m follow-up, VAI score persisted high in all 8 patients, with a worsening in 7 out 8 whereas, in the remaining 2 persisted a normal VAI score. Glycemia and blood pressure were in the normal range before and after 6 m IP-treatment. Our data confirm the alterations of lipidic profile in patients and suggests an increase CVR in treated and untreated with high VAI score despite young age. Furthermore, our study suggests VAI, among the most common indexes of adiposity assessment, is a very useful tool well mirroring "adipose tissue dysfunction" in HIV.

PP103

INSULIN AND BODY WEIGHT BUT NOT HYPERANDROGENISM, SEEM INVOLVED IN SEASONAL SERUM 25-OH-VITAMIN D3 LEVELS IN PCOS

M. Gallea[1], M. Granzotto[1], S. Leandri[1], S. Azzolini[1], F. Favaro[2], D. Faggian[2], R. Vettor[1], R. Mioni[1]

[1]Medicina - Clinica Medica 3 - Padova, [2]Medicina di Laboratorio - Padova

Background. 25OH-Vitamin D (VitD) deficiency is common in women affected by Polycystic Ovary Syndrome (PCOS). Serum levels of VitD are below 50nmol/l in approximately 67-85% of women with PCOS. Yet, low levels of VitD are also linked to obesity and insulin resistance, both frequently affecting women with PCOS. Finally recent studies have demonstrated the expression of VitD receptor in the ovaries, so suggesting a direct influence of this hormone in ovarian function. Aim: To evaluate the role of VitD in the etiopathogenesis and/or in the occurrence of metabolic complications linked to PCOS in two different group of young women affected or without PCOS. We also studied the role of insulin,androgen and body weight excess in PCOS patients respect with a group of normal insulinemic,androgenic and body weight state: [**PCOS** vs no-ovarian hyperandrogenic (**noPCOS**), Functional Hypothalamic Amennohrrea (**FHA**) and healthy women (**Con**)]. Patients and methods: 77 women 18-42 yrs old were studied. The group includes: 37 PCOS, 13 noPCOS , 12 FHA and 15 Con. According to their BMI, patients are divided into lean (**L**: 18.5 < BMI > 24.9) and obese (**O**: BMI > 30); after OGTT they are divided into normoinsulinemic (**nINS**:max peak $\leq$ 60mUI/L) and hyperinsulinemic (**hINS**:max peak $\geq$ 80mUI/L). According to the seasonal period of the observation, each patient was further divided into "**summer**" (July-November) and "**winter**" (December-June). Serum VitD levels of each subject were measured in the proliferative phase (1-7 day) of the cycle. Hormonal, metabolic and calcium homeostasis parameters were also taken into account. Results: Both in summer and in winter, patients with **PCOS** had basal levels of VitD significant lower than **Con** and similar to those of patients with **noPCOS**. Only in winter they were lower than those of patients with **FHA**. Yet, results referring to **LnINS** patients with PCOS were equal to **Con**, while results referring to **LhINS** PCOS patients and to **O** PCOS patients were significantly lower than **Con** in terms of VitD levels, although being similar to one another. Conclusions: In general, patients with PCOS had levels of VitD lower than controls. Weight and hyperinsulinaemia had a significant influence on these values, resulting in the lowest levels, close to deficiency, being recorded in obese and hyperinsulinemic patients. PCOS was not a prerequisite for VitD deficiency. Finally, over 70% of our healty patients had VitD definciency.

PP104

THYROID FUNCTION AND MATERNAL BODY WEIGHT: RISK OF ADVERSE NEONATAL OUTCOMES IN HYPOTHYROID PREGNANT WOMEN

M. Altomare[1], S. Tumino[1], S. La Vignera[1], A. Garofalo[2], R. Zappalà[2], E. Vicari[1], A. E. Calogero[1]

[1]Sc. Mediche e Pediatriche, Sez. Andrologia e Endocrinologia, DR Sc. Andrologiche, Riproduzione Umana e Biotecnologiche, Univ. di Catania - Catania, [2]UOC Ostetricia e Ginecologia, AOU Policlinico-V. Emanuele - Catania

Background: Hypothyroidism in pregnancy is associated to an increased risk of adverse obstetric and neonatal outcomes. Gestational obesity contributes to maternal and neonatal complications. Previous studies have shown a positive correlation between TSH and body mass index (BMI), and between fat accumulation and lower fT4. **Objective**: To evaluate the relationship between maternal thyroid function and BMI, and between maternal hypothyroidism (even if associated with obesity) and birth weight (BW). **Subjects and methods**: Thyroid function (TSH and fT4) and BMI (WHO criteria) was assessed in 1006 pregnant women at different gestational age. Hypothroidism was defined when TSH serum levels were >2.5 mIU/L (1st trimester) or >3 mIU/L (2nd and 3rd trimester). **Results**: fT4 levels were significantly lower in overweight women than underweight women, and in obese women compared to underweight or normoweight women ($P<0.05$, ANOVA followed by Duncan test). TSH levels did not differ significantly among pregnant women with various BMI classes. A positive correlation between BMI and TSH ($r=0.07$, $P<0.05$) and negative correlation between BMI and fT4 ($r=-0.15$, $P<0.001$) were found. The latter was confirmed in 1st ($r=-0.12$, $P<0.05$) or 2nd ($r=-0.11$, $P<0.05$) trimester pregnant women considered separately. One hundred thirty out of 1006 pregnant women (12.9%) had hypothyroidism, and 738 (73.4%) had normal TSH. In 48 out of 130 hypothyroid women, and 176 out of 738 normal women, offspring BW was reported. Offspring of hypothyroid women had a not significantly lower mean BW than the offspring of women with normal TSH (3.0 vs. 3.3 kg). The mean BMI of women with hypothyroidism at the mean gestation week (GW) 13.7 was higher, but not significantly, compared to that of women with normal TSH (GW 13) (25.0 vs. 23.8 kg/m2). Considering women up to the 20th GW, the BW was lower, but not significantly, in offspring of obese hypothyroid compared to normoweight hypothyroid women (2.8 vs. 3.1 kg). Interestingly, the mean BW of euthyroid obese women offspring was similar to that on euthyroid non-obese women (3.3 kg). **Conclusion**: These findings showed that BMI and thyroid hormonal parameters are strictly related in pregnant women. Thus, an impaired thyroid function may enhance weight gain throughout pregnancy. Furthermore, obesity may increase the risk of low BW related to hypothyroidism.

PP105

THE COMBINATION OF GENETIC VARIANTS IN THE FSHR AND FSHB GENES AFFECTS SERUM FSH IN WOMEN OF REPRODUCTIVE AGE

V. Moriondo[1], A. La Marca[2], E. Papaleo[3], C. Alviggi[4], G. Ruvolo[5], G. De Placido[4], M. Candiani[3], E. Cittadini[5], F. De Michele[3], V. Catellani[1], A. Volpe[1], M. Simoni[1]

[1]Unità e cattedra di Endocrinologia , Dipartimento di Biomedicina, Metabolismo e Neuroscienze, Università di Modena e Reggio Emilia - Modena, [2]Dipartimento Materno-Infantile, Università di Modena e Reggio Emilia - Modena, [3]Centro Natalità, Dipartimento Materno-Infantile, Università Vita-Salute, Ospedale S. Raffaele - Milano, [4]Dipartimento di Ostetricia, Ginecologia, Urologia e Medicina Riproduttiva, Università Federico II di Napoli - Napoli, [5]Centro di Medicina Riproduttiva, Università di Palermo - Palermo

Introduction: The relationship between SNPs of the FSHR gene and serum FSH has not been completely clarified. . Genetic variants of the FSHB gene have been associated to variation in gene transcription and to serum FSH levels in men. An interesting joint effect of both FSHB-211G>T and FSHR 2039 A>G on male reproductive parameters has been recentely observed. In the present study we investigated for the first time the effect of FSHB-211G>T SNP in conjunction with the FSHR 2039 A>G SNP on levels of serum FSH in women. **Description of methods:** To investigate the effect of FSHB -211G>T togheter with the FSHR 2039 A>G on serum FSH in women we conducted a prospective study including 193 healthy eumenorrhoic women of reproductive age. In all women early follicular phase FSH and AMH were measured by commercial assays and antral follicle count was measured by transvaginal ultrasound. Genomic DNA was purified from total peripheral blood and genotyping for the two SNPs was performed by HRM technique. **Results:** No significant trends day3 FSH across the FSHR 2039 (AA/AG/GG) and FSHB -211 (GG/GT/TT) genotypes, respectively, were observed. When women were stratified according to the FSHR 2039 and the FSHB -211 genotypes a statistically significant reduction of day3 FSH was shown in the group of women with the FSHB -211 GT+TT / FSHR2039 AA genotype compared to the FSHB -211 GG / FSHR2039 GG genotype. **Conclusion:** In this study, the FSH-211G/T and FSHR2039A>G polymorphisms alone were not associated to significant modification in day3 serum FSH in women of reproductive age. The reduction of d3 FSH evidenced in the group of women with the FSHB -211 GT+TT / FSHR2039 AA genotype combination demostrates an additive effect of the different SNPs in FSHR and FSHB on regulating serum FSH in women.

PP106

IDIOPATHIC HIRSUTISM: A NEW PLANT-BASED THERAPY.

G. Tirabassi[1], L. Giovannini[1], F. Paggi[1], G. Panin[2], N. delli Muti[1], F. Panin[2], R. Papa[3], M. Boscaro[1], G. Balercia[1]

[1]Division of Endocrinology, Department of Clinical and Molecular Sciences, Umberto I Hospital, Polytechnic University of Marche - Ancona, [2]Hulka S.r.l. - Rovigo, [3]Centre of Socio-economic Gerontological Research, Scientific-Technological Area, Italian National Institute on Aging - Ancona

Background. Hirsutism is defined as the presence of excessive terminal hair in androgen-dependent areas of a woman´s body. Regarding this it has been demonstrated that Lavender and Tea tree oils have antiandrogenic activities.**Aim**. To evaluate therapy based on Lavender and Tea tree oils in women suffering from mild idiopathic hirsutism. **Subjects and Methods**. A prospective, open-label, placebo controlled, randomized study was performed: women affected by mild idiopathic hirsutism were randomly assigned to receive oil spray containing Lavender and Tea tree oils (group T) (n=12) or placebo (group P) (n=12) twice a day for 3 months in areas affected by hirsutism. Evaluation of hirsutism was carried out at baseline and after 3 months by Ferriman-Gallwey score and by measuring hair diameter taken from some body areas. A hematological and hormonal evaluation was carried out at baseline and after 3 months. **Results**. No significant variations were found in any of the hormones studied in groups T and P between baseline and after 3 months. A statistically significant decrease of hirsutism total score and of hair diameter was found in group T, while no statistically significant difference in these two parameters was observed in group P; in group T percentual reduction of hair diameter was significantly greater than in group P. **Conclusions**. Lavender and Tea tree oils applied locally on skin could be effective in reducing mild idiopathic hirsutism; this treatment could represent a safe, economic and practical instrument in the cure of this disease.

PP107

THE MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF THE P.R329C MUTATION IN THE BMP15 GENE.

R. Rossetti[1], E. Beccaria[2], L. Persani[3]

[1]Dip. Scienze Cliniche e di Comunità, Università degli Studi di Milano - Milano, [2]Lab. Ricerche Endocrino-Metaboliche, Istituto Auxologico Italiano - Cusano Milanino, [3]Dip. Scienze Cliniche e di Comunità, Università degli Studi di Milano, e Lab. Ricerche Endocrino-Metaboliche, Istituto Auxologico Italiano - Milano

Several variations of the human BMP15 gene affecting the precursor domain of the translated protein have been described in women affected with primary ovarian failure (POF). The only one missense substitution (p.R329C) affecting the mature and biologically active domain of the human BMP15 gene was found in a 37-year-old woman who had secondary amenorrhea at the age of 27. She presented with elevated serum levels of FSH and LH and a family history of early menopause. This mutation results in the substitution of the polar/positive aminoacid Arginine to the nonpolar and neutral aminoacid Cysteine at residue 329, which is located next to residues important for the cysteine knot formation, a typical feature of the TGFbeta superfamily members. Molecular analysis on stably expressed recombinant BMP15 protein show a severe impairment of the mutant precursor and mature peptide production, both intracellular and in the culture medium, with respect to the wild type forms. The biological impact of the p.R329C mutation was further demonstrated by a reporter bioassay in COV434 granulosa cells, showing a significant reduction of the BMP-dependent signaling pathway activation compared to the wild type. No differences in the mRNA expression levels of the mutant and the wild type forms could be evidenced by quantitative Real Time-PCR and the non-reducing western blot analysis failed to show abnormal products of processing or dimerization. Therefore, we hypothesize a reduced precursor stability, consistent with an accelerated proteasome-proteolytic pathway. This could represent a novel mechanism leading to defective BMP15 secretion in the follicular fluid, which is critical for female fertility and hereby might predispose to augmented atresia and diminished ovarian follicle viability. In conclusion, present analysis elucidates the molecular mechanism underlying the onset of the fertility defect in presence of a mis-folded mature peptide and highlight the critical role of the BMP15 gene dosage for the normal ovarian folliculogenesis.

PP108

OVARIAN GERM CELL TUMOR SECRETING INSULIN: A CASE REPORT

M. Battocchio[1], A. Rebellato[1], E. Zanchetta[1], E. De Carlo[1], C. Martini[1], M. C. Zatelli[2], S. Chiarelli[3], C. Pasquali[4], R. Vettor[1], P. Maffei[1]

[1]Clinica Medica III, DIMED - Padova, [2]Dipartimento di Scienze Mediche - Ferrara, [3]Anatomia Patologica, DIMED - Padova, [4]Clinica Chirurgica III, DIMED - Padova

A 76-yr-old woman was admitted to our department for recurrent hot flushes, sweating and dizziness associated to fasting hypoglycaemia. Her medical history was relevant for type 2 diabetes mellitus, hypertension and atrial fibrillation. Physical examination was remarkable for abdominal obesity and ascites. On admission, tumor markers and hormonal assessment revealed abnormal levels of calcitonin, chromogranin A, CEA, AFP, CA 125, CA 19.9, testosterone, progesterone and 17-beta-estradiol. Hypokaliemia and hypocalcaemia were also evidenced. Seric and urinary cortisol, ACTH, thyroid hormones, LH, FSH, HCG, glucagon, free IGF-I, serotonin, NSE and CA 15.3 were in the normal range. The patient underwent fasting test, but it was stopped at the fifth hour for symptoms of neuroglycopenia. Blood samples were collected and hypoglycaemia was demonstrated; in addition, insulinemia was inappropriately elevated, as for C-peptide levels. Suspecting an insulinoma, abdominal US was performed as first-line morphological exam, showing a spiculated disomogeneous mass of 10 cm diameter in left adnexal region. CT confirmed the presence of a necrotic-colliquative expansive lesion in left adnexal and described ascites. A ^{18}FDG-PET was coherent with the suspect of malignancy, describing multiple broad areas of intense up-take (SUVmax 22) in left adnexal. Octreoscan® was negative for pathological uptake. The patient underwent isteroannessiectomy and she died few days later because of septic complications. Hystological examination was consistent with ovarian germ cell tumor presenting extensive areas of neuroendocrine differentiation (positive immunostaining for synaptofisin, chromogranin A and insulin) and areas of glandular type (either of intestinal and Yalk sac with liver-like differentiation tumor). We were able to detect a significant amount of insulin in the medium of ovarian cancer cell culture. Remarkably, a complete inhibition of insulin secretion was observed *in vitro* at maximal doses of both Pasireotide and Everolimus with no effect on cell vitality. Only 10 cases of insulin secretion by ovarian tumors have been reported so far in literature. To the best of our knowledge, this is the first *in vitro* analysis showing an insulin secretion by an ovarian tumor, assessing also the effect of SSA and everolimus on tumor cells.

PP109

POOR DIAGNOSTIC VALUE OF THE EUGLYCEMIC-HYPERINSULINEMIC CLAMP IN THE CLINICAL ASSESSMENT OF INSULIN RESISTANT WOMEN WITH PCOS.

M. C. Amato[1], C. Rizzolo[1], A. Magistro[1], R. Vesco[2], V. Guarnotta[2], A. Ciresi[2], C. Giordano[1]

[1]Sect of End, Diab and Met (Di.Bi.M.I.S) - Palermo, [2]Section of Endo - Palermo

Insulin resistance (IR) *per se* and/or the ensuing compensatory hyperinsulinism, have been always considered as a key point in the pathogenesis of PCOS. Aim of our study was to evaluate which of these two aspects plays a key role in the pathogenesis of PCOS. Using a cross-sectional study design, 15 PCOS (Rotterdam criteria) and 16 age/BMI-matched control women (with suspect of IR) underwent clinical measures of IR after a 3-month withdrawal of insulin sensitizers and oral contraceptive pills. In an academic clinic setting, average glucose infusion rate (M-value) (during an euglycaemic–hyperinsulinaemic clamp), AUCinsulin and AUCglucose (during an Oral Glucose tolerance Test), HOMA-IR, ISI-Matsuda, Oral Dispositional Index (DIo) and Visceral Adiposity Index (VAI) were investigated. The prevalence of IR (according the M-value cut-off of 4.7 mg/Kg/min proposed by Bergman et al.) in the two groups was comparable: 14/16 (87.5%) for control women and 15/15 (100%) for PCOS women (p=0.484). No significant differences were observed between the two groups for M-value (p=0.540), VAI (p=0.406) and DIo (p= 0.813). Women with PCOS showed significantly higher levels of fasting insulin [median (IQR): 22 (19-37) vs. 13.55 (10.25-18.67) mU/ml; p<0.001], 30' after OGTT insulin [96 (66-230) vs. 48 (26.25-83.25) mU/ml; p=0.003] and consequently significant differences in derived indexes (HOMA-IR, ISI-Matsuda, $AUC_{insulin}$). Furthermore, by performing a Kruskal-Wallis test between the four Rotterdam-PCOS phenotypes, the complete phenotype showed significantly higher levels of VAI (p=0.007). Our study suggests that for the assessment of IR in PCOS, the gold standard euglycemic-hyperinsulinemic clamp, an expensive method requiring an experienced operator to manage the technical difficulties, has also the limitation that utilizes steady-state insulin levels that may be supra-physiological. This finding may be explained in the reversal of normal portal to peripheral insulin gradient. Thus, the glucose clamp may not accurately reflect the ovarian insulin action under physiological conditions, nor gives us information about the compensatory hyperinsulinism. In non-diabetic women with PCOS, a dynamic test, such as OGTT (in particular the initial phase of stimulated insulin secretion), provides more useful information than the gold standard glucose Clamp.

PP110

HYPOTHALAMIC AMENORRHEA REVERSAL IN PATIENTS WITH EATING DISORDERS TREATED WITH OUTPATIENT COGNITIVE BEHAVIORAL THERAPY AND NUTRITIONAL REHABILITATION

D. Consoli[1], G. Borzì[1], S. C. Ingrilli[1], F. Guardo[1], P. Malandrino[1], G. Padova[1]

[1]Endocrinology, Garibaldi Nesima Hospital, Department of Clinical and Molecular Biomedicine, University of Catania - Catania

BACKGROUND: Eating Disorders (ED) are mental diseases characterized by extreme cravings or food refusal aimed at excessive weight control. Menstrual irregularities are a frequent complication of ED. According to current diagnostic criteria, anorexia nervosa (AN) is the only eating disorder subtype associated with amenorrhea. However, even among patients with bulimia nervosa (BN), binge eating disorder (BED) and ED not otherwise specified (EDNOS), menstrual irregularities may be present.**AIM**: To establish the long-term effects of cognitive behavior therapy (CBT) associated with a gradual nutritional rehabilitation (GNR) in patients suffering from ED. **METHODS**: 29 girls with amenorrhea and ED were enrolled in the study. All subjects met DSM IV-R criteria for ED. In addition, hormonal and clinical assessment confirmed the diagnosis of hypothalamic amenorrhea. AN was diagnosed in 72.5% of patients, while EDNOS, BN and BED were diagnosed in 20.8%, 3,4% and 3,4% respectively. The amenorrhea lasted for an average of 12,6±7,8 months. Body mass index (BMI), body fat percentage (%FM), were measured at baseline and every 6 months for 18 months. In addition, ED psychopathology was evaluated every 6 months with the Eating Disorders Examination-Questionnaire (EDE-Q). Each patient was followed for 18 months and offered 36 sessions of a combination of CBT and GNR aimed at weight gain, resolution of the underlying psychological conflicts and resumption of menses. Importantly, exercise was limited in all patients until the end of the study. Contiuous variables were analyzed using the Student's T-test, while Chi-Square test was used to analyze each categorical variable. **RESULTS**: The study was completed by 82,7% of patients. Among these, more than 65% of patients resumed menses. In these patients, the amenorrhea reversal was strongly associated with significant improvement of BMI, %FM and with a decrease of each of EDE-Q subscales scores (p<0.001). As expected, in patients remaining amenorrhoic, the improvement of all anthropometric variables was instead not statistically different compared to those evaluated at the beginning of the study. **CONCLUSIONS**: These results confirm that CBT associated with GNR remains the best treatment for ED and that menses resumption is strongly associated with increase not just in BMI but also in total body fat percentage.

PP111

FEMALE HYPERANDROGENISM ITALIAN SURVEY

E. Faloia[1], S. Paolini[1], L. Zanotti[2], V. Toscano[3], A. A. Sinisi[4], M. Boschetti[5], G. Targher[6], R. Pasquali[2]

[1]Division of Endocrinology, Department of Clinical Medicine and Applied Biotechnologies, Polytechnic University of Marche - Ancona, [2]Endocrinology Unit, Department of Internal Medicine, S.Orsola Malpighi Hospital - University of Bologna, [3]Endocrinology, Department of Clinical and Molecular Medicine, Sant'Andrea Hospital - Sapienza University of Rome, [4]Department of Clinical and Experimental Medicine and Surgery, Endocrine and Medical Andrology Unit. - Second University of Naples, [5]Department of Endocrinology and Medical Sciences. - University of Genova, [6]Division of Endocrinology and Metabolism, Department of Medicine. - University of Verona

Introduction: Hyperandrogenism is one of the most endocrinological disorder in women in reproductive age, with an incidence is about 6-8%. The Polycystic Ovary Syndrome (PCOS) represents the main syndrome due to androgen excess. The diagnostic criteria, are defined by the presence of almost two between the follow characteristics: oligo/amenorrhea, clinical and/or biochemical signs of hyperandrogenism, sonographic features of PCOS. Despite of the high incidence of disease, there are a lot of different therapeutical approaches but, currently, their use is off-label.

Objective: The aim of the survey was to collect the experience of Italian endocrinologists who deal with this disease daily, to investigate therapeutical approaches, effectiveness based on clinical experience of every specialist and the side effects of their use.

Design: Cross-sectional study, internet survey

Partecipants: 31 Endocrinologists, SIE membership

Results: A clear suggestion on choosing monoterapy or combined therapy does not exist. In clinical practice, 48% participants preferred both choices to carry out patient's needs. With respect to monotherapy, preferences are 73.7% for oral contraceptives (OCs), 68.2% for metformin, and 52.6% spironolactone. However, metformin is resulted the most frequently prescribed drug off-label (83,8%). The combined therapy (metformin + OCs) was largely preferred by 76.9% of Endocrinologists. The majority of participants (79%) found the combined treatment very effective, whereas 17% suggested poor efficacy or no efficacy (4%). A very high tolerability profile was reported.

Conclusion: These data support the need for a further evaluation, on national basis, on how to manage hyperandrogenic states, particularly PCOS, with available drugs, including oral OCs, antiandrogens and metformin. Due to their potential benefit and low cost, regulatory agencies should adequately support a strategy for their use, provided individual responsiveness and safety is adequately assessed.

PP112

EHTNIC DIFFERENCES IN WOMEN WITH PCOS LIVING IN THE MEDITERRANEAN AREA.

A. Repaci[1], A. Gambineri[1], A. Paccapelo[1], R. Mioni[3], F. Kelestimur[4], M. Pugeat[5], E. Diamanti-Kandarakis[6], R. Pasquali[1]

[1]Div. of Endocrinology, Dept. of Medical and Surgical Science - Bologna, Italy, [2]Div. of Endocrinology, Dept. of Medical and Surgical Science - Bologna,Italy, [3]Dept of Medicine, Clinica Medica 3 - Padova, Italy, [4]Department of Endocrinology - Kayseri, Turkey., [5]Fédération d'Endocrinologie, Groupement Hospitalier Est - Bron, France, [6]Medical School, University of Athens - Athens, Greece

The pathogenesis of Polycystic ovary syndrome (PCOS) is multifactorial, involving genetic, enviromental, and ethnic factor.In this multicentric study we investigate the differences in clinical phenotypes and metabolic parameters among 4 different population of women with PCOS that living different countries of the Mediterranean area.The study was designed to collect different cohorts around South-Europe with an adequate number of women with PCOS in order to investigate potential ethnic differences in the prevalence of features defining the disorder according to the NIH criteria. We are consecutively collected, from a pre-existing data-base, 409 women with PCOS from Italy, France, Greece, Spain,Turkey. Results. Their age ranged from 15 to 45 years and reasons for seeking medical visit were, collectively, mainly related to obesity (51,5%), oligomenorrhea (44,3%) or amenorrhea (10%), infertility (4,1%), hirsutism (42%), acne and/or alopecia (15.9%). From the initial population we are selected 359 women PCOS and the Spanish population was not included because of the inclusion of obese individuals only. After adjustment for age and BMI we found significantly differences in prevalence of acne among French women PCOS vs women PCOS of other countries (P<0,001), of oligomenorrhea between French vs Italian PCOS women PCOS (70,6 vs 57,7% P=<0,05), between Greek vs Italian PCOS women (86% vs 57,7% P<0,01) and between Greek women PCOS vs Turkey women PCOS (86% vs 53,5% P<0,01). Hirsutism did not differ among the countries. By contrast, significant differences in androgen levels, but not SHBG, were found (P<0,01). At metabolic levels we found significantly differences in total cholesterol (P=0,023), HDL (P<0,001), triglycerides (P<0,001), fasting glucose (P<0,001), glucose at 120 min after OGTT (P=0,001), fasting insulin (P=0,008), HOMA-IR (P=0,021). The prevalence of NGT (P<0,001) and IGT (P<0,001), but not T2D, was also significantly different, as did that of the IDF (P=0,011) but not the ATP- metabolic syndrome. Conclusions. These data suggest that in the Mediterranean area some difference may exist in the PCOS phenotype, as well as the degree of androgen excess and metabolic derangement, according the ethnicity. Further studies in larger population should be performed in order to investigate the role of both genetic and environmental factors.

PP113

RELATIONSHIPS BETWEEN INSULIN-RESISTANCE AND PITUITARY-THYROID AXIS IN POLYCYSTIC OVARY SYNDROME

D. Romualdi[1], S. Raimondo[2], C. Di Segni[2], M. Persano[2], M. Cammarano[2], S. De Cicco[1], A. Lanzone[1], A. Pontecorvi[2], A. Mancini[2]

[1]Istituto di Clinica Ostetrica e Ginecologica - Università Cattolica del Sacro Cuore - Roma, [2]Dipartimento di Scienze Mediche - Divisione di Endocrinologia - Università Cattolica del Sacro Cuore - Roma

It is known that thyroid hormones (TH) influence ovarian activity, via direct and indirect mechanisms; they are among main regulators of SHBG. Moreover, hypothyroidism is frequently characterized by insulin resistance (IR), which is also a metabolic feature of polycystic ovary syndrome (PCOS). However few data do exist about the relationships among TH, androgen secretion, nutritional status and IR in PCOS. Therefore, we studied 112 consecutive women, aged 18-35 ys, affected by PCOS, attending our divisional outpatient services; the diagnosis was established according to Rotterdam criteria. 44 were overweight or obese (BMI >27 kg/m2) and 68 normal weight. Anthropometric parameters, hormonal assays (fT4, TSH, testosterone, androstenedione, DHEAS, 17OH progesterone, AMH, sex hormone-binding-globulin), oral glucose tolerance test (OGTT) and euglycaemic-hyperinsulinaemic clamp were evaluated in all patients. TSH values resulted to be directly correlated with waist to hip ratio (WHR) (r: 0.22; p=0.03) and inversely correlated with M-clamp, a reliable measure of peripheral total body glucose utilization (r: -0.24; p=0.03). Neither basal nor after-load glycemia were correlated with TSH. No correlations were found between TSH levels and gonadotropins, plasma androgens, AMH, sex hormone-binding-globulin. Similarly, the hirsutism score and the menstrual pattern seemed not to be influenced by thyroid function. After dividing patients on the basis of BMI, we did not observed significant difference in TSH values (obese: 1.98 ± 0.80 µUI/ml; normal weight: 1.90 ± 0.76 µUI/ml) and in free-thyroxine levels (obese: 11.46 ± 1.24 pg/ml; normal weight: 11.75 ± 1.33 pg/ml). Insulin response after OGTT (AUC) was significantly different between the two groups as expected (obese: 13071.8 ± 8028.8 , normal-weight: 8991.5 ± 4346.6 µU/ml/180 min). TSH levels showed a significant direct correlation with both stimulated insulin secretion (r: 0.40; p=0.009) and peripheral insulin resistance (r: 0.35; p=0.04) in obese but not in normal-weight patients. In conclusion, thyroid function seems to be linked to insulin metabolism sensitivity in this syndrome. It remains to be determined the role of central and peripheral mechanism in TSH elevation and if it may be a cause, presumably via systemic oxidative stress, or a consequence of insulin resistance, as suggested by previous reports on TSH normalization after insulin-sensitizers administration.

PP114

ULTRASONOGRAPHIC VISCERAL FAT ASSESSMENT IN POLYCISTIC OVARY SYNDROME

L. Rizzo[1], G. Borzì[2], S. C. Ingrilli[2], F. Guardo[2], P. Tita[2], G. Padova[2]

[1]Diagnostic Ultrasound Center - Catania, [2]Endocrinology, Garibaldi Nesima Hospital, Department of Clinical and Molecular Biomedicine, University of Catania - Catania

INTRODUCTION - Polycystic ovary syndrome (PCOS) that affects 4% to 12% of women is associated with metabolic and cardiovascular risk. Pattern of fat distribution rather than obesity itself is an important factor for cardiovascular morbidity. Waist circumference (WC) and waist-to-hip ratio (WHR) can identify abdominal fat distribution, but they cannot differentiate subcutaneous from visceral fat. Various methods for the measurement of visceral fat are used, such as Computed tomography (CT), Magnetic resonance imaging (MRI), Dual energy x-ray absorptiometry (DEXA) and Bioelectrical impedence analysis (BIA), but they are expensive or not accurate. Ultrasonography (US) may be a valid alternative for visceral fat estimation; different US approaches were reported, but they seems poorly reproducible. **AIM - T**o correlate visceral fat measured by US with clinical and biochemical features in PCOS pts. **METHODS -** 13 PCOS pts (mean age 21.3 years ± SD 5.45). BMI and WC were measured and serum glucose, insulin, cholesterol and triglycerides levels were evaluated; OGTT was performed in 6 pts. US for the visceral fat assessment was performed, measuring the right renal adipose capsule thickness, at the level of the antero-lateral wall with Siemens S2000, probe Convex Multi-MHertz 4.5-4.3 with Hanafy Lens and Harmonic Imaging. **RESULTS -** BMI was 25-30 Kg/mq in 5 pts and >30 in 4 pts (mean 27.3 ± SD 5.4); 10 pts had a WC >80 cm and 8 had a WC >88 cm (mean 92 ± SD13). Two pts had an impaired fasting glucose (IFG) and 2 pts had an impaired glucose tolerance (IGT); 4 pts had dyslipidemia and 4 pts had hepatic steatosis. Mean US renal fat measurement was 0.45 cm (± SD 0.30). We found a high positive correlation not only between US parameters and BMI (r=0.79) and WC (r=0.77), but also with IFG, IGT, dyslipidemia and hepatic steatosis: 6 pts with at least one metabolic alteration (IFG, IGT, dyslipidemia and hepatic steatosis) had a higher visceral fat amount (0.65 cm ± SD 0.32) than PCOS pts with normal metabolic parameters (0.27 cm ± SD 0.14, P <0.05). Instead, we found a low positive correlation between US parameters and fasting glucose (r=0.35), insulin (r=0.57) and HOMA-IR (r=0.56). **CONCLUSION** – This US technique seems a suitable, noninvasive and reproducible method for the quantification of visceral fat amount and it correlates not only with BMI and WC but also with the most important biochemical markers of metabolic damage and therefore with cardio-vascular risk factors in PCOS pts.

PP115

ACCURACY OF INTROITAL ULTRASOUND IN PREDICTING VAGINAL ORGASM AND FEMALE EJACULATION

S. Di Sante[1], A. Tullii[2], G. L. Gravina[1], A. Marcozzi[2], E. Limoncin[1], G. Ciocca[1], E. Carosa[1], A. Lenzi[3], E. A. Jannini[1]

[1]Department of Biotechnological and Applied Clinical Sciences - L'Aquila, [2]Department of Obstetrics and Gynaecology, Teramo Hospital - Teramo, [3]Department of Medical Pathophysiology - Roma

Purpose. A clear anatomical structure of the urethrovaginal septum (UVS) that adequately stimulated can lead to vaginal orgasm has not been fully described yet. This study sought to identify which differences were present between women with or without experience of female ejaculation on the basis of thickness of UVS

Method. Introital ultrasound was performed to examine the thickness of UVS in a cohort of 129 women. Ultrasound analysis was performed measuring UVS obtained along a line between the border of the smooth muscle and mucosa-submucosa layer of the urethral wall and the border of the vaginal wall at 90th (distal) percentile of the urethra length. Two skilled sonographers analyzed UVS and inter-operator agreement was assessed by Pearson's correlation coefficient.

Results. Forty-eight women out of 129 had no experience of vaginal orgasm (Group 1), 44 women had vaginal orgasm (Group 2), and 37 women had both vaginal orgasm and ejaculation (Group3). The UVS was significantly different among the three groups as showed using ANOVA test (p=0.0001). Post-hoc analysis showed an higher UVS in Group 3 (9.3±3.1mm) compared to Group 2 (6.5±2.0mm; p=0.0001) and Group 1 (4.0±1.5mm; p=0.0001). UVS in group 2 was also significantly different compared to group 1(p=0.0001) and 3 (p=0.0001). The inter-operator agreement was high (R2=0.81, p=0.0001).

Conclusions. The presence of vaginal orgasm and female ejaculation were associated with higher thickness of UVS. The measure of UVS is highly reproducible.

PP116

CLINICAL CHARACTERISTICS AND GENETIC ANALYSIS IN WOMEN WITH PREMATURE OVARIAN INSUFFICIENCY.

L. Russo[1], E. Ferrarini[1], F. Fruzzetti[2], E. Gianetti[1], P. Agretti[1], T. Simoncini[3], P. Simi[4], F. Baldinotti[4], A. Pinchera[1], P. Vitti[1], M. Tonacchera[1]

[1]Endocrinologia e Malattie Metaboliche - Pisa, [2]Department of Reproductive Medicine and Child Development, Division of Obstetrics and GynecologyMedicine and Child Development - Pisa, [3]Department of Reproductive Medicine and Child Development, Division of Obstetrics and Gynecology - Pisa, [4]Mother and Child Department, Cytogenetics and Molecular Genetic Unit - Pisa

Objective: Premature Ovarian Insufficiency (POI) is defined as a primary ovarian defect characterized by absent menarche (primary amenorrhea) or premature depletion of ovarian follicles before the age of 40 (secondary amenorrhea) with hypergonadotropism and hypoestrogenism.Methods: We studied the clinical, biological, and genetic data related to 50 POI patients with a mean age of menopause of 29 years (94% with secondary amenorrhea, 6% with primary amenorrhea and 15% with a family history of POI). Seventeen patients were affected by endocrine autoimmune diseases, antral follicles were observed in 31 patients by ultrasonography.Results: Karyotype analysis did not show any abnormality of the X chromosome. No mutation in FSH receptor and GDF-9 genes was reported, while in one patient a variant of BMP-15 gene (A180T) was found. Four patients had Fragile X mental Retardation 1 gene (FMR1) premutation and one an intermediate sized CGG repeats of the same gene. Two patients with FMR1 premutation were sister and developed secondary amenorrhea at the age of 34 and 37 years. The other two patients presented with oligoamenorrhea at the age of 39 and 34 years. The patient harbored the intermediate sized CGG repeats developed secondary amenorrhea at the age of 33 years. Conclusions: The genetic analysis performed on a cohort of patients with POI revealed that 8% had FMR1 premutation and only one patient a previously known variant of BMP-15 gene. No alteration of the karyotype and FSH receptor and GDF-9 genes was evidenced.

PP117

PREDICTORS OF CARDIORESPIRATORY FITNESS IMPAIRMENT IN WOMEN WITH POLYCYSTIC OVARY SYNDROME

C. Negri[1], E. Bacchi[1], D. Di Sarra[1], C. Bonin[2], F. Zambotti[1], M. Dall'Alda[1], G. Spiazzi[1], F. Schena[3], F. Tosi[1], P. Moghetti[1]

[1]Endocrinologia, Diabetologia e Metabolismo, Università e AOUI di Verona - Verona, [2]Ostetricia e Ginecologia, Università e AOUI di Verona - Verona, [3]Dipartimento di Scienze Neurologiche, Neuropsicologiche, Morfologiche e Motorie, Università di Verona - Verona

Polycystic Ovary Syndrome (PCOS) is a common endocrine disorder characterized by chronic anovulation, hyperandrogenism and frequent insulin resistance. Preliminary data showed that subjects with PCOS may have an impaired cardiopulmonary capacity, which could be associated to metabolic abnormalities. To investigate this issue, 27 PCOS women (mean±SD: age 23.0±4.1 yr, BMI 24.8±4.5 kg/m2) and 10 healthy controls (age 29.0±3.4 yr, BMI 20.1±2.04 kg/m2) were studied, assessing anthropometric features, hormonal and metabolic profiles, insulin sensitivity (glucose clamp technique), cardiorespiratory fitness (maximal exercise ramp protocol of 15 Watt/min to evaluate peak oxygen consumption (VO2peak), oxygen uptake at aerobic threshold, and the maximal workload). BMI, waist circumference, and serum testosterone were higher in PCOS subjects than in healthy women, while insulin sensitivity was significantly reduced in PCOS women. VO2peak and maximal workload were also significantly reduced in PCOS women (25.6±5.3 vs 35.4±5.4 mL·kg-1·min-1, p<0.0001; and 136±21 vs 168±37 Watt, p=0.002, respectively). Similarly, oxygen consumption at aerobic threshold was reduced in PCOS subjects. Similar results were obtained when normal weight PCOS women (n=13) were compared with normal weight healthy subjects. In the entire cohort of subjects, VO2peak was negatively associated with BMI, serum testosterone and insulin levels, and fat mass, and positively associated with insulin sensitivity. In multivariate models, VO2peak was independently predicted by PCOS diagnosis, fat mass and insulin levels (R2=0.68, p<0.0001). In conclusion, our data show that PCOS subjects have a striking alteration of cardiorespiratory fitness. This abnormality is associated with PCOS status, independently of insulin resistance and obesity.

PP118

RELATIONSHIPS BETWEEN BODY FAT, FAT DISTRIBUTION AND PHENOTYPES OF POLYCYSTIC OVARY SYNDROME

M. Dall'Alda[1], F. Tosi[1], D. Di Sarra[1], F. Zambotti[1], M. Donati[1], R. Moretta[1], A. Sacco[1], C. Bonin[2], G. Spiazzi[1], V. A. Giagulli[3], J. Kaufman[4], P. Moghetti[1]

[1]Endocrinology, Diabetes and Metabolism, University and AOUI Verona - Verona, Italy, [2]Obstetrics and Gynecology, University and AOUI Verona - Verona, Italy, [3]Unit of Metabolic Diseases and Endocrinology - Conversano, Italy, [4]Laboratory for Hormonology and Department of Endocrinology, University of Ghent - Ghent, Belgium

PCOS is a heterogeneous disorder characterized by endocrine, reproductive and metabolic abnormalities. Adoption of the current Rotterdam diagnostic criteria has introduced different PCOS "phenotypes", named Classic (C-PCOS, characterized by hyperandrogenism and oligoanovulation, with or without micropolycystic ovaries, PCO), Ovulatory (OV-PCOS, characterized by hyperandrogenism and PCO), and Normoandrogenic (NA-PCOS, characterized by oligoanovulation and PCO). Recent data suggested that these phenotypes could differ in terms of metabolic abnormalities, possibly due, at least in part, to differences in adiposity. To further investigate this issue, 91 PCOS women underwent accurate clinical, endocrine and metabolic assessment. Serum free testosterone was measured by mass spectrometry and equilibrium dialysis, insulin sensitivity by the glucose clamp technique, and body composition and fat distribution by DXA. In these women mean BMI (±SD) was 27.8±7.2 Kg/m2; insulin resistance (M-clamp value <11.75 mg/Kg fat-free mass x min) occurred in 65.5% of subjects. According to diagnostic criteria, 59 patients (65%) were C-PCOS, 15 (16%) OV-PCOS, and 17 (19%) NA-PCOS. These subgroups differed in terms of total body fat, fat distribution, M-clamp value and HDL-cholesterol. In the whole population, multiple regression analysis showed that insulin sensitivity was predicted by body fat, particularly truncal fat. However, when lean women only were analyzed, serum free testosterone was an independent predictor of insulin sensitivity. In conclusion, our data confirm that PCOS is associated with insulin resistance and indicate that PCOS phenotypes differ in terms of body fat and insulin sensitivity. Body composition and fat distribution are associated with the metabolic abnormalities of PCOS. However, hyperandrogenism may contribute to these alterations, particularly in lean women.

PP119

AEROBIC PHYSICAL ACTIVITY IMPROVES PLATELET ABNORMALITIES IN PCOS WOMEN

G. Muscogiuri[1], S. Palomba[2], P. Putignano[3], S. Savastano[1], G. Colarieti[4], G. Lombardi[1], R. Pivonello[1], G. B. La Sala[2], A. Colao[1], F. Orio[5]

[1]Dipartimento di Medicina Clinica e Chirurgia, Università "Federico II" - Napoli, [2]Unità di Ginecologia ed Ostetricia, Arcispedale Santa Maria Nuova, Istituto di Ricovero e Cura a Carattere Scientifico, Università di Modena e Reggio - Reggio Emilia, [3]Ambulatorio di Endocrinologia e Diabetologia, Presidio territoriale Ospedale San Gerardo - Monza, [4]SSD Tecniche di Sterilità, PMA, AOU "S. Giovanni di Dio e Ruggi d'Aragona" - Salerno, [5]SSD Tecniche di Sterilità, Endocrinologia dell'età fertile, AOU "S. Giovanni di Dio e Ruggi d'Aragona" , Endocrinologia, Dipartimento di Scienze Mot - Napoli, Salerno

Recent researches suggest that the increased thrombotic risk in women with polycystic ovary syndrome (PCOS) is due to platelet abnormalities, i.e. increased platelet count and MPV. These hematological alterations have been reported to correlate with the severity of insulin resistance. The aim of this study was to investigate the platelet abnormalities of women with PCOS and their changes after insulin sensitizing therapy.Sixty consecutive obese PCOS women (cases) were studied. PCOS diagnosis was performed according to Rotterdam criteria. Clinical, hormonal and metabolic parameters were assessed in each subject. Platelet count and MPV were also evaluated. PCOS women were randomized to metformin (500 mg twice daily, n=30) or aerobic physical activity (6 hours weekly, n= 30), monitoring the changes on platelet parameters at baseline and after 24 weeks of treatment.At the baseline platelet count (255±65 x 10^3/ml *vs.* 245±82 x 10^3/ml) and MPV (9.7±1.1 fl *vs.* 9.5±0.9 fl) were similar both metformin and aerobic physical activity group, respectively.After 24 weeks aerobic physical activity was associated with significant (p<0.05) lower total platelet count (221±75 x 10^3/ml *vs.* 258±71 x 10^3/ml,) and MPV (9.1±1.0 fl *vs.* 9.8±1.3 fl) compared to metformin group, respectively. It was observed similar reduction in insulin resistance [(HOMA): 3.2±0.9 *vs.*2.8±0.8 mU/ml and Glucose-to-Insulin Ratio (GIR): 5.6±1.2 mg/10^{-4}U *vs.* 6.2±1.4 mg/10^{-4}U] for both metformin and aerobic physical activity group, respectively].The change of platelet abnormalities were not related to the improvement of insulin resistance. No changes of platelet parameters were observed in the metformin group.In conclusion aerobic physical activity may exert a beneficial effects on platelet abnormalities women, yet the effects of metformin appear to be missing.

PP120

HORMONAL PROFILE, METABOLIC PATTERN AND QUALITY OF LIFE IN OBESE AND NORMAL-WEIGHT PATIENTS WITH POLYCYSTIC OVARY SYNDROME

A. Panico[1], G. A. Lupoli[1], M. Cacciapuoti[1], L. Barba[1], R. Lupoli[1], L. Coviello[1], N. Verde[1], F. Romano[1], A. Tortora[1], G. Lupoli[1]

[1]Dipartimento di Medicina Clinica e Chirurgia, Università degli Studi Federico II – Napoli - Napoli

INTRODUCTION: PCOS is the most common endocrine disorder in adult women. About 50% of PCOS patients are overweight or obese. Although the pathophysiological role of obesity in PCOS is well acknowledged, poor attention has been focused on its real impact on hormonal profile, metabolic pattern and patients' quality of life. **AIM**: To evaluate how obesity contributes to worsen the endocrine-metabolic profile and the quality of life of PCOS patients. **METHODS**: We recruited 100 pts aged 23.1±5.9 yrs: 50 with BMI ≥25 (Overweight PCOS, O-PCOS; 22.8±5.6 yrs; BMI: 29.8 (26.4-35.4) kg/m2) and 50 with BMI <25 (Lean PCOS, L-PCOS; 23.3±6.1 yrs; BMI: 22.5 (20.9-23.7) kg/m2). L-PCOS were compared with a healthy control group of 50 patients, age-matched, with a similar socio-economic background (Lean Controls, L-C, 24.1±7.7 yrs; BMI: 22.3 (21.6-23.5) kg/m2). Another control group of 50 overweight/obese women (Overweight Controls, O-C, 23.3±6.2 yrs; BMI: 27.4 (26.4-28) kg/m2) was compared with O-PCOS. All subjects underwent an evaluation including: LH; FSH; free testosterone (T); DHEAS; D4-Antrostenedione (A); 17-OH-Progesteron; total, LDL and HDL cholesterol (C); triglycerides (TG); TG/HDL; Glycaemia (G); Insulin (I); G/I; HOMA-IR; pelvic ultrasound. The degree of hirsutism and acne were evaluated with Ferriman-Gallwey's and Cremoncini's score, respectively. Furthermore, the quality of life was evaluated with PCOSQ (Polycystic Ovary Syndrome Questionnaire). **RESULTS**: Compared with L-C, L-PCOS showed higher levels of DHEAS, T, total-C, TG , HOMA-IR, I; lower levels of HDL-C (p<0,05) and a worse quality of life. Compared with O-C, O-PCOS showed a more severe metabolic (p<0,05 for HOMA-IR, I, TG, TG/HDL) and hormonal (p<0,05 for T, A, and LH) profile. They also showed a more marked hirsutism, a more frequent acne and a worse quality of life than L-PCOS. Evaluating the PCOSQ data, the most frequently affected domains were: Body weight (22%), Hirsutism (19%), Emotions (18%), Acne (18%), Infertility (17%), Menstrual disorders (17%). The most significant differences (p<0.001) between O-PCOS and L-PCOS were evidenced for Weight and Acne. **CONCLUSIONS**: Our study shows that obesity contributes to get worsen not only the phenotypic and endocrine-metabolic spectrum but also the quality of life of PCOS patients.

PP121

POLYCYSTIC OVARY SYNDROME: LONG TERM EVALUATION OF BLOOD PRESSURE, HORMONAL AND METABOLIC PARAMETERS AFTER ESTROPROGESTINIC THERAPY SUSPENSION

P. Sartorato[1], C. Sabbadin[1], F. Manganello[2], P. Zanon[1], S. Dalla Pria[2], G. Donà[3], L. Bordin[3], G. Clari[3], D. Armanini[1]

[1]Medicina-Endocrinologia - Padova, [2]Scienze Ginecologiche e della Riproduzione Umana - Padova, [3]Medicina Molecolare-Chimica Biologica - Padova

Polycystic Ovary Syndrome (PCOS) is associated with metabolic and hormonal imbalances and oxidative stress. Several studies underline the main role of aldosterone in the pathogenesis of these alterations. We studied 26 women affected by PCOS and overweight (BMI>24), who were treated with oral contraceptives (OCPs) for more than 6 years. We evaluated the variations of some clinical (blood pressure, BMI, Ferriman-Gallwey's score) and biochemical parameters (OGTT, testosterone, upright standing aldosterone and PRA, total cholesterol and triglycerides) at the moment of diagnosis and after 15 years. At the time of sampling patients had stopped the use of OCPs for 3 months. The results have been compared with basal values measured before the treatment. We found an improvement over time of hirsutism (Ferriman-Gallwey's score from 12±3 to 6±2) and a decrease of testosterone (from 122±76 to 73±35 ng/dL). Total cholesterol and triglycerides have not shown significant variations. Also the average BMI value were unchanged, but the number of obese patients (BMI>30) increased from 8 to 11. At the end of the study all patients underwent an OGTT test for glucose and insulin: all women were insulin-resistant (basal insulin: 18±14; after 60': 76±38; after 120': 57±35 UI/L) and 3 of them glucose intolerance. Finally, we compared average blood pressure values, upright standing aldosterone and PRA with those of 20 healthy women, matched for age and BMI. At the moment of diagnosis patients'average blood pressure values were normal but significantly higher than in controls (PCOS 96±7; controls 85±4 mmHg). At the end of the study, patients'average blood pressure appeared significantly higher both than their baseline values and than in controls (PCOS 100±11; controls 87±3 mmHg). PRA at the end of the study was not significantly different in the two groups. Aldosterone and aldosterone/PRA ratio (ARR) resulted significantly higher in patients (Aldosterone 26±14 ng/dL, ARR 9±5) than in controls (Aldosterone 12±3 ng/dL, ARR 3±2), although within normal range. Conclusions: in PCOS women, long term estroprogestinic therapy improves clinical/biochemical signs of hyperandrogenism, but worsens the metabolic profile and exposes to a higher risk of developing mellitus diabetes. The increase of aldosterone/PRA ratio compared to basal value may play a role in the well-known cardiovascular risk of these patients.

PP122

PREDICTIVE MARKERS OF COMPLETE RECOVERY OF GONADAL FUNCTION IN YOUNG WOMEN AFFECTED BY ANOREXIA NERVOSA

B. Altieri[1], T. Schirò[1], A. Capozzi[1], M. C. Fabiano[1], A. Pontecorvi[1], S. Della Casa[1]

[1]UOC di Endocrinologia e Malattie del Metabolismo, Università Cattolica del Sacro Cuore - Roma

OBJECTIVE: Anorexia Nervosa (AN) is an eating disorder affecting lots of adolescent characterized by a distorted body image and a striking fear for obesity that leads to a severe self-induced weight loss (less than 85% of normal weight for age and height). It usually causes some typical metabolic and hormonal complications such as prepubertal pattern of gonadotropin secretion which are frequent in other forms of hypothalamic anovulation. Weight gain and improvement of hormones should not produce the normalization of gonadal function. The aim of our study is to evaluate the link between hormonal changes and resumption of menses. METHODS: We enrolled 30 adolescent females (mean age 20,03±3,60 DS BMI 17,97±3,38 DS) with active or past diagnosis of AN according to DSM-IV. They were divided into three main groups: the first one is composed by 10 patients affected by active AN and amenorrhea (group 1); second and third groups consisting in patients with an old diagnosis of AN, who restored healthy weight, presenting respectively amenorrhea (group 2) and normal menses (group 3). We evaluated hormonal parameters (sexual steroids, serum cortisol, thyroid hormones, IGF-1, PTH) and markers of bone turnover (vitamin D, osteocalcin, β cross-laps). Total body BMD was determined using Dual Energy x-ray absorptiometry (DEXA). RESULTS: In group 1 we found a positive correlation between IGF-1 and BMI (r=0,06), fat mass (r=0,06) and osteocalcin (r=0,11); in group 2, we observed a positive correlation between IGF-1 and osteocalcin (r=0,01), BMI (r=0,01) and area under curve of LH during GnRH test (AUC LH) (r=0,04). Besides, In group 3, there was a positive correlation between estradiol and fat mass/lean mass ratio (r=0,04), IGF1 (r=0,03) and osteocalcin (r=0,044); osteocalcin was also positively correlated with BMI (r=0,06), fat mass/lean mass ratio (r=0,025), IGF-1 (r=0,04) and T-score (r=0,07). CONCLUSION: It is well known anovulation persists in up to 50% of anorexic patients even after achieving normal weight. Our data showed that the weight gain alone, measured as BMI, does not mean normalization of all compromised parameters and does not represent recovery from AN. Markers of bone metabolism resulted positively correlated with restoring of normal nutritional conditions, in particular IGF1 was correlated with recovery of gonadal function. This evidence may suggest that BMI plus osteocalcin may be a predictive marker of complete healing in recovering of AN.

PP123

VIDEODERMOSCOPIC EVALUETION OF SKIN ANDROGEN EXCESS IN POLYCYSTIC OVARY SYNDROME

L. Guccione[1], P. Di Giacinto[1], L. Chioma[1], M. Colicchia[1], G. Vancieri[1], E. Schiano Moriello[1], C. Moretti[1]

[1]UOC of Endocrinology and Diabetes Univ. of Rome Tor Vergata, Sect. of Repr. Endocrinology S. Giovanni Calibita Fatebenefratelli Hosp. Tiber Island - Rome

Videodermoscopy is a technique used by dermatologist to evaluate areas of reduced hair density, usually at the level of the scalp at X20 to X160 magnification. We have hypothesized that this instrument can be useful in evaluating the androgen dependent areas of hair growth at X10 magnification in women affected by hirsutism, to create a new and more objective scoring system of this pathology, considering that the Ferriman scoring system is limited by its operator's dependence. We have evaluated 98 patients affected by polycystic ovary syndrome at the basal level (no therapy) and after six and twelve months of estro-progestin plus anti-androgen therapy, creating a new videodermoscopic scoring system and comparing it to the Ferriman scoring. For each patient we have taken videodermoscopic's pictures of five androgen dependent areas: chin, chest, white line 4 cm under the umbilicus, sacrum, at basal, +6 and +12 months of therapy always evaluating the same area using anatomic land marks and counting the hair' follicles in an area of 2x2 squared cm at X10 magnification. This method allowed us to objectively follow the reduction of the hair density in the same body areas performing a precise count of the follicles, and it has shown a major sensibility compared to the Ferriman&Gallway system in evaluating the degree of hirsutism. We think that this new technique can be a helpful instrument for the physicians to precisely calculate the hirsutism degree ant the effects of the therapy, considering the strong impact that this pathology has on the affected women's quality of life.

PP124

FUNCTIONAL CHARACTERIZATION OF A NEW VARIANT IN THE HUMAN LUTEINIZING HORMONE RECEPTOR (HLHR) CAUSING LEYDIG CELL HYPOPLASIA

P. Duminuco[1], A. Vottero[2], V. Vezzoli[1], S. Peverelli[1], R. Minari[2], E. Pignatti[3], M. Simoni[3], S. Bernasconi[2], L. Persani[4], M. Bonomi[5]

[1]IRCCS Ist Auxologico Italiano, Lab di Ricerche Endocrino-Metaboliche - Cusano Milanino, [2]Dip di Med Clinica e Sperim, Univ di Parma - Parma, [3]Dip di Med, Endocrinologia, Metabolismo e Geriatria, Univ di Modena - Modena, [4]Univ di Milano, Dip di Scienze Clinice e di Comunità e IRCCS Ist Auxologico, UO di Medicina e Lab Ric Endocrino-metaboliche - Milano, [5]IRCCS Ist Auxologico Italiano, UO di Medicina ad indirizzo Endocrino-metabolico, Lab di Ricerche Endocrino-metaboliche - Milano

The hLHR is a member of the family A G protein-coupled receptor, that mediates, in the testis, the effect of LH on Testosterone biosynthesis. Indeed, inactivating mutation of the hLHR in male are known to be a cause of hypergonadotropic hypogonadism (HH) due to the gonadal resistance to the LH action. Here we report the case of a 13 years old adolescent that come to the attention of the pediatricians with micropenis and cryptorchidism. Hormone evaluation showed LH=10.9 IU/L; FSH=6.5 IU/L; Te total=<0.69nM/L with a conserved response to GnRH test (LH net increase=60.4; FSH net increase=3.86) and a slight increase of Testosterone to hCG stimulation (Te pre=<0.69 nM; post=1.49nM). At the age of 15 years old he presented LH=40.8 IU/L; FSH=16.0 IU/L; Te total= 4.09 nM/L. No other hormone deficit were present. Testis biopsy showed Leydig cell absence and germinal line elements were not observed. The sequence analysis of the hLHR gene showed the presence of a compound heterozygosity, being one variation, c.1847C>A p.S616Y, already described in association to HH, and the other, c.29 T>C p.L10P, a new identified variant. We then decide to pharmacologically characterized the p.L10P new variant by in vitro experiments. We subcloned this variant in a plasmid vector and we tested its ability to stimulate cAMP accumulation in COS-7 transfected cells by performing concentration-effect curves. The results showed a reduced Emax and an equal EC50 in comparison with the wild-type hLHR. We then tested its expression level at the plasma membrane by two different methods. The FACS analysis confirmed a marked reduction of the p.L10P variant with only a partial recovery of the expression after cell permeabilization. The western blotting analysis was also confirming the reduced amount of the variant protein. Our in vitro results demonstrate the pathogenic role of the p.L10P variant, which is to be considered a novel loss-of-function mutation significantly contributing to the Leydig cell hypoplasia of this patient.

PP125

ANTI-INFLAMMATORY EFFECTS OF VDR AGONISTS IN HUMAN SKELETAL MUSCLE CELLS

C. Crescioli[1], M. Sottili[2], C. Antinozzi[1], G. B. Vannelli[3], F. Romanelli[4], S. Minisola[5], V. Riccieri[6], G. Valesini[6], A. Lenzi[4], L. Di Luigi[1]

[1]Dep Mov Human & Health Sc, Foro Italico - Rome, [2]Exc C for Research, Transf and High Ed - Florence, [3]Dep of Anat, Hist and Forensic Med - Florence, [4]Dep of Exp Med, Sapienza - Rome, [5]Dep of Int Med and Med Disciplines, Sapienza - Rome, [6]Dep of Int Med and Cl Spec, Sapienza - Rome

The mainstay therapies for inflammatory myopathies (IMs), Th1-driven autoimmnune diseases, are corticosteroids and second-line immunosuppressants, aimed at suppressing the immune system. About 25% of the patients are not responders and left with disability. Skeletal muscle cells are now accepted as proper immunoactive structures contributing to the disease throughout the production of inflammatory mediators, such as the chemokine CXCL10. New therapeutic agents, targeting also the muscular component, would be desirable. Non or less hypercalcemic vitamin D receptor (VDR) agonists retain the same immunomodulatory properties as the native hormone without the typical side effects; among them, BXL-01-0029 and elocalcitol have been previously shown be eligible as novel immunosuppressants in autoimmune diseases or organ transplantation. The aims of this study are: to investigate in vitro the effect of BXL-01-0029 and elocalcitol onto IFNγ and TNFα-induced CXCL10 protein secretion by human skeletal muscle cells (Hfsmc) in comparison with immunosuppressants currently used for IM treatment (Mep, MTX, CsA, Infliximab, Lef); to assess in vivo CXCL10 level in sera of subjects at the time of diagnosis with IMs and before therapy, together with other Th1 type cytokines involved in muscular inflammation, such as IL-8, IL-6, IFNγ, TNF-α, MCP-1, MIP-1?, in comparison with healthy subjects. BXL-01-0029, similarly to elocalcitol, impaired at the highest potency IFNγ and TNFα-induced CXCL10 secretion by Hfsmc; only the IC50 of BXL-01-0029 was significantly different vs. the IC50s of all the current immunosuppressants. BXL-01-0029 targeted intracellular pathway downstream of TNFα, such as JNK, NF-kB and ERK1/2, which appeared highly engaged in CXCL10 secretion. In sera of IM patients at time of diagnosis, CXCL10 level was the highest vs. the other cytokines, and it was the only one significantly different vs. the matched healthy controls. All IM patients show low vitamin D serum level. In conclusion, our in vitro and in vivo data confirm the relevance of CXCL10 in IMs and suggest BXL-01-0029 as a novel pharmacological tool for IM treatment, possibly to be used in combination with the current immunosuppressants to minimize side effects.

PP126

LONG-TERM METABOLIC EFFECTS OF GH THERAPY IN CHILDREN WITH IDIOPATHIC GH DEFICIENCY

A. Ciresi[1], V. Guarnotta[1], R. Modica[1], M. Leotta[2], S. Radellini[2], C. Giordano[2]

[1]Section of Endocrinology, Diabetology and Metabolism (Di.Bi.M.I.S) - Palermo, [2]Section of Endocrinology - Palermo

Background Increased insulin levels during GH replacement therapy are often reported both in adults and in children with GH deficiency (GHD), although discordant data fully describe the behavior of metabolic parameters during GH therapy in children and overall the majority of published data are limited to the first 12 months of follow-up and in small populations. **Aim** To evaluate the long-term metabolic influences of GH replacement in a large selected cohort of prepubertal children with idiopathic GHD during GH therapy. **Subjects and Methods** Data of 194 prepubertal GHD children (150 M, 44 F; age 10.85 ± 2.77 yrs) were analyzed. Before GH therapy and yearly up to 36 months we measured body mass index (BMI), waist circumference (WC), IGF-1, total-, HDL- and LDL-cholesterol, triglycerides, glucose and insulin levels, Homa-IR and HbA1c. In a subgroup of 34 patients (28 M, 8 F) we performed yearly OGTT and calculated ISI-Matsuda (ISI), Insulinogenic Index (InsIn) and Oral Disposition Index (DIo). **Results** No subject showed overt dysglycemia or dyslipidemia at baseline and during GH therapy. Compared to baseline (17.62 ± 3.14 Kg/m2), BMI significantly increased after 12 (17.81 ± 3.36 Kg/m2; p<0.001), 24 (18.24 ± 3.22 Kg/m2; p<0.001) and 36 months (18.43 ± 3.58 Kg/m2; p=0.014), without significantly changes in WC. Glucose (88.24 ± 9.09 vs 82.52 ± 9.00 mg/dl; p<0.001), insulin (7.41 ± 4.40 vs 4.96 ± 4.20 mg/dl; p<0.047), Hba1c (5.07 ± 0.43 vs 4.78 ± 0.52 %; p<0.001) and triglycerides (73.62 ± 34.88 vs 66.59 ± 34.30 mg/dl; p=0.029) were significantly higher after 1 year of therapy when compared to baseline, although within the normal values, without additional increase at 24 and 36 months. Homa-IR significantly increased from baseline to 12 months (5.07 ± 0.43 vs 1.03 ± 1.01; p<0.001) with a subsequent significant reduction after 24 months (2.36 ± 1.64; p=0.020), without further modifications at 36 months (2.06 ± 1.45; p=0.242). In the subgroup of 34 children, ISI (7.22 ± 0.38), InsI (0.75 ± 0.04) and DIo (5.52 ± 1.34) did not significantly change during the entire follow-up. **Conclusions** A slight deterioration of glucose and lipid metabolism seems to occur during the first year of GH treatment, with a re-establishment in the subsequent years of follow-up, although no overt worsening in insulin sensitivity occurs. A longer follow-up could clarify the real long-term metabolic influences of GH therapy in GHD children.

PP127

GH DEFICIENCY IN THE TRANSITION PERIOD: BODY COMPOSITION AND GONAD FUNCTION.

M. Spaziani[1], L. Giovannini[2], F. Paggi[2], N. Tahani[1], M. Boscaro[2], A. Lenzi[1], A. Radicioni[1], G. Balercia[2]

[1]Department of Experimental Medicine, Sapienza University - Rome, [2]Medical Andrology, Endocrinology, Dept of Internal medicine and Applied Biotechnologies, Politechnic University of Marche, Umberto I Hospital - Ancona

Recombinant growth hormone therapy is normally administered to GH-deficient children in order to achieve a satisfactory height - the main target during childhood and adolescence. However, the role of GH does not end once final height has been reached, but continues during the so-called transition period. In this phase of life the body undergoes several changes, that culminate in adulthood. During this period, GH has a part in numerous metabolic functions. These include the lipid profile, where it increases HDL and reduces LDL, with the global effect of cardiovascular protection. It also has important effects on body composition (improved muscle strength and lean body mass, and reduced body fat), the achievement of proper peak bone density and gonad maturation with an important role in both gametogenesis and steroidogenesis. Retesting during the transition period, involving measurement of IGF-1 plus a provocative test (insulin tolerance test or GHRH + Arginine Test), is thus necessary to establish any persistent GH-deficiency requiring additional replacement therapy. In GHD patients, GH treatment during the transition period is recommended to achieve not only a good peak bone density but also full reproductive maturation and an optimal lean body mass/body fat ratio. Once the final height has been reached, it is imperative to re-test the GH/IGF1 axis in order to identify any remaining GH deficiency requiring replacement therapy.

PP128

THYROID FUNCTION IN NORMAL PRE-PUBERTAL CHILDREN AND PUBERTAL ADOLESCENTS AND IN OVERWEIGHT ADOLESCENTS: REFERENCE RANGES' DETERMINATION FOR CHILDHOOD

N. Tahani[1], M. Spaziani[1], G. Ruga[1], S. Granato[1], A. Semeraro[1], A. Anzuini[1], C. Piccheri[1], L. Tarani[2], A. Lenzi[1], A. F. Radicioni[1]

[1]Section of Medical Pathophysiology and Endocrinology, Department of Experimental Medicine, Sapienza University of Rome, Italy - Rome, [2]Department of Pediatrics, Sapienza University of Rome, Italy - Rome

Thyroid hormones are essential for normal pubertal growth, sexual development and reproductive function. They act on bone formation and resorption, on metabolism and oxygen consumption. Moreover T3 regulates testicular development, controlling Sertoli and Leydig cells proliferation and differentiation. The elevated activity of the thyroid gland during the pubertal period leads to a progressive modification in TSH, FT3 and FT4 concentrations. For this reason, reference ranges for TSH, FT3 and FT4 normally used in adults can not be applied during childhood and adolescence.

Aim of this study is to establish thyroid hormone reference ranges in pre-pubertal children, pubertal adolescents and adults and to evaluate if there are differences in thyroid function between overweight and normal weight pubertal individuals.

Serum concentrations of TSH, FT3 and FT4 were analysed in samples from 508 children and adolescents aged 6 to 18 years and 100 healthy adults aged 30 to 60 years and from 82 overweight pubertal adolescents, followed as endocrinological outpatients. None of them was affected by acute or chronic diseases, endocrine disorders or were under any kind of therapy. As data were not normally distributed, we compared these values through non-parametric tests for independent samples and the reference ranges were assumed to lie between the 2.5th and 97.5th percentile.

A regular and statistically significant reduction of TSH, FT3 and FT4 was observed in all normal groups with increasing age. In overweight patients, TSH levels were significantly higher than in normal weight subjects. This study revealed the importance to use specific thyroid hormone reference ranges in clinical practice during childhood and puberty.

This study was in part funded by the Italian Ministry of Health and the Italian Medicines Agency (AIFA): research project MRAR08Q009 on rare diseases.

PP129

PREVALENCE AND RISK FACTORS FOR ARTERIAL HYPERTENSION IN A POPULATION OF ITALIAN SCHOOLCHILDREN: A LONGITUDINAL STUDY.

A. Marolda[1], F. Prodam[1], A. Rapa[1], A. Monzani[1], S. Bellone[1], N. Fuiano[2], G. Diddi[2], G. Bona[1]

[1]Divisione di Pediatria-Dipartimento di Scienze della Salute-Università del Piemonte Orientale "A. Avogadro" - Novara, [2]Unità di Pediatria, Prevenzione e Salute Pubblica-ASL FG - Foggia

Introduction: Aim of our study was to assess the prevalence of hypertension in a population of Italian schoolchildren and identify risk factors.

Methods: 516 subjects (M/F=281:235), median age 6.2, were enrolled for a longitudinal 5-year study. For each child we collected weight, height, systolic and diastolic blood pressure, waist circumference (WC), anamnestic data and laboratory parameters.

Results: Prevalence of systolic (SH) and diastolic hypertension (DH) increased from 2.3% to 14.0% and from 12.8% to 41.1%, while obesity (OB) and overweight (OW) increased from 6.4% to 8.5% and from 23.6% to 26.9%. OB and OW children had a greater prevalence of SH and DH compared to normal weight (NW) ($p<0.002$). Prevalence of SH was 3.4% in NW, 10.0% in OW and 27.7% in OB children, while DH was 17.2% in NW, 25.6% in OW and 50.2% in OB children. The presence of OW, OB and WC >90th centile, were independently related to SH and DH ($p<0.0001$). Insulin resistance ($p<0.0006$), high uric acid levels ($p<0.02$), high triglycerides ($p<0.006$) and maternal history of obesity ($p<0.03$) were independently related to SH and DH. Snack consumption was associated with DH ($p=0.009$).

Conclusions: Prevalence of hypertension is rising not only in OB and OW children, but also in NW, showing that other factors could be involved in childhood hypertension.

PP130

ROUTINE SERUM STEROID PROFILING USING LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY BASED IVD REAGENT KIT

F. Settanni[1], A. Nonnato[2], M. Lucchiari[2], P. Puccinelli[2], S. Einaudi[3], L. Ghizzoni[1], F. Guaraldi[1], R. Granata[1], E. Arvat[1], G. Mengozzi[2], E. Ghigo[1]

[1]Dip Scienze Mediche, Divisione di Endocrinologia, Diabetologia e Metabolismo - Torino, [2]Dip Diagnostica di Laboratorio, Molinette - Torino, [3]Dip Pediatrica Specialistica, OIRM-Sant'Anna - Torino

The measurement of a complete steroid profile rather than individual analytes is a powerful tool in the diagnosis of steroid metabolism disorders. The immunoassays are widely used in clinical laboratories for the quantitative measurement of steroids, but these methods are not very specific. We validated an isotopic dilution liquid chromatography–tandem mass spectrometry method for the simultaneous measurement of 11-deoxycortisol, 17α-hydroxy-progesterone, androstenedione, corticosterone, cortisol, dehydroepiandrosterone sulfate, progesterone and testosterone in serum, based on IVD reagent kit from Perkin Elmer (Turku, Finland). We tested the advantages of steroid profiling measurement in 123 pediatric patients (84 healthy subjects and 39 with endocrine pathologies). A 96-well plate based assay involves a easy sample preparation, where 100 µl of serum were spiked with stable-isotope-labeled internal standards (ISs) and extracted by protein precipitation. This method uses an ultra-performance liquid chromatography system and a tandem mass spectrometer equipped with electrospray ionization (ESI) source (Acquity UPLC with TQ detector WATERS, Milford-USA). Analytes were separated on UPLC® with Acquity column, during 11 min gradient run and they were revealed by Multiple Reaction Monitoring (MRM) analysis. The preliminar results obtained in terms of accuracy, precision and lower limit of quantification (LLOQ), allows the use of this method for a routinely measurement of a wide steroid profile. The intra-assay precision (n=2) was <15% for all steroids escluded testosterone and progesterone (<20%). The newly standardized LC-MS/MS assay in kit-format allows the simultaneous determination of a wide steroid profile by a single run, in clinically relevant ranges, both in pediatric and adult age with good sensitivity, accuracy and precision. The importance of steroid profile in clinical diagnosis and the limit of routine immunoassay at low concentration were also highlighted. The easy sample preparation makes this method suitable for routine use in clinical laboratories.

PP131

THYROID HEMIAGENESIS: VARIABILITY OF PHENOTYPIC CHARACTERISTICS

R. Strano[1], R. Reale[1], R. Gelsomimo[1], C. Pace[1], S. Salamone[1], P. E. Macchia[2], D. Leonardi[1], F. Calaciura[1]

[1]Endocrinology Unit, Department of Clinical and Molecular Bio-Medicine, University of Catania Medical School, Garibaldi Hospital, Catania - Catania, [2]Department of Molecular and Clinical Endocrinology and Oncology, University of Naples "Federico II", Naples - Napoli

Thyroid hemiagenesis (TH) is a congenital abnormality in which one thyroid lobe (mostly the left one) fails to develop. We previously reported a 0,05% prevalence of TH in school children population screened by thyroid ultrasound. TH can also be detected by neonatal screening as a mild form of congenital hypothyroidism. Aim of this work is to analyse the clinical characteristics of patients with TH. During the last 20 years of screening for CH, our group identified 22 patients with TH. Hemiagenesis occurred in 12/22 female children with a F:M ratio of 1.2:1.0. 19 patients were picked up at the screening: 12 newborns at diagnosis presented hypothyroidism or hyperthyreotropinemia with high TSH levels (mean 83.1±102.4 mU/L; range 11.1-385.5) and low or low-normal FT4 values (mean 0.8±0.4 ng/dl; range 0.3-1.6); 7 newborns presented only a short lasting hyperthyreotropinemia (mean TSH 8.4±1.8 mU/L; range 4.7-9.5). Three additional patients were false negative at screening and diagnosis of TH occurred at 2-3 months of life. Of note, 68.4% of newborns were identified after year 2000 when the TSH cut-off was reduced to 10 mU/L and the second screening was routinely introduced. Since year 2000 in our CH screening program TH prevalence is 0.006% (13/216,502) that is almost 1/10 of our previously reported observation. Thyroid hemiagenesis was due to absence of the left thyroid lobe in 15/22 (68.2%) and agenesis of the right lobe in the remaining 7/22 subjects. FT4 concentrations at diagnosis were lower in newborns with right lobe hemiagenesis (1.1±0.4 vs 0.7±0.3, $p<0,05$). At re-evaluation at 3 years most patients showed a persistent hyperthyreotropinaemia or mild hypothyroidism. In pre-screening period the absence of one thyroid lobe usually was considered not to cause clinical symptoms by itself. Our patients show a phenotypic heterogeneity with a wide spectrum of thyroid dysfunction. In contrast to the already reported observation and our previous epidemiological data, in these patients diagnosed at CH screening a high percentage of agenesis of right lobe was detected. A right lobe agenesis seems to be associated with a more severe neonatal thyroid function abnormalities. Subjects with hemiagenesis (in late childhood or puberty) show a higher TSH level than controls of same age. This may be the reason for the higher frequency of hypothyroidism or other thyroid diseases in adult patients with TH.

PP132

A SECOND SCREENING FOR CH IS REQUIRED IN NEWBORNS AT HIGH RISK FOR DELAYED TSH INCREASE

R. Gelsomino[1], C. Pace[1], A. Mirone[1], G. Parrinello[1], R. Reale[1], S. Salamone[1], R. Strano[1], R. Vigneri[1], F. Calaciura[1], D. Leonardi[1]

[1]Endocrinology Unit, Department of Clinical and Molecular Bio-Medicine, University of Catania Medical School, Garibaldi Hospital, Catania - Catania

Screening procedures for congenital hypothyroidism (CH) based on neonatal TSH measurement have improved sensitivity progressively decreasing the TSH cut-off level. Using TSH measurement the main reason for missed CH cases is the "delayed TSH increase" commonly found in preterm and LBW newborns. A second delayed measurement, however, is not routinely used because of cost and frequency of transient CH in these infants. Some programs have attempted to identify high risk newborns to be targeted by routine re-screening, other reports indicate that a second measurement may not be necessary when using a lower TSH cut-off (screening threshold of 6 mU/l). We report our data of routinely second screening program, performed in a high risk newborns. In the period 2000-2011 the 15% of the entire newborn cohort screened (216,502) in our Center was submitted to a second paper sample (at week 2-4 of life) because of LBW and/or preterm birth, antibiotic therapy, first test positivity to Cistic Fibrosis and/or PKU. The TSH cut-off was 10 mU/L. 219/216,502 CH were identified. Among them, 43 (19.6%) were detected only at the second screening (mean TSH 40.6±49.2 mU/L, range 10-277). At first screening all the 43 newborns had TSH<10 mU/L (mean 4.5±2.4), 28/43 (65%) had TSH<6 mU/L. At recall all these 43 children presented high TSH levels (mean 66.6±74.7) and low or very low FT4 (mean 0.68±0.26 ng/dl). Among the 43 newborns 31 were preterm, 22 LBW or VLBW, 7 discordant twinings; 4 had received antibiotic therapy and 4 had Down syndrome. Thyroid ultrasound examination showed a thyroid in situ in all but one who had thyroid hemiagenesis. In the newborn population re-screened the prevalence of CH was very high (1:601). Late re-evaluation at age 3 years in 17/43 indicated that all (17/17) had transient CH with normal TSH values or persistent mild hypertireotropinaemia.

Conclusions: a) 43/219 (19.6%) CH would have been missed because "false negative" at the neonatal screening; b) also a screening lower threshold (as 6 mU/L) would have identified only 15/43 (34.8%) "false negative"; c) although CH is transient in most of these newborns, the presence of low FT4 levels during the neonatal period is known to be associated with impaired long term cognitive outcome. Our data, therefore, support a second screening test in newborns at high risk for delayed TSH increase as a routine procedure in CH screening centers.

PP133

A PRECOCIOUS GH PEAK AT GHRH PLUS ARGININE TEST IN GH SUFFICIENT SHORT CHILDREN IS PREDICTIVE OF A LOWER GROWTH VELOCITY

S. Bellone[1], F. Prodam[1], M. Castagno[1], G. Genoni[1], C. Fiorito[1], S. Esposito[1], A. Petri[1], G. Aimaretti[2], G. Bona[1]

[1]Div. di Pediatria, Dip di Scienze della Salute, Univ del Piemonte Orientale - Novara, [2]Endocrinologia, Dip. di Medicina Traslazionale, Univ. del Piemonte Orientale - Novara

Introduction: In children, GH secretion is considered sufficient when at least one value is >20 ng/mL at the GHRH + Arginine (ARG) test. Because GH typically peaks at 45 minutes, we evaluated whether peak occurrence at one specific time is predictive of clinical outcomes in short stature children who are GH sufficient.

Subjects and methods: Children who performed a GHRH plus ARG test for short stature were retrospectively recruited. Inclusion criteria were: 1) a GH peak > 20 ng/ml; 2) Tanner stages within 1-3 stages; 3) 1 year growth velocity since the test execution; 4) born adequate for gestational age; 5) the absence of signs suggestive of syndromes. Primary outcomes were height standard deviation score (SDS), growth velocity (GV), GVSDS and IGF-I SDS.

Results: 228 subjects were recruited, by which 14 were excluded because they did not satisfy inclusion criteria. Of 214 subjects, 121 (56.5%) had a peak at 45' min, 55 (25.7%) at 30' min, and 38 (17.8%) at 60' min. Subjects presented a peak at 30 min had lower height SDS ($p<0.05$), growth velocity ($p<0.001$), growth velocity SDS ($p<0.001$), and GH peak ($p<0.05$) than those had a peak at 45' min. Subjects presented a peak at 30' min had lower GV ($p<0.001$), and GVSDS ($p<0.001$), but higher GH peak ($p<0.05$) than those had a peak at 60' min. No differences were shown between children with a peak at 45' or 60' min. No differences in Tanner stages, sex, IGF-I SDS were recorded among three groups.

Conclusion: A peak at 30 minutes at the GHRH + ARG test in children who are short and without GH deficiency may be predictive of lower growth velocity in the year of the test. Because arginine infusion stops at 30 minutes, a somatostatinergic higher tone could have a role in the clinical picture.

PP134

24-HOURS BLOOD PRESSURE PROFILE IN ADULT GH DEFICIENCY

C. Moroni[1], L. Valente[1], M. D. Tinti[1], F. Lopreiato[1], S. C. Ferracane[1], R. Iuorio[2], M. D'Armiento[2], C. Gaudio[1], P. Gargiulo[2]

[1]Scienze Cardiovascolari, Sapienza Univ. - Roma, [2]Medicina Sperimentale, Sapienza Univ - Roma

Background- Few reports in the literature deal with the blood pressure profile of adults with Growth Hormone Deficiency (GHD). In this case-control study we evaluated the effects of GHD on Blood Pressure (BP) by 24 hours Ambulatory Blood Pressure Monitoring (ABPM).

Methods and Results- 31 consecutive patients (mean age 42.16±18.38 years, 20 men), with severe GHD (peak GH response to provocative testing <9 mU/l; mean disease duration: 31±13 months), not in GH replacement therapy, were recruited. A control group of 30 healthy subjects (mean age 40±15 years, 20 men) was considered for comparison. Exclusion criteria were: hypertension, previous cardiovascular disease, heart failure, ischemic heart disease, dyslipidemia, cigarette smoking, diabetes mellitus, other neoplasia, family history of heart disease, chronic obstructive pulmonary disease, renal failure, anemia. Physical examination, blood pressure measurement, ECG and 24-hour Ambulatory BP Monitoring were obtained. ABPM recordings were conducted using an A&D TM2430 device (measurements every 15 min from 06.00 to 23.00 and 30 min for the remaining period). GHD patients showed a mean 24-h Systolic BP (SBP) and Diastolic BP (DBP) of respectively 124.78±12.29 mmHg and 74.53±9.01 mmHg; mean daytime SBP: 128.18±12.86 mmHg; mean daytime DBP: 77.24±9.17 mmHg; mean night-time SBP: 111.14±11.58 mmHg; mean night-time DBP: 63.82±8.67 mmHg. Controls mean 24-h SBP and DBP were respectively 126.10±7.20 mmHg (p: 0.614 vs GHD) and 73.45±6.21 mmHg (p: 0.591 vs GHD); mean daytime SBP: 128.63±7.16 mmHg (p: 0.868 vs GHD); mean daytime DBP: 75.20±6.08 mmHg (p: 0.314 vs GHD); mean night time SBP: 114.72±9.15 mmHg (p: 0.189 vs GHD); mean night time DBP: 65.63±6.82 mmHg (p: 0.373 vs GHD). A BP nocturnal fall by 10 to 20% was detected in 35.5% GHD patients; 32.25% showed an "extreme dipping" pattern (nocturnal fall >20%) and 32.25% a non dipping pattern (nocturnal fall <10%). In the control group a non dipping pattern was observed in 10% subjects.

Conclusions- When comparing BP mean values, no significant differences were found between the two groups. By contrast, a remarkable prevalence of "unusual" BP profiles (involving prognostic implications) characterized by "non-dipping" and "extreme dipping" patterns was highlighted in adults with GHD ($p<0.03$ vs Controls).

PP135

ADULT GH DEFICIENCY AFTER MILD TRAUMATIC BRAIN INJURY

S. Giuliano[1], F. Nicoletti[2], C. Ceccotti[2], L. Bruno[1], S. Talarico[1], A. Mazza[1], A. Belfiore[1]

[1]Dipartimento della Salute U.O. Endocrinologia - Catanzaro, [2]Neurochirurgia Ospedale Pugliese - Catanzaro

Traumatic brain injury (TBI) is a worldwide health problem and a major cause of disability and death among young adults, as well as a cause of neuroendocrine dysfunction. It has been recently demonstrated that subjects evaluated several months or years following moderate/severe TBI may show hypopituitarism. In particular, growth hormone deficiency (GHD) occurs in 25-40% of these patients.

We aimed at evaluating whether mild (Glasgow Coma Scale score of 13-15) TBI may also cause GHD, and whether GHD occurrence changes with time after TBI.

Methods: Twenty patients (5 females; 15 males) were evaluated 7 years after mild TBI (group A) and 21 patients (5 females; 16 males) were evaluated 1 year after mild TBI (group B). In all patients, basal measurements of IGF-I, TSH, free thyroxine (FT4), free triiodothyronine (T3), LH, FSH, prolactin (PRL) and testosterone (in men) or estradiol (in women) were performed, as well as a combined growth hormone releasing hormone (GHRH) + arginine test. Glycemia and lipid profile were also evaluated.

Results: We found that severe GHD occurred in 5/20 patients (25%) of group A and in 6/20 patients (30%) of group B. Patients with GHD showed a deteriorated metabolic profile that was more pronounced in group A patients.

Conclusions: Our data indicate that even mild TBI may cause severe GHD in 25-30% of patients, with no significant difference between subject evaluated either 1 or 7 years after TBI. Therefore, screening of pituitary function is crucial even after mild TBI.

PP136

THE ITALIAN REGISTRY OF GH-TREATMENT (RNAOC): SETTING UP AN E-CRF AND A WEB-BASED PLATFORM FOR THE NATIONAL DATABASE OF GH PRESCRIPTIONS

F. Pricci[1], E. Agazio[1], D. Rotondi[1], C. Fazzini[1], F. Maccari[2], P. Roazzi[2], P. Panei[3]

[1]Dip. Biologia Cell. e Neuroscienze-Ist. Superiore di Sanità - Roma, [2]SIDBAE-Ist. Superiore di Sanità - Roma, [3]Dip. Farmaco-Ist. Superiore di Sanità - Roma

Background. Recombinant Growth Hormone (rGH) therapy is applied both in GH- and in non-GH-deficiencies. However, this therapy has not been exstensively analyzed by large clinical studies, in part because of lacking exhaustive databases. In Italy, a national registry (RNAOC) was established at the National Institute of Health (ISS), by a Ministerial Decree (GU n290/1993), and has been collecting all the national rGH therapies since late '80s, with the aims of exerting pharmacosurveillance. Nevertheless, comprehensive data are still incomplete, so that a computerized registry was planned at ISS by the Italian Agency of Medicines (AIFA). **Objective**. To set up a database capable of collecting accurate and complete data about GH treatment in Italy, dedicated form and web-based platform were configured. **Methods**. Since 1983 to 2009, the former non-electronic RNAOC collected autonomous medical records from clinical centers, in absence of specific forms or electronic submission. These data were archived in a dedicated electronic database. The novel computerized RNAOC was based firstly on the definition of the electronic-Case Report Form (e-CRF), based on a minimum data set, identified by a national expert panel relying on the scientific literature (relevant clinical trials or international guidelines) and on the warnings by AIFA. This e-CRF contains the selected information, with tutoring about data format, and multiple options and controls everywhere these could be done. Subsequently, the e-CRF was developed in a web-based protocol, offered to specialist clinical units and Regions in the 2010 and at present is operative. The data management is in respect of privacy law. **Results.** The non-electronic database included 4371 total GH-treated patients, lacking diagnosis in 81.6% and domicile in 53% of the records. Reliable clinical and pharmacological data on GH-treatment for the evaluation of efficacy or safety of rGH-treatment were really scarce. The current web-based e-CRFs include 866 GH-treated patients, with missing data about diagnosis in 17.2% and about domicile in 2% of the records. These data are quite complete with the specific diagnostic and clinical markers as requested by AIFA regulation. **Conclusions.** The web-based Italian Registry of GH-Treatment offers the opportunity of exerting adequate public health surveillance on this treatment, allowing to monitor its use, in terms of adequacy and safety, and its abuse, in terms of appropriateness.

PP137

MODERATE HYPONATREMIA IS ASSOCIATED WITH AN INCREASED RISK OF OVERALL MORTALITY: A COMPREHENSIVE META-ANALYSIS

G. Corona[1], C. Giuliani[2], G. Parenti[2], D. Norello[2], J. G. Verbalis[3], G. Forti[2], M. Maggi[4], A. Peri[2]

[1]U.O Endocrinologia, Ospedale Maggiore - Bologna, [2]U.O Endocrinologia, Università di Firenze - Firenze, [3]Division of Endocrinology and Metabolism, Georgetown University - Washington, [4]U.O di Medicina della sessulità e Andrologia, Università di Firenze - Firenze

Introduction. Hyponatremia is the most common electrolyte disorder in clinical practice, and evidence to date indicates that severe hyponatremia is associated with increased morbidity and mortality. The aim of our study was to perform a comprehensive meta-analysis that included all the published studies that compared mortality rates in subjects with or without hyponatremia of any degree. Methods. An extensive Medline, Embase, and Cochrane search was performed to retrieve all studies published up to October 1, 2012 using the words “hyponatremia” and “mortality”. Results. Eighty-one studies satisfied inclusion criteria encompassing a total of 850,222 patients, of whom 147,948 (17.4%) were hyponatremic. Across all 81 studies, hyponatremia was significantly associated with an increased risk of overall mortality (RR=2.60[2.31-2.93]). Hyponatremia was also found to increase the risk of mortality in patients with multiple diseases, including myocardial infarction (RR=2.83[2.23-3.58]), heart failure (RR=2.47[2.09-2.92]), cirrhosis (RR=3.34[1.91-5.83]), pulmonary infections (RR=2.49[1.44-4.30]), mixed diseases (RR=2.50[1.97-3.18]), and in hospitalized patients in whom the diagnosis was not specified (RR=2.48[2.09-2.45]). A mean difference of serum [Na+] of 4.8 mmol/L was found in subjects who eventually died compared to survivors (130.1±5.6 vs 134.9±5.1 mmol/L, p<0.001). Furthermore, a meta-regression analysis showed that the hyponatremia-related risk of overall mortality was inversely correlated with serum [Na+] (S=-0.096[-0.114;-0.077]; I=13-710[12.258-16.161];both p<0.0001). This association was confirmed in a multiple regression model after adjusting for age, sex, and associated morbidities such as diabetes mellitus. Conclusions This meta-analysis shows for the first time that even moderate serum [Na+] decreases are associated with an increased risk of mortality in commonly observed clinical conditions across large numbers of patients.

PP138

EFFECTS OF GROWTH HORMONE THERAPY ON GLUCOSE METABOLISM IN ADULT PATIENTS WITH PRADER-WILLI SYNDROME

G. Grugni[1], A. Crinò[2], A. Sartorio[1]

[1]Division of Auxology - IRCCS Istituto Auxologico Italiano - Piancavallo (VB), [2]Unit of Autoimmune Endocrine Diseases - IRCCS Osp. Pediatrico Bambino Gesù - Rome

INTRODUCTION: The clinical picture of Prader-Willi syndrome (PWS) in adulthood strongly supports the presence of GH deficiency (GHD). Apart from short stature, both PWS and GHD are characterized by impaired physical strength, decreased left ventricle mass, reduced bone mineral density and an abnormal body composition. Moreover, reduced GH stimulated levels has been reported in a significant proportion of these patients. Consequently, GH therapy (GHT) is actually suggested as a possible treatment option in PWS adults. In such patients, however, it is necessary to consider the potential diabetogenic effect of GHT, since they tend to develop type 2 diabetes.
OBJECTIVE: The aim of this study was to investigate the effects of GHT on glucose and insulin homeostasis in a group of PWS adults. PATIENTS AND METHODS: Thirteen subjects with genetically confirmed PWS, 9 males and 4 females, aged 19-35 years, participated to the study. After baseline evaluation, the experimental protocol encompassed a 24-month GHT. Before starting GH treatment (BAS), and after 12 and 24 months of treatment, glucose, insulin, HOMA-IR, glycosylated hemoglobin (HbA1c), and IGF-I levels were determined. RESULTS: Between BAS and after 12 and 24 months of GHT, IGF-I increased significantly (103.5+/-14.3 mcg/L vs 318.9+/-38.8 mcg/L vs 245.2+/-28.4 mcg/L, respectively: p<0.0001) (mean+SE). On the other hand, no significant change was observed for glucose (80.5+/-2.7 mg/dl vs 86.2+/-2.7 mg/dl vs 81.0+/-2.9 mg/dl, respectively), insulin (12.4+/-1.7 mU/L vs 16.4+/-3.0 mU/L vs 14.1+/-2.1 mU/L, respectively), and HOMA-IR (2.50+/-0.34 vs 3.62+/-0.75 vs 2.93+/-0.53, respectively) values. At one year of GHT HbA1c levels were significantly decreased when compared to BAS (5.4+/-0.1% vs 5.6+/-0.1%: p<0.04), but at two years were unchanged (5.5+/-0.2%). Glucose levels remained within the normal range in all of our PWS at all times, with the exception of one subject showing impaired fasting glucose at one year. At baseline, 5 patients had values indicating increased insulin resistance. After 12 and 24 months of GHT, 6 individuals had a HOMA-IR above 2.77. CONCLUSION: Our data seem to support the view that GHT exerts few negative effects on carbohydrate metabolism in PWS adults. However, the high prevalence of insulin resistance makes vigorous monitoring of glucose and insulin homeostasis in these patients mandatory.

PP139

EFFECTS OF GENDER AND BODY COMPOSITION ON GH RESPONSE TO GHRH PLUS ARGININE (GHRH+ARG) IN HIV-LIPODYSTROPHIC PATIENTS

G. Brigante[1], C. Diazzi[1], G. Ferrannini[1], A. Ansaloni[1], L. Zirilli[1], G. Guaraldi[2], V. Rochira[1]

[1]Chair and Unit of Endocrinology & Metabolism, Department of Biomedical, Metabolic and Neural Sciences, University of Modena & Reggio Emilia - Modena, [2]Metabolic Clinic, Infectious and Tropical Disease Unit, Department of Medicine and Medical Specialties, University of Modena & Reggio Emilia - Modena

Background: GH response to GHRH+Arg is impaired in HIV-infected men and women, compared to gender matched controls. Moreover, reduced GH secretion seems to occur more frequently in HIV-infected males than females. **Methods:** To determine gender effects on GH secretion in HIV-infected patients with lipodystrophy, we compared GH/IGF1 status and body composition in 103 males and 97 females. A standardized GHRH+Arg test was performed. Anthropometric measurements were obtained by means of BMI, waist/hip, Dual-Energy-X ray-Absorptiometry (DEXA) and abdominal CT scan.
Results: Considering the threshold of GH peak of 7.5 mcg/L, 21% of women and 38% of men demonstrated an impaired GH peak. Comparing males and females with insufficient GH peak, they did not differ with regard to BMI, fat mass measured by DEXA (total, at arm, at leg, at trunk) and VAT, SAT and TAT measured by CT. However, men showed higher values of VAT/SAT and VAT/TAT ratios (p<0.05). The intra-gender comparison showed that body composition was not significantly different between women with GH peak≤7.5 and >7.5 mcg/L. Conversely, men with GH deficiency had higher values of trunk fat mass at DEXA and of VAT and TAT at CT (p<0.05), compared to men with normal GH peak. **Conclusions:** Impaired GH response to GHRH+Arg is very common in HIV-lipodystrophic subjects. Men demonstrate a higher rate of GH deficiency compared to women. Adipose tissue seems to influence GH peak in males more than in females. However, distribution of adipose tissue more than fat mass *per se* seems to have a role in the upset of GH/IGF1 status in these patients. Both in men and women body composition changes alone do not fully account for gender differences in GH secretory response in HIV-infected patients. Thus, an impairment of hypothalamic-pituitary function due to other factors (eg. viral infection, antiretroviral drugs) could not be ruled out.

PP140

THE INTRACELLULAR ENERGY SENSOR AMP-ACTIVATED PROTEIN KINASE (AMPK) IN GH-SECRETING PITUITARY TUMOR CELLS

G. Tulipano[1], L. Faggi[1], A. Cacciamali[1], M. Spinello[2], M. Losa[3], D. Cocchi[1], A. Giustina[4]

[1]Medicina Molecolare e Traslazionale, Univ. di Brescia - Brescia, [2]Novartis Farma - Origgio (Va), [3]Neurochirurgia, Istituto Scientifico S.Raffaele - Milano, [4]Endocrinologia, Dipartimento Scienze Cliniche e Sperimentali - Brescia

AMP-activated protein kinase (AMPK) is a cellular sensor of low energy stores and has a role in the regulation of cell growth. The efficacy of mTOR inhibitors (rapamycin and everolimus) is being actively studied in neuroendocrine tumors, including pituitary GH-secreting tumors. Reportedly, AMPK activators may represent an alternative way to inhibit mTOR in tumor cells. Our research group has recently investigated the effects of the AMPK activator AICAR on cell viability and GH secretion in rat pituitary tumor cells (GH3 and GH1 cells) and in primary cultures from human GH-secreting pituitary adenomas. In GH3 and GH1 cells, we showed an inhibitory effect of AICAR on cell viability and cell proliferation. AICAR decreased p70S6-kinase activity, as assessed by phospho-S6 levels. Noteworthy, p70S6K is one of the main downstream effectors of mTOR. The effect of AICAR did not result in cross-reactivation of ERK1/2 pathway. In fact, AICAR caused a marked decrease in serum-induced ERK activation after treatment of GH3 cells with serum. somatostatin-14 did not affect significantly p70S6K activity but it was able to enhance the inhibitory effect of AICAR on phospho-S6 levels. Moreover, AICAR and SS-14 reduced ERK phosphorylation with a different time course and the combined treatment reduced phospho-ERK levels at any time point. As to the regulation of AMPK activity by metabolic and endocrine factors, we showed that inhibition of glycolysis by 2-deoxyglucose induced AMPK activity whereas treatment with the estrogen receptor antagonist fulvestrant reduced AMPK activity in GH3 cells. Primary cultures of somatotrophs from human pituitary tumors were less sensitive to AICAR inhibitory activity on cell viability vs GH3 cells. Indeed, four adenomas out of 15 were responsive to AICAR (0.4 mM) and five adenomas were responsive to SS-14 (100 nM). One adenoma was responsive to both somatostatin and AICAR. Interestingly, in two adenomas which were not responsive to either AICAR or SS-14, the cotreatment was able to reduce cell viability. As to the effects on GH secretion, nine adenomas out of 15 were responsive to AICAR. Twelve adenomas were responsive to SS-14. Cotreatment exceeded the effect of single treatments in 4 out of 10 adenomas.

PP141

MUSCLE SYMPATHETIC NERVE ACTIVITY IN ACROMEGALY: EFFECTS OF SOMATOSTATIN ANALOGUE TREATMENT

C. Carzaniga[1], G. Seravalle[2], R. Attanasio[3], G. Grassi[4], R. Cozzi[5], L. M. Fatti[2], M. Montini[6], G. Vitale[1], G. Sciortino[1], S. Damanti[7], M. Scacchi[1], G. Mancia[8], F. Cavagnini[9], L. Persani[1]

[1]Dip.to di Scienze Cl. e di Comunità, Univ. di Milano - Milano, [2]Ist. Auxologico Italiano IRCCS - Milano, [3]Endocrinologia, Ist. Galeazzi - Milano, [4]Dip.to di Medicina Clinica e Prevenzione, Univ. di Milano Bicocca - Milano, [5]Div. di Endocrinologia, Osp. Niguarda Cà Granda - Milano, [6]Humanitas Gavazzeni - Bergamo, [7]Scuola di Spec. di Geriatria, Univ. di Milano - Milano, [8]Dip.to di Med. Cl. e Prevenzione, Univ. di Milano Bicocca - Milano, [9]Lab. di Ric. Neuroendocrinologiche, Ist. Auxologico Italiano - Milano

We have recently described a marked sympathoinhibition in newly diagnosed acromegalic patients (Seravalle et al., Clin End 77:262, 2012). We have interpreted this unexpected finding, rather surprising in a clinical condition characterized by increased cardiovascular mortality, as an adaptive phenomenon. In rodents, central administration of somatostatin (SS) has been shown to inhibit peripheral sympathetic outflow (Rettig et al., Am J Physiol 257:R588, 1989). Based on the above, we elected to study muscle sympathetic nerve activity (MSNA) in acromegalic patients before and during treatment with SS analogues (SSA). Study. MSNA was directly measured by microneurography in the following groups of subjects: a) 24 newly diagnosed acromegalics (13 men & 11 women, mean age 45.5±13.0 years); b) 22 patients on SSA, 11 of whom (7 men & 4 women, mean age 52.4±13.9 years) attaining biochemical control according to the currently accepted criteria and 11 (5 men & 6 women, mean age 56.4±17.5 years) not attaining biochemical control; c) 17 normal weight healthy subjects serving as controls (11 men & 6 women, mean age 49.1±15.6 years). Results. As expected, mean MSNA was significantly lower in untreated acromegalic patients than in control subjects (18.3±8.39 vs 37.8±6.60 bursts/min, $p < 0.01$). Patients on SSA, either with controlled or uncontrolled disease, displayed mean MSNA values (27.4±8.24 and 31.6±3.27 bursts/min, respectively) significantly lower than those shown by controls ($p < 0.01$) but significantly higher than those found in untreated acromegalics ($p < 0.05$). Mean MSNA values were not significantly different between controlled and uncontrolled SSA-treated patients. Comment. The present study has confirmed the profound sympathoinhibition characterizing untreated acromegaly and has shown the reversibility of this alteration with the improvement of the disease. These preliminary data do not allow to unveil a possible role of SSA in these changes.

PP142

ANTERIOR PANHYPOPITUITARISM DUE TO NEUROSARCOIDOSIS UNMASKED BY SEXUAL DYSFUNCTION IN A YOUNG ADULT MAN

S. Vezzani[1], C. Diazzi[1], V. Rochira[1]

[1]Chair and Unit of Endocrinology & Metabolism, Department of Biomedical, Metabolic and Neural Sciences, University of Modena & Reggio Emilia - Modena

INTRODUCTION: Brain localization of sarcoidosis is rare (5-15%). The involvement of the hypothalamic-pituitary (HP) unit occurs in less than 1% of patients, causing often visual defects and LH, FSH and GH deficiency. TSH and ACTH are relatively preserved. Male sexual dysfunction (SD) is often related to hypogonadism (H), but is more frequently due to other causes. Neurosarcoidosis (NS) diagnostic categories (definite, probable, possible) depend from neurologic features, results of neuro-diagnostic evaluation and histological confirmation. CASE REPORT: A 35 years old man with SD (loss of libido and erectile dysfunction dated 6 years). The past history was uneventful, with only chronic rhinitis. At physical examination eunuchoid habitus, small testes and reduced virilization were detected. Biochemical and hormonal investigation showed hypogonadotropic H, hypocortisolism, central hypothyroidism, low IGF1, leukocytopenia and mild normocytic anemia. Brain MRI showed a solid lesion at the proximal side of the pituitary stalk, a mild edema of hypothalamic region and a lesion at the bulbo-medullary site. ACE serum levels were elevated. Enlarged hyilary limph nodes, vertebral skeletal lesions and diffuse linfoadenomegaly were detected at radiological examination. The histological examination of a hilary lymph node and the bone marrow were in line with a possible sarcoidosis, but not diagnostic. After excluding lymphoma, the patient was treated ex-juvantibus with oral prednisone. Glucocorticoid, levothyroxine and androgen replacement therapy were progressively started. RESULTS: After 3 months libido improved and chronic rhinitis disappeared. A normalization of blood count parameters and ACE serum levels was obtained. A severe GH deficiency was detected at GHRH+Arg test after normal serum testosterone restoration. At MRI: quite complete remission of the neurological lesions. CONCLUSIONS: The diagnosis of NS is not simple and this case report is anecdotal since possible NS was unmasked by signs and symptoms of H, more specifically by SD. As NS has often a worse prognosis than systemic sarcoidosis, early diagnosis and precocious steroid treatment are challenging. In this patient, NS caused a complete loss of function of pituitary. The beneficial effects of corticosteroid treatment on brain NS lesions, however, leaves unsolved the possibility of HP axes recovery, that will be retested after progressive reduction of replacement treatments. Patient's fertility will require gonadotropins treatment in the case of lack of gonadal function recovery.

PP143

ADULT-ONSET GH DEFICIENCY IN PATIENTS WITH ADDISON'S DISEASE

M. Mendola[1], E. Passeri[1], A. Dolci[2], M. Minotti[2], A. Tufano[1], M. Arosio[2], S. Corbetta[1], B. Ambrosi[1]

[1]Endocrinology and Diabetology Unit, Dept. of Biomedical Sciences for Health, University of Milan, IRCCS Policlinico San Donato - San Donato M.se, Milano, Italy, [2]Endocrinology Unit, Dept. of Clinical Sciences and Community Health, University of Milan, Ospedale San Giuseppe - Milano, Italy

Introduction: while excessive glucocorticoids undoubtedly inhibit GH secretion, the exact role played by chronic hypoadrenalism on GH secretion is not well defined. The aim of the present study is to evaluate GH secretory status in patients with Addison's disease (AD).

Materials and methods: we studied 14 AD patients (6M and 8F, age 49.9±13.7 years, mean+SD, BMI 24.4±3.2 kg/m²). Adrenal autoantibodies were positive in 12 of them. 11 AD patients were affected with autoimmune polyendocrine syndrome (APS) type 2; 3 patients were affected with isolated AD (i-AD). All AD patients were treated with cortisone acetate (25-37.5 mg/day) or hydrocortisone (10-25 mg/die); 12 of them were also given fludrocortisone (0.05-0.1 mg/day). 9 patients were given L-T4 or sex steroid as needed. In all subjects GH responses after GHRH+Arginine stimulation were evaluated. IGF-1 levels were calculated as SD score (IGF-1 SDS).

Results: 7 out of 14 AD patients showed an impaired GH response after GHRH+Arginine stimulation. In 3 cases there was a partial GH deficiency (GHD; GH peak 10.7 ± 0.3 ng/ml), while 4 patients had a severe GHD (GH peak 4.2 ± 1.1 ng/ml). 5 of the non-responder patients had APS type 2, while 2 were i-AD. As expected, GH peaks negatively correlated with BMI ($r = -0.57$; $p<0.01$). No correlation was found between GH peak and glucocorticoid doses. IGF-1 SDS in GHD patients were lower than in those with normal GH secretion (-3.7 ± 2.5 vs -1.1 ± 1.5, $p < 0.04$): 6 out of 7 GHD patients showed IGF-1 SDS<-2, while only 2 patients with a normal GH response had low IGF-1 SDS levels. IGF-1 SDS significantly correlated with peak GH levels ($r=0,55$; $p<0.01$). No correlation between cortisol and ACTH levels and GH response to the stimulus was found.

Conclusions: the present study demonstrates that GH insufficiency may occur in Addisonian patients, independently from the etiology of the disease, even if the extent of the defect is different. Thus, a dynamic evaluation of GH secretion, besides a complete hormonal and autoimmune study, is needed in order to evaluate the possible benefits of rh-GH therapy in Addisonian patients with GHD.

PP144

DIAGNOSIS AND COMPLICATIONS OF CUSHING'S DISEASE: GENDER-RELATED DIFFERENCES.

M. Zilio[1], V. Camozzi[1], N. Albiger[1], S. Koutroumpi[1], F. Ceccato[1], M. Barbot[1], L. Mazzai[1], F. Mantero[1], C. Scaroni[1]

[1]Dipartimento di Medicina DIMED, Università di Padova - Padova

Introduction: Cushing's disease (CD) presents a remarkable preponderance in female gender, with a female to male ratio of 3-8:1. The aim of this study was to evaluate gender-related differences in the presentation of CD, as regards: biochemical indices of hypercortisolism; sensitivity of diagnostic tests; clinical features and complications of disease.

Methods: We retrospectively studied 84 aduly patients with CD, 67 women and 17 men (mean age: 42 years, range: 15 - 70), evaluated during active phase of disease. We compared male and female patients both as a whole and after dividing them according to age (less or more than 50 years).

Results: We observed no differences between male and female patients as regards age at diagnosis, disease duration and BMI. Men, compared to women, presented higher urinary free cortisol values (median: 4.27 vs 2.35 times the upper limit of normality, $p<0.001$) and ACTH values (median: 66.8 vs 43.2 ng/L, $p<0.05$). As regards the tests performed to establish the diagnosis of CD, men presented a significantly lower ACTH response to DDAVP stimulation (responsive patients: 47% vs 77%, $p<0.05$). The pituitary tumor itself was less easily visualized by pituitary MRI in males compared to females (percentage of visible microadenomas: 46% vs 79%, $p<0.05$). Furthermore, some complications of disease were more frequent or more severe in men, in particular hypokalemia (41% vs 12%, $p<0.05$) and osteoporosis at lumbar spine (59% vs 20%, $p<0.01$), with consequent higher risk of vertebral fractures. Among younger patients (< 50 years), males also presented a more severe degree of hypertension and a higher prevalence of carotid atherosclerotic plaques.

Conclusions: Although CD is less frequent in male patients, in this gender it presents with more florid clinical manifestations and may imply more diagnostic difficulties. Gender differences are especially evident among patients aged less than 50 years.

PP145

IMPACT OF VITAMIN D DEFICIENCY AND OF GH/IGF-I AXIS ON CARDIOVASCULAR RISK IN HYPOPITUITARIC PATIENTS

M. C. Savanelli[1], E. Scarano[1], V. Brunelli[1], A. Colao[1], C. Di Somma[1]

[1]Medicina Clinica e Chirurgia Sezione di Endocrinologia Università "Federico II" - Napoli

Background: Experimental and epidemiological studies in healthy people have shown an association between the axis GH-IGF-I and the vitamin D. Aim: To assess a possible correlation between GH/IGF-1 axis, vitamin D and cardiovascular risk. Patients and methods: 41 hypopituitary patients with GHD (22 M, 19 F, age 18-84 years) and 41 controls comparable to patients by sex, age and BMI. In all patients and controls, we determined: height, weight, waist circumference, BMI, blood pressure (BP), blood glucose, HbA1c, total, HDL- and LDL-cholesterol, triglycerides, PTH, 25-OH-vitamin D, the use of specific drug therapy for hypertension, hypercholesterolemia and / or hypertriglyceridemia and diabetes mellitus (DM), GH peak after GHRH + ARG and IGF - 1. The presence of metabolic syndrome (MS) was evaluated by the IDF criteria. Results: The vitamin D were lower in patients than in controls (21.3 ± 12.3 vs 28.2 ± 9.4, p = 0.006). A deficiency of vitamin D (<20 ng / ml) was found in 51% vs. 14.6% (p = 0.000), insufficiency (20-30 ng / ml) in 26.8% vs 41.4% (p = 0.27) and normal vitamin D (> 30ng/dl) in 21.9% vs 43.9% (p = 0.06), respectively in patients and controls. The prevalence of dyslipidemia was 51.2% vs. 12.1% (p = 0.09), DM was 7.3% vs 17% (p = 0.292) of hypertension was 44% vs 22% (p = 0.06), MS was 17% vs. 14.6% (p = 0,957) respectively in patients and controls. In both groups there was a significant correlation between IGF-I, age, vitamin D and SBP. At the multiple regression, in both groups, the greater predictor of high values of SBP were the levels of IGF-I (t = -2.69, p = 0.011, t = -0.18, p = 0.018; respectively). At logistic regression only in patients we found a significant association between IGF-1 and vitamin D deficiency and dyslipidemia and hypertension, but not with DM. The MS was significantly associated only with vitamin D. At the multiple logistic regression, vitamin D was associated with dyslipidemia and hypertension. Conclusion: The GHD hypopituitaric patients have lower vitamin D levels and a more severe vitamin D deficiency compared with control subjects. Also, in these patients, the vitamin D deficiency is more associated with the presence of cardiovascular such as dyslipidemia, hypertension, and metabolic syndrome. Thus, it can be assumed that the vitamin D deficiency may represent an additional risk factor to the already known effects of hypopituitarism for cardiovascular diseases.

PP146

A RARE CASE OF SOMATOTROPIN AND THYREOTROPIN SECRETING PITUITARY ADENOMA

A. Tumminia[1], M. Russo[1], D. Maiolo[1], P. Tita[1], G. Parrinello[1], S. Squatrito[1], D. Gullo[1]

[1]Endocrinology, Dept of Clinical and Molecular Biomed., Garibaldi-Nesima Hospital - Catania

Introduction. TSH-secreting tumours account for less than 1% of all pituitary tumours. About 18 % of TSHomas co-secrete GH and show variable clinical features ranging from clinically silent to the expression of both acromegaly and thyrotoxicosis signs and symptoms. We report a rare case of a mixed somatotropin and thyreotropin macroadenoma with prominent clinical and biochemical features of acromegaly apparently unassociated with hyperthyroidism. **Case report.** A 48-year-old man was admitted with symptoms and clinical signs of acromegaly. In recent years he had noticed a gradual acral growth and erectile disfunction, whereas no complaint of sweating, palpitation, tremors or other symptoms of hyperthyroidism was reported. Physical examination revealed acromegaloid habitus, macroglossia, prognathism and acral enlargement. He had a pulse rate of 72 bpm, blood pressure 125/75 mmHg, dry skin and a palpable goiter. Laboratory data showed failure of GH suppression during OGTT (GH nadir 9.8 ng/ml) and normal glucose tolerance; IGF-1,217 ng/ml (54-336), cortisol 1.0 µg/dl (3.7-19.4), LH 0.5 mUI/ml (1.2-10), testosterone 0.3 ng/ml (2.7-9.6), PRL 14.6 ng/ml (1.2-22.9), TSH 2.2 µU/ml (0.3-4.9), FT3 4.2 pg/ml (1.7-3.7), FT4 1.6 ng/dl (0.7-1.5). Pituitary MRI revealed a 38x40x43 mm mass with supra-infra and parasellar involvement, optic chiasm compression and cavernous sinus invasion. Visual field examination was normal. Thyroid ultrasound demonstrated a diffuse goiter. Administration of 100 µg octreotide resulted in a reduction of 81.7% and 73% in GH and TSH levels, respectively. A diagnosis of GH/TSH cosecreting pituitary macroadenoma with partial hypopituitarism (hyposurrenalism and hypogonadism) was therefore made. Surgical removal of the tumor was not indicated and medical treatment with lanreotide 120 mg s.c. every 28 days plus cortisone acetate and depo-testosterone was started. After three months the following results were obtained: IGF-1 866 ng/ml (54-336), TSH 0.5 µU/ml (0.35-4.9), FT3 3.26 pg/ml (1.7-3.7), FT4 0.84 ng/dl (0.7-1.5). Pituitary RMI showed a significant reduction in tumor size. **Conclusions.** This is a rare case of pituitary macroadenoma co-secreting somatotropin and thyreotropin with clinical predominance of acromegaly overshadowing thyroid symptoms. In a preliminary evaluation at 3 months, lanreotide 120 mg sc showed a rapid efficacy on normalizing central hyperthyroidism and a partial effect on IGF-1 levels. If this treatment brings long-lasting disease remission remain to be determined at much longer follow-up.

PP147

THE GH RELEASING EFFECT OF ACYLATED GHRELIN IN NORMAL SUBJECTS IS REFRACTORY TO GH ACUTE AUTO-FEEDBACK BUT IS INHIBITED AFTER SHORT-TERM GH ADMINISTRATION

S. Belcastro[1], A. Benso[1], E. Gramaglia[1], I. Olivetti[1], M. Tomelini[1], V. Ramella Gigliardi[1], E. Calvi[1], E. Ghigo[1], F. Broglio[1]

[1]Divisione di Endocrinologia, Diabetologia e Metabolismo - Dipartimento di Medicina Interna - Università di Torino - Torino

Objective: Growth hormone (GH) secretion is regulated by an interplay between GH-releasing hormone (GHRH), somatostatin (SST) and other central and peripheral signals. Acylated ghrelin (AG) amplifies GH pulsatility acting, at least partially, independently from GHRH and SST. The GH response to GHRH is inhibited by recombinant human GH (rhGH), likely due to a somatostatin-mediated negative GH auto-feedback. The effect of exogenous rhGH on the GH-releasing effect of AG has never been tested.

Design and Methods: In 6 healthy volunteers we studied the GH response to acute AG administration (1.0 µg/kg i.v.) during saline or rhGH infusion (4.0 µg/kg/h i.v.) or after 4-day rhGH (10.0 µg/kg s.c.) administration.

Results: Compared to saline, rhGH infusion increased GH levels ($p<0.01$). During saline, acute i.v. AG induced a marked increase ($p<0.01$) of GH levels similar to that observed after AG administration during rhGH infusion. During s.c. rhGH, IGF1 levels rose from day 0 to day 5 ($p<0.01$). After 4-day s.c. rhGH, i.v. AG increased ($p<0.01$) GH levels, though significantly ($p<0.05$) less than on day 0.

Conclusions: the marked somatotroph-releasing effect of AG is refractory to a direct GH auto-feedback whereas is markedly inhibited after 4-day rhGH administration, suggesting the possibility of a selective IGF1-mediated inhibitory feedback.

PP148

IGF(CA)19 GENE POLYMORPHISMS IN ACROMEGALY.

E. Sala[1], E. Ferrante[1], A. M. Barbieri[1], E. Malchiodi[1], E. Verrua[1], C. Giavoli[1], P. Bellaviti Buttoni[1], M. Filopanti[1], P. Beck-Peccoz[1], A. Spada[1], G. Mantovani[1]

[1]Department of Clinical Sciences and Community Health, University of Milan, Endocrinology and Diabetology Unit, Fondazione IRCCS Cà Granda Ospedale M - Milan

INTRODUCTION In the promoter region of IGF-I gene a highly polymorphic microsatellite has been identified, comprising a variable length of a Cytosine-Adenosine (CA) repeat sequence. It normally ranges between 10 and 24 and the most common allele in the Caucasian population contains 19 CA (192 bp) repeats.

We aimed to investigate IGF-1 gene polymorphisms in patients with acromegaly because several studies investigated in the past the relationship between this polymorphism and IGF-I levels, with conflicting results. We included 88 patients with acromegaly and 98 healthy subjects.

MATERIALS AND METHODS Different genotypes were studied by microsatellite method. We divided patients in 3 groups: group A, homozygous for 192 bp allele (n=26, 29.2%), group B, with a number of repeats ≥19 (n=36, 40%,) and group C, with a number of repeats ≤19 (n=27, 30%).

RESULTS We did not observe difference in the frequency of alleles between healty and acromegalic patients. In the acromegalic population the genotype did not influence IGF-I level at diagnosis. Moreover a significant increase (p=0.01) in HOMA-IR was observed in group B (6.2±5.9) compared with group A (5.0±3.3) and C (4.0±3.1). we demonstrated also higher levels of total cholesterol and LDL (p=0.01 and p=0.01 respectively) in group B (233±49 and 168.5±46.6 respectively) compared to group C (175.3±43.4 and 104.0±38.2 respectively). Furthermore, the number of discrepant patients (high IGF-I and normal GH levels) during medical therapy was significantly higher in group B compared to groups A (p=0.02) and C (p=0.05).

CONCLUSION These data suggest that the genotype did not influence IGF-I levels at diagnosis in the acromegalic population. Interestingly, a worse glucidic and lipidic metabolism and a partial disease control during medical treatment may be related to a number of CA repeat higher than 19 .

PP149

AN UNUSUAL ASSOCIATION OF PITUITARY ADENOMA, HASHIMOTO THYROIDITIS AND MULTICENTRIC CARPOTARSAL OSTEOLYSIS

E. Malchiodi[1], M. F. Bedeschi[2], M. R. Calvello[2], E. Profka[1], E. Sala[1], E. Verrua[1], P. Bellaviti Buttoni[1], M. Filopanti[1], E. Ferrante[1], G. Mantovani[1], A. Spada[1], P. Beck-Peccoz[1], U. Verga[1]

[1]Department of Clinical Sciences and Community Health, University of Milan, Endocrinology and Diabetology Unit, Fondazione IRCCS Cà Granda - Milan, [2]Medical Genetic Unit, Fondazione IRCCS Cà Granda - Milan

Introduction: Multicentric carpotarsal osteolysis (MCTO) is an autosomal dominant syndrome, characterized by progressive destruction of the carpal and tarsal bone. MCTO is caused by heterozygous mutation in the MAFB gene on chromosome 20q12. Chronic renal failure is a frequent component of the syndrome. Mental retardation and minor facial anomalies have been noted in some patients. Association with endocrine diseases are not reported. **Case report:** We describe the case of a 36 years old woman who came to our observation for the finding of a non functioning pituitary microadenoma (6 mm) on an RMN study performed for headache. She had no signs or symptoms related to endocrine diseases. One year before, she developed autoimmune primary hypothyroidism and started replacement therapy with levothyroxine. Hormonal evaluation revealed the presence of central hypoadrenalism (serum cortisol peak during insulin tolerance test: 14 µg/dl) that has been replaced. Since she also had uterus bicornis, mitral valve prolapse and osteolysis we required genetic counseling suggestive for multicentric carpotarsal osteolysis. Genetic analysis confirmed the presence of a MAFB gene mutation. Family history was negative for endocrine and skeletal diseases. Genetic analysis of parents and complete study of syndromic context are in progress. **Conclusions:** In this report we emphasize the uncommon presence of pituitary adenoma and Hashimoto thyroiditis in a patients affected by multicentric carpotarsal osteolysis.

PP150

PITUITARY INCIDENTALOMAS: EXPERIENCE OF A SINGLE CENTRE

E. Malchiodi[1], A. Toini[1], E. Ferrante[1], E. Sala[1], E. Verrua[1], P. Bellaviti Buttoni[1], C. Giavoli[1], M. Filopanti[1], P. Beck-Peccoz[1], A. Spada[1], G. Mantovani[1]

[1]Department of Clinical Sciences and Community Health, University of Milan, Endocrinology and Diabetology Unit, Fondazione IRCCS Cà Granda - Milan

INTRODUCTION: With the development of sensitive neuroimaging techniques, pituitary lesions are being discovered incidentally with increasing frequency (0.2-38%). Aim of the study is to investigate clinical and biochemical characteristics of 205 consecutive patients (30% male, mean age at diagnosis 53.6±18.2 years) with incidental pituitary adenoma (IPA) followed at our center from 1990 to present.

MATERIALS AND METHODS: All patients were studied with basal e dynamic tests of pituitary function and pituitary imaging at baseline, 6 months later and then annually if there was no other specific indication. 70 patients were also screened for subclinical hypercortisolism with cortisol after 1-mg overnight dexamethasone suppression test (1mg-DST), late-night salivary cortisol and 24-hours urinary free cortisol.

RESULTS At diagnosis, radiological imaging revealed a micro-adenoma in 61 % and a macro-adenoma in 39 % of cases, respectively. 14.3% of patients (macro 28.6% vs micro 5.6%, P<0.05) had one or more pituitary deficiencies. 13.9% of patients (macro 15.5% vs micro 12.8%, P NS) had hyperprolactinemia. Subclinical hypercortisolism was found in 3/70 (4.2%) patients studied, all with macroadenomas. The mean follow-up time was of 4.9 years, in particular 110 patients had a follow-up longer than 12 months. At last control, radiological evaluation revealed a significant increase of tumor mass in 19/110 patients (17.3%, 13 macro vs 6 micro, P NS) and a reduction in 5.4% (all microadenomas). The volumetric increase occurred in 88% of patients during the first two years after diagnosis. Additional pituitary deficiencies were observed in 3% of patients during follow-up. Overall 18% of patients were treated with trans-sphenoidal adenomectomy owing to initial mass size or for their rapid increase.

CONCLUSIONS Our data confirm the importance of a close radiological and hormonal follow-up in patients with IPA. In addition, we suggest to exclude the presence of subclinical hypercortisolism in such patients.

PP151

CLINICAL, BIOCHEMICAL AND ANTHROPOMETRIC FEATURES IN ADULT ONSET, TRANSITION ONSET AND CHILD ONSET GH DEFICIENT PATIENTS

E. Scarano[1], M. C. Savanelli[1], V. Brunelli[1], A. Colao[1], C. Di Somma[1]

[1]Dipartimento di Medicina Clinica e Chirurgia, Sezione di Endocrinologia, Università "Federico II" - Napoli

Background: In literature only few data comparing child onset GHD (COGHD) patients and transition onset GHD (TOGHD) patients in the transition period are present; they have shown a similar response to GH replacement treatment (GHRT) in both groups and the age of onset has not appeared to be a significant predictor of response in any clinical, biochemical and anthropometric outcome. Aim: To compare clinical, biochemical and anthropometric variables in COGHD, TOGHD and young adult onset GHD (AOGHD) patients. Patients and methods: 53 GHD patients were divided in three groups: 19 young AOGHD patients (age 26-40, 8 M, 11 F), 16 COGHD patients during transition period (age 15-25, 8 M, 8 F), 18 TOGHD patient (age 15-25, 8M, 10 F). In all patients we determined: height, weight, BMI, waist and hip circumference, waist/hip ratio, systolic (SBP) and diastolic (DBP) blood pressure, total-, HDL- and LDL-cholesterol, triglycerides, blood glucose, HbA1c and IGF-I before and after 2 years of GHRT and GH peak after GHRH + ARG. Result: At baseline, SBP and DBP were lower in COGHD than AOGHD (115 ± 7.9 vs 124 ± 9.9, p=0.047; 66 ± 8.8 vs 74 ± 6.6, p=0.004; respectively). No significant differences were found in SBP and DBP between TOGHD and others groups and after 2 years of GHRT among the 3 groups of patients. At baseline and after 2 years of GHRT, no significant differences were found for the other parameters in all patients. At baseline, there was a significant correlation between GHD age of onset, SBP and DBP. GH peak after GHRH + ARG test was significantly correlated to waist and hip circumference and IGF-I (r= -0.272, p= 0.049; r= -0.274, p= 0.049; r= 0.300, p= 0.029; respectively). After 2 years of GHRT, the GHD age of onset was significantly correlated with waist/hip ratio and DBP (r= -0.265, p= 0.50; r= -0.341, p= 0.012; respectively). Conclusion: CO-, AO- and TO-GHD patients do not differ for any clinical, biochemical and anthropometric features, except for SBP and DBP between CO- and AO-GHD groups at baseline. Then, our results confirm past aknowledgements. However, the importance of GHD onset on these parameters requires further investigation.

PP152

CLINICAL PRESENTATION AND SURGICAL THERAPY OF ACROMEGALIC PATIENTS OVER THE LAST THREE DECADES

E. Ferrante[1], E. Sala[1], M. Locatelli[2], P. Rampini[2], G. Mantovani[1], M. Filopanti[1], E. Verrua[1], E. Malchiodi[1], G. Carrabba[2], M. Arosio[3], P. Beck-Peccoz[1], A. Lania[4], A. Spada[1]

[1]Dept of Clinical Sciences and Community Health, University of Milan, Endocrinology and Diabetology Unit, IRCCS Ca' Granda Osp. Maggiore Policlinico - Milan, [2]Unit of Neurosurgery, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico - Milan, [3]Dept of Clinical Sciences and Community Health, University of Milan, Unit of Endocrine Diseases and Diabetology, San Giuseppe-Multimedica group - Milan, [4]BIOMETRA Dept University of Milan, IRCCS Istituto Clinico Humanitas - Rozzano

Background: Trans-sphenoidal (TNS) surgery remains the primary therapeutic option for acromegaly, a rare and insidious disease associated with an increased morbidity and mortality. Objective: Aims of this study were to verify the impact of TNS surgery on treatment of acromegaly before and after the identification of a dedicated neurosurgical team, and to describe diagnostic features of the disease over three decades. Methods: 41 patients (group A) who underwent TNS surgery by a dedicated neurosurgical team between 2000 and 2008, and 126 patients (group B) who underwent TNS approach by a not selected operator between 1979 and 1999 were retrospectively analyzed. Results: Overall remission rate after surgery was 58.5% for group A (75% in microadenomas and 48% in macroadenomas, P=NS) and 37% for group B (P<0.05 vs group A; for microadenomas, 34% vs 75% of group A, P<0.05, for macroadenomas, 36% vs 48% of group A, P=NS). The mean delay of diagnosis was 4.9 and 5.9 years in group A and B, respectively (P=NS). No significant differences were observed between the two groups in terms of mean basal GH levels, mean GH nadir values, prevalence of hypopituitarism and hypertension. IGF-I SDS were significantly higher, while BMI and prevalence of IGT/diabetes were significantly lower in group B than in group A. Conclusion: our data confirm that a dedicated neurosurgical team is necessary in order to improve remission rates in acromegalic patients. No changes in biochemical, clinical and neuroradiological presentation of disease were observed over the last three decades. Since the high prevalence of macroadenomas negatively influences surgical cure of these patients, perform an earlier diagnosis should be considered crucial in order to achieve an even better outcome.

PP153

THE HEMOCROME IN PATIENTS WITH WITH CUSHING'S SYNDROME

A. Cozzolino[1], C. Simeoli[1], M. De Leo[1], P. Vitale[1], D. Iacuaniello[1], A. Albano[1], A. Colao[1], R. Pivonello[1]

[1]Dipartimento di Medicina Clinica e Chirurgia, Sezione di Endocrinologia, Università Federico II - Napoli

Introduction: Glucocorticoids (GC) are well known to have a stimulatory effect on neutrophils count and an inhibitory effect on the other categories of leukocytes. On the other hand, the effect on erythropoiesis have not been completely clarify, despite some evidence of stimulation in count of erythroid series. The aim of our study was to evaluate the hemochrome in patients with endogenous GC excess, namely Cushing's syndrome (CS).

Patients and methods: Fifty-eight patients with CS (19 M, 39 F; 6-73 yrs; 51 pituitary CS; 4 adrenal CS; 3 ectopic CS) and 58 healthy gender-, age- and BMI-matched controls entered the study. Hemochrome was evaluated in both patients and controls. In patients, hemochrome was correlated with the hormonal pattern.

Results: Medium corpuscular volume (MCV, p=0.000), hemoglobin (Hb, p=0.004), hematocrit (HCT, p=0.000) and mean corpuscolar hemoglobin (MCH, p=0.005) were significantly higher in patients than controls. A significantly higher number of white blood cells (WBC, p=0.000) was also observed in patients than controls, associated with a significantly higher count of neutrophils (p=0.000) and lower count of eosinophils (p=0.000) as well as linphocytes (p=0.006). Moreover, a significantly higher prevalence of macrocytosis (17.2% vs 0%, p=0.002), leukocytosis (24.1% vs 3.3%, p=0.002) and neutrophilia (36.4% vs 5%, p=0.000) was found in patients than controls. These parameters seemed to be not correlated with serum iron, ferritin, transferrin, folate and vitamin B12 levels as well as serum and urinary cortisol levels or disease duration.

Conclusions: In conclusion, CS seems to affect hemochrome parameters, inducing increase in erythrocytes volume and hemoglobin content. CS also determines increase in neutrophils count, commonly inducing a neutrophil leukocytosis associated to an inhibition of eosinophils and linphocytes. These results suggest that peculiar hemochrome alterations could be useful as markers of CS, if confirmed in a larger cohort of patients.

PP154

EFFECT OF SHORT AND LONG-TERM TREATMENT WITH PASIREOTIDE ON HEMOCHROME IN PATIENTS WITH CUSHING'S DISEASE (CD).

A. Cozzolino[1], C. Simeoli[1], L. Trementino[2], M. De Leo[1], P. Vitale[1], D. Iacuaniello[1], A. Albano[1], M. Boscaro[2], A. Colao[1], G. Arnaldi[2], R. Pivonello[1]

[1]Dipartimento di Medicina Clinica e Chirurgia, Sezione di Endocrinologia, Università Federico II - Napoli, [2]Divisione di Endocrinologia, Università Politecnica delle Marche - Ancona

Introduction: Glucocorticoids (GC) have a stimulatory effect on neutrophils and an inhibitory effect on the other leukocyte subpopulations. A potential stimulatory effect on erythropoiesis has been also hypothesized. The aim of our study was to evaluate the effect of pasireotide treatment on hemochrome parameters in patients with CD.

Patients and Methods: Fifteen patients with CD (19-57 yrs, 14 F, 1 M) and 45 sex, age and BMI-matched healthy controls entered the study. Hemochrome evaluation has been assessed at baseline and after 3 and 6 months of pasireotide treatment (dose 1200-2400 µg/day).

Results: Significantly higher levels of hematocrit (HCT) (p=0.019) and neutrophils (p=0.002) and a significantly lower number of lymphocytes (p=0.000) and eosinophils (p=0.009) have been observed in patients than controls. After 3 months of treatment, 8/15 patients (53.3%) normalized urinary free cortisol (UFC) levels and the percentage of reduction in UFC levels ranged from 25 to 96%; a significant decrease in platelets (PLT) (p=0.002) and neutrophils (p=0.017) and also a significant increase in lymphocytes (p=0.03), eosinophils (p=0.035) and basophils (p=0.011) have been observed. After 6 months, 5/13 patients (38.5%) had a persistent UFC normalization and the percentage of reduction in UFC levels ranged from 30 to 91%; so as after 3 months, a significant decrease in PLT (p=0.015) and neutrophils (p=0.023) and a significant increase in lymphocytes (p=0.002) and basophils (p=0.034) have been observed. No significant correlation was found between UFC levels and the different parameters of hemochrome at baseline and no significant correlation was found between their changes after 3 and 6 months of treatment.

Conclusions: Medical therapy with pasireotide is able to improve hemochrome parameters commonly altered in CD. These findings are probably due to a significant decrease in cortisol secretion, although a contributing or determinant role of different factors cannot be ruled out.

PP155

SALIVARY CORTISOL IS A USEFUL TOOL TO ASSESS THE IMMEDIATE RESPONSE TO PASIREOTIDE IN PATIENTS WITH CUSHING'S DISEASE

L. Trementino[1], M. Cardinaletti[1], C. Concettoni[1], G. Marcelli[1], F. Agostini[1], B. Polenta[1], M. Boscaro[1], G. Arnaldi[1]

[1]Clinica di Endocrinologia, Università Politecnica delle Marche - Ancona

Introduction: Pasireotide is a promising treatment option for patients with Cushing's disease (CD). The measurement of salivary cortisol is useful for diagnosing hypercortisolism and monitoring patients with CD following pituitary surgery. It may also be a better index of cortisol secretion than serum cortisol or urinary free cortisol (UFC). We investigated the value of salivary cortisol in monitoring short-term efficacy of pasireotide in patients with CD.

Methods: Seven patients (five females, two males; mean age 35.3±7.4 years) received pasireotide 600µg bid for 15 days in the Phase II study CSOM230B2208. Morning and midnight salivary cortisol, ACTH and morning serum cortisol were assessed at baseline and after 1, 5, 12 and 15 days of treatment. UFC was determined at baseline and day 15.

Results: On day 15, morning salivary cortisol had decreased in all patients; overall mean decrease from baseline was 70% (27.7±30.8 to 8.2±7.7 nmol/L). Midnight salivary cortisol had decreased in six patients and normalized in two; overall mean reduction from baseline was 50% (27.2±38.6 to 13.4±15.4 nmol/L). Decreases in morning and midnight salivary cortisol were observed from day 1 (mean reduction from baseline of 34% and 20%, respectively) and persisted until day 15; the greatest decrease was on day 5 (mean reduction of 70% and 58%, respectively). At day 15, mean UFC had decreased from baseline by 65% (1711±1941 to 593±360 nmol/24h). UFC was normalized in one patient (14%), who also had normalized midnight salivary cortisol, thereby restoring cortisol rhythm. Changes in ACTH and serum cortisol were similar to those of salivary cortisol.

Conclusions: Pasireotide rapidly reduced and normalized salivary cortisol. Salivary cortisol may be a simple, non-invasive biomarker to assess immediate response to pasireotide in patients with CD, particularly to determine whether cortisol rhythm is normalized in patients with normalized UFC levels. More studies are necessary to confirm these preliminary results.

PP156

PROGNOSTIC RELEVANCE OF KI-67 LI IN PITUITARY ADENOMAS: A RETROSPECTIVE OBSERVATIONAL STUDY

S. Chiloiro[1], A. Bianchi[1], A. Giampietro[1], A. Fusco[1], D. Iacovazzo[1], L. Tartaglione[1], F. Di Nardo[2], C. de Waure[2], F. Signorelli[3], F. Doglietto[3], L. Lauriola[4], C. Anile[3], G. Rindi[4], G. Maira[3], L. De Marinis[1], A. Pontecorvi[1]

[1]Section of Endocrinology, Department of Internal Medicine, Università Cattolica del Sacro Cuore, Policlinico Gemelli - Rome, [2]Institute of Hygiene, Università Cattolica del Sacro Cuore, Policlinico Gemelli - Rome, [3]Institute of Neurosurgery, Università Cattolica del Sacro Cuore, Policlinico Gemelli - Rome, [4]Institute of Anatomic Pathology, Università Cattolica del Sacro Cuore, Policlinico Gemelli - Rome

Introduction: Pituitary adenomas are usually benign. However these tumors can infiltrate surrounding structures. Ki-67 Labeling Index is an immunocytochemical marker of cell proliferation. Some authors report a positive correlation between Ki-67 expression and pituitary adenoma invasiveness and recurrence. The aim of this study is to investigate the possible prognostic value of the Ki-67 LI in predicting the clinical outcome of patients who underwent surgery for pituitary adenomas.

Methods & Materials: We retrospectively reviewed 212 consecutive patients who referred to the Hypothalamic-Pituitary Disease Unit at the Catholic University of Rome in the last 7-years. All patients underwent surgery for a pituitary tumor in the Department of Neurosurgery of the same institution. All patients underwent endocrine evaluation of the hypothalamic-pituitary function, ophthalmologic and neuro-radiological examinations, during the preoperative period and periodically during postoperative follow-up. All tissue specimens were examined for anterior pituitary hormones by immunohistochemistry and for proliferative activity by anti-Ki-67 monoclonal antibody.

Results: Recurrence was observed in 62 cases (29.2% of the patients who underwent radical excision). Optional cut-off value was identified at Ki-67-LI 1.25%. A Ki-67-LI value above 1.25% was associated with worse disease-free survival time, even after correction for age at treatment, gender, positivity to p53, functional classification, Knosp and Wilson's classifications of cranial invasion (adjusted HR: 2.54, 95%CI: 1.36-4.76). In our cohort, we didn't find any disease recurrence after 96 months.

Conclusion: Ki-67 LI may be useful in postoperative management. We suggest a Ki-67 LI cut-off value of 1,25% to plan a more adequate short-term follow-up.

PP157

EVALUATION OF RENAL MORPHOLOGY AND FUNCTION IN PATIENTS WITH ACTIVE OR CURED CUSHING'S SYNDROME

D. Iacuaniello[1], C. Simeoli[1], A. Cozzolino[1], F. Fonderico[1], R. Carotenuto[2], M. De Leo[1], P. Vitale[1], A. Boemio[2], T. Pellegrino[2], A. Cuocolo[2], A. Colao[1], R. Pivonello[1]

[1]Department of Clinical Medicine and Surgery-Section of Endocrinology, Federico II University - Naples, [2]Department of Advanced Biomedical Sciences, Federico II University - Naples

INTRODUCTION: Cushing's Syndrome (CS) is a rare endocrine disorder associated with several systemic complications including hypertension, diabetes mellitus, dyslipidemia and nephrolithiasis, which might affect renal function.

AIM: The aim of this study is two-fold: 1) to evaluate the morphology and renal function; and 2) to correlate morphology and renal function alterations with renal risk factors in patients with active or cured CS.

PATIENTS AND METHODS: Twenty-three patients (4 males and 19 females; 21-73 years) have been compared with twenty-three sex- and age-matched controls. The evaluation of renal morphology by ultrasound, and renal function, by measurement of glomerular filtration rate (GFR) through calculation of creatinine clearance (Clcr) and sequential dynamic renal scintigraphy with 99mTc-DTPA, was performed in all patients and controls and correlated with clinical and metabolic parameters.

RESULTS: Biochemical study showed higher level of HbA1C and lower Clcr in patients with active ($p=0.019$;$p=0.042$) and cured disease ($p<0.001$;$p=0.04$) than controls. Ultrasound study showed higher prevalence of nephrolithiasis and lower renal transverse diameter in patients with active ($p=0.001$;$p=0.001$) and cured disease ($p<0.001$;$p<0.001$) than controls. Scintigraphic study displayed lower GFR and cortical concentration of tracer in patients with active ($p=0.039$;$p=0.009$) and cured disease ($p<0.001$;$p=0.002$) than controls. No differences were recorded in the other parameters evaluated. No correlations between renal risk factors and renal function indices were found in patients with active disease, whereas, in patients with cured disease an inverse correlation was found between GFR and tryglicerides ($p=0.008$;$r=-0.636$), total cholesterol ($p=0.019$; $r=-0.578$), HbA1C ($p<0.001$;$r=-0.873$), systolic blood pressure ($p<0.001$;$r=-0.77$) and plasma ACTH ($p=0.025$;$r=-0.558$). HbA1C was the parameter most independently correlated with GFR ($p<0.001$;$B=-0.873$).

CONCLUSION: The results of the current study demonstrated that patients with active disease showed a renal damage which persists in cured stage of disease where it seems to depend on the persistence of risk factors for renal damage.

PP158

GROWTH HORMONE RECEPTOR ISOFORMS AND FRACTURE RISK IN ACROMEGALY

M. Mormando[1], A. Bianchi[1], L. A. Nasto[2], E. Pola[2], A. Giampietro[1], G. Mazziotti[3], A. Giustina[4], L. De Marinis[1], A. Pontecorvi[1]

[1]Endocrinology Catholic University - Rome, [2]Orthopaedic and Traumatology Catholic University - Rome, [3]Endocrinoology,Azienda Ospedaliera Carlo Poma - Mantua, [4]Medical and Surgical Sciences, Endocrine Service - Brescia

Introduction: acromegaly is a condition associated with an increased prevalence of vertebral fractures (VF) both in men and women. Bone fragility in these patients doen't be explained by bone mineral density (BMD). Both high serum IGF1 levels and longer duration of active disease have been linked to an increased risk of VF. Matherial and methods: a cross-sectional study was designed to investigate the impact of the deletion of exon 3 of GH receptor (d3GHR) on the prevalence of VFs in a population of acromegalic patients. We studied 109 acromegalic patients (M/F: 48/61); 57 with controlled disease, 36 with active disease and 16 healed patients. GHR genotype (full length isoform: flfl; full length-d3 deletion: fld3; or d3-deletion d3-deletion: d3d3) was assessed in each patient. All patients were screened for vertebral fractures (by quantitative morphometric analysis) and BMD at lumbar spine and hip (by dual energy X ray absorptiometry). Serum IGF1 levels and bone metabolism markers were also measured in all patients. Results: although BMD was not significantly associated with VFs in our population, we found that male sex (fractured 27M / 20F; not fractured 21M / 41F; p<0,012); IGF1 levels (416 ng/ml vs 294 ng/ml; p<0,01); length of active disease (91 vs 72 months; p<0,001) and disease activity (22 vs 14 patients; p<0,01) were significantly associated with higher prevalence of vertebral fractures. Levels of serum PTH, vitamin D and beta-CTX were not significantly different in fractures vs non fractured patients. Nevertheless higher osteocalcin (OC) levels (30,8 vs 20,7 ng/ml; p<0,05) were significantly higher in fractured patients. Female patients with fractures had significantly lower estradiol levels (10,8 vs 37,5 ng/ml ; p<0,01). The flfl genotype was detected in 54 patients (49,5%); the fld3 genotype was detected in 37 patients (34%), whereas the d3d3 genotye was expressed in 18 patients (16,5%). Carriers of at least one d3 allele showed a significantly increased prevalence of VFs (35 patients with fractures in the fl3d3+d3d3 group vs 12 patients with fractures in the flfl group; p<0,001). Conclusions: our data have shown that male sex, active disease, length of active disease, IGF1 and OC serum levels, and d3GHR isoform are significant risk factors for VFs in acromegalic patients. Moreover, higher estradiol serum levels had a protective effect against VFs only in female patients. The correlation between the d3GHR isoform and an higher risk of VFs in our population was particularly interesting. To our knowledge this is the first report of this association.

PP159

INVOLVEMENT OF HYPOTHALAMUS AUTOIMMUNITY IN PATIENTS WITH AUTOIMMUNE HYPOPITUITARISM: ROLE OF ANTIBODIES TO HYPOTHALAMIC CELLS

A. De Bellis, A. A. Sinisi, E. Pane, A. Dello Iacovo, G. Bellastella, E. Aitella[2], A. Falorni[3], C. Giavoli[4], V. Gasco[5], R. Giordano[5], M. Ambrosio[6], A. Colao[7], A. Bizzarro[8], A. Bellastella[1]

[1]Department of Cardiothoracic and Respiratory Sciences - Napoli, [2]Department of Clinical and Experimental Medicine and Surgery "F. Magrassi, A. Lanzara" - Napoli, [3]Department of Internal Medicine, Section of Internal Medicine and Endocrine & Metabolic Sciences - Perugia, [4]Department of Medical Sciences - Milano, [5]Department of Internal Medicine - Torino, [6]Department of Biomedical Sciences and Advanced Therapies - Ferrara, [7]Department of Molecular and Clinical Endocrinology and Oncology - Napoli, [8]Department of Clinical and Experimental Medicine and Surgery "F. Magrassi, A. Lanzara - Napoli

Antipituitary (APA) are usually searched for in autoimmune hypopituitarism.Aim of this study was to search for AHA and characterize their hypothalamic target in patients with autoimmune hypopituitarism, to clarify, on the basis of the cells stained by these antibodies, the occurrence of autoimmune central diabetes insipidus (CDI) and/or possible joint hypothalamic contribution to their hypopituitarism.95 APA positive patients with hypopituitarism were studied: 60 without (group 1) and 35 with (group 2) lymphocytic hypophysitis (LYH) ,20 patients with post-surgical hypopituitarism and 50 normal subjects. AHA by immunofluorescence and post-pituitary function were evaluated; AHA positive sera were retested by double immunofluorescence to identify the hypothalamic cells targeted by these antibodies. AHA were detected at high titer in 12 patients in group 1 and in 8 in group 2. They immunostained AVP-secreting cells in 9 in group 1 and in 4 in group 2 and different hypothalamic cells in 7 in group 1 and in 6 in group 2; of them, 4 patients with GH-ACTH deficiency showed AHA staining CRH-secreting cells. All AVPcAb positive patients presented with CDI. Conclusions: CDI in our patients with LYH seems due to an autoimmune hypothalamic involvement rather than an expansion of the pituitary inflammatory process. To search for AVPcAb in these patients could help to reveal CDI. Instead, the detection of AHA targeting CRH-secreting cells in some patients with GH/ACTH deficiency indicates that an autoimmune aggression to hypothalamus is responsible for their hypopituitarism.

PP160

THE SWITCH FROM DAILY TO TWICE A WEEK ADMINISTRATION OF PEGVISOMANT TREATMENT IN PATIENTS WITH ACROMEGALY: COMPARISON OF EFFICACY AND SAFETY

L. F. S. Grasso[1], L. Ferreri[1], M. Galdiero[1], T. Mannarino[1], C. Pivonello[1], A. Colao[1], R. Pivonello[1]

[1]Dipartimento di Medicina Clinica e Chirurgia, Sezione di Endocrinologia, Università Federico II - Napoli, Italia

Pegvisomant (PEG) is an alternative medical therapy indicated in patients fully or partially resistant to somatostatin analogue (SSA) therapy. PEG is approved as once daily administration at the dose of 10-30 mg/day, however previous studies demonstrated that alternate day administration of PEG normalized IGF-I levels in 50% of active acromegalic patients. The aim of the present study was to investigate the efficacy and safety of combined therapy with SSAs and twice a week dosing PEG in acromegalic patients previously treated with a stable daily dose of PEG.

Thirteen acromegalic patients biochemically controlled on combined therapy with SSAs and once daily dose of PEG by at least 6 months and two uncontrolled patients (9 women and 6 men, aged 40-73 years, 10/15 previous surgery), were evaluated 6 and 12 months after switching therapy to twice a week dose administration of PEG. At each visit vital signs, weight, liver function test and fasting glucose levels were monitored. All patients were converted from a mean dose of 15 mg daily (range 10-25 mg) to a mean weekly dose of 75 mg (range 40-140 mg) in combination with maximal dose of SSAs (9/15 lanreotide autogel 120 mg, 6/15 octreotide LAR 30 mg). With twice a week PEG treatment, all controlled patients maintained IGF-I levels unchanged within reference range (235±56 ng/ml at baseline, 204±68 ng/ml after 6 months, 207±76 ng/ml after 12 months) with no statistically significant difference after 6 and 12 months of therapy. The two uncontrolled patients normalized IGF-I levels after 6 months with a subsequent increase in one of them after 12 months, probably due to a bad compliance. PEG dose wasn't changed during the study. During combined therapy with twice a week PEG serum transaminases, fasting glucose and weight were unchanged. All 15 patients documented a similar or better compliance with the twice a week dose, and during the study everyone elected to continue with twice a week administration of PEG. In conclusion, twice a week administration of PEG in combined therapy with SSAs is equally effective in controlling acromegaly with no requirement for an increase in PEG dose during the study. Moreover, safety parameters remained stable during the study, and all patients elected to continue weekly dose, suggesting this regimen would improve patients compliance.

PP161

ANALYSIS OF SHORT- AND LONG-TERM METABOLIC EFFECTS OF GROWTH HORMONE REPLACEMENT THERAPY IN ADULT PATIENTS WITH CRANIOPHARYNGIOMA AND NON-FUNCTIONING

E. Profka[1], C. Giavoli[1], S. Bergamaschi[1], E. Ferrante[1], E. Malchiodi[1], E. Sala[1], E. Verrua[1], A. Spada[1], P. Beck-Peccoz[1]

[1]Dpt of Medical Sciences and Community Health, University of Milan, Endocrinology and Diabetology Unit, Fond. IRCCS Ca' Granda Ospedale Policlinico - Milano

Adult patients operated for craniopharingioma frequently develops GH deficiency (GHD) and tends to be more obese and dyslipidemic than patients with non-functioning pituitary adenoma (NFPA). Data on metabolic effects of rhGH replacement in these subjects are limited, a previous report showing less reduction in body fat (BF%) in CP patients than in those with NFPA after 2-yrs therapy. Aim of the study was to compare both short- (12 months) and long-term (5 years) effects of rhGH on IGF-I levels, BF%, lipid profile and glucose metabolism in 36 GHD adult patients, 18 operated for CP and 18 for NFPA. At baseline no difference between the two groups was observed, apart from a higher prevalence of diabetes insipidus in CP patients (83% vs 22%). After 12 months, IGF-I SDS normalized and BF% significantly decreased in both groups. During long-term treatment, increase in IGF-I levels was maintained, while decrease in BF% was persistent only in NFPA group (from 32±10.6 to 30±10%, P<0.05 in NFPA and form 33.4±8% to 31.7±7%, P=NS, in CP). Only in CP patients a long-term worsening of insulin sensitivity, documented by increase in insulin levels and HbA1c (from 10.1±6.2 to 13.5±9.4 uIU/ml and from 5.0±0.6 to 5.9±0.99%, respectively, P<0.05) and decrease of QUICKI (from 0.36±0.04 to 0.33±0.03, P<0.05) was observed. On the contrary, a significant improvement in lipid profile shown by reduction in total cholesterol and LDL-cholesterol (from 225±61 to 209±52 mg/dl and from 148±44 to 122±41 mg/dl, respectively, P<0.05) was present only in NFPA group. In conclusion, present data suggest that patients with CP are less sensitive to the positive rhGH effects on lipid profile than patients with NFPA. Moreover, we confirm that BF% reduction in CP is less than in NFPA patients also during long-term treatment, a condition that might explain the long-term worsening of insulin sensitivity observed only in the former group.

PP162

PRELIMINARY DATA ON AIP AND AHR GENOTIPIZATION IN APPARENTLY SPORADIC ACROMEGALIC PATIENTS

M. Ragonese[1], P. D. Romeo[2], S. Puglisi[1], M. L. Torre[1], F. Ferraù[1], A. Albani[1], O. R. Cotta[1], C. Salpietro[2], F. Trimarchi[1], S. Cannavò[1]

[1]Department of Clinical and Experimental Medicine – Endocrinology - Messina, [2]Department of Pediatric, Gynecological, Microbiological and Biomedical Sciences – Genetics and Immunology Pediatrics - Messina

Context: Germline mutations of the AIP (aryl-hydrocarbon receptor interacting protein) gene are associated with a predisposition to pituitary adenomas. Such mutations are found in about half of patients with familial acromegaly, but penetrance is incomplete.
Objective: This is a preliminary study concerning genotipization of AIP and AHR genes in a small group of acromegalic patients with sporadic pituitary adenoma.
Patients and Methods: The entire coding sequence of the AIP and AHR genes were screened for germline mutations in 18 patients (M= 8, age 57.5±12.8 yrs±SD) with diagnosis of acromegaly in adult age. Genotyping was performed by direct automated sequencing on ABI3130.
Results: A missense AIP mutation, R304Q, whose pathogenic role has already been confirmed, was found in 1 (5.5%) of 18 patients. Furthermore, in all patients we found 2 polymorphic AIP variants, rs641081 (c.682C>A) and rs4930199 (c.920A>G) in homozygosity, with uncertain pathological role. Concerning AHR gene, 15 (83%) out of the 18 patients showed a major polymorphic variant rs2066853 in heterozygous state, 1 (5.5%) showed the rs2066853 (c.1661G>A) and the rs4986826 (c.1708G>A) variants in compound heterozygosity and in 1 patient (5.5%) showed both variants in homozygosity and heterozygosity state, respectively. The pathogenic role of AHR gene variants is still unconfirmed.
Conclusion: On the basis of this preliminary data, we can conclude that AIP mutations with potential pathogenetic role are present in 5.5% of subjects with adult onset acromegaly due to apparently sporadic GH-secreting pituitary adenoma. On the contrary, no AHR gene mutations have been detected in this small group of acromegalic patients.

PP163

BMI AND DURATION OF TREATMENT INFLUENCE THE PEGVISOMANT DOSE IN ACTIVE ACROMEGALY AFTER LONG-TERM FOLLOW-UP

A. Bianchi[1], P. Maffei[2], S. Grottoli[3], G. Arnaldi[4], M. Ragonese[5], M. R. Ambrosio[6], T. Porcelli[7], A. Giampietro[1], M. C. Zatelli[6], S. Cannavò[5], M. Boscaro[4], C. Martini[2], A. Giustina[7], E. Ghigo[3], A. Pontecorvi[1], L. De Marinis[1]

[1]Medicina Interna e Scienze Specialistiche, Università Cattolica del Sacro Cuore, Policlinico Universitario "A. Gemelli". - Roma, [2]Dipartimento ad Attività Integrata Sperimentale (DAIS) di Medicina, Università di Padova - Padova, [3]Dipartimento di Medicina Interna, AUO San Giovanni Battista di Torino, Università di Torino - Torino, [4]Dipartimento di Scienze Cliniche e Molecolari, Università Politecnica delle Marche - Ancona, [5]Dipartimento Clinico-Sperimentale di Medicina e Chirurgia, AOU Policlinico di Messina, Università di Messina - Messina, [6]Dipartimento di Scienze Mediche, AOU di Ferrara, Università di Ferrara - Ferrara, [7]Dipartimento di Scienze Cliniche e Sperimentali, Ospedale di Montichiari, Università di Brescia - Brescia

In acromegaly, various factors may affect dosing of pegvisomant (PEGV). In patients with active disease we investigated which factors may influence the final dose of pegvisomant in a long-term study. Data from 128 subjects (72 F) evaluated in a retrospective cross-sectional study in 7 italian hospitals were collected. At study entry, 82% had undergone neurosurgery, 24% radioterapy and 91% was treated with somatostatin analogs (SSA). PEGV was added to SSA in 42% of patients. Patients initially received 10 mg/d and PEGV dose was adjusted by 5 mg every 8 week until serum IGF-I was normalized. The mean follow-up was 37.6+/-22.8 months. At baseline and after treatment, no differences in IGF-I levels were found according to sex and previous radiotherapy. At the end of follow-up, no differences were found in IGF-I levels and PEGV final dose between patients treated with PEGV alone or in combination with SSA. A stepwise multiple linear regression analysis showed a strong direct correlation between BMI, duration of PEGV therapy and final dose of PEGV required, regardless the treatment regimen (PEGV alone or in combination). In conclusion, BMI and duration of PEGV treatment influence the dose of PEGV required to normalize serum IGF-I in patients with active acromegaly during long-term PEGV therapy.

PP164

A PATIENT AFFECTED BY DERCUM'S DISEASE AND ADULT-ONSET IDIOPATHIC HYPOGONADOTROPIC HYPOGONADISM TREATED WITH HUMAN CHORIONIC GONADOTROPIN

G. Ruga[1], E. Miraglia[2], N. Tahani[1], M. Spaziani[1], S. Granato[1], G. Tabacco[1], G. Papi[1], S. Calvieri[2], A. Lenzi[1], S. Giustini[2], A. F. Radicioni[1]

[1]Center for Rare diseases, Department of Experimental Medicine, Section of Medical Pathophysiology, Food Science and Endocrinology, Sapienza - Rome, [2]Department of Dermatology and Venereology, Sapienza University of Rome - Rome

Dercum's disease or adiposis dolorosa (OMIM:103200) is a rare autosomic dominant disease characterized by the presence of multiple painful subcutaneous lipomas. The trunk and the proximal part of the limbs are mainly interested associated with characteristic signs and symptoms as obesity, asthenia, weakness, fatigability and mental disturbances. This disease was described for the first time in 1892 by Francis Xavier Dercum and its prevalence is about 1/200,000.
The development of this pathology is slow and gradual with the appearance of nodular lesions in several body regions which increase in size causing discomfort and pain associated with functional impotence. Pain increases with BMI and patients are usually 50% above normal weight.
We present a case report of a 42 year-old male patient affected by Dercum's disease, who came to our attention for decreased libido and erectile dysfunction. Physical examination revealed the presence of cutaneous multiple and widespread soft swellings which caused spontaneous and palpatory pain. During the andrological evaluation a mild degree of beard and pubic hair reduction was evidenced; both testes were painful during palpation.
The patients underwent blood tests that revealed low testosterone, FSH and LH levels. According to clinical manifestations, endocrinological and laboratoristic evalutation, adult-onset hypogonadotropic hypogonadism (IHH) diagnosis was performed, probably due to an isolated pituitary gonadotropin defect.
The patient was treated with human chorionic gonadotropin and a fast improvement of sexual function was described during the next clinical evaluation.

PP165

ROLE OF GROWTH HORMONE-RELEASING HORMONE (GHRH) ON CARDIAC HYPERTROPHY AND MIR-133 EXPRESSION

L. Trovato[1], M. Taliano[1], E. Ghigo[1], R. Granata[1]

[1]DIVISIONE DI ENDOCRINOLOGIA, DIABETOLOGIA E METABOLISMO, DIPARTIMENTO SCIENZE MEDICHE, SCUOLA DI MEDICINA, UNIVERSITA' DEGLI STUDI DI TORINO - TORINO

We and others have shown that the hypothalamic hormone growth hormone-releasing hormone (GHRH) exerts cardioprotective effects both *in vitro* and *in vivo*. Moreover, GHRH acts as pro-survival factor for cardiomyocytes, suggesting a role in preventing cardiac cell loss in pathological conditions, such as myocardial infarction and ischemia/reperfusion injury. To date, the role of GHRH in cardiac hypertrophy is unknown. Conversely, it has been demonstrated that several microRNAs (miRs), small endogenous non-coding RNAs that repress mRNA transcription, play a pervasive role in the control of cardiac hypertrophy. Among these, cardio-specific mirR-1 and -133 are key regulators of cardiac hypertrophy and recent studies revealed an inverse correlation between their expression and myocardial hypertrophy. Therefore, we investigated the effect of GHRH on hypertrophy induced by the α_1-adrenergic receptor agonist phenylephrine (PE) in rat H9c2 cardiomyocytes. The role of the hormone on mirR-1 and miR-133 expression and the underlying signaling pathways were also studied. H9c2 cells were cultured in serum deprived medium for 24 h and incubated in the presence or absence of either PE (10 μM and 25 μM) or GHRH (0.5 μM) for 24h. Immunofluorescence analysis for α-actinin showed that GHRH reduced PE-induced increase of cell area, an hypertrophic index, whereas GHRH alone had no effect. Moreover, real-time PCR results showed that GHRH counteracted PE-induced hypertrophy, by decreasing atrial natriuretic peptide (ANP) and increasing miR-133 gene expression. Western blot results showed that GHRH inhibited ERK1/2 phosphorylation in the presence of PE. Interestingly, both GHRH and PE were found to increase this survival and prohypertrophic pathway when given alone, acting through different G proteins. The adenylyl cyclase (AC)/cAMP/protein kinase A (PKA) pathway has been shown to regulate cardiac hypertrophy. We found here that forskolin (FSK), a potent cAMP activator, inhibited PE-induced ANP increase. Moreover, in PE-treated cells the antihypertrophic effect of GHRH was further increased by FSK, suggesting a synergistic effect of GHRH and FSK. In conclusion, these results show that GHRH exerts antihypertrophic effects in cardiomyocytes, by counteracting PE-induced increase of ANP, cell area, and miR-133 reduction.

PP166

GROWTH HORMONE DEFICIENCY AND CARDIOMYOPATHY: A CASE REPORT

S. Raimondo[1], C. Di Segni[1], M. Persano[1], C. Ierardi[2], A. Mancini[1], A. Pontecorvi[1]

[1]Dip di Scienze Mediche - Divisione Endocrinologia - Università Cattolica del Sacro Cuore - Roma, [2]Dip di Cardiologia - Università Cattolica del Sacro Cuore - Roma

It is known that there is a relationship between chronic heart failure and growth hormone deficiency(GHD); abnormalities of the GH–IGF-I axis in adults are associated with a number of harmful factors for the cardiovascular system. We report here the emblematic history of a patients with a peculiar association of the illnesses. A 66y. man had a long history of cardiomyopathy: non-obstructive hypertrophic cardiomyopathy was diagnosed in 1978; in 2006 he reported palpitations during exercise for which he performed a cardiological work-up, showing the presence of premature ventricular contractions and right bundle branch block. In 2007 a worsening was observed, with onset of mitral annular dilatation, mitral valve regurgitation and left atrial enlargement, intermittent complete left bundle branch block, development of frequent ventricular and supraventricular arrhythmias culminating in the appearance of high frequency atrial fibrillation; so the patient at the end of 2007 was treated with cardiac resynchronization therapy and then had been subjected to several cardioversions. One year ago, the patient started to complain a decrease in visual acuity. A visual field was performed, showing a bitemporal quadrantanopsia. For this reason, the patient came to our observation; we performed a CT scan of the hypothalamic-pituitary region, which showed the presence of a mass the size of 20x15 mm, characterized by a slightly inhomogeneous enhancement after intravenous administration of contrast medium, localized in the sellar region, with enlargement of the parasellar floor. Hormone evaluation did not show pituitary hyperfunction. The dynamic tests showed a significant reduction of the functionality of the GH/IGF-I: the value of IGF-I was 48 ng/ml (normal levels 80-330 ng/ml) and the GH peak after GHRH+bioarginine infusion was 4.49 ng/ml. The patients underwent transsphenoidal adenomectomy; the histological examination confirmed the diagnosis of pituitary adenoma. In particular, it was a tumor with moderate cellularity, diffusely positive for synaptophysin and focal positive for cytokeratin with a proliferative index (Ki67) of about 2%. The control TC, 6 months after surgery, showed the absence of residual disease. Then a replacement therapy with rh-GH was started. The temporal relationships between the worsening of cardiomyopathy and the clinical manifestation of the non-secreting tumor strongly suggested a role of GHD to modify the course of cardiac dysfunction. Experimental models, preliminary human studies and a meta-analisys that included many trials about the effect of GH replacement on the heart have demonstrated that GH administration may have beneficial cardiovascular effects, especially on the heart, even if more experimental and clinical studies are necessary to clarify the mechanisms that determine the variable sensitivity to GH and its positive effects in the failing heart.

PP167

CORRELATION BETWEEN ANTIOXIDANTS AND METABOLIC PARAMETERS IN ADULT GROWTH HORMONE DEFICIENCY

A. Mancini[1], S. Raimondo[1], C. Di Segni[1], M. Persano[1], G. Gadotti[1], J. Pareo[1], R. Festa[2], S. Silvestri[3], P. Orlando[3], L. Tiano[3], A. Pontecorvi[1]

[1]Dip di Scienze Mediche - Divisione di Endocrinologia - Università Cattolica del Sacro Cuore - Roma, [2]Dip di Scienze Cliniche e Molecolari - Università Politecnica delle Marche - Ancona, [3]Ist di Biochimica - Università Politecnica delle Marche - Ancona

It is known that growth hormone deficiency (GHD) is associated with oxidative stress, but discordant data concern GH effects on antioxidants; GHR-ko mice have decreased antioxidants (with differential pattern in relation to sex and tissues), while in df/df a discrepancy has been demonstrated between the increased enzymatic antioxidants and scavengers, which are on the contrary decreased. In humans, GH treatment seems to increase antioxidant capacity, but other studies show reduced lipid peroxidation in GHD adults, reverted by GH treatment. We have evaluated total antioxidant capacity (TAC) and the levels of total, reduced and oxidized CoQ10, a lipophilic antioxidant, in 16 patients with GHD, diagnosed by GHRH+arginine test, due to empty sella or previous transsphenoidal surgery; it was not associated to other hormone deficiency (gonadotropin or thyroid) known to influence antioxidant levels. Moreover, metabolic parameters (glycemia, insulinemia, HOMA-index, total-LDL and HDL-cholesterol, uric acid) were evaluated; 6 patients were also studied before and after a 6 months treatment with recombinant human GH (between 1 and 2 mg/weekly). A control group of 10 normal, aged-matched, subjects was also studied. TAC was determined using the system H2O2-metmyoglobin as source of radicals and a chromogen (ABTS); the latency time (LAG) in the accumulation of ABTS.+, spectroscopically detectable, is proportional to antioxidants concentration. CoQ10 was evaluated by HPLC-electrochemical method. Despite LAG and CoQ10 levels showed a not significant trend to lower levels in GHD vs controls (LAG:66.33±4.46vs75±6.02sec, CoQ10: 0.65 ± 0.26 vs0.68±0.04μg/mL; respectively), the ratio oxidized/total CoQ10 was significantly higher (13.60±4.72%vs4.0±1.0%,p<0.05) and slightly decreased after treatment (11.83±4.70%); treated GHD also showed a greater CoQ10 levels, when corrected for cholesterol levels (171.11 ± 48.20vs153.09±53.37nmol/mmol). In untreated population, IGF was inversely correlated with LAG (r=-0.32,p<0.05). Finally, when evaluated patients with normal or high cholesterol levels, we found significantly higher ratio oxidized/total CoQ10 in hypercholesterolemic patients (14.8±5.6%vs9.8±3.2%) suggesting increased oxidative stress. These preliminary data, in the context of metabolic alterations and cardiovascular risk of these subjects, seem to indicate that oxidative stress could be a link between hormone deficiency and cardiovascular complications; a slight increase in CoQ10, corrected for cholesterol values, is induced by rhGH treatment; however, more data and a more prolonged period of observation is mandatory to draw conclusions on this topic.

PP168

FACTORS AFFECTING PROGNOSIS IN A SERIES OF ACROMEGALIC PATIENTS

F. Lugli[1], A. Fusco[1], A. Bianchi[1], D. Iacovazzo[1], A. Giampietro[1], D. Milardi[1], S. Chiloiro[1], S. Piacentini[1], L. Tartaglione[1], F. Doglietto[2], C. Anile[2], G. Maira[2], L. De Marinis[1]

[1]Policlinico A. Gemelli, Endocrinologia - Roma, [2]Policlinico A. Gemelli, Neurochirurgia - Roma

Introduction: the main goal in the treatment of acromegaly is achieving biochemical control, as to prevent the wide range of cardiovascular, respiratory and metabolic comorbidities. Few data are available about factors affecting prognosis and response to medical treatment.

Materials and methods: we describe a series of 118 patients diagnosed with GH-secreting pituitary adenoma (24 microadenomas, 94 macroadenomas), all submitted to surgery as first line treatment. All patients with persistent disease after surgery have been treated with somatostatin analogues (SSA). We analyzed GH and IGF-1 levels, tumor size and invasiveness, Ki-67 labeling and correlated these findings with prognosis and response to medical treatment.

Results: twenty-eight/118 patients (23.7%) were considered biochemically cured after surgery: these patients had more frequently microadenomas (65 vs 18%) with lower Ki-67 (1.2 vs 1.7) and cavernous sinus involvement (4 vs 46%) compared to patients with persistent disease after surgery. There were no differences among these two groups of patients regarding basal GH and IGF-1 levels. Among the 90 patients treated with SSA, 64 (71.1%) were biochemically controlled. Patients with disease resistant to SSA presented more frequently cavernous sinus involvement (65 vs 29%) and higher Ki-67 (2.4 vs 1.5%) compared to SSA responsive patients. GH and IGF-1 levels did not differ significantly between SSA responsive and SSA resistant patients.

Conclusion: our data show that tumor size, local invasiveness and Ki-67 labeling are all prognostic factors in pituitary GH-secreting adenomas. The apparently low percentage of patients with biochemical remission after surgery probably reflect the high proportion of macroadenomas in our series, considering that our is a tertiary referral center for pituitary diseases.

PP169

A CASE OF CLINICALLY SILENT ACROMEGALY WITH ERECTILE DYSFUNCTION

S. Puglisi[1], R. Vita[1], M. Ragonese[1], A. Albani[1], S. Benvenga[1], F. Trimarchi[1], S. Cannavò[1]

[1]Department of Clinical and Experimental Medicine – Endocrinology, University of Messina - Messina, Italy

Clinically silent Acromegaly is a condition of elevated GH/IGF1 serum concentrations without clinical features of GH excess. Several recent studies suggested that it is probably more common than previously assessed, and its prevalence is evaluated about one-third of all somatotroph adenomas. We report the case of a 77 year old patient, who complained only of erectile dysfunction, started five years before and partially responsive to 50 mg of tadalafil on demand. He did not present any clinical features of acromegaly and he did not complain of headache nor visual field loss. However, acromegaly had likely started many years before, since there were several complications of the disease (multinodular goiter, obstructive sleep apnea syndrome, colorectal polyps, diabetes mellitus, high blood pressure) in his past history. Genital exam was unremarkable. IIEF-5 score was 14 (equivalent to a mild to moderate erectile dysfunction). Serum total and free testosterone as well as prolactin levels were normal, whereas FSH, LH, GH and IGF-1 levels were above normal range. GH excess was confirmed based on the lack of pulse and a nadir > 2 ng/ml when serial blood samples were drawn every 30 minutes for 2 hours (we could not perform OGTT because the patient was diabetic). At MRI, in the pituitary gland there was an oval-shaped lesion of 21x14 mm. In conclusion, acromegaly may be a rare cause of erectile dysfunction, which, as shown here, may be the first sign of a long-standing condition of GH/IGF-1 excess. Its prompt identification is mandatory for an appropriate surgical and/or medical treatment.

PP170

BILATERAL INFERIOR PETROSAL SINUS SAMPLING IN CUSHING'S SYNDROME: COMPARISON BETWEEN AN OLD AND A NEW TECHNIQUE IN NAPLES EXPERIENCE

M. De Leo[1], F. Tortora[2], A. Cozzolino[1], C. Simeoli[1], D. Iacuaniello[1], A. Albano[1], S. Cirillo[2], F. Briganti[3], A. Colao[1], R. Pivonello[1]

[1]Dipartimento di Medicina Clinica e Chirurgia, Sezione di Endocrinologia, Università "Federico II" - Napoli, [2]Dipartimento di Neuroradiologia, Seconda Università degli Studi - Napoli, [3]Dipartimento di Neuroscienze, Università "Federico II" - Napoli

Bilateral inferior petrosal sinus sampling (BIPSS) is the test that offers the highest diagnostic accuracy in the differential diagnosis between pituitary and ectopic Cushng's syndrome (CS). The aim of this study was to compare the diagnostic accuracy of BIPSS performed in the last six years, after the change in the technical procedure with that performed in the past period in Naples centre. Seventeen patients with CS followed-up in our institution between 2007 and 2012 were compared with 10 retrospective patients with pituitary-dependent CS subjected to BIPSS. The change in technical procedure was the use of 4-french (instead of 5-french) hydrophilic-coated vertebral catheters introduced into femoral veins using the Seldinger technique. In the 10 historical patients BIPSS yielded three (30%) false-negative, together with a discordant result between baseline and post-CRH stimulation in one case, whereas side-to-side (S/S) gradient indicating a correct lateralization in nine patients (90%). In the 17 recent patients, no false-negative or false positive cases were observed, although in one case an IPS/periphery (P) gradient was found only at baseline evaluation. Moreover, a S/S ratio correctly indicated the lateralization of the lesion in 100% cases. The mean post-CRH IPS/P ($p<0.01$) gradient was significantly higher in recent than historical patients, although no significant difference was found in mean IPS/P and S/S gradient at baseline evaluation. Sensitivity, specificity and predictive value of BIPSS in the recent group was of 100%, whereas sensitivity and positive predictive values were 70%, and 100% in the historical group. In Naples experience the diagnostic value of BIPSS has been improved in the last years likely due to the introduction of new catheters, permitting to reach a diagnostic accuracy and a predictivity of 100% cases.

PP171

EVALUATION OF THE OXIDATIVE STRESS IN PATIENTS WITH CUSHING'S SYNDROME AT DIAGNOSIS.

I. Karamouzis[1], R. Berardelli[1], B. Fussotto[1], V. D'Angelo[1], C. Zichi[1], R. Giordano[2], E. Ghigo[1], E. Arvat[1]

[1]Scienze Mediche, Div. Endocrinol. Diab. e Metab. - Torino, [2]Scienze Cliniche e Biologiche - Torino

Introduction: Studies in adults have clearly demonstrated that Cushing Syndrome(CS) is a main factor implicated in increased cardiovascular disease (CVD) leading to increased morbidity and mortality. Furthermore, different studies have reported that oxidative stress (OS) and platelet activation (PA) are associated with several pathological conditions and their co-existence increase the risk of CVD.

However, scarce data on the presence of OS among patients with CS exist. The aim of this study was to clarify and determine the oxidant-antioxidant balance in patients affected by Cushing's syndrome at the diagnosis.

Subjects and methods: We evaluated in 11 normal subjects (NS, age mean ±SEM:41.09±1.0years, BMI 23.04±0.92kg/m2) and 11 patients affected by CS (7 of pituitary origin, 1 ectopic and 3 of adrenal origin, age 48.33±3.89years, BMI 28.54±4.31kg/m2). OS was evaluated by measuring plasma 15-F2t-Isoprostane levels (15-F2t-IsoP), highly accurate measure of OS in humans and PA by thromboxane B2 levels (TXB2), which is stable but inactive metabolite of thromboxane A2 (TXA2), representing a reliable indicator of its biosynthesis. We also estimated the antioxidant reserve using plasma total antioxidant capacity (TAC) and serum vitamin E.

Results: The 5-F2t-IsoP levels in CS (10.20±2.43 ng/ml) were significantly elevated ($p=0.002$) respect to NS (1.51±0.06) as also the TXB2 levels in CS (2.96±1.36ng/ml) were significantly ($p=0.002$) higher than what observed in NS (0.35±0.13). On the other hand, their Vitamin E levels (13.30±1.12µg/ml) were significantly lower ($p=0.022$) vs NS (15.48±0.79 ng/ml). It was further observed a significant increase of isoprostane and a clear decrease of TAC levels ($p= 0.006$, $p=0.021$, respectively) in CS with several complications compared to CS without any complication. Finally, a positive correlation between Vitamin E and UFC levels was observed in CS ($p=0.042$, $r=0.542$).

Conclusions: This study demonstrates the impairment of the normal homeostatic balance between oxidant-antioxidant state and the PA in CS. Furthermore, as OS and PA contribute to the pathogenesis of atherosclerosis, it could be further assumed that their markers could be considered as further CVD risk factors.

Finally, the further increase of OS and the further reduction of the antioxidant reserve in CS with several complications, may further affect the increased CVD risk and possibly amplify the mortality in these patients.

PP172

CUSHING-SCORE: A NEW TOOL FOR SUSPECTED EARLY DIAGNOSIS OF CUSHING SYNDROME.

R. Berardelli[1], I. Karamouzis[1], I. Floriani[2], V. D'Angelo[1], C. Zichi[1], B. Fussotto[1], A. Picu[1], S. Grottoli[1], F. Broglio[1], R. Giordano[3], M. Spinello[4], E. Ghigo[1], E. Arvat[5]

[1]Scienze Mediche, Div. Endocrinol. Diab. and Metab. - Torino, [2]Lab. Clinical Trials, Dip. Oncol., Ist. Ric. Farmacol. Mario Negri - Milano, [3]Scienze Cliniche e Biologiche - Torino, [4]Novartis Farma - Origgio (VA), [5]Div. Endocrinol. Oncol. - Torino

Introduction: The Cushing's Syndrome (CS) is a rare disease burdened by high morbidity and mortality. The clinical symptoms often are not diriment, resulting in a delay in diagnosis. To date, in relation to poor knowledge of the disease and the symptomatology often deficient in specific signs and symptoms, the diagnostic latency is very high. Therefore, there is urgent need for a tool able to identify as early as possible the CS. It was created a simple and effective mathematical model (CUSHING-SCORE) aiming to identify patients with a high probability of being affected by CS. Subjects and Methods: In 48 patients with ACTH-secreting pituitary or adrenal cortisol-secreting adenoma (CS, median age 43yrs, range 17-73, 5?, 43?) were recorded signs/symptoms/comorbidities (SSC) present/complained at the diagnosis; it was administrated a specific questionnaire in which were reported all SSC typical of the CS. The same methodology was used in 52 patients, control group, with no ACTH-secreting pituitary or adrenal non-secreting adenoma (non-SC 58yrs 12-83, 26?, 26?). For dichotomous SSC, the frequencies were described by proportions, the comparison between the groups was summarized in terms of odds ratios and tested with the x2. For SSC expressed continuously (eg, age), were used median and range. Using multivariate analysis, the SSC have been associated with SC with statistical significance <0.10. The regression coefficients of the selected variables were used as weights for the production of the score. The cut-off to discriminate patients according to the likelihood of having CS were identified with a recursive division. Results: The elements identified were: age, abnormal glucose and calcium phosphate metabolism, facies rubra, centripetal obesity, muscle atrophy. The product score is derived from equation: score =5(if facies rubra)+4(if altered glucose metabolism)+3(if altered bone metabolism)+1(if central adiposity)+3(if muscular atrophy)-2xdecade of age +17. On this basis, patients with score <7 had a low probability of CS (2.3%), with a score of 8-10 31%, with 11-13 of 73% and if>14 100%. Conclusions: The score, at the time being validated, could become an important tool, simple and practical, for early detection of patients with a high probability of suffering from CS. Positive patients to CUSHING-SCORE, could then be candidate to the biochemical screening, with a possible significant early diagnosis of Cushing's syndrome.

PP173

EFFECTS OF CABERGOLINE TREATMENT ON METABOLIC SYNDROME AND VISCERAL ADIPOSITY INDEX IN PATIENTS WITH HYPERPROLACTINEMIA.

Y. Perone[1], R. S. Auriemma[1], L. Granieri[1], M. Galdiero[1], L. F. Grasso[1], C. Simeoli[1], C. Pivonello[1], M. Gasperi[2], C. Giordano[3], A. Colao[1], R. Pivonello[1]

[1]Dipartimento di Medicina Clinica e Chirurgia, Section of Endocrinology, University "Federico II" - Napoli, [2]Department of Medicine and Health Sciences, University of Molise - Campobasso, [3]Dipartimento di Oncologia Sperimentale ed Applicazioni Cliniche, University of Palermo - Palermo

Introduction: Hyperprolactinemia is reportedly associated with an impaired metabolic profile, particularly in patients with concomitant hypogonadism. The current study aimed at investigating the effects of short (12 months) and long (60 months) treatment with cabergoline (CAB) on metabolic complications, metabolic syndrome (MS) prevalence and visceral adiposity index (VAI) in hyperprolactinemic patients (pts). Patients and Methods: Seventy-one pts (51 F, 20 M, aged 35.4±11.7 yrs), including 36 with microprolactinomas, 32 with macroprolactinomas and 3 with non-tumoral hyperprolactinemia, entered the study. In all pts, PRL and metabolic parameters (BMI, waist circumference, lipid and glucose profile, insulin, VAI) were assessed at diagnosis and after 12 and 60 months of continuous CAB treatment. MS was evaluated in line with NCEP-ATP III criteria. Results: Compared to baseline, CAB induced a significant decrease in PRL levels after 12 months (p=0.000) and a further decrease after 60 months (p=0.000) with complete normalization in 93% of pts. At baseline, MS prevalence was significantly higher in pts with PRL above than in those with PRL lower than the median (187 µg/L, p=0.02). MS prevalence significantly decreased after 12 (12.6%, p= 0.009) and 60 (7%, p=0.000) months of treatment compared to baseline (32.4%). Total cholesterol and triglycerides were significantly reduced after 12-month CAB compared to baseline (p=0.03), and further decreased (p=0.000) after 60-month follow-up. HDL cholesterol resulted significantly increased after 60-month CAB compared to baseline (p=0.000) and 12 months (p=0.000). Glucose and insulin significantly decreased after 12 months of CAB (p=0.001), and were further improved after long-term CAB (p=0.03 and p=0.000 respectively) compared to short-term therapy. Compared to baseline, a slight but not significant decrease in VAI was found at 12-month evaluation, whereas VAI was significantly decreased after 60 months of treatment (p=0.000). Conclusions: Short-term CAB treatment significantly improves metabolic profile, so that to reduce MS prevalence, whereas longer treatment is required to achieve a significant improvement of VAI.

PP174

THE PARADOX OF LOWER THAN EXPECTED CARDIOVASCULAR RISK IN ACROMEGALY: ROLE OF IGF-1

G. Bellastella[1], M. I. Maiorino[1], R. Pivonello[2], L. F. Grasso[2], A. A. Sinisi[3], A. Colao[2], D. Giugliano[1], K. Esposito[3]

[1]Dipartimento di Scienze Mediche, Chirurgiche, Neurologiche, Metaboliche e dell'Invecchiamento, Seconda Università di Napoli - Napoli, [2]Dipartimento di Endocrinologia ed Oncologia Molecolare e Clinica, Università di Napoli Federico II - Napoli, [3]Dipartimento di Scienze Cardio-Toraciche e Respiratorie, Seconda Università di Napoli - Napoli

Background: Endothelial progenitor cells (EPCs), involved in the repairing mechanisms of vascular damage, are positively correlated to IGF-1 concentrations in healthy adults. EPCs may be involved in the lower than expected cardiovascular risk in acromegaly, but this has never been investigated.

Aim: We conducted a cross-sectional study in order to assess the levels of the different phenotypes of circulating EPC in acromegalic patients.

Subjects and Methods: We studied 55 acromegalic patients and 65 matched controls. EPCs were assessed by flow cytometry and IGF-1 by IRMA. Carotid sonography was performed on the same ultrasound machine for the assessment of carotid intima-media thickness (CIMT).

Results: Compared with subjects of the control group, acromegalic patients showed significantly higher levels of EPCs phenotypes expressing KDR antigen, (KDR+, cells per 10 6 events, median and interquartile range, 44 [28-67] vs 23 [13-40] P=0.006; CD34+KDR+ 25 [18-38] vs 12 [8-17] P <0.001; CD133+KDR+ 17 [13-30] vs 8 [6-12] P <0.001; CD34+KDR+CD133+ 16 [12-25] vs 8 [6-10] P <0.001). There was a positive correlation (r = 0.79, P <0.001) between IGF-1 levels and CD34+KDR+CD133+ cells in patients, and an inverse correlation between CD34+KDR+CD133+ cells and CIMT in patients (r = -0.57, P = 0.01), which persisted in multivariate analysis.

Conclusions: Acromegalic patients show higher circulating EPCs levels expressing KDR, positively correlated with IGF1; moreover we found and an inverse correlation between CD34+KDR+CD133+ cells and CIMT. These results suggest that KDR+ EPCs phenotype found in our patients may be involved in the less than expected atherosclerotic burden of acromegaly.

PP175

THE PASIREOTIDE IN THE CUSHING'S DISEASE:EXPERIENCE OF THE SCHOOL OF NAPLES

M. De Leo[1], A. Cozzolino[1], C. Simeoli[1], P. Vitale[1], A. Albano[1], D. Iacuaniello[1], A. Colao[1], R. Pivonello[1]

[1]Dipartimento di Medicina Clinica e Chirurgia, università "FedericoII" - Napoli

Introduction. Pasireotide is a multireceptor-targeted somatostatin analogue, with a high binding affinity to somatostatin receptor subtypes 5, particularly expressed in ACTH-secreting pituitary adenomas, indeed it has been proposed as a potentially effective therapy in Cushing's disease. The purpose of this study is to describe our experience with the use of pasireotide in patients with CD as part of a multicenter, double-blind, phase III study.

Patients and methods. Twenty-five patients (23 F, 2M) with CD were screened, and 14 patients (12 F, 2M) have begun treatment with pasireotide dose 900 µg twice daily. Urinary free cortisol (CLU) was used as markers of disease and evaluated together with clinical parameters expression of the complications of the disease.

Results. Eight patients (57.1%) were treated for 6 months, and seven patients (50%) completed the study up to the twelfth month. In the first three months of treatment, 6 patients left the study due to adverse events (4) or withdrawal of consent (2), at the sixth month another patient left the study for unsatisfactory response to treatment. After 3 months of treatment was observed a significant reduction of the values ??of CLU (p = 0.008) and of ACTH (p = 0.028); 6 patients continued treatment with the initial dose of pasireotide, while the remaining two increased the dose to 1200 µg twice daily. After 6 months of treatment, 37.5% (3/8) of the patients showed normalization of the levels of CLU with a decrease of the average values ??of CLU by over 60% compared to baseline; in this phase of treatment, along with a decrease in the CLU (p = 0.018) was evident reduction in BMI (p = 0.025) and an increase in HbA1c (p <0.018) associated with a non-significant increase in blood glucose (P = 0.09). After 12 months, 28.5% (2/7) of patients maintained a normalization of CLU, with a reduction of the average values ??of about 70% compared to baseline. It is observed, moreover, a decrease of the values ??of ACTH (p = 0.042), an improvement of the score of BDI (p = 0.046) even if associated with an increase in HbA1c (p = 0.028). The most frequent adverse events were hyperglycemia (35.7%) and diarrhea (21%).

Conclusions. Our experience shows that the pasireotide is effective in controlling the secretion of cortisol in 30-40% of patients who continue treatment for long-term, even if hyperglycemia is a common side effect, which requires specific management during treatment.

PP176

FUNCTIONAL STUDIES OF PROKR2 ALLELIC VARIANTS IN ICH PATIENTS HIGHLIGHT THE IMPORTANCE OF BOTH SIGNAL TRANSDUCTION PATHWAYS COUPLED WITH THIS RECEPTOR

D. V. Libri[1], G. Kleinau[2], M. Busnelli[3], F. Guizzardi[1], A. Sinisi[4], A. I. Pincelli[5], A. Mancini[6], G. Russo[7], P. Beck-Peccoz[8], S. Loche[9], M. Maghnie[10], C. Krausz[11], L. Persani[1], M. Bonomi[1]

[1]Lab. di Ric. Endocrino-Metaboliche, IRCCS Ist. Auxologico It. - Milano, [2]Inst. of Exp. Pediatric Endocrinology, - Berlino, [3]CNR - Milano, [4]Dip. di Sc. Cardiotoraciche e Respiratorie - Napoli, [5]Clin. Med., AO San Gerardo - Monza, [6]Div. di Endocrinologia, Dip. di Med. Int. - Roma, [7]Dip. di Pediatria, Osp. San Raffaele - Milano, [8]Dip. di Sc. Cliniche e di Comunità - Milano, [9]Serv. Endocrinologia Pediatrica - Cagliari, [10]Dip. di Pediatria, IRCCS G. Gaslini - Genova, [11]Dip. di Fisiopat. Clin. - Firenze

Idiopathic central hypogonadism (ICH) is a rare and heterogeneous disease with a strong genetic component.Among the 15 genes to date linked to this disease the prokineticin system (PROKR2 and PROK2) is one of the main genetic component.To date all functional studies carried out on PROKR2 variants have focused exclusively on Gq-associated transduction pathway.Here, we present the results of the genetic screening in the largest series of ICH Italian patients (n = 246) where we found 4 novel (p.V158I, p.T260M, p.V334M, p.N15TfsX30) and 5 already known (p.L173R, p.R268C, p.V274D, p.V331M, p.H20MfsX23) germline variants in PROKR2 gene. We evaluated for the first time effects of these alterations on the two different PROKR2-mediated signalling pathways: IP3-Ca2+ (Gq-coupling) and cAMP (Gs-coupling).All the mutated receptors, transfected in HEK293 cells, showed a 50% reduction in membrane expression levels compared to the wild-type (wt), except for p.V158I, p.V331M, p.V334M.Variants p.T260M, p.R268C and p.V331M showed no remarkable changes in cAMP EC50 while the IP3 signalling appeared strongly affected; p.L173R and p.V274D presented no virtual response in terms of cAMP accumulation. Moreover, p.L173R had an IP3 response (EC50) similar to wt and p.V274D presented a 10-fold increase of IP3 EC50. Variant p.V334M lead to a 3-fold increase of EC50 for both cAMP and IP3. We also made an homology model of PROKR2 in order to provide structural insights on the transducing mechanisms of this GPCR. Our PROKR2 mutations are located at different spatial receptor regions and might cause modifications of receptor functions by diverse mechanisms, such as the alteration of binding surface with G-proteins or the active/inactive transition of the receptor.Concluding, PROKR2 variants cause inability to stimulate one and/or two signal transduction pathways, suggesting that the integrity of both ways is necessary for development and function of GnRH-secreting neurons.

PP177

MANAGEMENT OF CRANIOPHARYNGIOMA IN CHILDHOOD AND ADULTHOOD: THE ROLE OF ENDOSCOPIC ENDONASAL SURGEY.

M. Faustini-Fustini[1], M. Zoli[2], D. Mazzatenta[2], G. Frank[2], E. Pasquini[3]

[1]Dipartimento Medico. Unità di Endocrinologia. Ospedale Bellaria - Bologna, [2]IRCCS Istituto delle Scienze Neurologiche. Centro di Chirurgia dei Tumori Ipofisari e di Chirurgia Endoscopica della Base Cranica. Ospedale Bellaria. - Bologna, [3]Dipartimento Chirurgico, Unità ORL. Azienda USL Bologna - Bologna

Introduction. To date, a number of endocrinologists and neurosurgeons still believe that the vast majority of patients affected with craniopharyngioma (CR) have to be treated by craniotomy. The introduction of endoscopic technique in the surgical approach to the sellar/suprasellar region would have profound implications for treatment. Aim of the study was to analyze results, advantages and limits of endoscopic endonasal approach (EEA). *Design/Methods*. Ninety-five patients underwent surgery for CR from 1998 to 2012. Trancranial approach was chosen for 33 patients (35%) on the basis of shape, size, and location. The remaining 62 (52% sellar and suprasellar, 45% purely suprasellar, 3% purely intrasellar; male to female ratio: 0.73; median age: 46 yrs, range, 3-83 yrs; 26% aged 18 years or younger) were operated by EEA, for a total of 71 surgical procedures, including 9 recurrences during the follow-up period (mean: 59 months, range, 3-98). *Results.* At presentation, visual impairment was detected in 77%, hypopituitarism in 54%, isolated diabetes insipidus (DI) in 3%, panhypopituitarism coupled with DI in 24%. Endocrine function became further worse postoperatively, as expected (novel cases of DI and hypopituiraism occurred in 12.6% and 15.4%, respectively); conversely, after EEA visual function returned to normal in 35%, improved in 47%, and remained unchanged in 18%. Gross tumor removal was obtained in 80%. Morbidity consisted in post-operative CSF leak (18%) and chronic subdural haematoma (1,5%); one acute post-operative hydrocephalus (1,5%) was fatal. Weight gain occurred in 6%. Recurrence rate was 14.5%. Quality of life was preserved in 85% of cases, a moderate worsening (social reintegration at a lower level) occurred in 10% and a heavy worsening (semi- or totally dependent) in 5%. *Conclusions.* Our data shows that EEA was a reliable approach in the majority of patients (65%) referred to us. The technique, safe and well tolerated, provides a direct approach along the way of the tumour growth and allows the surgeon to remove the lesion, avoiding brain retraction and vascular-nervous structures manipulation. Practical matters, particularly shape and location of the tumour, are likely to be critical in the choice of the better approach, making the design of randomized trials quite difficult.

PP178

RATHKE CLEFT CYSTS: SURGICAL OUTCOME- THE EXPERIENCE OF ITALIAN COOPERATIVE STUDY GROUP

D. Mazzatenta[1], G. Frank[1], M. Faustini Fustini[1], M. Zoli[1], E. Pasquini[2]

[1]IRCCS ist delle scienze neurologiche - Bologna, [2]ORL-Area metropolitana - Bologna

Patients and Methods:all the consecutive cases of RCCs operated on since 1998 to August 2010 in 10 italian neurosurgical centers (Bologna, Napoli, Pavia, Treviso, Pisa, Cagliari, Firenze, Livorno, L'Aquila) has been retrospectively collected. Medical reports, surgical records, radiological charts and follow-up data have been evaluated. Results:113 patients have been enrolled. The more common symptoms were endocrinological dysfunction (40 cases, 43%), headache (26 cases, 28%), visual disturbances (25 cases 27%) and CN III palsy (1 case) and CN VI palsy (1 case). RCC were purely intrasellar in 29(26%) patients, intra/suprasellar in 79(70%) and purely suprasellar in 5(4%). The endoscopic transsphenoidal approach was performed in 109 patients; one case was treated with an endoventricular endoscopic cystic drainage, and 3 others with a craniotomic approach. Among patients treated by endoscopic endonasal surgery radical resection was achieved in 45 cases (40%), while in the remaining the biopsy of the wall and the drainage of the content with or without the marsupialitation in the sphenoidal sinus was performed. Hyperprolactinemia normalized in 17 patients (85%). No improvement in DI or anterior pituitary insufficiency was observed. Headache disappeared in all cases. Visual acuity improved or normalized in 10 cases (83%), and remained unchanged in 2(17%). Visual field defects improved or normalized in 21(77%) and remained unchanged in 6 patients (23%). In one case visual function (both visual acuity and visual field) worsened. 2 major complications were observed, one fatal pulmonary embolism and one thalamic ischemia, resulting in mild cognitive impairment. The more common complications was CSF leak, which occurred on in 8 cases (7%), requiring re-intervention for plastic repair. Endocrinological complications consisted in 6 cases of permanent DI, 4 of SIADH, 10 of new pituitary insufficiency. One epistaxis occurred, one overpacking caused a visual worsening, requiring reintervention after which visual function normalized. Eleven recurrences (2 in the same patient) were observed (9%) but only 8 were symptomatic requiring re-intervention. Mean time for recurrence was 4.2 years (range 1-8). Of these 11 recurrences 8 occurred on after radical resection and 3 after cyst drainage. There is no statistical differences between the radical removal and drainage of the cyst for ophtalmological, endocrinological outcome, or recurrence and complication rate. Conclusion:only symptomatic or growing Rathke's cleft cysts have been considered for surgery. In such a case the endoscopic endonasal treatment has been the more commonly therapeutic option. No difference in clinical outcome has been observed between radical cyst resection and simple biopsy with drainage of the cyst. Thus, aggressive surgical strategies seem to be unjustified, and a more conservative attitude (biopsy of the wall and drainage of the cyst's content) should be considered the gold standard of treatment.

PP179

CORONARY FLOW RESERVE IN ACROMEGALY

F. Dassie[1], S. Tellatin[2], G. Famoso[2], E. Zanchetta[1], C. Martini[1], A. Paoletta[3], N. Sicolo[1], F. Fallo[1], R. Vettor[1], F. Tona[2], P. Maffei[1]

[1]Clinica Medica III, DIMED - Padova, [2]Department of Cardiological, Thoracic and Vascular Sciences - Padova, [3]Endocrinology Outpatient Service - Cittadella

Background: Acromegaly (AC) increases the risk of cardiovascular diseases. We evaluated coronary flow reserve (CFR) by transthoracic Doppler echocardiography (TDE), as an index of coronary microvascular function, in AC. Methods: We studied 39 AC patients (pts) (22 M, aged 52±11 years); 9 pts were medically controlled (5 pts with somatostatin analogs and 4 with GH receptor antagonist), 2 pts were cured and 28 have active AC; pts have not clinical evidence of heart disease. AC pts were compared with 48 controls matched for age and gender. Coronary flow velocity in the left anterior descending coronary artery was detected by TDE at rest and during adenosine infusion. CFR was the ratio of hyperaemic diastolic flow velocity (DFV) to resting DFV. A CFR≤2.5 was considered abnormal. The median time between the onset of symptoms and CFR assessment was 5 years (interquartile range 2-10 years). In AC pts anthropometric and biochemical profile were collected. Results: In AC pts, CFR was lower than in controls (2.9±0.8 vs 3.8±0.7, p<0.0001). CFR was ≤2.5 in 13 (33.3%) pts compared with controls (0%) (p<0.0001). CFR was inversely related to insulin-like growth factor 1 (IGF-1) levels. In pts with CFR≤2.5, IGF-1 was higher (756 [381-898] vs 246 [186-484] mg/L, p<0.004) while growth hormone (GH) levels were similar (6.3 [2.8-13.7] vs 5 [2.8-8.9] mg/L, p=0.8). At multivariable analysis adjusted for age, gender and other cardiovascular risk factors, IGF-1 was an independent determinant of CFR (b=-0.527, p<0.0001) and a predictor of CFR≤2.5 (p=0.01). Conclusions: Microvascular function is impaired in AC and is correlated with IGF-1 independently of GH levels, suggesting a negative effect of IGF-1 on coronary microcirculation that may contribute to the increased cardiovascular risk in AC.

PP180

BLOOD PRESSURE VARIABILITY IN ACROMEGALY

F. Dassie[1], A. Grillo[2], R. Carretta[2], B. Fabris[2], L. Macaluso[2], M. Bardelli[2], A. Rebellato[1], C. Martini[1], A. Paoletta[3], R. Vettor[1], N. Sicolo[1], F. Fallo[1], P. Maffei[1]

[1]Clinica Medica III, DIMED - Padova, [2]Department of Medical, Surgical and Health Sciences - Trieste, [3]Endocrinology Outpatient Service - Cittadella

AIM: Blood pressure variability (BPV) has been more recently shown to represent an additional correlate and possibly a causal factor of the hypertension related cardiovascular complications. The aim of this study was to investigate short term systolic and diastolic variability indexes in 96 patients with active acromegaly in comparison with 35 aged matched normotensive control subject (CTRNT). PATIENT AND METHODS: Patients underwent 24 hour ambulatory blood pressure monitoring (ABPM) and BPV was calculated as follows: systolic and diastolic standard deviation of 24 hour (SD_S, SD_D), day-time (SD_S1, SD_D1) and night-time (SD_S2, SD_D2); weighted SD of 24 hour systolic and diastolic (wSDS, wSDD) and systolic and diastolic average real variability (ARV_S, ARV_D). According to 2007 ESH-ESC guidelines, 34 acromegalic subjects were hypertensive (ACROHYP>130/80 mmHg) at ABPM (M15/F19, MBP 101.1±7.2 mmHg, mean age 54.8±12.8 yr), and 62 were normotensive (ACRONT M31/F31, MBP=85.2±5.6 mmHg, mean age=46.5±14.0 yr). In acromegalic patients anthropometric and biochemical profile were collected. RESULTS: Acromegalic patients, either normotensive or hypertensive, had higher SD_S, SD_D, SD_S1, SD_D1, SD_S2, wSDS, wSDD, ARV_S and ARV_D when compared against controls (all variables of ACRONT and ACROHYP vs CTRNT, P<0.01); only SD_D2 did not differed from the control population (ACRONT vs CTRNT, P<0.059; ACRONT vs CTRNT, P<0.186). CONCLUSION: BPV is increased in acromegalic population. It may represent an additional cardiovascular risk factor in this disease. The role of GH/IGF-I axis has to be further clarified.

PP181

THALASSEMIA MAJOR, GH DEFICIENCY, DIABETES AND CARDIAC IMAGING

F. Dassie[1], G. Geranio[2], L. Frizziero[2], P. Melacini[3], A. Pepe[4], R. Vettor[1], M. C. Putti[2], P. Maffei[1]

[1]Clinica Medica III, DIMED - Padova, [2]Department of Pediatrics - Padova, [3]Department of Cardiological, Thoracic and Vascular Sciences - Padova, [4]Magnetic Resonance Imaging Unit, Fondazione G. Monasterio C.N.R - Pisa

Background: In thalassemia major (TM) regular transfusion therapy leads to iron overload-related systemic complications, including endocrine alterations such as diabetes mellitus (DM) and pituitary insufficiency, in particular growth hormone deficiency (GHD). These complications could have a role in worsening iron overload-related cardiac dysfunction. Methods: We studied 18 patients with TM (M6; mean age36±7.4yrs). Biochemical GHD was tested with growth hormone-releasing hormone + arginine. All patients were studied by echocardiogram (ECHO), cardiac magnetic resonance imaging (MR) and cardiac MR T2* (CMRT2*). We also evaluated their glycemic profile. Results: 8 patients were diabetic and 5 patients had a GHD condition. Patients with DM had greater intracellular iron stores than those without DM (noDM) (mean septumT2*: DM 21.1±8.3 vs noDM 40.4±12.4 P=0.002; mean globalT2*: DM 23.3±9.5 vs noDM 39.2±11.2 P=0.005) at CMRT2* and a reduced E/A (DM 1.53±0.4 vs noDM 2.13±0.7 P=0.041) at ECHO. In GHD patients CMRT2* was not different in comparison to noGHD. Patients with GHD had a greater mass (LVM) and dilated (LVV) left ventricle with reduced ejection fraction (EF) than those without GHD (GHD vs noGHD: LVM 100.7±48.9gr/m2 vs 62.4±10.2 gr/m2 P=0.021; LVV 80.4±18.4ml/m2 vs 62.6±9.6 ml/m2 P=0.021; EF 51±7.2% vs 58.2±4% P=0.035) at ECHO. Conclusions: This study has highlighted an adding role for diabetes mellitus and GH deficiency in the origin of cardiac dysfunction in thalassemia.

PP182

EFFICACY AND SAFETY OF RHGH THERAPY IN ADULT POLYTRANSFUSED ß-THALASSEMIC PATIENTS

M. POGGI[1], V. BISOGNI[1], S. MONTI[1], V. TOSCANO[1]

[1]Cattedra di Endocrinologia, II Facoltà di Medicina, Azienda Ospedaliera Sant'Andrea - Roma

Introduction. Impairment of GH/IGF-I axis has been recently described in patients affected by β-Thalassemia Major (TM). The presence of a state of GH deficiency could be an important actor in the induction and progression of bone and cardiac diseases in these kind of patients.

Aim. To report results of short term treatment with recombinant human GH (rhGH) therapy in TM affected by GH deficiency (GHD).

Subjects and Methods. We studied a large population (122) adult TM. A state of GH deficiency was detected in a relevant part of entire group (35/122 = 28.6%). We planned a short term treatment (1 year) with rhGH in 18 adult TM patients at a starting dose of 0.2 mg/die. During treatment we evaluated clinical (waist circumference, body mass index) hematochimical (glycemic and lipid profile) and psychological parameters (by ad hoc validated questionnaire on quality of life – QoL-H). Moreover we observed, during the whole period, the bone mineral density detected by DEXA. All these values were compared with those of a similar group (16 adult TM) affected by GH deficiency but not enrolled in treatment with rhGH.

Results. In the group treated with rhGH we observed, at the end of follow up period, a significant increase in IGF-1 values (120.71 ± 48.08 ng/ml vs 81.47 ± 36.27 ng/ml, p = 0.007) and an important reduction of waist circumference (87.61 ± 7.42 cm vs 89.53 ± 0.11 cm, p = 0.003). The entire group showed a remarkable improvement in sense of well being detected by specific questionnaire (QLS-H score: 53.25 ± 11.31vs 38 ± 7.07, p = 0.048; Z score – 0.50 ± 0.02 vs – 0.98 ± 0.67, p = 0.047). No one reported any side effects during treatment. Moreover during follow up period the group planned to treatment with rhGH showed a fair improvement in bone mineral density while the control group showed an outstanding deterioration of the bone status in association with a clear worsening of metabolic profile.

Conclusions. Our work firstly shows results of short term treatment (1 year) with rhGH in TM. Treatment seems to be safe and well tolerated. rhGH therapy seems to be able to achieve good results in terms of metabolic profile, perceived sense of well being and bone mineral density. In the future rhGH therapy could be a promising approach in the treatment of TM affected by GHD, specially regarding metabolic profile, bone disease and quality of life.

PP183

REDUCTION OF PRIMARY MOTOR CORTEX THICKNESS IPSILATERALLY TO INVOLUNTARY HAND MOVEMENTS IN MALES WITH KALLMANN SYNDROME AND MIRROR MOVEMENTS

R. Manara[1], A. Salvalaggio[2], V. Palumbo[3], G. Bonanni[4], N. Greggio[5], E. Napoli[6], A. D'Errico[7], E. Cantone[8], G. Ottaviano[9], D. Visconti[3], A. Favaro[10], A. Brunetti[7], F. Di Salle[1], A. A. Sinisi[3]

[1]Neuroradiologia Univ Salerno - Salerno, [2]IRCCS San Camillo - Venezia, [3]Endocrinologia II Univ Napoli - Napoli, [4]Endocrinologia, Univ Padova - Padova, [5]Pediatria Univ Padova - Padova, [6]Medicanova - Battipaglia, [7]Radiologia, Univ Federico II - Napoli, [8]ORL, Univ Federico II - Napoli, [9]ORL, Univ Padova - Padova, [10]Dpt Neuroscienze, Univ Padova - Padova

Involuntary hand movements that mirror intentional movements of the opposite hand (bimanual synkinesis) might be present in male Kallmann syndrome (KS) patients, but the underlying anatomo-functional mechanism is unknown. Either abnormal development of the ipsilateral corticospinal tract or lack of contralateral motor cortex inhibitory mechanisms through the corpus callosum were hypothesized. This study aimed to investigate the anatomo-functional basis of mirror movements with recent MRI techniques specifically addressing regional cortical thickness and white matter integrity. ***Methods***: presence and side of mirror movements were assessed in 38 KS male patients (mean age 29.5 yrs). All pts underwent a brain MRI protocol that included T1-weighted volumetric and diffusion tensor images with a 1.5T scanner (Achieva, Philips). MR images were processed using FreeSurfer and FSL to estimate cortical thickness and to evaluate white matter integrity measures (fractional anisotropy, mean diffusivity), with particular attention to corpus callosum and cortico-spinal tracts. As controls we used 16 healthy volunteers and subjects with headache. ***Results***:10 KS pts (26%) with mirror movements showed decreased primary motor cortex cortical thickness compared to healthy controls and to KS pts without mirror movements ($p<0.05$). In two KS pts with unilateral mirror movements, the cortical thickness was decreased ipsilaterally to involuntary movements. Integrity of white matter myelination was preserved. ***Conclusions***: the significant reduction of primary motor cortex thickness ipsilaterally to involuntary movements suggests that defects in neuronal migration and inhibitory trans-callosal connections between primary motor cortices of the two hemisphere may be involved in mirror movements elicitation during voluntary movements execution of the opposite hand.

PP184

PSYCHOPATHOLOGY, SEXUALITY AND THE ROLE OF PROLACTIN IN A GROUP OF FIRST-EPISODE PSYCHOSIS PATIENTS

G. Ciocca[1], J. Usall[2], M. Dolz[3], E. Limoncin[1], G. L. Gravina[1], E. Carosa[1], B. Sánchez[3], A. Barajas[2], I. Baños[2], E. Huerta[2], A. Farreny[2], E. A. Jannini[1], S. Ochoa[2]

[1]Department of Biotechnological and Applied Clinical Sciences - L'Aquila, [2]Parc Sanitari Sant Joan de Déu - Barcelona, [3]Sant Joan de Déu Hospital - Barcelona

Introduction and Aim: This study mainly aims to investigate sexual dysfunctions , psychopathological symptoms and the role of prolactin in people with first episode psychosis in the initial phase of pharmacological treatment.

Materials and Methods: 40 males and 37 females with first episode psychosis took part in the study. We administered a psychiatric protocol composed of PANSS and UKU along with SCID- DSM-IV diagnosis. We also took into account the influence of antipsychotic treatment and prolactinemia. All patients were of reproductive age and had received pharmacological treatment for less than three months.

Results: 42.5% (17/40) of men and 37.8% of women (14/37) were found to be affected by sexual dysfunction. In addition, both the groups had a hyperprolactinemic mean score: 743.6 mIU/L in males, 1541.92 mIU/L in females.

Logistic regression showed that the influence prolactin levels on sexual dysfunctions was not statistically significant in both the groups, in particular in male: erectile dysfunction (p=.359; OR=1.002), ejaculatory alteration (p=.774; OR=1.000), decrease of libido (p=.859; OR=1.000), orgasmic alteration (p=.426; OR=1.001); in female: alteration of vaginal lubrication (p= .14; OR= 1.001), decrease of libido (p= .08; OR= 1.000), orgasmic alteration (p=.1; OR=1.001).

We did not find any correlation between psychopathology and sexual dysfunctions in males . In females, general psychopathology and positive symptoms were linked to the alteration of vaginal lubrication: (r=.547; p=0.011) and (r=.485; p=0.003), and general psychopathology was linked also to the orgasmic alteration (r=.500; p=0.013).

Conclusion: These results revealed that in the initial phase of pharmacological treatment high prolactin levels did not seem to be linked to and to influence sexual dysfunctions. In particular, in the female group sexual dysfunctions were more linked to psychopathology than hyperprolactinemia.

PP185

SALIVARY CORTISOL IN THE DIFFERENTIAL DIAGNOSIS OF CUSHING SYNDROME, PSEUDO-CUSHING STATE, ADRENAL INCIDENTALOMA AND ADRENAL INSUFFICIENCY

F. Ceccato[1], M. Barbot[1], M. Zilio[1], S. Ferasin[1], G. Occhi[1], S. Rizzati[1], N. Albiger[1], L. Mazzai[1], M. Iacobone[2], L. Alessio[3], L. Denaro[3], F. Mantero[1], C. Betterle[1], C. Scaroni[1]

[1]Endocrinology Unit, Department of Medicine DIMED, University Hospital of Padua - Padua, [2]Minimally Invasive Endocrine Surgery Unit, Department of Surgery, University Hospital of Padua, Italy - Padua, [3]Neurosurgery Division; University Hospital of Padua - Padua

Objective: Salivary cortisol has been recently proposed as a simple tool to study Hypothalamic-Pituitary-Adrenal (HPA) axis: lack of circadian rhythm is a peculiar marker of Cushing Syndrome (CS), and some authors reported that low salivary cortisol levels could be a marker for adrenal insufficiency.

Aim: to study the performance of salivary cortisol for the diagnosis of HPA axis diseases.

Subjects and Methods: We analyzed Morning Salivary Cortisol (MSC) and Late Night Salivary Cortisol (LNSC) in 406 consecutive subjects: 52 Cushing Disease (CD), 13 Ectopic-CS, 17 adrenal-CS, 27 CD in remission, 45 adrenal incidentaloma, 73 Pseudo-Cushing, 75 patients with adrenal insufficiency and 104 healthy subjects. We analyzed samples by RIA assay (sensitivity of 0.5 ng/mL and intra-assay and inter-assay variation of 3% and 9%).

Results: LNSC was higher in CS than in control population, a threshold value of 5.24 ng/mL provided high sensitivity and specificity to screen for CS in healthy subjects and in Pseudo-Cushing group. We documented higher LNSC in ectopic-CS than CD, a value of 24.17 ng/mL reached the best accuracy. We found no difference between CD in remission and healthy subject. LNSC was higher in adrenal incidentaloma than in healthy controls. MSC below 2.65 ng/mL revealed high sensitivity and specificity between healthy subjects and adrenal insufficiency.

Conclusions: salivary cortisol is a useful tool to assess endogenous hypercorticism and able to provide a differential diagnosis of the ACTH-dependent forms and from the Pseudo-Cushing state, moeover it is useful to identify adrenal insufficiency and to follow-up patients in remission of CD.

PP186

ACROSCORE: A NEW TOOL FOR EARLY DETECTION OF ACROMEGALY DESEASE

N. Prencipe[1], I. Floriani[2], S. Di Giacomo[1], F. Guaraldi[1], A. Berton[1], V. Gasco[1], M. Spinello[3], E. Arvat[4], E. Ghigo[1], S. Grottoli[1]

[1]Divisione di Endocrinologia, Diabetologia e Metabolismo - Torino, [2]Dipartimento di Oncologia, Istituto di Ricerche Farmacologiche Mario Negri - Milano, [3]Novartis Farma, Origgio - Varese, [4]Divisione di Endocrinologia Oncologica - Torino

Acromegaly in a rare disease due to hypersecretion of GH/IGF-I. It's a systemic disease and multi-organ complications, somatic and acral modifications, headache, hyperidrosis and menstrual disorders are principal features. Clinical effects of GH excess may occur insidiously over many years. That's why a great diagnostic delay from the onset of signs and symptoms (about 5–8 y) occurs. It is associated to higher mortality and duration of disease is one of most important factors. Therefore, early recognition is necessary to obtain a high rate of treatment success and to avoide long-term comorbidities. General practitioners (besides endocrinologist) are ones that mainly should suspect acromegaly. Aim of study: to create a simple and effective tool (ACROSCORE) that could be used by any physician to identify acromegalic patient. Methodes: we recorded signs and symptoms and comorbilities (SSC) in 120 acromegalic patients and 62 patients with non GH-secreting tumor, representing the control group (non-ACRO) by using an interview and data collected in clinical records.. For dichotomous SSC: we calculated the prevalence rate in cases and control group, used the odds ratio as summary measure of association and tested the null hypothesis of no association with the ?2 test. For continuous features (e.g. age): range and median were used as summary measures. We developed the statistical model for ACROSCORE by selecting SSC associated with acromegaly (cut-off value set = 0.10) through logistic multivariate analysis. The regression coefficients of selected variables model were used to derive weighting factors of the diagnostic index: they were 'normalised' dividing by the smallest one and rounding the resulting ratios to the nearest integer value. We selected more relevant score cut-offs for the diagnosis of acromegaly by a "regression-tree analysis". Results: features of subjects: ACRO: 72F, age: 49.0 y, 18-83; non-ACRO: 37 F, 49.0 y, 19-77. Selected SSC: acral and somatic modifications, goiter, presence of bowel polyps and reduction of acromegaly rate in older patients. Final score derived from the equation: Score=8+6(if polyps)+6(if goitre)+12(if acral/somatic modifications)-(decade of age). Probability to being acromegalic patient: score <10=5.6%; score 10-16=74.4%, score >16=98.9%. Therefore, after external validation, ACROSCORE could be a new , pratical and simple tool to early diagnose acromegaly for a better prognosis

PP187

ACUTE HYPOPITUITARISM IN A CASE OF PLACENTA ACCRETA

M. G. Sama[1], P. Cenni[2], F. D. Baccarini[3], C. Cappi[1], S. Senni[1], S. Versari[1], B. Di Deco[1], F. Bondi[1]

[1]Endocrinologia - Ravenna, [2]Radiologia - Ravenna, [3]Anestesia e Rianimazione - Ravenna

UM, primipara, 31 years old, gave birth to 40 ° w (natural childbirth, healthy male of 3.5 kg weight); 2 hours after birth the patient was affected by severe postpartum hemorrhage that necessitated finally hysterectomy.

Meanwhile, the bleeding determined a severe anemia (hemoglobin up to 3.6 mg / dl) and severe hypovolemia, with signs of low flow (Pa max 70, 25 Fr, pallor, sweating icy), with moreover intact sensory (GCS 15); the patient had also a severe alteration of electrolytes (Na + 122 mEq / L and K + 3 mEq / L), and hypoglycemia (65 mg / dl).

After three days, with clinical conditions apparently stabilized, the patients presented a generalized tonic clonic seizure; the EEG showed only non specific alterations and a CT scan excluded brain ischemic events or bleeding but showed a clear aspect of cerebral oedema; a subsequent MRI demonstrated acute ischemia of pituitary gland without intra- and supra-sellar lesions, and chiasmatic impairment; the cavernous sinuses were intact and signs of ophthalmoplegia were not found.

A steroid therapy, with dexamethasone at the beginning and with hydrocortisone subsequently, was administered; the hormonal values, carried out in conditions of urgency before the introduction of steroid therapy, showed a condition of severe hypopituitarism (TSH 1.6 ?U / ml, FT4 5.8 pg / ml?; ACTH 8.1 pg / ml, Cortisol 19 ng / ml; 0:34 GH ng / ml, IGF-1 55 ng / ml; 0.3 FSH IU / l, LH 0.1 IU / L, E2 9 ng / ml; Prolactin 8.4 ng / ml).

After 10 days of hospitalization in intensive care unit the patient was sent home with steroid and levothyroxine replacement therapy.

After 3 months it was possible to suspend the therapy considering the full recovery of the anterior-pituitary axis.

The patient was affected by placenta accreta, a condition characterized by abnormal coupling of chorionic villi in the myometrial thickness for partial or total absence of the basal decidua; if demonstrated in the prenatal period, the uterus can be saved by means of cesarean section or elective embolization of the uterine arteries; this affection is associated with maternal and fetal mortality in 19% of cases.

The Sheehan's syndrome is a rare cause of hypopituitarism, leading to ischemic necrosis of the pituitary gland during or shortly after childbirth, when an event, usually bleeding, results in a critical reduction of blood supply to the gland that during gestation shows an increase of size and of metabolic needs.

PP188

EFFICACY AND TOLERABILITY OF LOW DOSE, LONG TERM TREATMENT WITH TOLVAPTAN IN HYPONATREMIC PATIENTS WITH SIADH

P. Razzore[1], J. Ramunni[1], E. Gamarra[1], F. Ragazzoni[1], G. Magliona[1], F. Garino[1], P. Limone[1]

[1]Div. Endocrinology AO Mauriziano - Turin

Hyponatremia (HYPO) is the most frequent electrolyte abnormality occuring in clinical practice and is associated with increased morbidity and mortality. The syndrome of inappropriate secretion of antidiuretic hormone (SIADH) is the most common cause of euvolaemic HYPO. Hypertonic saline infusion is the indicated treatment for acute symptomatic HYPO, while fluid restriction and correction of the underlying disorders is the mainstay of treatment for mild and moderate HYPO. However, fluid restriction is often difficult to obtain in SIADH pts. Tolvaptan (TOLV) is an oral non-peptide selective V2 vasopressin receptor antagonist recently become available at a dosage of 15-30 mg once daily (OD) for hyponatremic pts with SIADH. Only case reports are present in literature on long term low dose treatment with TOLV in SIADH pts. We report data on the use of TOLV in 6 SIADH pts (2M, 4F, mean age 69.1, range 45-87). Two pts were affected by idiopathic SIADH, one patient had pulmonary fibrosis, one developed a paraneoplastic syndrome in association with NHL and developed SIADH as a result of neurosurgery for a subdural haematoma and an extensive parasellar meningioma respectively. The latter of the two was on antiepileptic drugs, non suitable for substitution. All pts were hospitalized prior to starting treatment due to symptomatic HYPO and clinical and biochemical data were in accordance with the diagnosis of SIADH. TOLV was started at 15 mg OD in all but two patient (which started at 7.5 mg OD). Baseline serum [Na+] levels were 126.8±2.3 mmol/l (mean ± SD), raising to 134.3 ± 4 mmol/l after 24 hours and 136.8 ± 4 after 48 hours, with an increase at 24 and 48 hours respectively of 7.5 and 10 mmol/l. After normalization of sodium levels TOLV was titrated in order to mantain sodium levels in a safety range above 133 mmol/l. The mean weekly dosage was 18.43 mg/weekly (range 4.55 – 35 mg/week). Data from one, two, three, six, twelve and eighteen months therapy in 5, 5, 3, 2, 1, 1 pts respectively were 137±3.3, 135.8±3.8, 133±1.4, 139.3±4.5, 137 and 136 mmol/l. No significant adverse effects were reported, except for diuresis increase and slight headache in 2 out of 5 pts in the first 24 hours; only patient #1 needed admission to the emergency department due to symptomatic HYPO (120 mmol/l) after 12 months of treatment, probably due to suboptimal compliance towards treatment; after hypertonic infusion and [Na+] normalization she was restarted on low dose TOLV with no further adverse events. Our data suggest that individually titrated low dose treatment with TOLV is effective and safe in normalizing serum [Na+] levels in hyponatremic pts with SIADH and could therefore significantly reduce the overall cost of treatment for these patients.

PP189

OLFACTORY FUNCTION IN MALE KALLMANN SYNDROME: CORRELATION WITH OLFACTORY BULBS VOLUME AND FRONTAL CORTEX GIRI'S DEPTH

A. Salvalaggio[1], A. D'Errico[2], R. Manara[3], V. Citton[1], V. Palumbo[4], G. Bonanni[5], N. Greggio[6], E. Napoli[7], E. Cantone[8], G. Ottaviano[9], A. Favaro[10], A. Elefante[2], A. Brunetti[2], F. Di Salle[3], A. A. Sinisi[4]

[1]IRCCS San Camillo - Venezia, [2]Radiologia Univ. Federico II - Napoli, [3]Neuroradiologia Univ. Salerno - Salerno, [4]Endocrinologia II Univ. Napoli - Napoli, [5]Endocrinologia Univ Padova - Padova, [6]Pediatria Univ Padova - Padova, [7]Medicanova - Battipaglia, [8]ORL Univ Federico II - Napoli, [9]ORL Univ Padova - Padova, [10]Neuroscienze Univ Padova - Padova

Background: sensorial deficit and structural alterations of olfactory bulbs (OB) and sulci (OS) are present in Kallamn syndrome (KS). **Aim**: to measure OB volume and OS depth using magnetic resonance imaging (MRI) sequences in KS patients with a well established olfactory function. **Methods**: olfactory function was assessed in 41 KS male pts (mean age 29.5 yrs) using the "Sniffin' Sticks" test battery (Burghart Medical Technology). Odor threshold, discrimination, and identification were evaluated by three subtests, and results were then summed up to a composite a score (TDI score). Pts were divided into 2 groups, anosmic (if TDI score was <15,5) and hyposmic (if TDI score was >15,5 and <30). All patients underwent a brain MRI protocol that included T1-weighted volumetric images with a 1.5T scanner (Achieva, Philips). Control group (ctrls) consisted of 16 healthy volunteers and subjects with headache. OB volume was measured manually on coronal T1-weighted images, with "pencil function" both in pts and ctrls. All MRIs were processed using FreeSurfer to estimate sulci depth focusing on frontal cortex. **Results**: All ctrls had normal OB (mean volume 1,02 mm3, range 0,33-1,46 mm3). MRI revealed OB aplasia in 31 KS pts (71%, bi- and 5%, mono-laterally) or hypoplasia (mean volume 0,47 mm3, range 0,16-1,20 mm3) in 10 (24%). KS had lower OB volumes ($p<0.05$) and decreased OS depth compared to ctrls, with a positive correlation between OB volume and OS depth. KS showed deeper medial orbitofrontal giri when compared with ctrls. **Conclusions**: functional and anatomic changes of the orbito-frontal region are frequent in KS patients, reflecting a primary role of mutated genetic mechanisms, involved in olfactory structures development and CNS embryogenesis.

PP190

KI-67 LABELING INDEX: AN USEFUL PROGNOSTIC INDICATOR IN PITUITARY ADENOMAS? OUR EXPERIENCE.

C. P. Delfinis[1], V. Mercuri[2], N. Mecca[2], G. Lapadula[1], A. Santoro[1], P. Gargiulo[2]

[1]Div. di Neurochirugia, Dip. di Sc. Neurologiche Università "Sapienza" di Roma - Roma, [2]Dip. di Medicina Sperimentale Università "Sapienza" di Roma - Roma

Pituitary adenomas are benign slow-growing tumours, but can behave in a very aggressive invasiveness of parasellar regions and in relapse.The prognostic indicator of disease is absolutely the degree of removal,but the determination of the degree of biological aggressiveness is still a matter of research and debate.The study of Ki-67 LI is one of the less complex and more reliable methods to determinate the degree of proliferation in tissues.The Ki-67(an antigen protein contained in cells nuclei under mitotic activity)is present indifferently both in tumour and normal cells, therefore represents an excellent marker for the rate of growth of a cell population and proliferation and invasiveness of the tumour. The Ki-67 LI is used in a variety of brain tumours since 1983. The first report of an evaluation in pituitary adenomas dates back to 1986 with values ??between 0.2 and 1.5%.We included in the study 213 pituitary adenomas and analyzed the?? Ki-67LI values.The results showed a variation from 0.1% to 11%(average 1.34%),with a value slightly higher in women(0.84%) than in males(0.77%) which was not statistically significant. The Ki-67 LI value was higher in subjects younger than 30 years (mean of 1.1%) as in macroadenomas (2.24%).In order to assess the significance of the index Ki-67 as a prognostic index of relapse,we selected patients undergoing surgery for recurrent adenomas, which fulfilled the criterion of total or subtotal removal (where you want to subtotal excision> 80%) at the first operation.To reduce the bias, we selected only patients with recurrences followed by the same specialist endocrinologist and operated by the same surgeon.In our series, there were 27 recurrences that underwent reoperation.The analysis of the data collected showed that the recurrent adenomas have a Ki-67LI equal to 1.67%-higher than non-recurrent ones(0.65%), and than the average for the entire series(1.34%)-which is also slightly modified to the evaluation of the second intervention (Ki-67 LI = 1.86%,P<0.01).The Ki-67LI cannot be used as an independent index of determining the degree of biological aggressiveness in pituitary adenomas but it can be considered useful in the clinical practice. In our opinion, there is no doubt that the finding of a high value of Ki-67LI is associated with an increased risk of early recurrence, and in consideration of a poor prognosis,it can affect the timing of the follow-up and the choice of medical treatment and/or radiotherapy.

PP191

COMPARING PROGRESSION OF PITUITARY TUMOURS IN PATIENTS WITHOUT OR WITH GH DEFICIENCY, DURING OR NOT LONG-TERM GH REPLACEMENT THERAPY

V. Gasco[1], F. Guaraldi[1], C. Baldini[1], N. Prencipe[1], S. Di Giacomo[1], A. Berton[1], M. Maccario[1], E. Ghigo[1], S. Grottoli[1]

[1]Endocrinologia, Diabetologia e Metabolismo - Torino

Introduction: One concern of long-term GH replacement treatment (GHT) in adults with a history of pituitary tumour is the risk of an increased rate of tumour recurrence or enlargement.

Aim: To clarify whether GHT is associated with an increased prevalence of recurrence or enlargement of pituitary tumours.

Methods: We retrospectivelly reviewed data from 232 patients (117 M, 115 F) with a history of a tumour of the hypothalamo-pituitary area (175 adenomas, 39 craniopharyngiomas or Rathke's cystic lesions, 9 meningiomas, 9 tumours of other histotype). 161 patients had been treated with neurosurgery (NCH) alone, 65 with NCH + radiotherapy (RT), 4 with RT alone, and 2 patients were treatment naïve.

Results: 165 patients were affected by severe growth hormone deficiency (GHD) and 85 out of these were under GHT. Mean follow-up was similar in patients without GHD (70.6 ± 67.1 months) and in patients with GHD but not under GHT (89.9 ± 88.8 months), while it was greater in GHD patients under GHT (149.4 ± 104.2 months, $p < 0.0001$). The GHT duration was 75.7 ± 44.7 months. The progression rate of tumours of the hypothalamo-pituitary area was 9.8% in patients without GHD. Even if mean follow-up was greater in patients under GHT, there wasn't difference in relapse or progression of tumour between patients with GHD under GHT (10.4%) and patients with GHD but not under GHT (11.1%). The progression rate in GHD patients, under or not GHT, was similar to that one in patients without GHD.

Conclusion: In conclusion our study confirm that GHT is safe also in term of progression rate of the tumours of the hypothalamo-pituitary area even in the long-term.

PP192

EVALUATION OF COGNITIVE FUNCTION IN ADULT GHD PATIENTS

F. Rota[1], R. Baldelli[2], M. Maschio[3], A. Barnabei[2], V. Adinolfi[1], A. Paoloni[1], L. Rizza[1], L. Dinapoli[3], M. Appetecchia[1]

[1]Endocrinology, "Regina Elena" Italian National Cancer Institute - Rome, [2]Endocrinology, "Regina Elena" Italian National Cancer Institute - Rome, [3]Center for brain tumor related epilepsy "Regina Elena" Italian National Cancer Institute - Rome

In the past years, a small number of studies on cognitive performance in adults with growth hormone deficiency (GHD) have been performed. These studies indicate that cognitive function might be impaired. Cognitive performance was studied especially by neuropsychological tests. Only three studies reported assessment of cognitive function by measuring P300 event-related potentials (ERPs) in few adult patients with GHD (only 9 pituitary adenoma mixed Sheehan's Syndrome). The present study was designed to investigate the effects of GHD on cognitive function by using neuropsycological tests and P300 ERPs in patients underwent surgery for pituitary adenoma. We have studied an homogeneous population of six patients underwent surgery for pituitary adenoma (3 M, 3F, mean age 43.8 +/- 7.9 years). Four patients had hormone deficiencies other than GH and, before undergoing GH testing, they were under stable replacement therapy. The diagnosis of GHD was established by GHRH+ARG test and was defined as a peak GH level <9 ng/ml. Cognitive performance was studied by Raven Standard Progressive Matrices Test, Trail Making Test, Rey Auditory Verbal Learning Test, a Verbal Fluency Test. It was also assessed the Karnofsky performance status (KPS), wich is an index of motor function. Cognitive function were assessed also by measuring P300 ERPs. The mean peak level of GH after GHRH+ARG was 3.8+/-2.5 ng/ml (range 0.6-7.1 ng/ml) and all patients had a peak GH level <9 ng/ml. The mean serum IGF-1 concentration in the patients before GHRH+ARG test was 86.1+/-29.7 (range 52-136 ng/ml). All patients had a KPS normal (maximum score), indicating no impairment of neuromotor function. The neuropsycological evaluation showed values lower than normal only for the test of verbal fluency in two of the six patients with GHD. Also P300 ERPs latencies were prolonged in two of the six patients, and specifically in those patients who experienced abnormalities in neuropsychological test. In conclusion, we observed a direct correlation between abnormalities of P300 ERPs (potential recorded on the fronto-central brain regions) and abnormalities in a Verbal Fluency Test (suggestive of impairment of the same brain regions). Of course, these are preliminary data, obtained in a small number of patients. However, if these data are confirmed in a wide range of cases, it is useful to evaluate the effects of GH replacement therapy and neurocognitive rehabilitation on cognitive function in these patients.

PP193

PREVALENCE OF HYOPITUITARISM IN POST-ACUTE AND CHRONIC PHASE OF HEMORRHAGIC STROKE

M. Bondanelli[1], A. Malaspina[1], A. Montis[2], S. Lavezzi[2], M. C. Zatelli[1], A. Guerra[1], N. Basaglia[2], M. R. Ambrosio[1], E. C. degli Uberti[1]

[1]Section of Endocrinology, University of Ferrara - Ferrara, [2]Dept of Rehabilitation Medicine, St Anna Hospital - Ferrara

Stroke is a leading cause of death and represents the main cause of severe long-term disability. Ischemic stroke may be associated with hyopituitarism possibly influencing neurological recovery. Aim of the study was to evaluate the prevalence of pituitary dysfunction in a cohort of adult patients with moderate to severe hemorrhagic stroke (intraparenchymal hemorrhage) in comparison with a cohort of ischemic stroke patients. We evaluated pituitary function in 73 hemorrhagic patients (HE) (47 M; mean age 55.5 ±14.0 yr) during the post acute phase (after 1-6 mo) and 28 HE (14M; mean age 48.5 ±15.2 yr) during the chronic phase of stroke in comparison with 117 ischemic patients (IS) (75 M; mean age 63.6 ±13.8 yr) studied during the post acute phase and 37 IS (20 M; mean age 54.0 ±14.5 yr) studied during the chronic phase of stroke. Hypopituitarism was detected in 18/73 HE (24.6%) during the post-acute phase and in 4/28 HE (14.3%) during the chronic phase. In the long term, HE showed a trend to a reduction in hypopituitarism prevalence. However, this prevalence did not significantly differ from that observed in IS [32/117 (29.9%) in the post acute phase and 8/37 (23.8%) in chronic phase, respectively. The following defects were observed in HE: 9 GH, 9 LH/FSH, 1 ACTH, 2 TSH (including 3 cases of combined deficiencies: 2 GH+ LH/FSH , 1 GH + TSH) in the post.-acute phase; 3 GH, 2 LH/FSH (including 1 case of GH+ LH/FSH defect) in chronic phase. A similar distribution of defects was observed in IS: 23 GH, 9 LH/FSH, 1 ACTH, 1 TSH (2 combined deficiencies: 1 GH+ LH/FSH , 1 GH + ACTH) in the post.-acute phase; 3 GH, 2 LH/FSH (1 case GH+ LH/FSH) in chronic phase. Severity of stroke (measured by National Institutes of Health Stroke Scale), did not significantly differ in patients with hypotuitarism, both HE and IS, compared with normal patients. By considering all hypopituitaric patients (both HE and IS), pituitary dysfunction was associated with lower cognitive levels (measured by Functional Independence Measure and Mini Mental Test Examination). Moreover, the presence of GHD, both in the post acute phase and long term period, was associated with higher prevalence of diabetes mellitus and unfavourable metabolic profile (higher glucose and HbA1c, and lower HDL cholesterol, $p<0.05$). Hypopituitarism may be frequently discovered also in hemorrhagic stroke, persisting in the long term period in 15% of cases. Pituitary dysfunction in stroke is associated with unfavourable metabolic profile and may impair recovery. An endocrine assessment should be performed in all stroke patients.

PP194

COMPARISON OF PRIMARY SOMATOSTATIN ANALOGUE THERAPY AND PITUITARY ADENOMECTOMY ON SURVIVAL IN PATIENTS WITH ACROMEGALY: A RETROSPECTIVE COHORT STUDY.

M. Lombardi[1], G. Rossi[2], C. Urbani[1], C. Sardella[1], A. Iannelli[3], I. Scattina[1], L. Manetti[1], S. Del Sarto[2], R. Pivonello[4], L. F. S. Grasso[4], I. Lupi[1], R. S. Auriemma[4], G. Lombardi[4], E. Martino[1], A. Colao[4], F. Bogazzi[1]

[1]Department of clinical and experimental medicine, University of Pisa - Pisa, [2]Unit of Epidemiology and Biostatistics, Institute of Clinical Physiology, National Research Council - Pisa, [3]Department of Neurosciences, University of Pisa - Pisa, [4]Department of Molecular and Clinical Endocrinology and Oncology, University Federico II, Naples - Napoli

Acromegalic patients have increased mortality. To compare the effect of different therapies for acromegaly on mortality 438 consecutive acromegalic patients referred at two University Centers were retrospectively analyzed. Patients' mortality was compared to that of the general population by standardized mortality ratio (SMR); predictive factors for mortality and the effect of different therapies on survival were evaluated by Cox regression analysis. Twenty patients (4.5%) died between 1996 and 2009. Age and sex-adjusted SMR was 0.70 (95% C.I., 0.43-1.08). Death occurred in 2.4% (adenomectomy), 2.6% (adenomectomy plus SSA) and 11.4% (SSA alone) of patients. Hazard risk (HR) was higher in patients receiving SSA (5.09, 95% C.I., 1.04-24.86, p=0.044) than in all patients submitted to pituitary neurosurgery (adenomectomy and adenomectomy plus SSA); in particular, the increased HR was observed in diabetic patients (22.295, 95% CI, 0.938-530.106, p=0.055). HR of patients receiving SSA following adenomectomy (0.322, 95% C.I., 0.044-2.355, p=0.264) did not differ from that of cured patients by pituitary adenomectomy. Multivariate Cox regression analysis revealed that, in the whole population, both general risks factors (age, and the physical status at diagnosis) and specific factors for acromegaly (macroadenoma, hypopituitarism and uncontrolled disease) predicted death. Radiotherapy was associated with increased mortality, which occurred in patients with the more locally advanced disease. The most compromised patients at diagnosis had higher mortality (p=0.001), which occurred even though acromegaly was controlled. Conclusions: therapies of acromegaly and comorbidities have lowered mortality to the level of the general population; SSA following pituitary adenomectomy was comparable to curative neurosurgery on survival; whether SSA as primary medical therapy reduces mortality, requires further studies.

PP195

THE OUTCOME OF ENDOSCOPIC ENDONASAL SURGERY IN 229 PATIENTS WITH ACROMEGALY.

M. Faustini-Fustini[1], M. Zoli[2], D. Mazzatenta[2], E. Pasquini[3], G. Frank[2]

[1]Dipartimento Medico, Unità di Endocrinologia, Ospedale Bellaria - Bologna, [2]IRCCS Istituto delle Scienze Neurologiche, Centro di Chirurgia dei Tumori Ipofisari e di Chirurgia Endoscopica della Base Cranica, Ospedale Bellaria - Bologna, [3]Dipartimento Chirurgico, Unità di PORL, Azienda USL - Bologna

Introduction. Surgery has a key role in the treatment of acromegaly. However, long-term medical therapies have been available for many years, thus leading to a wide use of these treatments also as the first-line therapy in a large number of acromegalic patients, apart from those with microadenomas or enclosed macroadenomas. Aim of the study was to analyse the outcome of endoscopic endonasal approach (EEA) in a large cohort of acromegalic patients who underwent surgery in a single centre with a dedicated team.

Design/Methods. Between 1998 and 2012, 229 patients (36% microadenomas, 4.8% of whom invasive with parasellar extension; 64% macroadenomas, 13.7% of whom enclosed intrasellar, 46.57% with suprasellar extension and 39.72% invasive with parasellar and/or sphenoidal extension; male to female ratio: 0.75; mean age: 46.8 +/- 12 yrs) were consecutively operated by EEA, for a total of 240 surgical procedures, 12% of whom secondary (repeat) surgery. The mean follow-up period was 34 months (+/- 28). Statistical analysis was performed using the SPSS software (vers. 21).

Results. At presentation, visual impairment was evident in 14%, pituitary apoplexy in 2%, III cranial nerve palsy in 2%. The rate of biochemical 'cure' (normalisation of both basal GH levels and IGF-I levels) correlated with invasion (intraoperative findings). The overall remission rate was 70%. There were 10 new cases (4.5%) of postoperative hypopituitarism. Transient diabetes insipidus occurred in 6% and SIAD in 4.5%. The overall surgical complication rate was 2%. Mortality rate was 0%. Six months after surgery, improvement of visual function occurred in 76% of patients with visual impairment at presentation, while in the remaining 24% visual damage remained stable.

Conclusions. Surgery by EEA led to the biochemical 'cure' in the majority of patients (70%), not only in those with microadenomas or enclosed macroadenomas, which encompassed for 45% as a whole. Parasellar and/or sphenoidal invasion (intraoperative findings) significantly affected the remission rate. The technique was safe and well tolerated with low morbidity. Visual function improved in the vast majority (76%) of patients with visual impairment. In conclusion, our data provide support for confirming surgery, particularly EEA, as the primary treatment of acromegaly, with a rapid normalisation of GH hypersecretion in a large number of patients.

PP196

THYROID DISORDERS IN ACROMEGALY: A SINGLE CENTER EXPERIENCE

C. Urbani[1], M. Lombardi[1], C. Sardella[1], I. Scattina[1], C. Cosci[1], E. Dell'Unto[1], L. Manetti[1], E. Martino[1], F. Bogazzi[1]

[1]Department of Clinical and Experimental Medicine - University of Pisa

Objectives: Thyroid diseases are a frequent finding of acromegaly, although their prevalence is not known. The aim of the study was to evaluate the prevalence of thyroid disorders and the effects of biochemical control of acromegaly on thyroid status. **Methods:** Clinical records of 209 consecutive acromegalic subjects were analyzed. Patients affected by non functioning (n=150) or PRL-secreting (n= 178) pituitary adenoma served as control. **Results:** Five patients with a co-secreting TSH pituitary adenoma were excluded from the analysis. No differences were found between control and acromegaly group for age of diagnosis or sex. At the end of follow-up (mean duration 7.8 ± 5.2 yrs) 67% of patients had a controlled disease. The great majority of patients (n=181, 86.6%) was affected by a thyroid disorder at diagnosis ($p<0.0001$ vs. controls): 98 (51.7%) had a non-toxic nodular goiter, 32 (15.3%) a non-toxic diffuse goiter, 9 (4.3%) a toxic nodular goiter and 4 patients had Graves' disease. All subjects with Graves' disease had a moderate-severe ophthalmopathy requiring combination of glucocorticoids, external radiotherapy or orbital decompression. Thyroid autoimmunity was present in 26.3% patients and in 20.8% of controls (p=NS). 19 patients (9.1%) presented a papillary thyroid cancer at diagnosis or during follow-up. Any of these subjects at the end of follow-up had biochemical or morphological persistence of thyroid cancer. Mean thyroid volume at diagnosis was higher in acromegalic patients than controls ($p<0.0001$), and was related to the estimated duration of acromegaly ($r=0.50$, $p<0.0083$), age ($r=0.25$, $p=0.0128$) and IGF1-index ($r=2,16$, $p=0.0239$) at diagnosis. **Conclusion:** Acromegaly is characterized by an increased prevalence of thyroid disorders, particularly non-toxic nodular goiter and thyroid carcinoma in comparison to the general population. In addition, in our series, acromegalic subjects with concomitant Graves' disease were affected by a moderate-severe ophthalmopathy.

PP197

WRIST ACTIGRAPHY DETECTS SLEEP DISORDERS IN PATIENTS WITH CUSHING'S SYNDROME

V. D'Angelo[1], G. Beccuti[1], R. Berardelli[1], I. Karamouzis[1], A. Picu[1], E. Marinazzo[1], C. Zichi[1], B. Fussotto[1], R. Giordano[2], E. Ghigo[1], E. Arvat[3]

[1]Dipartimento di Scienze Mediche, Divisione di Endocrinologia, Diabetologia e Metabolismo - Torino, [2]Dipartimento di Scienze Cliniche e Biologiche - Torino, [3]Dipartimento di Oncologia, Divisione di Endocrinologia Oncologica - Torino

Interrelationships between HPA axis and sleep architecture are well documented. However, the knowledge about sleep parameters in chronic hypercortisolism in humans is scanty. Our aim was to evaluate sleep efficiency in patients with Cushing's syndrome (CS) in active phase and after remission, using wrist actigraphy, a wristwatch-like device that provides a good estimation of sleep architecture, by detecting motor activity. Actigraphic evaluation was performed on 3 consecutive days under free living conditions in 12 patients with active CS (A) without ongoing specific therapy (11F, 1M; 40.0±10.9 yrs; 8 with ACTH-secreting pituitary adenoma, 4 with cortisol-secreting adrenal adenoma), in 12 healthy control subjects (N) (11F, 1M; 44.0±11.0) and in 12 patients in remission from CS (R) (10F, 2M; 54.8±11.9 yrs; 4 with ACTH-secreting pituitary adenoma, 7 with cortisol-secreting adrenal adenoma, 1 with ectopic secretion). Data were analysed using Actiware®-Sleep Software. **Results**: wrist actigraphy revealed a significant increase ($p<0,05$) of actual wake time ([mean±SD] 43'±10' vs 33'±12' vs 34'±11'), fragmentation index (19.37±0.90 vs 13.67±1.3 vs 17.21±6.01), total activity score (8318±4308 vs 4971±2372 vs 5340±2261), mean activity score (8.67±4.25 vs 5.44±2.16 vs 6.03±2.61) and mean score in active time (104.83±39.23 vs 74.81±23.15 vs 75.93±31.85) in A patients compared to N and to R subjects. No differences were found between N and R subjects. On the contrary, time in bed, actual sleep time, sleep efficiency and sleep latency resulted similar in the 3 groups. No positive correlation was found between deranged parameters in A patients and urinary free cortisol, neither between actigraphic parameters in R subjects and time free from disease. In conclusion, these preliminary results indicate that hypercortisolism is associated in a dose-independent manner with sleep alterations that seem to disappear after remission. Further studies in a larger cohort of patients and the use of more accurate instruments, such as in-home polysomnography, are needed to confirm these findings.

PP198

LIMITATIONS OF LATE NIGHT SALIVARY CORTISOL IN THE SCREENING OF CUSHING'S SYNDROME

G. Marcelli[1], M. Brugia[2], C. Concettoni[1], G. Tirabassi[1], L. Trementino[1], F. Agostini[1], V. Scocco[2], M. Boscaro[1], G. Arnaldi[1]

[1]Division of Endocrinology, Polytechnic University of Marche - Ancona, [2]Laboratory Analysis - Ancona

CONTEXT Late-night salivary cortisol (LNSC) measurement has been promoted as an ideal screening test for the diagnosis of Cushing's Syndrome (CS). However, its performance using commercially available assays has not been widely evaluated and limited data are available on its use in populations with chronic medical conditions.

AIM To compare the diagnostic performance of LNSC (routine use) in patients with CS and in patients with different medical conditions.

METHODS We studied 26 patients with active CS, 73 normal weight healthy volunteers, 21 patients with uncontrolled diabetes, 43 obese subjects and 13 anorexic women. Each subject provided bedtime (h 23:00) saliva sample for LNSC determination. LNSC has been assessed using a commercially chemiluminescence immunoassay (CLIA, Access Beckman Coulter).

RESULTS The mean LNSC concentrations were significantly higher ($P<0,001$) in CS (1,72±0,6 mcg/dl; range: 0,63-4,33) compared with healthy subjects (0,309±0,13 mcg/dl; range: 0,018-0,62). The optimal LNSC cut-off value derived from ROC analysis for the differentiation between patients with and without CS was achieved at the level of 0,58 mcg/dl (SE 100%, SP 98,6%). However, this cut-off showed a lack of specificity when used in obese subjects, diabetic patients and in anorexic women, respectively 67%, 57% and 30%. LNSC of obese subjects (0,583±0,5 mcg/dl; range: 0,06-2,36), diabetic patients (0,900±0,67 mcg/dl; range: 0,26-3,0) and anorexic women (0,67±0,23 mcg/dl; range: 0,23-1,09) remained yet significantly lower than LNSC of CS ($P<0,001$).

Reapplying ROC analysis to our data, the most optimal cut-off value to differentiate patients with CS from those with different medical conditions was 1,02 mcg/dl (SE 80%, SP 81,4%) for obese subjects, 1,01 mcg/dl (SE 80%, SP 80%) for diabetic patients and 0,86 mcg/dl (SE 96%, SP 95%) for anorexic women respectively.

CONCLUSIONS This study confirms the value of LNSC for the diagnosis of CS, even using a routinary method as CLIA. However, the study underlines the need to fix the different diagnostics cut-off locally, especially in the presence of comorbidity that can lead to an overactivity of the hypothalamic-pituitary-adrenal (HPA) axis.

PP199

THYROID DISORDERS IN CUSHING'S SYNDROME

V. Raffaelli[1], L. Manetti[1], I. Scattina[1], I. Lupi[1], M. Gasperi[2], M. Cosottini[3], A. Iannelli[3], F. Bogazzi[1], E. Martino[1]

[1]Endocrinology - Pisa, [2]Chair of Endocrinology - Molise, [3]Neuroscience - Pisa

Cushing's syndrome (CS) may alter the performance of the immune system and hypothalamic-pituitary-thyroid axis in several ways. Few case reports show that resolution of hypercortisolism may be followed by the emergence or worsening of autoimmune thyroid disease (AITD). It also be suggest the CS patients display an higher prevalence of nodular thyroid disease compared with general population.

Objective of this historical prospective study was to evaluate the prevalence of primary thyroid disorders (AITD and nodular disease) and positive thyroid antibodies in patients with active and remission CS and compare this result with a control group. Ninenty-two patients with active CS were enrolled in the study. Sixty-six patients were affected by an ACTH-secreting pituitary adenoma, 2 had an ectopic ACTH secretion and 24 had an adrenal adenoma. All these patients, after surgery and/or radiotherapy or medical treatment, were in remission of disease during a mean follow up period of 64.6±45.8 months (6-168 months). Three hundred and twenty two subjects affected by non-functioning and PRL-secreting pituitary adenomas, matched for sex and age, served as control group. Free thyroid hormones (fT4, fT3), TSH, antithyroglobulin (TgAb), antithyroperoxidase (TPOAb) and thyroid ultrasound were performed in all patients at baseline and after the cure. The prevalence of AITD in the active phase (8.7%) is similar to remission CS (9.8%) (p=NS). The control subjects had higher prevalence of AITD than CS (p=0.001). Thirty-nine active CS (42.4%) had nodular thyroid disease, in remission phase the patients were 40 (43.5%). The prevalence of nodular disease in this two group was not significant (p=NS). Moreover the active CS had higher prevalence of nodular disease than control group. The prevalence of positive TgAb titre was higher in remission CS than in active phase, but not significantly (p=0.169), while positive TPOAb titre was similar in the two groups. Serum TSH concentrations were significantly lower during hypercortisolism than in remission (0.87±0.88 vs 1.36±0.96 mg/ml, p=0.0005). In conclusion, patients with CS showed a high prevalence of thyroid disorders. Moreover, patients successfully treated for CS did not show an increased prevalence of AITD compared to active patients. However there was an increased concentration of thyroid antibodies after the remission of hypercortisolism. CS patients showed higher prevalence of nodular thyroid disease than controls. As known TSH levels were significantly lower in active than in remission patients.

PP200

OVERNIGHT 1MG DEXAMETHASONE SUPPRESSION TEST HAS A PERFORMANCE SIMILAR TO MIDNIGHT SERUM CORTISOL IN THE DIAGNOSIS OF CUSHING'S SYNDROME

F. Agostini[1], G. Tirabassi[1], L. Trementino[1], G. Marcelli[1], C. Concettoni[1], M. Boscaro[1], G. Arnaldi[1]

[1]Clinica di Endocrinologia e Malattie del Metabolismo, Università Politecnica delle Marche - Ancona

Purpose: The diagnosis of Cushing's Syndrome (CS) is still a challenge since there is not a single test with optimal sensitivity (Se) and specificity (Sp). This study is aimed to: 1) Evaluate accuracy and diagnostic performance of overnight 1mg dexamethasone suppression test (DST) in the screening of a population with a suspect of hypercortisolism; 2) Compare DST to midnight serum cortisol (F24); 3) Define new cut-off values for these tests; 4) Analyze which clinical factors could affect their diagnostic performance.

Methods: Retrospective Case-Control Study on 110 patients with confirmed CS and 58 patients with unconfirmed clinical suspect of CS (Pseudo-Cushing). Serum cortisol after DST and F24 were measured and compared. Receiver operating characteristic (ROC) analysis has been performed to define the best cut-off values.

Results: DST and F24 showed a good diagnostic performance. For F24 the best cut-off value is 7.5 mcg/dl in terms of Se and Sp. For DST a cut-off value at 2.65 mcg/dl (Se 96.7%; Sp 97.67%) was better than the conventional cut-off value of 1.8 mcg/dl (Se 98.9%; Sp 93.02%). DST was an accurate test even in mild forms of hypercortisolism. In male patients DST appeared more effective than in females. There were no differences between the tests when analyzing the different aetiologies of hypercortisolism.

Conclusions: There is not a single test able to diagnose or exclude all cases of SdC. DST gives a diagnostic performance similar to F24 if we consider the 2.65mcg/dl cut-off value. These data confirm the necessity of a complex diagnostic procedure, even if the clinical suspicion of CS is high.

PP201

EXPRESSION OF GH CLUSTER AND GHRH-R SPLICE VARIANTS IN PITUITARY TUMORS

G. Appolloni[1], R. A. Ricciuti[2], F. Rychlicki[2], M. Losa[2], M. Scarpelli[4], L. Trementino[1], G. Marcelli[1], F. Agostini[1], M. Boscaro[1], G. Arnaldi[1]

[1]Scienze Cliniche e Molecolari - Ancona, [2]Neurochirurgia - Ancona, [3]Neurochirurgia - Milano, [4]Anatomia Patologica - Ancona

It is well known that growth factors and in particular the members of the GH/IGF1 axis are involved in pituitary and extrapituitary tissues tumor development. Although the oncogenic effects are mainly attributed to the action of IGF-I and IGF-II, we can not exclude that in pituitary the tumorigenic action can be exercised by GH and its regulators in an autocrine/paracrine manner. Furthermore, as demonstrated for other human tumors, also the GHRH and the splicing variants of its receptor may be involved in the development of tumors of the pituitary.

The aim of this work was to evaluate the expression of the GH cluster to look for a possible aberrant expression of placental members, in particular hGH-V variant, in GH secreting and non-functioning pituitary tumors. We also evaluated the gene expression of GHRH-R and its splice variants (SV) always in pituitary tumors. Our results confirmed the expression of hGH-N both in normal pituitary than in all GH secreting and non-functioning pituitary adenoma whereas no PCR products were found for the other four genes, hCS-L, hCS-A, hGH-V, and hCS-B. The presence of hCS-A and hCS-B genes was identified only in the placenta used as a positive control. The expression of GHRH-R and its SVs was assessed by nested PCR in 11 GH secreting and 14 non-functioning human pituitary adenoma. Furthermore as positive control we evaluated a sample of placenta and a postmortem normal pituitary specimen. The wild type receptor was expressed in all pituitary adenomas except in 3 non-secreting samples. Concerning the splice variants, SV1 was expressed in 5 GH secreting pituitary adenoma. In 4 of these samples, we amplified SV2 and among these only 1 was positive for SV4 showing the simultaneous presence of the 3 variants. Among non-functioning adenoma, SV1 was positive in 6 samples, and only 2 of these were positive even for SV2. No PCR products for SV3 was found in all 25 pituitary adenoma.

The presence of hGH-V both in GH secreting that in non-functioning pituitary tumors, suggests a potential involvement of this growth factor in the development and progression of these disorders and thus its potential involvement in pituitary oncogenesis. These conclusions are further confirmed by the results obtained from the study of GHRH-R SV.

PP202

EFFECTS OF A FISH-ENRICHED DIET ON ENDOTHELIAL PROGENITOR CELL NUMBER: A CROSSOVER INTERVENTION STUDY

V. Spigoni[1], M. Cito[2], M. Pinna[2], R. Aldigeri[2], N. Anelli[3], G. Lanati[2], I. Zavaroni[2], R. Raddino[1], A. Dei Cas[2]

[1]Department of Medical and Surgical Specialties, Radiological Sciences, and Public Health. University of Brescia - Brescia, [2]Endocrinology, Department of Clinical and Experimental Medicine. Unit of diabetes and prevention of associated diseases. University of Parma - Parma, [3]Dietetic Service. Azienda Ospedaliero-Universitaria di Parma - Parma

Omega-3 fatty acids exert beneficial effects on cardiovascular (CV) system due to their ability to reduce oxidative stress and inflammation. Circulating endothelial progenitor cell (EPC) number is considered a new CV risk marker since it is reduced in the presence of CV risk factors and diseases.

We recently demonstrated that omega-3 improved EPC number and function in vitro by reducing pro-inflammatory response, suggesting a beneficial effect of omega3 on the CV system, but in vivo data are lacking.

Aim: to evaluate the effects of a fish-enriched diet on EPC number, omega-3 plasma level, omega-6/omega-3 ratio and plasma inflammatory and oxidative stress markers.

Methods: we recruited, in a cross-over intervention study, 21 healthy subjects (10F/11M, 34±5yrs), normotensive, and not taking any medication to received, in a randomized 1:1 order, 120g of mackerel or sardine 4 times per week for 6 weeks with a 2-week wash-out in between. EPCs were identified as cells positive for CD34, CD133 and Kinase insert Domain Receptor (KDR) cell-surface antigens by flow cytometry analysis. Specific immunoglobulins were used as control.

Results: EPC number was comparable at baseline between the two groups and it significantly improved after mackerel ($p<0.005$) and sardine–enriched ($p<0.05$) diet (paired t-test). Repeated measures ANOVA highlighted a significant increase in EPC number after 6 weeks of treatment ($p<0.001$), although this effect was reduced and no more significant at the end of the study (14 weeks). We are currently analyzing omega-3 levels, omega-6/omega-3 ratio, lipid profile and inflammatory and oxidative stress parameters.

Conclusion: These data confirm our in vitro results showing an improvement in EPC number following a fish-enriched diet with potential favorable CV effects.

PP203

PLASMA CORTISOL TREND IN OBESE AND SUPEROBESE SUBJECTS, WITH AND WITHOUT TYPE 2 DIABETES

D. Capoccia[1], M. Del Giudice[1], F. Coccia[1], L. Alessandroni[1], V. Paglia[1], B. Ferretti[1], F. Leonetti[1]

[1]Medicina Sperimentale - Roma

In literature, not recent studies showed increased cortisol plasma levels (CPL) in obese diabetic and non diabetic patients. Aim of this study was to examine the trend of CPL and ACTH related to anthropometric parameters in a large population from normal-weight to superobese patients (BMI 20.3-75.9 Kg/m2), with and without diabetes. 573 patients (430 women; 15-80 years) were divided in diabetic (n=129, diagnosed according to ADA guidelines) and non-diabetic subjects and then divided in 7 groups according to BMI (GroupA BMI 20-24.9; GroupB 25-29.9; GroupC 30-34.9; GroupD 35-39.9; GroupE 40-49.9; GroupF 50-50.9; GroupG ≥60). All the patients were evaluated for anthopometric parameters (BMI, percentage of fat mass [%fat], waist circumference) and fasting plasma levels of cortisol, ACTH and HbA1c. CPL in non-diabetic patients resulted significantly lower ($p=0.0034$) in obese (GroupC 12.1±5.3 µg/dl; GroupD 11.4±4.0; GroupE 12.1±5.3; GroupF 12.2±4.4; GroupG 12.8±5.3) than in normal-weight (15.6±7.3 µg/dl) and overweight subjects (14.8±5.7 µg/dl); no correlation was found with waist circumference. ACTH levels, specularly, rised proportionally to BMI ($r=0.2$, $p=0.0015$). Differently, in diabetics, CPL and ACTH levels were not significantly related to BMI (p=ns), resulting meanly higher than in the corresponding BMI group of non-diabetics. In diabetic patients, CPL trend showed a positive correlation ($r=0.18$) to HbA1c levels ($p=0.01$). ACTH levels, instead, were not related to HbA1c (p=ns). These unexpected results of decreased CPL in obesity could be explained by the hypothesis of adipose tissue is an active endocrine organ, responsible of the increased peripheral clearance of cortisol followed by the consequent increase of the plasmatic ACTH levels. The lack of CPL reduction in diabetic obese patients could be related to metabolic stress, as confirmed by the positive relation between CPL and HbA1c levels.

PP204

ALTERATION OF HUMAN OSTEOBLASTIC CELLS HOMEOSTASIS UPON EXPOSURE TO SERA OF OBESE SUBJECTS: IN VITRO CHARACTERIZATION

F. Wannenes[1], V. Papa[1], E. A. Greco[2], R. Fornari[2], C. Marocco[2], C. Lubrano[2], C. Baldari[1], G. Emerenziani[1], P. Sgrò[1], L. M. Donini[2], L. Di Luigi[1], L. Guidetti[1], A. Lenzi[2], S. Migliaccio[1]

[1]Dip Scienze Motorie, Umane e della Salute - Roma, [2]Dip Medicina Sperimentale, Università Sapienza - Roma

Osteoporosis and obesity are two important global health problems with high impact on both morbidity and mortality. Interestingly, obesity has been considered a risk factor for cardiovascular and metabolic diseases, nevertheless a protection factor for osteoporosis. However, recent epidemiologic and clinical studies indicate that high level of fat mass might be a risk factor for osteoporosis and fragility fractures.

Aim of our study was to characterize the effects of the exposure of osteoblasts to the sera of obese subjects to evaluate potential changes in skeletal homeostasis.

Obese subjects with normal BMD (OB), obese with low BMD (OB/OS), subjects with sarcopenia (OB/SAR) and obese with low BMD and sarcopenia (OB/OS/SAR) were evaluated. Patients were investigated for metabolic and bone markers, body composition and sera used to culture osteoblasts in vitro to evaluate changes in cells homeostasis.

Expression of IL-18 and OPG, but not IL-6, was significantly reduced in human osteoblasts (hSAOS) upon incubation for 24 hrs with sera from OB/OS, OB/SAR and OB/SAR/OS subjects as compared to hSAOS incubated with sera of OB subjects, suggesting a potential alteration in the secretion of cytokines involved in the modulation of osteoclasts activity, likely increasing resorbing activity. Markers of osteoblastic cell activity were evaluated to depict potential alteration in the differentiation of bone matrix forming cells. Alkaline phosphatase, osteopontin and BMP4 were reduced in osteoblasts incubated with sera from OB/OS, OB/SAR and OB/SAR/OS subjects as compared to cells incubated with sera of OB subjects, while collagen levels did not appear to be modulated by exposure to the sera. Likewise RANKL/OPG ratio was increased, suggesting the induction of an osteoclastogenic environment by OB/OS, OB/SAR and OB/SAR/OS sera.

These results show that sera of obese subjects with low BMD and sarcopenia alter osteoblasts homeostasis in vitro, suggesting potential detrimental effects on skeletal homeostasis (Research funded by PRIN 2009-2009KENS9K_004). *Equal contribution

PP205

VERY LOW CARBOHYDRATE KETOGENIC DIET (VLCKD) IN OBESE PATIENTS CANDIDATE TO LAPAROSCOPIC BARIATRIC SURGERY: PROSPECTIVE EVALUATION OF AN ORIGINAL SEQ

F. Coccia[1], D. Capoccia[1], M. Del Giudice[1], L. Alessandroni[1], S. Caponigro[1], V. Paglia[1], G. Silecchia[1], F. Leonetti[1]

[1]Università Sapienza - Roma

BACKGROUND: Preoperative weight loss may reduce operative risk. Aim of the study was to evaluate the effect of an original diet schedule (Obese Pre-Operative Diet–OPOD) in morbidly obese and super-obese, diabetic (DM2) and non diabetic (noDM2) patients, candidate to laparoscopic bariatric surgery. PATIENTS AND METHODS: 40 patients with BMI 53.5±8.4 kg/m2, age 47.7±11.2 years, 14 of which affected by type 2 diabetes (DM2), as defined by the ADA guidelines, were prospectively evaluated. OPOD implies for the first ten days a very low carbohydrate ketogenic diet (VLCKD); at the eleventh day they stopped it and started a traditional very low calorie diet (VLCD) until the twentieth day; afterwards they begun a low calorie diet (LCD) that was maintained until surgery (total treatment period 30 days). Patients were evaluated at recruitment (T0), after 10 (T1), 20 (T2) and 30 days (T3). Five patients underwent liver ultrasound (US) at T0 and T3. RESULTS: At T1, T2, T3 the reductions in body weight (from 150,4 ± 26,3 to 137,6 ± 22,5 Kg), BMI (from 53,5 ± 8,4 to 49,2 ± 8,7 Kg/m2), waist circumference (WC) (from 145,0 ± 15,6 to 125,9 ± 16,3 cm) and neck circumference (NC) (from 44,0 ± 3,3 to 41,1 ± 5,2 cm) were statistically significant in all patients but two, who discontinued OPOD after 4-7 days (drop-out 5%). In DM2 patients, fasting plasma glucose (FPG) significantly decreased (from 197,4 ± 89,2 to 147,8 ± 77,4 mg/dl) allowing either reduction or discontinuation of diabetic medications. Plasma and urine ketones increased significantly at T1 with values always <1 mmol/L and hunger degree markedly decreased during the entire treatment. Steatosis pattern was improved and liver volume reduced by 30% (limited to 5 patients). No relevant side effects were recorded. CONCLUSIONS: In conclusion, preoperative 30 days diet including 10 days of VLCKD (OPOD original regimen) is safe and effective to achieve a mean 8% weight loss with NC and hepatomegaly reduction in morbid obese and superobese patients. Furthermore, it is quicker and cheaper than other pre-operative treatments and it shows a high satisfaction index. It seems to offer a proper preoperative treatment in morbid obese/super-obese (diabetic and non-diabetic) patients candidated to any type of surgery, including laparoscopic bariatric surgery.

PP206

THE ENDOCANNABINOID SYSTEM (ECS) RESPONSE TO AN ORAL GLUCOSE TOLERANCE TEST (OGTT) IS INFLUENCED BY DYSMETABOLISM PARAMETERS

F. Fanelli[1], S. Garelli[1], M. Mezzullo[1], G. Di Dalmazi[1], J. Manso[1], F. Ponti[1], A. Bazzocchi[1], V. Vicennati[1], G. Battista[1], R. Pasquali[1], U. Pagotto[1]

[1]Scienze Mediche e Chirurgiche - Università di Bologna - Bologna

The ECS is involved in the regulation of food intake and energy expenditure. Increased level of EC lipid mediators anandamide (AEA) and 2-arachydonoylglycerol (2AG) and/or a higher expression of the cannabinoid receptor type1 in central and peripheral districts are supposed to contribute to the development and maintenance of obesity and related dysmetabolism. To understand how the ECS response to an insulin signal is influenced by anthropometric and biochemical parameters, we evaluated the effect of the OGTT on circulating ECs in five fasted obese females (age: 41.4±3.6y, BMI: 32.5±4.2kg/m2, waist circumference: 103.4±11.1cm). Blood samples were collected before and after 30, 60, 90 and 120min from a 75g glucose ingestion. Plasma AEA, related N-acylethanolamines (NAEs) palmitoylethanolamide (PEA) and oleoylethanolamide (OEA), 2AG and the inactive isomer 1AG were measured by LC-MS/MS. A composition analysis was performed on the whole body and specific region by dual-energy X-ray absorptiometry. Mean basal insulin and glucose levels were 9.4±1.1uU/ml and 91.0±5.4mg/dl, and the calculated area under curve (AUC) were 9,777±2,601 and 15,375±4,044, respectively. AEA, PEA, OEA, 2AG and 1AG mean basal levels were 1.280±0.498, 15.48±3.70, 4.244±1.450, 1.498±0.465 and 0.588±0.192 pmol/ml, respectively. AEA, PEA and OEA significantly decreased along the OGTT (p=0.004, p=0.001 and p=0.003, respectively). At 60min their level (Δ(t0-t60)/t0%) reduced to 0.639±0.340 (51.4%), 9.16±3.69 (41.2%) and 2.276±0.906 pmol/ml (44.9%). Conversely, 2AG and 1AG levels did not significantly change. AEA, PEA and OEA reduction (Δ(t0-t60)/t0%) negatively correlated with glucose AUC (r=-0.895, p=0.040; r=-0.929, p=0.022; r=-0.948, p=0.014, respectively) and positively with whole body (r=0.882, p=0.048; r=0.910, p=0.032; r=0.944, p=0.016, respectively) and gynoid lean mass (r=0.957, p=0.010; r=0.967, p=0.007; r=0.951, p=0.013, respectively). No significant correlations were observed for BMI, waist circumference, basal glucose and insulin, insulin AUC and blood lipids. Our preliminary data indicated that a glucose load is able to differentially modulate blood EC levels, by suppressing AEA and other NAEs but not 2AG and 1AG levels. Interestingly, the extent of NAE suppression is promoted by lean mass and affected by increasing glucose AUC. This research was supported by the European Union grant (NEUROFAST FPVII-KBBE-2009-3-245009).

PP207

DYSLIPIDEMIA AND OBESITY IN ADULT SURVIVORS OF CHILDHOOD CANCER

F. Felicetti[1], F. D'Ascenzo[2], F. Lazzarato[3], A. Corrias[4], E. Biasin[5], N. Fortunati[6], F. Gaita[2], E. Brignardello[6]

[1]SCDU Endocrinologia Oncologica, Università di Torino - Torino, [2]SCDU Cardiologia, Università di Torino - Torino, [3]SCDU Epidemiologia dei Tumori, Università di Torino e CPO Piemonte - Torino, [4]SS Endocrinologia Pediatrica, AO Città della Salute e della Scienza - Torino, [5]SC Oncoematologia Pediatrica e Centro Trapianti, AO Città della Salute e della Scienza - Torino, [6]SSCVD Unità di Transizione per Neoplasie Curate in Età Pediatrica, AO Città della Salute e della Scienza - Torino

Introduction. It has been reported that childhood cancer survivors (CCS) have an increased risk of overweight and dyslipidemia, but the distribution and the potential relationships between anticancer therapies and these cardiovascular (CV) risk factors have been heterogeneously described. Aim of this study is to evaluate the influence of previuos treatments in the developement of obesity, hypercholesterolemia and hypertriglyceridemia in a cohort of CCS. **Methods.** In the setting of an outpatient clinic for CCS, we analyzed the clinical data of 340 consecutive patients (M 197; F 143). The most common cancer diagnosis were hematological malignancies (n=227) and brain tumors (n=51). Hypercholesterolemia and hypertriglyceridemia were defined as total cholesterol >200 mg/dl or triglycerids>200 mg/dl; patients with a Body Mass Index >30 kg/m2 were considered obese. Cox Multivariate adjustment were performed to account for differences in neoplasia and treatment. **Results.** After a median follow-up of 16.1 years, hypercholesterolemia was diagnosed in 87 CCS (25%), hypertriglyceridemia in 20 (6%) and obesity in 28 (8%). At multivariate adjustment, total body irradiation (TBI) and growth hormone deficiency (GHD) increased the risk of both hypercholesterolemia (HR 2.7: 1.2-4.4 and 2.3: 1.1-4.9; all p<0.05) and hypertriglyceridemia (HR 6.5:1.4-31 and 7.2: 1.1-43; all p<0.05). The risk of hypercholesterolemia was also higher in CCS submitted to autologous hematopoietic stem cell transplantation (HSCT; HR 3.2: 1.7-5.9; p<0.001) or platinum-based chemotherapy (HR 2.7: 1.5-4.9; p<0.001), whereas a previous diagnosis of brain tumor (HR 10: 1.2-45; p<0.05) and anthracyclines (HR 1.3: 1.2-26; p<0.05) significantly predicted obesity. **Conclusion.** Survivors of childhood cancer who developed GHD and/or were exposed to TBI reported the highest risk of dyslipidemia. Also brain tumor survivors and CCS previoulsy treated with anthracyclines or platinum-based chemotherapy show an increased risk of hypercholesterolemia and obesity. These patients need an accurate and tailored long-term control of CV risk profile.

PP208

PREVALENCE OF ABDOMINAL OBESITY IN CHILDHOOD VARIES WIDELY ACCORDING TO THE CONSIDERED WAIST CIRCUMFERENCE PERCENTILES

A. Monzani[1], F. Prodam[1], A. Rapa[1], N. Fuiano[2], G. Diddi[2], A. Petri[1], R. Ricotti[1], S. Bellone[1], G. Bona[1]

[1]Divisione di Pediatria - Dipartimento di Scienze della Salute - Università del Piemonte Orientale "A. Avogadro" - Novara, [2]Unità di Pediatria, Prevenzione e Salute Pubblica - ASL FG - Foggia

Waist circumference (WC) is a good proxy measure of visceral adiposity. WC cut-off values for Italian children are not available yet. Aim of our study was to compare the prevalence of abdominal obesity in two populations of Italian schoolchildren according to the available WC charts. In a total of 1062 schoolchildren aged 7-14 years, 499 children (M/F=243/256) living in Northern Italy (Piemonte Region) and 563 children (M/F= 306/257) living in Southern Italy (Puglia Region), WC was measured. Abdominal obesity was defined as WC ≥ 90th percentile for gender and age according to eight available charts including 90th percentile values for children aged 7-14 years (made in USA, England, Pescara, Poland, Turkey, Cypro, Australia, Hong Kong). In overall children, abdominal obesity prevalence was 25.8%, 61.4%, 9.1%, 41.1%, 50.7%, 25%, 47.7%, 50.8% according to USA, England, Pescara, Poland, Turkey, Cypro, Australia, Hong Kong cut-offs, respectively. Abdominal obesity prevalence ranged from 2.4% to 35.7% in Northern children (according to Pescara and England cut-offs, respectively) and from 15.1% to 84.2% in Southern children (according to Pescara and England cut-offs, respectively). This discrepancy highlights the need for Italian national-specific WC cut-offs estimated by a national survey that could be really representative of the whole pediatric population. Moreover, it would be required to calculate pragmatic WC cut-points associated with clustered cardio-metabolic risk, as already performed in adults. In conclusion, national-specific and risk-weighted cut-off values for WC are needed for an accurate and reliable estimate of abdominal obesity in pediatric population.

PP209

INFLUENCE OF EARLY LIFE FACTORS AND PARENTAL WEIGHT STATUS ON CHILDHOOD OVERWEIGHT AND OBESITY

C. Parrino[1], N. La Spina[1], R. Vigneri[2], L. Frittitta[3]

[1]Dipartimento di Biomedicina Clinica e Molecolare Università di Catania - Catania, [2]Ospedale Garibaldi - Catania, [3]Dipartimento di Biomedicina Clinica e Molecolare Università di Catania/Ospedale Garibaldi - Catania

Neonatal and early life factors such as birth weight and breast-feeding are important for the development of childhood and adulthood obesity. Moreover, the complex relationship between parental and child weight is influenced by sharing both genetic and environmental habits. To analyze the role of newborn- and parent-related risk factors we studied 1607 Sicilian schoolchildren (795/812 F/M) aged 8–14 y attending primary or secondary schools. Weight (W), height (H), waist circumference (WC) and fat mass (FM, by bioelectrical impedance, BIA) were measured. Thinness, normal weight, overweight, obesity and Body Mass Index z-score (BMI z-score) were defined according to IOTF (International Obesity Task Force). Birth weight (BW), breast feeding (Bf), and parental W and H were obtained by self-report instruments; parental BMI was calculated. Children were classified as follows: 2.8% thin, 55.9 % normal weight, 27.2 % overweight and 14.1 % obese. The prevalence of a high BW (≥ 4 kg) and of non breast-fed was significantly ($p<0.05$) greater among overweight/obese in respect to normal weight children. Obese children also had a significantly higher percentage of obese mothers in respect to normal weight (15.4% vs. 4.5%, $p<0.0001$) and thin children (15.4% vs. 2.7 %, $p<0.05$) and a significantly higher percentage of obese fathers (32.7%) in comparison to overweight, normal weight and thin children (respectively 16.2%, 10.4%, 0.0%, $p<0.0001$). Both the mother and the father's BMI significantly ($p< 0.0001$ for both) correlated with the child's BMI z-score ($r=0.27$), WC ($r=0.25$) and FM ($r=0.24$). Logistic regression analysis indicated that a higher risk of being overweight or obese, after adjusting for age and gender, was associated with a high BW (OR: 1.83; 95% CI: 1.04–3.25; $p <0.05$), no breast feeding (OR: 1.56; 95% CI: 1.24–1.97; $p <0.0005$), and having an obese mother (OR: 2.31; 95% CI: 1.43–3.73; $p <0.005$) or an obese father (OR: 2.61; 95% CI: 1.86–3.67; $p <0.0001$). Our study indicates that BW, breast feeding and parental weight exert critical influences on the onset of overweight and obesity in children and adolescents.

PP210

TYPE 2 DIABETES REMISSION AFTER MINI-GASTRIC BYPASS OR GASTRIC BANDING

V. Tropea[1], F. Vinciguerra[1], A. Lomonaco[1], N. La Spina[1], A. Bellia[2], L. Piazza[2], S. Squatrito[1], L. Frittitta[1], R. Baratta[1]

[1]Dipartimento di Biomedicina Clinica e Molecolare, Università - Catania, [2]UO Chirurgia, Ospedale Garibaldi - Catania

Bariatric surgery, recently renamed “metabolic”, currently represents an effective treatment for morbid obesity and type 2 diabetes (T2D). The laparoscopic mini-gastric bypass (LMGB), conceived to maintain the efficacy of conventional gastric bypass and reduce its surgical complications, is an alternative approach to other bariatric surgical procedures, but little is known on its efficacy regarding T2D remission.

Aim of this study was to compare LMGB and laparoscopic adjustable gastric banding (LAGB) efficacy on T2D remission after 1-year follow-up.

Thirty-six consecutive patients with severe obesity and T2D (ADA criteria) were addressed to either LMGB (n=15, M/F: 4/11, age 50.1±8.8 years, BMI 52.1±10.9 kg/m^2) or LAGB (n=21, M/F: 4/17, age 41.1±8.4 years, BMI 45.6±6.2 kg/m^2) according to the NIH criteria for bariatric surgery. Anthropometric parameters, fasting blood glucose and HbA1c were evaluated in all patients before and 1 year after surgery; OGTT was carried out 1 year after surgery only in patients with fasting glucose <126 mg/dl.

Percent of excess BMI lost (%EBL) was 55.2±16.7% in LMGB group and 43.5±18.2% in LAGB group ($p=0.1$ after adjusting data for age, gender and basal BMI). At multiple logistic regression analysis, T2D complete remission (fasting glucose <126 mg/dl, 2-h OGTT glucose <200 mg/dl and HbA1c <6.5% in the absence of T2D treatment) was 66.7% (10/15) in LMGB group and 14.3% (3/21) in LAGB group ($p<0.01$ after adjusting data for age, gender and T2D duration and treatment). Interestingly, the different effect of LMGB and LAGB in remitting T2D remained significant also after further adjustment for %EBL ($p<0.05$). Therefore, in the LMGB group factors other than weight loss may be involved in T2D remission.

In conclusion, after 1-year follow-up LMGB causes a significantly higher rate of T2D remission in respect to LAGB, independently of weight loss.

PP211

ROLE OF LOCAL INFLAMMATORY RESPONSE IN ADIPOSE TISSUE DYSFUNCTION

G. Cantini[1], M. Francalanci[1], A. Di Franco[1], S. Baglioni[1], G. Poli[1], J. Samavat[1], V. Querci[2], F. Liotta[2], G. Perigli[3], M. Lucchese[4], G. Forti[1], M. Luconi[1]

[1]Clinical and Experimental Biomedical Sciences, University of Florence - Florence, [2]Clinical and Experimental Medicine - Florence, [3]General Surgery, University of Florence - Florence, [4]Bariatric and Metabolic Surgery, AOU Careggi - Florence

Chronic low-grade inflammatory condition is associated with obesity. However, the role exerted by local inflammation occurring in different fat depots, such as subcutaneous (SAT) versus visceral (VAT) adipose tissue (AT), is still under evaluation. Aim of the present study was to characterize the inflammatory state in adipose tissue of obese (OB: n=58, mean±SD BMI=47±9) versus lean (LEAN: n=28, mean±SD BMI=24±2) patients subjected to bariatric/general abdominal surgery at our University Hospital.

Blood, VAT and SAT biopsies were collected at surgery. Immunophenotyping of blood cells and stromal vascular fraction from AT revealed a higher presence of gammadelta lymphocytes in blood and AT from OB vs LEAN. Moreover, TaqMan analysis of gene expression confirmed a significant increase in specific inflammatory markers in AT of OB (n=38) vs LEAN (n=26), being VAT from OB interested by a significantly worse inflammation characterized by M1 macrophage polarization (CD40±SE expression fold increase=3.76+0.68 in VAT of OB/LEAN, $P<0.005$; CD206±SE expression fold increase=0.39±0.04 in VAT of OB/LEAN, $P<0.05$) and decreased regulatory components (IL10 VAT/SAT±SE expression=10.6±3.9 in LEAN and 1.6±0.4 in OB, $P<0.05$; foxp3/CD3±SE expression: 27.7±12.3 SAT and 5.5±1.5 VAT of LEAN, 17.4±1.8 SAT and 3.2±0.5 VAT of OB; $P<0.05$ VAT LEAN vs OB and $P<0.001$ OB SAT vs VAT). This condition was associated with a reduced VAT functionality in OB vs LEAN evaluated by adiponectin expression (adiponectin±SE expression: 29.1E6±10.1E6 SAT and 18.0E6±.9E6 VAT in 17 LEAN, 10.2E6±1.7E6 SAT and 6.5E6±1.1E6 VAT in 26 OB; $P<0.01$ SAT and VAT LEAN vs OB, $P<0.05$ OB SAT vs VAT).

In conclusion, these data suggest that obese AT is characterized by a local inflammation associated with functional metabolic alterations, more evident in VAT than SAT, which may play a pivotal role in the development of obesity.

PP212

SERUM ACID URIC LEVELS AND ALBUMINURIA-TO-CREATININURIA RATIO IN OBESE CHILDREN ARE ASSOCIATED WITH OBESITY COMORBIDITIES

F. Prodam[1], S. Bellone[1], A. Marolda[1], M. Castagno[1], A. Monzani[1], R. Ricotti[1], E. Giglione[1], G. Genoni[1], E. Altieri[1], G. Bona[1]

[1]Dipartimento di Scienze della Salute, Università del Piemonte Orientale "A. Avogadro" - Novara

Background. Elevated serum uric acid (SUA) is common in subjects with metabolic alterations and cardiovascular diseases, but is not considered a risk factor by all. Some studies have shown that elevated SUA is associated with microalbuminuria which conversely is a proved cardiovascular risk factor. Few data on childhood obesity are present.

Aim. Aim of this study was to investigate the association between SUA and albuminuria-to-creatininuria ratio (ACR) with metabolic alterations in a cohort of obese children.

Subjects and methods. 430 obese children and adolescents (age, mean±SD: 10.7±3.2 yrs) were recruited. Pathological cut offs for SUA and ACR were considered at 5.5 mg/dl, and 30 mg/g, respectively. Because no accordance on SUA distribution in childhood exists, SUA was also categorized in quartiles. Altered glucose levels according to ADA criteria, systolic (SBP) and diastolic blood pressure (DBP) > 90° percentiles, HDL-cholesterol < 40 mg/dl (males) or <50 mg/dl (females), and triglycerides > 150 mg/dl were considered for metabolic alterations. Glucose and insulin levels during an oral glucose tolerance test (OGTT), and HOMA-IR were also evaluated.

Results. Obese children with altered glucose levels had higher ACR ($p<0.03$), but similar SUA levels, respect to those euglycemic. Subjects with higher SBP or DBP, or lower HDL-cholesterol had higher SUA levels ($p<0.0001$ for both) but similar ACR respect to those normal. Being positive for ACR increased the risk to have altered glucose levels (OR: 6.821, IC95% 1.325-35.104; $p<0.02$) also when corrected for confounding factors (age, sex, BMI). Being in the highest quartiles of SUA increased the risk to have hypertension (OR: 1.860, IC95% 1.026-3.370; $p<0.04$) and low HDL-cholesterol levels (OR: 1.917, IC95% 1.079-3.757; $p<0.05$) also when corrected for covariates. Fasting insulin, insulin during OGTT, HOMA-IR were positively predicted by SUA, independently by covariates, but not by ACR.

Conclusion. Serum acid uric and albuminuria-to-creatininuria ratio are already increased in obese children and adolescents. They may predict different metabolic alterations in childhood obesity. Further studies are needed to understand their clinical significance in this age.

PP213

EFFECT OF EXERCISE AND WEIGHT LOSS THERAPY ON OSTEOPROTEGERIN LEVELS, ARTERIAL STIFFNESS, AND ENDOTHELIAL ACTIVATION IN OBESE OLDER ADULT

N. Napoli[1], S. B. Briganti[1], P. Pozzilli[1], R. Armamento-Villareal[2]

[1]Area di Endocrinologia, Università Campus Bio-Medico - Roma, [2]University of New Mexico - Albuquerque, USA

BACKGROUND AND OBJECTIVE: Osteoprotegerin (OPG), a decoy receptor for the RANKL, known osteoclast differentiation inhibitor and modulator of bone resorption is a novel and promising biomarker cardiovascular disease (CVD). However, the common presentation of osteoporosis and atherosclerosis has long been observed with increasing incidence in the aging population. While age is a factor linking the two diseases, multiple studies now propose a common mechanism of pathogenesis involving OPG. Aim of this study was to evaluate the effect of lifestyle intervention on serum OPG , pulse wave velocity (biomarker of arterial stiffness), serum s-vascular cell adhesion protein 1 (sVCAM-1) and E-selectin levels (biomarkers of endothelial activation) in a population of obese older adults.

METHODS: Twenty-six obese (BMI ≥ 30 kg/m2) older (age ≥ 65 yrs) adults were randomly assigned to 6 months of: 1) Healthy lifestyle (Control group; n = 8); 2) Exercise (combined aerobic/resistance exercise) (EX group; n = 9); 3) Diet-induced weight loss + exercise (WL+EX group; n = 9). Serum OPG (Biomedica) and serum s-VCAM-1 and E-selectin (R & D systems) were measured by ELISA. Pulse wave velocity was measured by using transcutaneous dopplers at the right common carotid and right femoral arteries

RESULTS: In the WL+EX group, serum OPG levels decreased (-0.6±0.3 pmol/L [-10%] ; P = 0.05); pulse wave velocity decreased (-1.8 ± 0.7 m/s [-20%]; P = 0.04) as well as serum E-selectin (-0.9 ± 0.3 ng/ml [-23%]; P = 0.04). There was also a trend for a reduction in s-VCAM in the WL+EX group. In the WL+EX group, body weight decreased (-8.5±1.2 kg). Weight was stable (1.0 ±0.9 kg) in the EX group and Control group (0.6 ± 1.5 kg).

There were no changes in these CVD markers in the EX group and Control group.

CONCLUSIONS: Lifestyle intervention via combined weight loss and aerobic/resistance training can decrease multiple biomarkers associated with atherosclerosis and aging simultaneously in obese older adults. Our study is the first study to show a statistically significant correlation between exercise induced weight loss in the elderly obese and serum levels of OPG.

PP214

AEROBIC PHYSICAL EXERCISE, BEHAVIOURAL COMPENSATIONS AND PLASMA ADIPOKINES IN POSTMENOPAUSE

A. Di Blasio[1], S. Melanzi[1], I. Bucci[1], M. Olivieri[1], A. Sagazio[1], M. Carpentieri[1], C. Giuliani[1], G. Napolitano[1]

[1]Medicina e Scienze dell'Invecchiamento, Università di Chieti - Chieti

INTRODUCTION Even if aerobic physical exercise has been widely indicated as effective intervention to improve plasma levels of adipokines, literature is reporting negative behavioural compensations, concerning dietary habits and spontaneous physical activity (SPA), in sedentary person who start training. Aim of the study was to determine whether aerobic physical exercise elicits positive effects on adipokines plasma levels independently from the effects of the training on behavioural variables. METHODS. Thirty-six postmenopausal women (55.94±4.22 yrs), without history of diabetes mellitus, pulmonary, myocardial and orthopedic diseases and without any pharmacological treatment were enrolled and trained four days per week, for 14 weeks: participants walked at moderate intensity. Aerobic fitness, body composition, plasma values of leptin, adiponectin, resistin, and visfatin were assessed. Daily physical activity (DPA) was objectively recorded through a multisensor device (Bodymedia armband, Sensewear). RESULTS. Adhesion rate to the programmed volume of physical exercise was 86.88±9.69 %. Paired-samples t-test after the training program revealed no significant variations of both body composition and DPA variables, while a significant increase of energy intake was detected. Also when normalized for kg of fat mass, plasma leptin (p=0.001), resistin (<0.001) and adiponectin (<0.001) have been found modified: leptin and resistin decreased, while adiponectin increased. Maximal aerobic capacity of participants increased (p<0.001). Although no significant variation has been shown for visfatin mean levels, we detected individual modification of its value. As in the sample were detected participants differently compensating the introduction of the training (i.e. increase or reduction of energy intake; increase or reduction of SPA) we used the Pearson's correlation analysis to investigate if any variable was correlated to plasma visfatin variation: the delta of visfatin to FMkg ratio has been found negatively correlated with daily minutes of moderate to vigorous physical activity variation (r = -0.373; p = 0.02) and with energy expenditure derived from them (r = -0.339; p = 0.04). CONCLUSIONS. Aerobic training improved aerobic fitness and plasma levels of adiponectin, leptin, and resistin independently from body composition variation and both energy intake and spontaneous physical activity compensation. On the contrary, visfatin modification seems to be influenced by total volume of daily physical activity practiced at moderate to vigourous intensity instead of physical exercise alone.

PP215

CHANGES IN THIGH MUSCLE VOLUME PREDICT CHANGES IN FEMORAL BMD IN SARCOPENIC ELDERLY OBESE ADULTS UNDERGOING LIFESTYLE THERAPY

N. Napoli[1], S. Briganti[2], P. Pozzilli[1], R. Armamento-Villareal[3]

[1]Area di Endocrinologia, Campus Bio-Medico di Roma - Roma, [2]Area di Endocrinologia, Campus Bio-Medico di RomaArea di Endocrinologia, Campus Bio-Medico di Roma - Roma, [3]University of New Mexico - Albuquerque, USA

Purpose: Loss of femoral BMD and lean body mass are major complications of weight loss (WL) from lifestyle therapy in frail sarcopenic elderly obese (EO); but these adverse effects can be attenuated by exercise (EX). We hypothesize that the negative effects of WL and the protective effect of exercise (EX) on femoral bone is modulated by changes in thigh muscle volume (TMV)leading to differences in mechanical strain and sclerostin production. The objective of this study is to examine changes in TMV and muscle strain and their effects on femoral BMD in EO subjects undergoing WL, EX and WL+EX.

Methods: 107 obese (BMI>30), older (age ≥ 65) subjects were randomized for 1 yr to control (CTRL) (n=27), WL (n=26), EX (n=26) and WL+EX (n=28) groups. WL was by dietary/behavioral intervention designed to induce and maintain WL of 10% of body weight; EX was by 3 supervised resistance/aerobic exercise sessions/wk and CTRL was to continue usual dietary and activity habits. TMV was measured using magnetic resonance imaging of both thighs at 0 and 12 mos; while BMD by DXA, sclerostin by Elisa, strength (knee extension and flexion by BIODEX) and physical function by Physical Performance Testing (PPT), were measured at 0, 6 and 12 mos.

Results: Significant reduction in TMV relative to CTRL and EX w as observed in the WL group while reduction in TMV in the WL+EX was not different from the CTRL. These changes followed the same pattern as the changes in total hip BMD. Both knee extension and flexion increased in the EX and WL+EX and were unchanged in the CTRL and WL . TMV changes correlated with: total hip BMD changes (r=0.55, p=0.000), changes in sclerostin (r=-0.26, p=0.03) and with a borderline correlation with changes in knee flexion (r=0.23, p=0.06). Multivariate analysis showed TMV and PPT changes as predictors for hip BMD changes.

Conclusion: Changes in TMV modulate femoral BMD changes in frail EO patients undergoing lifestyle therapy. WL-induced thigh muscle loss is attenuated by EX resulting in similar improvement in strength in WL and WL+EX groups. This positive effect of EX when added to WL may in part account for the relative reduction in WL- associated bone loss in the WL+EX patients.

PP216

USEFULNESS OF VISCERAL ADIPOSITY INDEX (VAI) IN THE EVALUATION OF ALTERATIONS IN SEMEN PARAMETERS AND CARDIOMETABOLIC RISK IN YOUNG OBESE MALES

G. Pizza[1], L. Barrea[1], V. Nedi[1], E. Guerra[1], P. Vitale[1], A. Inverardi[1], R. Pivonello[1], S. Savastano[1], A. Colao[1]

[1]Department of Clinical Medicine and Surgery, Division of Endocrinology. Federico II University - Naples

Background: Obesity is related to reproductive disorders in both males and females. Little information is available on the relationships between obesity, alterations in semen parameters, and cardiometabolic risk. Aim: To investigate the relationships between obesity, body composition, alterations in semen parameters, and cardiometabolic risk in young obese men. Materials and Methods: Thirty men with severe obesity (BMI 43.2±5.7 kg/m2, age 36.2±10.7 yrs) were recruited. Antropometric parameters, FSH, LH, testosterone (T), 17β-estradiol levels were evaluated. The HoMA (Homeostasis Model Assessment) index and Visceral Adiposity Index (VAI), were calculated. Metabolic Syndrome (MS) was diagnosed according to ATP III criteria. Semen samples were taken from each patient by masturbation after 2-5 days of abstinence and assessed according to the WHO criteria. Body composition was performed with bioimpedance analysis and biavector analysis. Results: MS was diagnosed in 54.5%. Normospermia was present in 43.3% of the study population, and oligoazoospermia in 56.7%. T levels were positively correlated with total sperm count (SC), sperm motility (SM), and sperm motility rapid progression (SMRP) (r=0.658, p<0.001, r=0.464, p=0.001; r=0.492, p=0.006, respectively). BMI was negatively correlated with total SC and T levels (r=-0.547, p=0.002; r=-0.558, p=0.001, respectively). A negative correlation was observed between % fat mass and total SC, SM, and SMRP (r=-0.408, p=0.025; r=-0.425, p=0.019; r=-0.471, p=0.009). Fat mass was negatively correlated with T levels (r=-0.460, p=0.011), while free fat mass was positively correlated total SC, SM, SMRP and T levels (r=0.575; p=0.001; r=0.388; p=0.034; r=0.551; p=0.002; r=0.677, p<0.001, respectively). VAI was positively correlated with HoMA index (r=0.506, p=0.004) and negatively with SM and SMRP (r=-0.435, p=0.016 and r=-0.388, p=0.034, respectively). At multiple regression analysis, T was the major determinants of total SC (β=0.497; p=0.017), while VAI well predicted SM and SMRP (β=-0.391, p=0.02, and β=-0.352, p=0.028, respectively). Conclusions: Visceral adipose tissue dysfunction, expressed by VAI, might affect the semen plasma quality and, consequently, male infertility in young males with severe obesity, likely through the increased release of inflammatory adipokines. The evaluation of VAI, apart from the cardiometabolic risk, could help to better identify the young obese men at risk of impaired semen plasma quality.

PP217

IN OVERWEIGHT AND OBESE CHILDREN THE MHO PHENOTYPE IS ASSOCIATED WITH A LOWER PREVALENCE AND LESS SEVERE NAFLD

F. Vinciguerra[1], M. Russello[2], V. Tropea[1], M. L. Arpi[1], D. Maiolo[1], A. Nigro[1], S. Squatrito[1], L. Frittitta[1]

[1]Endocrinology Unit, Department of Clinical and Molecular Bio-medicine, University of Catania, Garibaldi Hospital - Catania, [2]Liver Unit, Garibaldi Hospital - Catania

In obese children metabolic complications, including non-alcoholic fatty liver disease (NAFLD), are present. Among adult obese patients there is a subgroup without the metabolic complications of obesity and therefore defined "metabolically healthy obese" (MHO). Aim: in a cohort of 164 overweight (BMI z-score >85 th) or obese (BMI z-score >95 th) children (M/F=83/81, mean age 13.3 ± 2.7) we evaluated the prevalence of the MHO phenotype and the presence of NAFLD in this subgroup. The MHO phenotype was defined as the absence of at least 4 out of 5 of the following abnormalities: HOMA-IR > 2.5, fasting blood glucose > 100 mg/dl, triglycerides ≥ 95 th percentile, HDL-Cholesterol ≤ 5 th percentile and blood pressure ≥ 90th percentile. The MHO phenotype was present in 85/164 (51.8%) while "at risk obese" (ARO) phenotype in 79/164 (48.2%) overweight/obese children. The presence and the severity of NAFLD were determined by ultrasound scan carried out by a single operator.

NAFLD was detected in 52.9% of MHO and in 73.4% of ARO subjects ($p<0.01$). Children with NAFLD had higher BMI than those without NAFLD both in the MHO (BMI z-score 2.4±0.08 vs 2.03±0.08, $p<0.005$) and in the ARO children (BMI z-score 2.6±0.08 vs 2.19±0.13, $p<0.05$). In addition, in ARO children with NAFLD, GPT levels were higher in comparison to the other 3 groups ($p<0.05$). At multiple logistic regression, NAFLD was independently associated with BMI z-score, GPT levels and ARO phenotype ($p\leq0.05$). The prevalence of mild and moderate NAFLD was respectively 38.8% (33/85) and 14.1% (12/85) in MHO group vs 36.7% (29/79) and 24.0% (19/79) in ARO phenotype (p=n.s. between groups). Severe NAFLD was present in 15.2% (12/79) of ARO group and, in contrast, absent in MHO phenotype ($p<0.0001$).

In conclusion, the prevalence of NAFLD is higher in overweight and obese children with the ARO phenotype and NAFLD is independently associated with BMI, GPT levels and ARO phenotype. The assessment of MHO phenotype is a simple way to select overweight or obese children with a low risk of severe NAFLD.

PP218

A PROTEOMIC APPROACH TO EVALUATE THE ROLE OF COCOA CONSUMPTION IN THE INFLAMMATORY PATHWAY.

M. Roccio[1], F. Prodam[1], G. E. Walker[1], S. Moia[1], S. Bellone[1], M. Arlorio[2], G. Bona[1]

[1]Dipartimento di Scienze della Salute, Università del Piemonte Orientale "A. Avogadro" - Novara, [2]Dipartimento di Scienze del Farmaco, Università del Piemonte Orientale "A. Avogadro" - Novara

Background. Diet has been demonstrated to play a crucial role not only for the reduction of excessive energy intake but also for the normalization of tissue hyper-inflammation, that is characteristic of obesity. Dietary components such as polyphenols present in cocoa (Theobroma cacao L.) have important health properties including modulation of immune function and inflammation. Monocytes are key to this inflammatory status and offer an important model in investigating the role of dietary components and their metabolites.

Objectives. The main target of this research was to study the key molecular mechanisms through which the bioactive components of cocoa may modulate the inflammatory state in healthy subjects.

Methods and Results. At present, there is no specific report on the efficacy of bioavailable flavanols on inflammation in humans, despite promising data in animal models. For this reason a liquid chromatography-mass spectrometry (LC-MS) analysis was performed to evaluate the bioavailability of epicatechin, in particular the glucuronide metabolite, in the plasma of four healthy volunteers (young men aged 22-27 years) treated with acute and chronic levels of cocoa. Cocoa was administered in the form of chocolate bars, derived from two batches of chocolate with different percentages of cocoa (40% and 80%).

At the same time, peripheral mononuclear cells were isolated from the blood of the same subject, before and after administration. Cells were lysed and analyzed by 2D-electrophoresis with IPG strips 5-8 pH, with the respective proteomic profiles compared using PDQuest statistical software. Overall, the analysis showed an average total of 705 spots (n=4) present within the monocytes isolated. Following a deeper analysis, our attention was drawn to a cluster of spots localized between 20 to 60 KDa, where we observed differences in protein expression after three hours of administration of cocoa, when compared with the basal profile.

Conclusions. The data obtained from the bioavailability experiments and proteomic analyses represent the initial phase of an ongoing study that will contribute to understanding the role of diet on inflammatory mechanisms linked to cocoa consumption. In this regard confirmational experiments will be performed to investigate the hypothetical role of these proteins with inflammatory pathways.

PP219

IMPACT OF MEDICAL AND SURGICAL TREATMENT ON WEIGHT LOSS AND METABOLIC PATTERN IN SEVERE OBESITY

M. Tomelini[1], S. Boschetti[1], M. Maccario[1], F. Broglio[1], E. Ghigo[1], A. De Francesco[1], D. Boggio Bertinet[1], A. Palmo[1]

[1]Scienze Mediche - Torino

Severe obesity (i.e. BMI≥ 40 kg/m2) is associated with an increased risk of numerous comorbidities including diabetes, hypertension and cardiovascular disease. Lifestyle or pharmacologic interventions are usually not successful and, bariatric surgery is the only available treatment able to achieve and maintain substantial weight loss, decrease incidence of comorbidities, and improve overall quality of life. This study examined 4-year changes in weight, diet, lipid levels and glycemic control in 100 subjects who underwent bariatric surgery (BSP; of which 50 subjects after Gastric Bypass procedure [GBP] and 50 subjects after Vertical Banded Gastroplasty [VBG]) and in 50 subjects in Conventional Therapy (CT; i.e. dietary and behavioral program) followed in the Department of Dietetic and Clinical Nutrition, S. Giovanni Battista Hospital of Turin, from 2005 to 2010. The two groups were matched for age, BMI and caloric intakes. Aim of the study was to find a correlation between weight loss and improvement of metabolic data. All the groups achieved their maximal weight loss in the second year of follow up. BSP lost more ($p<.05$) weight (-33% of the initial weight) than CT (-15%). Blood lipids and fasting plasma glucose (FPG) improved in all the groups during the first two years; with a greater extent ($p<0.05$) in BSP (FPG: -22 mg/dL; Triglycerides: -55 mg/dL; LDLc: -24 mg/dL; HDLc: +18 mg/dL) than in CT (FPG: -11 mg/dL; Triglycerides: -14 mg/dL; LDLc: -9 mg/dL; HDLc: +7 mg/dL). Among BSP, patients undergoing GBP showed a greater extent ($p<0.05$) of reduction in FBG (-24 mg/dL), Triglycerides (-51 mg/dL), LDLc (-35 mg/dL) and of increase of HDLc (+20 mg/dL) than in VBG (FPG: -21 mg/dL; Triglycerides: -14 mg/dL; LDLc: -15 mg/dL; HDLc: +16 mg/dL). The lowest energy intake was observed during the first months being more marked ($p<.0001$) in the BSP (-990 kcal/d in VBG group; -1120 kcal/d in GBP group) than in CT (1560 kcal/d). However, after the first year GBP subjects showed to eat the same amount of kilocalories (1660 kcal/d) as CT group (1604 kcal/d), greater than ($p<.005$) in VBG group (1161 kcal/d). Multiple regression analyses suggested that weight loss had a significant ($p<.002$) and positive effect on glycemic reduction, while bariatric surgery is related to other metabolic effects ($p<.005$); energy intakes seemed to have no effects on metabolic and weight changes. In conclusion, this study suggests that weight loss is associated to metabolic improvement during the first year after both medical and surgical treatment for severe obesity with bariatric surgery apparently inducing more positive effects on glucose and lipid metabolism as well as on weight loss.

PP220

FOOD ADDICTED OR NOT FOOD ADDICTED: THAT IS THE QUESTION

S. Garelli[1], A. Agostini[2], G. Di Dalmazi[1], J. Manso[1], C. Cavazza[1], V. Vicennati[1], R. Pasquali[1], U. Pagotto[1]

[1]U.O. di Endocrinologia, Dipartimento di Medicina e Chirurgia, Policlinico S.Orsola-Malpighi, Alma Mater Studiorum - Bologna, [2]Dipartimento di Scienze Biomediche e Neuromotorie, Policlinico S.Orsola-Malpighi, Alma Mater Studiorum - Bologna

Background: Food addiction (FA) shares some features with other addictive behaviors: loss of control, tolerance, continuative use despite negative consequences. Its definition, however, is under intense scrutiny, as well as its distinction from binge eating disorder (BED). In previous studies the prevalence of FA was about 20-25% in obese people and 57% among patients with BED.

Objective: The aim of this study was to describe the prevalence of FA in a population of overweight/obese Italian women attending to our Endocrinology Unit for a consult.

Method: We translated in Italian language the Yale Food Addiction Scale (YFAS), a questionnaire recently proposed by Gearhardt and colleagues (J Addict Med, 2009), that applies the diagnostic criteria for substance dependence to eating behavior and provides a FA "symptoms" count and a FA "diagnosis". We administered YFAS to 98 overweight/obese women in pre-menopause age, in which any secondary cause of obesity was preliminary excluded by hormonal examination.

Results: The mean symptoms count was 26.1 (SD=1.6) and within our group 20 subjects (20.4%) resulted food addicted while 78 subjects (79.6%) were not-food addicted.

Discussion: In a population of overweight/obese women attending our Centre the prevalence of FA was 20.4%. The overlap between our results and those from previous studies found in similar populations with the YFAS suggests that this questionnaire is a valid tool for measures in this area.

Acknowledgments: This research was supported by a grant from the European Union (NEUROFAST FPVII-KBBE-2009-3-245009).

PP221

CORRECTION OF INTERMITTENT HYPOXIA WITH CPAP IMPROVES BLOOD PRESSURE AND METABOLIC ABNORMALITIES IN OBESE PATIENTS WITH OBSTRUCTIVE SLEEP APNEA.

M. Barbaro[1], S. Perrini[1], A. Ciavarella[1], S. Kounakis[1], A. Falcone[2], V. Quaranta[2], P. Nigro[1], R. Ficarella[1], A. Cignarelli[1], A. Natalicchio[1], L. Laviola[1], G. De Pergola[3], O. Resta[2], F. Giorgino[1]

[1]Endocrinologia, Dip. Emergenza e Trapianti di Organi, Università degli Studi di Bari "Aldo Moro" - Bari, [2]Pneumologia, Dip. Neuroscienze, Università degli Studi di Bari "Aldo Moro" - Bari, [3]Oncologia Medica, Dip. DIMO, Università degli Studi di Bari, "Aldo Moro" - Bari

Obstructive sleep apnea (OSA) is associated with obesity, insulin resistance, dyslipidemia, hypertension, and diabetes. It is unclear whether treatment of OSA with continuous positive airway pressure (CPAP) can modify these comorbidities. Sixty-two obese patients with OSA were studied at baseline and after three months of therapeutic CPAP treatment, and compared with twenty matched subjects who had a negative single-night polysomnogram. Both the OSA and control groups received general dietary and exercise advice throughout the study. Measurements of anthropometric variables, blood pressure, fasting blood glucose levels, insulin resistance (HOMA model), fasting blood lipid profile, HbA1c, C-reactive protein and fibrinogen were obtained at baseline and after three months. At baseline, neck circumference (44.7 vs. 40.5 cm; p=0.005), diastolic blood pressure (83.4 vs. 76.1 mmHg; p=0.017), HbA1c (5.9 vs. 5.4%; p=0.02) and white blood cells (7,823 vs. 6,349 per µL; p=0.008) were significantly increased in the OSA compared to control group. 50 of 62 OSA and 15 of 20 control subjects, respectively, completed the study. After 3 months, CPAP treatment resulted in significant mean decreases in BMI (-4 kg/m2; p=0.003), waist (-10 cm; p=0.01) and neck (-3 cm; p=0.006) circumferences, systolic (-10.0 mmHg; p=0.02) and diastolic (-7.0 mmHg; p=0.03) blood pressure, and serum total cholesterol (-26.0 mg/dL; p=0.02) in the OSA group, whereas no significant changes in these parameters were observed in the control group. In conclusion, 3 months of CPAP therapy in a group of obese patients with moderate-to-severe OSA is associated with improved control of blood pressure and amelioration of anthropometric variables and metabolic abnormalities, suggesting a favorable effect of correcting intermittent hypoxia on metabolic and cardiovascular outcomes in obesity.

PP222

GROWTH HORMONE IS NECESSARY FOR THE P53-MEDIATED OBESITY-INDUCED INSULIN RESISTANCE

F. Raggi[1], C. Urbani[1], D. Russo[1], C. Sardella[1], M. Lombardi[1], S. Brogioni[1], A. Campomori[1], I. Lupi[1], E. Martino[1], F. Bogazzi[1]

[1]Department of Clinical and Experimental Medicine - University of Pisa

Objectives: Insulin resistance is a key marker of both obesity and GH excess. The molecular pathways involved in insulin resistance induction in obesity and GH excess are not fully understood. The aim of the study was to investigate the molecular interplay between p53 and the GH-IGF1 system in diet induced obesity. **Methods:** The study was carried out using mice with obesity induced by diet (Obe) or with overexpression of bovine GH (Acro). Adipose protein or RNA expression was evaluated by Western Blot or quantitative Real Time PCR (qRT-PCR), respectively. **Results:** Mice with obesity, induced by high fat diet, had insulin resistance, which is sustained by a selective increased expression of p53 in adipose tissue. Normal insulin sensitivity was restored and adipose p53 expression normalized when the GH pathway was blocked. Only the adipose p53 expression was sensitive to the GH blockage, which occurred through the p38 pathway. Adipose tissue of Obe mice had a coordinate overexpression of SOCS1-3 and STAT1, 3 and 5b, not different from that of acromegalic mice (Acro), suggesting an increased sensitivity of adipose tissue to GH; this condition was linked to the increased expression of adipose inflammatory cytokines, including IL6. **Conclusions:** In conclusion, GH seems to be necessary for the increased adipose p53 expression and for insulin resistance of obese mice. Adipose GH pathways might be target for future therapies of insulin resistance.

PP223

HUMAN WHITE ADIPOCYTES EXPRESS THE TRPM8 RECEPTOR WHICH ACTIVATION INDUCES A "BROWN-LIKE" PHENOTYPE

M. Rossato[1], M. Granzotto[1], V. Macchi[2], A. Porzionato[2], L. Petrelli[2], A. Calcagno[1], R. Rizzuto[3], V. Vindigni[4], R. De Caro[2], R. Vettor[1]

[1]Department of Medicine-University of Padova - Padova, [2]Department of Molecular Medicine - University of Padova - Padova, [3]Department of Biomedical Sciences - University of Padova - Padova, [4]Department of Neurosciences - University of Padova - Padova

Introduction. The uncoupling protein 1 (UCP1) is a hallmark of brown adipocytes and the pivotal player for cold-induced thermogenesis in these cells. Recently “browning” of white adipose tissue (WAT), with massive up-regulation of UCP1 has been confirmed after physiological and pharmacological manipulations. We aimed to investigate the expression of the cold-sensing receptor TRPM8 in human WAT and the effects of its activation by natural and synthetic agonists. **Materials and Methods**. The stromal vascular fraction from human white adipose tissue was isolated and pre-adipocytes were differentiated to mature adipocytes in adipogenic medium. We investigated the expression of TRPM8 receptor in human adipocytes together with the effects of its activation by natural (menthol) and synthetic agonists (icilin) on adipocyte cytoplasmic calcium concentrations, UCP1 expression, genes regulating mitochondrial biogenesis, glucose uptake and cell ultrastructure with TEM. **Results**. TRPM8 receptor is expressed in human white adipocytes and its activation induces a rise in [Ca2+]i along with the induction of UCP1 expression and increased glucose uptake. The induction of “brown-like” phenotype in human white adipocytes after TRPM8 activation is further supported by ultrastructural morphological changes of mitochondrial morphology and intracellular localization around lipid droplets, with no changes in the expression of the master genes regulating mitochondrial biogenesis. **Discussion**. Our findings provide evidence that human white adipocytes express the cold receptor TRPM8 which activation induces a cellular phenotype resembling that of brown adipocytes thus providing support for this cold-sensing receptor in the control of adipose tissue metabolism and whole body energy balance.

PP224

MTOR, AKT, P70S6K AND ERK1/2 LEVELS PREDICTIVE MARKERS TO MTOR AND PI3K/MTOR INHIBITORS SENSITIVITY IN HUMAN BRONCHIAL CARCINOIDS

T. Gagliano[1], M. Bellio[1], E. Gentilin[1], D. Molè[1], F. Tagliati[1], M. Schiavon[2], N. G. Cavallesco[3], F. Calabrese[2], M. R. Ambrosio[1], F. Rea[2], E. degli Uberti[1], M. C. Zatelli[1]

[1]Dipartimento di Scienze Mediche, Sez. Endocrinologia - Ferrara, [2]Dipartimento di Scienze Cardiologiche, Toraciche e Vascolari - Padova, [3]Dipartimento di Morfologia, chirurgia e medicina sperimentale - Ferrara

Background: Bronchial carcinoids (BCs) are rare neuroendocrine tumors that are still orphan of medical treatment. Human BC primary cultures may display resistance to Everolimus, an inhibitor of the mammalian target of rapamycin (mTOR), in terms of cell viability reduction.

Aim: to assess whether the novel dual PI3K/mTOR inhibitor, NVP-BEZ235, may be effective in Everolimus-resistant human BC tissues and cell lines. In addition, we search for possible markers of mTOR inhibitors efficacy, that may help in identifying the patients that may benefit from mTOR inhibitors treatment, sparing them from ineffective therapy.

Results: NVP-BEZ235 is twice as potent as Everolimus in reducing cell viability and activating apoptosis in human BC tissues that display sensitivity to mTOR inhibitors, but is not effective in Everolimus-resistant BC tissues and cell lines, that by-pass cyclin D1 down-regulation and escape G0/G1 blockade. Rebound AKT activation was not observed in response to treatment with either mTOR inhibitor in “resistant” BC cells. We also show that, in addition to total mTOR levels, putative markers of BC sensitivity to mTOR inhibitors are represented by higher AKT, p70S6K and ERK1/2 protein levels.

Conclusion: These data indicate that the dual PI3K/mTOR inhibitor NVP-BEZ235 is more potent than Everolimus in reducing human BC cell proliferation. “Resistant” cells display a lower levels of mTOR, AKT, p70S6K and ERK1/2, indicating that these proteins may be useful as predictive markers of resistance to mTOR and PI3K/mTOR inhibitors in human BC.

PP225

EGFR ACTIVATION ENHANCES ACC CELL PROLIFERATION BY INDUCING VEGF SECRETION

T. Gagliano[1], D. Molè[1], E. Gentilin[1], M. Bellio[1], E. degli Uberti[1], M. C. Zatelli[1]

[1]Dipartimento di Scienze Mediche, Sez. Endocrinologia - Ferrara

Adrenocortical cancer (ACC) is a rare and aggressive malignancy. Currently the main therapeutic option is surgery, but due to difficult and delayed diagnosis and to the onset of metastases, medical therapy is often tried. ACC treatment is mainly represented by Mitotane alone or in association with chemotherapy, with variable results. Understanding the molecular mechanisms that regulate ACC proliferation could be useful to identify new therapeutic options. Aim of our study is to identify growth factors that may regulate ACC proliferation, using two human ACC cell lines, the SW13 and the NCI-H295 cells. Our data show that Epidermal Growth Factor (EGF) and Transforming Growth Factor (TGF)-α enhance SW13 cell proliferation and reduced apoptosis, while had modest effects on NCI-H295 cells. Sunitinib, an EGF receptor (EGFR) inhibitor, and NVP-BEZ235, a PI3K/mTOR inhibitor, reduced cell viability in both cell lines, being counteracted by both EGF and TGF-α in SW13 cells. Since in other settings EGF regulates cell proliferation by inducing VEGF, we investigated VEGF secretion by the two cells lines. EGF and TGF-α enhanced VEGF secretion only in SW13 cells while had no effects on NCI-H295. In addition, a VEGF receptor blocking antibody significantly reduced EGF and TGF-α induced cell proliferation.

We investigated in both cell lines the expression of EGFR, which is higher and ubiquitous in SW13 cells, while it is weaker and sparse in NCI-H295 cells, where it is present only on the membrane.

These data demonstrate that EGF and TGF-α are important in regulating Sw13 cell proliferation, also by modulating VEGF secretion. In conclusion our data suggest that EGF pathway could represent a new molecular target in drug design for treatment of ACC that display enhanced EGFR expression.

PP226

EGF PATHWAY AS A POSSIBLE TARGET IN THE PHARMACOLOGICAL THERAPY OF BRONCHIAL CARCINOIDS

T. Gagliano[1], M. Bellio[1], M. Minoia[1], K. Benfini[1], E. degli Uberti[1], M. C. Zatelli[1]

[1]Dipartimento di Scienze Mediche, Sez. Endocrinologia - Ferrara

Bronchial carcinoids (BC) are rare tumors originating from endocrine cells dispersed in the respiratory epithelium. Currently, the main BC treatment is surgery, that can be curative in most of the cases, but is not feasible for large, infiltrating and metastatic disease. In these settings, medical therapy is often tried, being mainly represented by chemotherapy and radiation in the attempt to reduce tumor mass, while somatostatin analogues are employed for symptomatic control. Therefore it is important to identify new therapeutic targets and new molecules capable of providing adequate medical treatment for patients with BC for which surgical removal is not feasible. Growth factors which are important in experimental models of neuroendocrine tumors include EGF (epidermal growth factor), TGF (transforming growth factor) α, TGFβ. EGF and TGFα bind to the EGF receptor to stimulate the PI3K/RAS/RAF/MAPK pathway, leading to the transcription of genes associated with cell proliferation, invasion and metastasis.

Our aim is to evaluate the effects of Sunitinib, a multi-targeted receptor tyrosine kinase (RTK) inhibitor, and NVP-BEZ235, a PI3K/mTOR inhibitor, on human primary BC cells cultures in order to verify the involvement of the EGF pathway in regulating crucial cellular processes.

Human BC primary cultures were treated with Sunitinib or NVP-BEZ235, alone or in combination with EGF. EGFR expression, cell viability and caspase 3/7 activation were evaluated.

By immunofluorescences we found that EGFR is expressed in all primary cultures. In addition, 100 nM NVP-BEZ235 and 10 μM Sunitinib inhibit cell viability by 30% and 20% ($P<0.01$), respectively. Both NVP-BEZ 235 and Sunitinib promote apoptosis (+100%). 100 ng/ml EGF impairs the antiproliferative and pro-apoptotic effects of both Sunitinib and NVP-BEZ 235.

These data suggest a possible role for EGFR pathway as molecular target in the medical treatment of BC. Further studies are necessary to understand the molecular basis of this mechanism.

PP227

1,25-DIHYDROXYVITAMIN D INHIBITS PROLIFERATION OF ADRENOCORTICAL CANCER NCI-H295R CELLS BY PROMOTING CELL CYCLE ARREST

C. Pilon[1], S. Vettore[1], R. Sirianni[2], V. Pezzi[2], T. S. Williams[3], P. Mulatero[3], A. Fassina[1], H. Sasano[4], R. Urbanet[1], F. Fallo[1]

[1]Dpt of Medicine - University of Padova, Italy, [2]Dpt of Pharmaco-Biology - University of Calabria, Italy, [3]Dpt of Medical Sciences - University of Torino, Italy, [4]Dpt of Pathology - Tohoku Univ School of Medicine, Sendai, Japan

The active form of vitamin D, 1,25-Dihydroxyvitamin D [1,25(OH)D] can, in general, inhibit the growth of normal and malignant cells. The aim of our study was to investigate the molecular mechanisms of a potential anti-proliferative action of 1,25(OH)D in the human adrenocortical cancer NCI-H295R cell line. mRNA levels for CYP2R1, CYP27B1 and vitamin D receptor (VDR) were measured by RT-PCR and/or Western blotting in NCI-H295R cells. DNA synthesis was evaluated by [3H]TdR cell incorporation after 96 h cell treatment with 1,25(OH)D at increasing doses, compared to untreated control cells. The effect of 1,25(OH)D on cell cycle and apoptosis was analyzed with a flow cytometer. Cyclin-dependent kinase 4 (CDK4) gene expression, a molecular marker of G1-S cell cycle transition phase, was evaluated in NCI-H295R cells treated with 1,25(OH)D, before and after VDR gene silencing. Western blotting and immunohistochemistry for vitamin D metabolic cascade-related genes were also performed in normal and tumorous adrenocortical tissues. 1,25(OH)D treatment inhibited cell proliferation by 20% at a dose of 10-8 M and led to a concomitant decrease in cortisol, aldosterone and DHEA-S. 1,25(OH)D induced cell cycle arrest, promoting accumulation of cells in G1 phase without inducing apoptosis. In NCI-H29R cells, activation of CDK4 was reduced by 1,25(OH)D treatment but was not affected by 1,25(OH)D after VDR gene silencing. CYP2R1, CYP27B1 and VDR were expressed in NCI-H295R cells and adrenal tissues (non-functioning adenomas, n=5; cortisol/aldosterone-secreting adenomas, n=9; cortisol-producing carcinoma, n=7). Conclusions: 1,25(OH)D has an anti-proliferative effect on NCI-H295R cells by promoting cell cycle arrest. This suggests a potential role of 1,25(OH)D for treatment of adrenocortical cancer. Expression of vitamin D metabolism genes in NCI-H295R cells and in normal/tumorous adrenals may indicate an autocrine activity of 1,25(OH)D in regulating adrenal growth and function.

PP228

EXPRESSION OF AURORA KINASES IN ADRENOCORTICAL TUMORS

R. Pezzani[1], B. Rubin[1], M. V. Cicala[1], M. Salvà[1], M. Iacobone[2], S. Ulisse[3], F. Mantero[1]

[1]Department of Medicine - Padova, [2]Department of Surgical and Gastroenterological Sciences - Padova, [3]Department of Experimental Medicine - Roma

Background: Both secreting and non-secreting adrenocortical tumors (ACT) are frequently found and are mostly benign, but among them, adrenocortical carcinomas (ACC), although rare, show poor prognosis and metastatic potential. Aurora kinase (AK) family members are serine/threonine kinase involved in the regulation of mitosis.

Aim: To investigate the expression of Aurora kinase A, B, C (AKA, AKB, AKC) in adrenocortical tumors and to evaluate the pan-Aurora kinase inhibitor, MK-0457, in adrenocortical cell lines.

Materials and methods: 12 ACT were analyzed: 4 ACC, 3 aldosterone producing adenoma (APA), 3 cortisol producing adenomas (CPA) and 2 non-secreting adenomas (NSA). Also 3 normal adrenal tissues and SW13 and H295R cells were studied. All the samples were evaluated by quantitative RT-PCR for *AURKA*, *AURKB*, *AURKC*. MTT test and ^{3}H thymidine assay were performed in SW13 and H295R cells after treatment with MK-0457.

Results: All tissues and cell lines expressed AKA, AKB and AKC. ACC samples overexpressed AKA and AKB, while among ACT only CPA showed increased AKA. MK-0457 inhibited SW13 cell viability at 72h with IC_{50} of 85nM. Furthermore we observed a significant time-dependent reduction in cell proliferation for SW13 cells at 24 and 72h. No appreciable change was perceived in H295R cells.

Conclusions: our preliminary results demonstrated AKA, AKB and AKC expression in ACT. AKA overexpression in ACC may suggest the potential anti-mitotic effect of AK inhibitor in adrenocortical cells. Nevertheless MK-0457 seems to act only in SW13 cells. Further analysis are needed to substantiate these data.

PP229

A SINGLE-CENTER, OPEN-LABEL, PHASE II, PROOF-OF-CONCEPT STUDY WITH PASIREOTIDE LAR IN PATIENTS WITH PROGRESSIVE MEDULLARY THYROID CANCER (MTC): CLINIC

M. Del Prete[1], P. Ferolla[2], G. Vitale[3], V. Ramundo[4], V. Marotta[4], F. Marciello[4], L. Pezzullo[5], M. G. Chiofalo[5], L. Camera[6], R. Fonti[6], B. Biondi[4], A. Colao[4], A. Faggiano[4]

[1]Department of Clinical Medicine and Surgery, Federico II University - Naples, [2]Department of Medical Oncology, Santa Maria della Misericordia Hospital, Azienda Ospedaliera di Perugia - Perugia, [3]Ospedale San Luca IRCCS, Istituto Auxologico Italiano, Milan; Department of Medical Sciences, University of Milan - Milan, [4]Department of Clinical Medicine and Surgery, Federico II University, - Naples, [5]Surgery, National Cancer Institute, "Fondazione G. Pascale" - Naples, [6]Department of Biomorphological and Functional Sciences, Federico II University - Naples

Introduction: in MTC patients (pts) somatostatin receptor (sst) expression is higher for sst1 and sst5 than for sst2. This may explain why the available sst2–selective analogues do not work in these pts and why pasireotide (SOM230), a novel, multi-receptor targeted somatostatin analogue with high-binding affinity for sst1,2,3 and sst5 could be effective.**Aim**:To evaluate clinical response and safety of SOM230 long-acting release (LAR) in MTC pts with progressive disease.**Patients-Methods**:At now, 11 (7 male and 4 female; mean age: 57, range 28-80 years old) consecutive pts with progressive metastatic or persistent postoperative MTC have been enrolled and received SOM230 LAR 60 mg/i.m.every 28 days.**Results**: At baseline, among 11 pts, 8 had diarrhea which was scored as grade 1 in 2 pts, grade 2 in 5 and grade 3 in one. One pt had back pain grade 1, one had constipation grade 2 and 2 pts hyperglycemia grade 1, which was treated with hypoglycemic diet. After treatment, a significant improvement of diarrhea was observed at 3 month evaluation: 7 pts had diarrhea of grade 1 and none had diarrhea grade 2-3. One pt had headache grade 1 and one hypertransaminasemia grade 1; 6 pts had hyperglycemia grade 2, which was treated with oral hypoglycemic drugs. Of the 9 pts undergone 6 month evaluation, only 4 had diarrhea grade 1, while hypertransaminasemia, headache and constipation were no longer observed. Hyperglicaemia was not further impaired and remained under pharmacological control in 3 and diet control in 3 others.**Conclusions**: SOM230 LAR is effective on clinical symptoms and well tolerated in MTC pts. After six months of treatment, there was improvement of diarrhea and mild worsening of glucose homeostasis which was controlled by low-carbohydrate diet and therapy with oral hypoglicemic drugs.

PP230

MECHANISMS OF RESISTANCE TO IGF-IR TYROSINE KINASE INHIBITION IN HUMAN THYROID ANAPLASTIC CANCER CELLS

R. MALAGUARNERA[1], A. SACCO[1], A. PALUMMO[1], A. MORCAVALLO[1], M. NICOLOSI[2], F. FRASCA[2], A. BELFIORE[1]

[1]Department of Health Science - Catanzaro, [2]Department of Clinical and Molecular Biomedicine - Catania

Introduction: Anaplastic thyroid cancer (ATC) is a lethal disease, characterized by almost complete refractoriness to currently available anti-cancer therapies. ATC cells overexpress the insulin receptor isoform A (IR-A), the IGF-I receptor (IGF-IR) as well as cognate ligands, IGF-II and IGF-I, suggesting an important role of overactivation of the IGF axis. These findings raise the possibility that ATC could be a candidate for IGF-IR targeted therapies. However, in vivo studies have shown that tumors may develop resistance to IGF-IR inhibition.

Aim: We aimed at evaluating molecular mechanisms of resistance to IGF-IR inhibition in human ATC cells.

Methods and Results: We first selected sensitive and resistant ATC cells by measuring cell proliferation/viability after exposure to NVP-AEW541, a specific IGF-IR TKI. Western blot analysis was used to study IGF-IR phosphorylation and the activation of receptor downstream signaling. We found that NVP-AEW541 abrogated IGF-IR phosphorylation in all cell lines studied. In sensitive cells, NVP-AEW541 inhibited AKT phosphorylation (pAKT) but marginally affected ERK phosphorylation status. Conversely, in resistant ATC cells, expressing high IR-A:IGF-IR ratio and high EGF receptor (EGFR) content, NVP-AEW541 was less effective in inhibiting pAKT and was able to stimulate both ERK and EGFR phosphorylation.

Conclusion: In human ATC cells, high IR-A and EGFR expression is associated to refractoriness to IGF-IR inhibition and to activation of the ERK pathway. Combination therapy with ERK and/or EGFR inhibitors, may overcome these mechanisms of drug resistance.

PP231

CERVICAL LYMPH NODE METASTASES FROM THYROID CANCER: USEFULNESS OF THYROGLOBULIN AND CALCITONIN MEASUREMENT IN FINE NEEDLE ASPIRATES

E. Baldini[1], S. Sorrenti[2], C. Di Gioia[3], C. De Vito[4], A. Antonelli[5], L. Gnessi[1], G. Carbotta[1], A. Nesca[1], S. Carocci[1], L. Paone[1], P. Miccoli[6], E. De Antoni[2], M. D'Armiento[1], S. Ulisse[1]

[1]Dipartimento di Medicina Sperimentale, "Sapienza" Università di Roma - Roma, [2]Dipartimento di Scienze Chirurgiche, "Sapienza" Università di Roma - Roma, [3]Dipartimento di Scienze Radiologiche, Oncologiche ed Anatomo-Patologiche, "Sapienza" Università di Roma - Roma, [4]Dipartimento di Sanità Pubblica e Malattie Infettive, "Sapienza" Università di Roma - Roma, [5]Dipartimento di Medicina Interna, Università di Pisa - Pisa, [6]Dipartimento di Chirurgia, Università di Pisa - Pisa

Thyroglobulin (Tg) protein measurement in the washout of the needle employed for fine needle aspiration biopsy cytology (FNAB-C) has been report to augment the diagnostic accuracy of FNAB-C in identifying cervical lymph node (CLN) metastasis from differentiated thyroid cancer (TC). We here evaluated the ability of routine measurement of Tg protein (FNAB-Tgp), Tg mRNA (FNAB-Tgm) and calcitonin (CT) mRNA (FNAB-CTm) in the FNAB washout of CLN to improve the accuracy of FNAB-C in the diagnosis of suspicious metastatic CLN. To this end, 35 CLN from 28 patients were evaluated. Histology demonstrated the presence of metastatic papillary TC (PTC) in 26 CLN, metastatic medullary TC (MTC) in 3 CLN, metastatic anaplastic TC (ATC) in 3 CLN and 3 metastatic CLN from extra-thyroidal cancers. The overall accuracy of FNAB-C range from 84.4% to 95.7% when the analysis was restricted to PTC. Both FNAB-Tgp and FNAB-Tgm compared favorably with FNAB-C and shown diagnostic performances not statistically different from that of FNAB-C. However, FNAB-Tgp and FNAB-Tgm/FNAB-CTm were found clinically valuable only in cases in which FNAB-C outcome were inadequate or provided diagnosis inconsistent with patient's clinical parameters.

In conclusion, FNAB-C, Tg/CT mRNA and Tg protein determination in the fine-needle washout showed similar accuracy in the diagnosis of metastatic CLN from TC. The obtained results suggest that samples for Tg protein and Tg/CT mRNA measurements from CLN suspicious for metastatic TC should be collected, but their measurements restricted to cases in which FNAB-C provides uninformative or inconsistent diagnosis with respect to patient's biochemical and clinical parameters.

PP232

CHARACTERIZATION OF THE MTOR PATHWAY IN HUMAN NORMAL ADRENAL AND ADRENOCORTICAL TUMORS

M. C. De Martino[1], R. A. Feelders[1], W. W. de Herder[1], P. van Koetsveld[1], J. A. Janssen[1], M. Waaijers[1], C. Pivonello[2], S. W. J. Lamberts[1], A. Colao[2], R. R. de Krijger[3], R. Pivonello[2], L. J. Hofland[1]

[1]Department of Internal Medicine, Erasmus MC - Rotterdam, [2]Deparment of Endocrinology & Oncology, "Federico II" University - Naples, [3]Department of Pathology, Erasmus MC - Rottedam

New treatment options are required for patients with adrenocortical carcinoma (ACC) and the mTOR-pathway have been recently suggested as a new potential target for therapy in ACC.

To describe the expression of the mTOR-pathway in normal adrenal (NA), hyperplasia (AH) and in adrenocortical tumors, we evaluated the mRNA expression levels of mTOR, S6K1 and 4EBP1 in 10 NA, 10 AH, 17 adrenal adenomas (ACA) and 17 ACCs by qPCR and the protein expression of total/phospho-mTOR; total/phospho-S6K and total/phospho-4EBP1 in 3 NAs, 3 AHs, 6 ACAs and 20 ACCs by immunohistochemistry (IHC).

In the NA and HA we observed a layer specific expression of total and phospho mTOR, S6K1 and 4EBP1. S6K1 mRNA levels were lower in ACCs compared with NA, HA and ACA ($p<0.01$). A subset of ACCs and ACAs presented a moderate-high staining of total-mTOR (60%; 100%); phospho-mTOR (10%; 33%); total-4EBP1 (75%; 100%); phospho-4EBP1 (60%; 100%); total-S6K1 (25%; 67%) and phospho-S6K1 (30%; 50%). The median IHC score of total-S6K1 in ACCs was significantly lower than ACAs ($p<0,01$). A moderate-high staining of phospho-S6K1 and/or phospho-4EBP1 was observed in 80% of ACCs and all ACAs. Tumors not having this moderate-high staining had significantly higher Weiss's score than others ($p<0,05$).

In conclusion this study suggests 1) a potential layer-specific role of the mTOR-pathway in normal adrenal; 2) the presence of an activated mTOR-pathway in a subset of adrenal tumors and a possible down-regulation of the mTOR-pathway in tumors with higher Weiss's score.

PP233

EFFECTS OF SELECTIVE AURORA-A AND AURORA-B INHIBITORS ON ANAPLASTIC THYROID CANCER DERIVED CELL LINES

E. Baldini[1], S. Sorrenti[2], A. Antonelli[3], S. Morrone[1], A. Catania[2], E. De Antoni[2], M. D'Armiento[1], S. Ulisse[1]

[1]Dipartimento di Medicina Sperimentale, "Sapienza" Università di Roma - Roma, [2]Dipartimento di Scienze Chirurgiche, "Sapienza" Università di Roma - Roma, [3]Dipartimento di Medicina Interna - Pisa

Anaplastic thyroid carcinomas (ATC) are highly aggressive tumors unresponsive to any available radio- or chemotherapeutic protocol, with a median survival rate of 4-5 months from the time of diagnosis. We previously demonstrated that ATC are characterized by increased expression of the kinases Aurora-A, -B and -C, involved in the regulation of multiple steps of the mitotic phase, and that ATC cells are sensitive to Aurora kinase pan-inhibitors. In this study, we evaluated the efficacy of small molecule inhibitors selective for Aurora-A (MLN8237/Alisertib) and Aurora-B (AZD1152-HQPA/Barasertib) in suppressing cell growth and inducing apoptosis of four different ATC derived cell lines: CAL-62, 8305C, 8505C and BHT-101. The cells were treated with each inhibitor or with the sole vehicle DMSO, and examined by means of cell proliferation assay, immunofluorescence, cytofluorimetry, time lapse microscopy, and soft-agar colony formation. Both inhibitors were able to impair proliferation in a time- and dose-dependent manner, with IC50 comprised between 50 nM and 100 nM. Time-lapse video-microscopy performed on CAL-62 cells showed that the administration of either Alisertib or Barasertib prevented the completion of mitosis with cytokinesis leading to polyploidy. Immunofluorescence experiments evidenced in all the cells typical mitotic features of Aurora-A and Aurora-B inhibition, that is lack of histone H3 phosphorylation on Ser10, multipolar spindles with shorter microtubules, misalignment of chromosomes at the metaphase plate, and aberrant post-mitotic nuclei. The final outcome of such endoreplication was the induction of apoptosis, as demonstrated by the augmentation of sub-G0 cell population after 24 h exposition to each inhibitor. Finally, the two molecules were also capable to block anchorage-independent cell growth.

In conclusion, our results demonstrated that cell proliferation and tumorigenicity of different ATC derived cell lines can be prevented by selective inhibitors of Aurora-A or -B. At present these inhibitors are both undergoing clinical trials for hematologic cancers, and they may represent a new therapeutic option for the ATC treatment.

PP234

CUTANEOUS MELANOMA AS AN ENDOCRINE TUMOR: EVALUATION OF SOMATOSTATIN AND DOPAMINE RECEPTOR EXPRESSION IN MELANOMA CELL LINES.

G. Cuomo[1], C. Pivonello[1], M. Sarnataro[1], M. Negri[1], F. Cariati[1], G. Coppola[1], A. Colao[1], R. Pivonello[1]

[1]Medicina Clinica e Chirurgia, Università "Federico II" - Napoli

Background: Cutaneous melanoma is a highly aggressive malignant skin cancer with heterogeneous aetiology arising from neoplastic transformation of melanocytes. The incidence of cutaneous metastatic melanoma continues to increase and treatment has long been a challenge. Evidence is accumulating that skin is as a peripheral endocrine organ and melanocytes an endocrine cell. As a consequence, an intriguing hypothesis has been postulated that consider melanoma as a peculiar endocrine tumor. The aim of this study was to characterize melanoma for somatostatin and dopamine receptors, generally expressed in endocrine tumors, and to evaluate the *in vitro* effect of somatostatin and dopamine analogues on cell viability in melanoma cell lines.

Methods: The dopamine receptor (D2) and somatostatin receptors (SSTR1,2,3,5) messenger expression was evaluated in two melanoma cell lines (A375, HMCB) by real time PCR whereas the protein expression of D2 and SSTR1 and SSTR2 were explored by immunocitochemistry (ICC). The effectiveness of D2 agonist (DA) cabergoline and the somatostatin analogs ocreotide and lanreotide on cell proliferation was evaluated by MTT.

Results: At real time PCR, D2, SSTR1, SSTR2 and SSTR5 messengers were found to be significantly expressed in both melanoma cell lines, being SSTR2 prevalent of the other receptors. D2, SSTR1 and SSTR2 expression was also confirmed at protein level, since all three receptors were localized both at membrane and cytoplasmic levels of the melanoma cells. Unfortunately, no significant inhibition of cell proliferation was observed after treatment with cabergoline and somatostatin analogues.

Conclusions: The presence of somatostatin and dopamine receptors confirmed a typical endocrine pattern of receptor expression in melanoma cell lines, although the preliminary results of this study suggest that these receptors seem not to mediate an inhibition of cell viability. Further experiments are needed to evaluate a possible role of somatostatinergic and dopaminergic system in melanoma.

PP235

THE SYNERGISTIC ACTION OF DOPAMINE AGONISTS AND SOMATOSTATIN ANALOGUES WITH MTOR INHIBITORS: IN VITRO EFFECTS IN A BRONCHIAL CARCINOID CELL LINE

M. Sarnataro[1], C. Pivonello[1], C. Sciammarella[1], F. Cariati[1], G. Cuomo[1], M. Negri[1], G. Coppola[1], A. Faggiano[1], A. Colao[1], R. Pivonello[1]

[1] Medicina Clinica e Chirurgia, Università "Federico II" - Napoli

Bronchial carcinoids, are rare tumors accounting for 25% of all neuroendocrine tumors (NET). The mammalian target of Rapamycin (mTOR) pathway plays a pivotal role in proliferative signaling in NET. mTORC1 inhibition leads to a feedback activation of Akt, which in turn attenuates the mTOR inhibitor's (mTORi) anticancer efficacy. Somatostatin analogs (SA) are currently used in medical therapy for NET since they inhibit the hormone secretion and cell proliferation. NET express dopamine D2 receptor, and D2 agonists are effective in regulating hormone secretion and cell proliferation. The aim of this study is to investigate the antiproliferative effects of the SA octreotide (OCT) and the dopamine agonist cabergoline (CAB) in a typical bronchial H727 cell line and to evaluate whether these drugs regulate the Akt pathway and strengthen the effects of the mTORi rapamycin (RAP) and everolimus (EVE).Transcript levels of somatostatin receptors (SSTRs), D2 and mTOR system (mTOR, 4eBP1 and p70S6K) have been assessed by RT-qPCR; localization of D2, SSTR2 and mTOR proteins has been evaluated by immunocytochemistry and immunofluorescence. Cell viability is examined by MTT assay. WB is performed to investigate Akt phosphorylation. Graded doses of mTORi, CAB and OCT are tested.SSTRs, D2 and mTOR pathway are expressed in the H727 cell line. After 3, 6, 9 days of treatment, mTORi induced a significant time- and dose-dependent inhibition of cell proliferation with IC50 of 10-9 M. CAB and OCT did not inhibit cell growth. The co-treatment at equimolar concentration (10-8M) of mTORi with OCT and CAB induced a significantly higher inhibition of cell proliferation after 6 days (~40%) and after 9 days (~50%) compared to the effect of mTORi as single agents (~30%). As expected, RAP and EVE induced an increase of pAkt phosporylation, however co-treatment with CAB and OCT decreased the activation of pAkt compared to mTORCi as single agents.Dopaminergic and somatostatinergic agents synergistically potentiate the inhibitory effects of mTORi in H727 cell line. A combined treatment with these drugs might represent a promising approach in the management of NET.

PP236

ER STRESS AND GRP78/BIP PLAY A ROLE IN ENDOMETRIAL CANCER

L. Ulianich[1], G. Calì[1], D. Conza[1], F. Zatterale[1], A. Leone[1], I. Prevenzano[2], B. Di Jeso[3], F. Fiory[1], C. Miele[1], P. Mirra[1], L. Parrillo[1], G. Bifulco[2], C. Nigro[1], F. Beguinot[4]

[1]IEOS-C.N.R. - Napoli, [2]Università "Federico II" - Napoli, [3]Università del Salento - Lecce, [4]Università "Federico II" e IEOS-C.N.R. - Napoli

Background. Recent studies have indicated that Endoplasmic Reticulum (ER) stress, the Unfolded Protein Response (UPR) activation and altered GRP78/BiP expression and function can play an important role in a variety of tumors development and progression. Furthermore, the ER chaperone GRP78/BiP has been detected also on the surface of different cancer cells, where it mediates oncogenic signals. However, informations are lacking about a possible role for ER stress and GRP78/BiP in endometrial cancer. **Methods**. We evaluated BiP/GRP78, ATF6 and GADD153/CHOP mRNA levels in tissue specimens of endometrioid endometrial adenocarcinomas by Real Time-PCR analysis. Protein expression levels and protein localization have been evaluated, in the same specimens, by Western Blot and immunohistochemical analysis, respectively. Proliferation and migration of the Ishikawa adenocarcinoma cell line have been evaluated by both proliferation curves analysis and wound healing experiments. GRP78/BiP membrane localization has been evaluated by both biotinilation/immunoprecipitation and immunofluorescence experiments. **Results**. GRP78/BiP, ATF6 and GADD153/CHOP levels are significantly increased in the pathological tissues analyzed. In Ishikawa cells, the inhibition of GRP78/Bip by the use of a specific short hairpin (sh)-RNA, markedly reduced both cell growth and migration. Ishikawa cells displayed a cell membrane localization of GRP78/BiP and the amount was increased by treating cell with tunicamycin, an ER stress inducer. **Conclusions**. These data suggest that activation of ER stress and GRP78/BiP expression and, possibly, its membrane localization might play a role in endometrial cancer development and progression.

PP237

CUSHING'S SYNDROME IN A PATIENT WITH LEYDIG CELL TUMOR: A CASE REPORT

F. Lolli[1], L. Rizza[1], M. R. Di Giorgio[1], C. Tosti-Croce[1], L. Gandini[1], A. Lenzi[1]

[1]Dipartimento di Medicina Sperimentale-Sezione di Fisiopatologia Medica ed Endocrinologia, Sapienza Università di Roma - Roma

A 36 year old man referred to an emergency room complaining abdominal and left testis pain in 2008. A scrotal-US showed an hypoechoic round mass of left testis of 14 mm in diameter. The tumour markers were negative, no hormonal exams were performed. The patient underwent left orchiectomy. The histological exam proved a Leydig cell tumour. After two months, the patient was admitted to another hospital for asthenia and dyspnoea. A total body TC was performed and a left adrenal mass of 30 x 28 x 22 mm was discovered. The patient was sent to our Unit for adrenal incidentaloma in 2009. The patient was sent to our Unit for adrenal incidentaloma in 2009. The patient presented an history of different episodes of renal stones from 1998 to 2000; no history of chronic stable hypertension. The patient referred increased irritability, interacting difficulty, mental fatigue, decreased mood and asthenia. Clinical examination showed abdominal fat and high blood pressure (BP: 140/105 mmHg). A complete blood count and tests of liver function, coagulation, and kidney function were normal, as were the serum levels of electrolytes included potassium. Hormonal results were: UFC: 155 mcg /24h (26.2-134.8), F: 932 nmol/l (am 66-720), A: 168 pg/mL (35-300), PRA: 2.86 ng/ml/h (1.5-5.7), T: 2.93 ng/ml (2.8-9.0), 17OHP: 4.6 ng/ml (0.61-3.34), DHEA-S: 70.5 µg/ml (120-360), A4: 1.36 ng/ml (0.61-3.71), LH: 15.9 mIU/ml (1.5-9.2), FSH: 22.8 mIU/ml (1.0-14), E2: 42.4 pg/ml (20-60), PRL: 22.2 ng/ml (2.0-14.0), ACTH: 17.3 pg/ml (10-60), PTH: 35 pg/ml (10.6-54), Metanephrine: 112 mcg/24h (20-345). After LDDS test the F level was 2,9 mcg/dl. MRI of adrenal gland confirmed a nodule in the left adrenal gland with an inhomogeneous signal intensity. The patient underwent left laparoscopic adrenalectomy for Cushing's Syndrome; pathological examination revealed a neoplasia constituted by cells with eosinophilic and vacuolated cytoplasm, round nucleus. On the 7th days post-surgery, after discontinuation of cortone acetate, F level was 190 nmol/l. After 3 months the higher 17OHP levels fell in the normal range. A scrotal-US showed no lesions in the right testis and the revision of testis histological exam confirmed a Leydig cell tumour. The 17OHP levels were probably connected to the presence of the adrenal adenoma. To our knowledge, this is the first case report of a Leydig cell tumour and Cushing's Syndrome. The adrenal rest should be considered in the differential diagnosis of Leydig cell tumour since the histological and echographic features are similar in both condition.

PP238

PROGNOSTIC PARAMETERS OF POOR OUTCOME IN A CONSECUTIVE SERIES OF 26 PATIENTS WITH ADRENAL CORTICAL CARCINOMA.

M. Russo[1], G. Pellegriti[1], P. Malandrino[1], R. Terranova[1], G. Giannone[2], R. Vigneri[1], C. Scollo[1]

[1]Endocrinology, Department of Clinical and Molecular Biomedicine, University of Catania, Garibaldi-Nesima Medical Center - Catania, [2]Department of Surgical Oncology, Garibaldi-Nesima Medical Center - Catania

Adrenal cortical carcinoma (ACC) is a rare tumor with an aggressive behavior. A combined treatment approach can ameliorate progression-free and overall survival which is also influenced by individual factors. **Objectives**: To define risk factors at presentation that may influence outcome in ACC patients. **Patients and Methods**: A consecutive series of 26 ACC patients (18F/8M, median age at diagnosis 53 yrs, range 19.9-78.9 yrs), came to our observation in the years 1997-2012. At presentation 12 patients (46%) had hypersecreting tumors (2 cortisol alone, 4 cortisol and androgens, 5 androgens alone and 1 aldosterone alone). At surgery radical resection was obtained in 20/26 patients. Median tumor size was 8.5 cm (range 3.5-25 cm) and tumor stage (according to WHO classification) was as follow: 13 low stage tumors (2 stage I, 11 stage II) and 13 high stage tumors (6 stage III, 7 stage IV). All patients at stage I, II, III and 1 patient with stage IV were cured after surgery (R0); in the remaining 6 patients with stage IV surgery was not curative. Mitotane as adjuvant treatment was administered to 12/20 patients R0; 6 patients with persistent disease were given mitotane and/or chemiotherapy (cysplatin/etoposide/doxorubicin). During follow up 8/20 R0 patients had disease recurrence, and 2 of them underwent repeated surgery. At last control, after a mean follow up of 40 months, 14 patients (2 stage I, 10 at stage II, 1 stage III, 1 stage IV) are free of disease, 1 patient (at stage III) is alive with progression and 11 patients (1 stage II, 4 stage III, 6 stage IV) died for disease. **Results**: At univariate analysis, factors associated with poor prognosis (disease progression/death) were: tumor size (p=0.03), stage III/IV (p=0.0002) and residual cancer after surgery (p=0.004). Sex, age at diagnosis and secreting tumor didn't predict outcome. Median progression free survival (PFS) was 25.7 months and median overall survival (OS) was 53.1 months. OS was significantly shorter in patients at stage III-IV versus stage I-II (mean OS 47.1 vs 92.1 months, p=0.008) and also in patients without radical resection (median OS 7.7 vs 82.7 months, p=<0.001). In the 20 R0 patients median disease-free survival (DFS) was 78.7 months. **Conclusions**: Our data indicate that in ACC patients, size, cancer stage at presentation and the consequent possibility of radical surgery are the major factors for predicting outcome and survival.

PP239

THYROID CANCER EPIDEMIOLOGY IN YOUNG PATIENTS: DATA FROM THE SICILIAN REGIONAL REGISTRY FOR THYROID CANCER (SRRTC)

M. Russo[1], C. Scollo[1], P. Malandrino[1], M. A. Violi[2], G. Dardanoni[3], A. Maniglia[4], P. Richiusa[5], R. Vigneri[1], G. Pellegriti[1]

[1]Endocrinology, Department of Clinical and Molecular Biomedicine, University of Catania, Garibaldi-Nesima Medical Center - Catania, [2]Endocrinology, University of Messina - Messina, [3]Sicilian Regional Epidemiology Observatory - Palermo, [4]Endocrinology, Cervello Hospital - Palermo, [5]Endocrinology, University of Palermo - Palermo

Background: Thyroid carcinoma (TC) is rare in children and adolescents. In 0-14 y children the incidence rate in females (F) is 0.3 and 0.2 per 100.000 inhabitants in USA and in Europe respectively, and in males (M) is 0.1 in both geographical areas. At 15-19 y, incidence rates are 0.4 in M and 2.6 in F in USA and 0.4 in M and 1.1 in F in Europe (SEER, ACCIS). The Sicilian Regional Register for Thyroid Cancer (SRRTC) indicates a higher incidence of TC in residents of Catania province (volcanic area of Mt.Etna). **Objectives:** To analyze all incident cases of TCs in patients aged 0-19 yrs living in Sicily in the period 2002-2006. **Methods:** Epidemiological, clinical, hystopathological and demographic characteristics were evaluated. The age specific incidence rate (ASR) was calculated and expressed as the incidence of TCs per 100.000 residents/year. **Results:** In 2002-2006, 34 incident TCs (26F/8M, F/M ratio 3.3) were diagnosed in Sicily among young population (0-19 years). ASR was 0.6 (0.9 F, 0.3 M). No TC occurred among children aged 0-4, 3 cases (all medullary histotype) in the age group of 5-9 y (ASR 0.2; 0.3 in F, 0.1 in M), 5 cases in the age group 10-14 y (ASR 0.3; 0.5 in F, 0.1 in M) and 26 cases among 15-19 y adolescents (ASR 1.6; 2.6 in F and 0.7 in M). The histotype was differentiated thyroid cancer (DTC) in 88.2% of cancers (30/34 cases: 24F/6M, F/M ratio 4/1, five cases in the 10-14 and 25 in the 15-19 age groups), 90% (27/30) of these were papillary. Mean DTC size was 1.6 cm (range 0.2-4.5 cm) and microcarcinomas accounted for 46.7%. Multifocality was observed in 30% of cases, extrathyroidal invasion in 26.7% and lymphnode metastasis in 23.3%. DTC incidence was higher in Catania than in the rest of Sicily (ASR 1.6 in F and 0.5 in M vs 0.6 in F and 0.1 in M). Incidence difference was more marked in the 15-19 y group: F=5.2 and M=1.1 in Catania vs F=1.8 and M=0.5 in the rest of Sicily. **Conclusions:** SRRTC data show that: 1. DTC is very rare before the age of 10 yrs; 2. Its incidence increases with age and is higher among F and in the age group of 15-19 yrs. 3. Also in childhood and adolescence papillary thyroid carcinoma is the most frequent histotype. 4. Residents in the Catania province volcanic area have a higher incidence of TC also at young age. Further studies are warranted to assess risk and prognostic factors for TC in young patients.

PP240

COULD THE LONG-ACTING SOMATOSTATIN ANALOGUES MODIFY THE THERAPEUTIC STRATEGY IN PATIENTS WITH EARLY STAGE MEN1-RELATED DUODENO-PANCREATIC NETS?

V. Ramundo[1], M. Del prete[1], V. Marotta[1], F. Marciello[1], L. Camera[2], V. Napolitano[3], L. De Luca[4], A. C. Carratù[1], R. Esposito[1], C. de Luca di Roseto[1], A. Colao[1], A. Faggiano[1]

[1]Clinical Medicine and Surgery, "Federico II" University of Naples - Naples, [2]Biomorphologic and Functional Sciences, "Federico II" University of Naples - Naples, [3]Internal Medicine, Second University of Naples - Naples, [4]Gastroenterology and Digestive Endoscopy, Pellegrini Hospital ASL NA1 - Naples

Background: Somatostatin analogues (SSA) represent one of the main therapeutic option in patients affected with functioning well-differentiated neuroendocrine tumors (NETs). There are no studies specifically focusing on NETs associated to Multiple Endocrine Neoplasia type 1 (MEN1).

Aim: To evaluate the efficacy of long-acting somatostatin analogues in MEN1 patients affected with duodeno-pancreatic NETs.

Patients & Methods: All first-degree relatives of MEN1 subjects, genetically diagnosed for MEN1 before the clinical diagnosis of NETs and with evidence of one or more duodeno-pancreatic NETs <15 mm in size were enrolled. Twenty-two patients with MEN1-related duodeno-pancreatic NETs (age range 21-42 yrs) were treated with octreotide LAR (30 mg / 28 days). Treatment duration ranged 1-7 yrs. At the radiological evaluation (performed by multidetector-row computed tomography and endoscopic ultrasound), multiple duodeno-pancreatic NETs (range 1-8), sized 3–14 mm, were detected.

Results: An objective tumor response was observed in 18%, stable disease in 78% and progression of disease in 4% of cases. In 5 patients with abnormally increased chromogranin-A and/or gastrin serum concentrations, a significant hormonal response occurred in 100% cases and was stable along the time.

Conclusions: Therapy with SSA is highly effective in patients with early stage MEN1 duodeno-pancreatic NETs, resulting in long-time suppression of tumor and hormonal activity and 18% objective response. This suggests a change in therapeutic strategy in patients with early stage MEN1-related NETs.

PP241

EVEROLIMUS TREATMENT IN A SERIES OF PATIENTS WITH ADVANCED NEUROENDOCRINE TUMORS

D. Iacovazzo[1], F. Plastino[2], R. Barile[2], G. Schinzari[2], A. Bianchi[1], G. Rindi[3], A. Pontecorvi[1], L. De Marinis[1], C. A. M. Barone[2]

[1]Endocrinologia - Università Cattolica del Sacro Cuore Policlinico "A. Gemelli" - Roma, [2]Oncologia Medica - Università Cattolica del Sacro Cuore Policlinico "A. Gemelli" - Roma, [3]Anatomia Patologica - Università Cattolica del Sacro Cuore Policlinico "A. Gemelli" - Roma

Introduction: everolimus is an oral mTOR inhibitor that exerts antineoplastic effects inhibiting cell proliferation, survival and angiogenesis. Its activity in advanced neuroendocrine tumors (NETs) has been demonstrated in controlled trials and everolimus was approved by the FDA for the treatment of progressive, advanced pNETs in May 2011.

Materials and methods: we treated with everolimus, at the dosage of 10 mg once daily, 14 patients with advanced, progressive, low or intermediate-grade NETs for a mean period of 11 months. Somatostatin analogues treatment was continued in all patients. 12/14 patients had previously undergone Peptide Receptor Radionuclide Therapy (PRRT).

Results: according to RECIST criteria, stable disease was observed in 9/14 patients and partial response was achieved in 2/14 patients. Median progression-free survival was 12.0 months. Drug-related adverse events included stomatitis (7/14), hyperglycaemia (7/14), hypertriglyceridemia (5/14), pneumonitis (4/14), hematologic toxicity (4/14), peripheral oedema (4/14) and rash (2/14). Grade 3 and 4 adverse events included pneumonitis (3 cases) and thrombocytopenia (2 cases). Dose reduction was required in 5/14 patients.

Conclusion: our data confirm the efficacy of everolimus in the treatment of progressive, advanced NETs. The apparently higher rate of grade 3 and 4 adverse events is probably related to the high proportion of patients in our series that had previously undergone PRRT, as it may enhance everolimus potential mielotoxicity.

PP242

PROGNOSTIC ROLE OF KI-67 LABELING INDEX IN NEUROENDOCRINE TUMORS OF HETEROGENEOUS ORIGIN: EXPERIENCE IN A SINGLE CENTER

A. Bianchi, D. Iacovazzo, F. Lugli, F. Plastino[2], G. Schinzari[2], G. Treglia[3], V. Rufini, F. Inzani[4], A. Larghi[5], M. G. Brizi[6], P. Granone[7], D. D'Ugo[8], G. B. Doglietto[9], C. A. M. Barone, G. Rindi[4], L. De Marinis[1], A. Pontecorvi[10]

[1]Endocrinologia - Roma, [2]Oncologia Medica - Roma, [3]Medicina Nucleare - Roma, [4]Anatomia Patologica - Roma, [5]Endoscopia Digestiva - Roma, [6]Diagnostica per Immagini - Roma, [7]Chirurgia Toracica - Roma, [8]Chirurgia Generale - Roma, [9]Chirurgia Digestiva - Roma, [10]Endocrinologia - Università Cattolica del Sacro Cuore Policlinico "A. Gemelli" - Roma

Many studies concerning prognostic factors in NETs have been done, but there are no data about patients with NETs of heterogeneous origin. The aim of our study was to evaluate the prognostic factors for NETs in a center with integrated multidisciplinary approach to these tumors.

We report the results of a retrospective and prospective study regarding 55 patients (27 M, 28 F, median age 60 years) affected by neuroendocrine tumor, that came to our observation consecutively between 2005 and 2009. Twenty-six were affected by GEP-NETs, 27 by lung NETs and 2 patients presented with metastases from occult primary tumor. Treatment options and response to treatment have been evaluated according to the new classification of NETs (WHO 2010), that provides a grading system based on Ki-67 labeling index and mytotic count. The study population, according to this classification, has been divided into three distinct categories: G1 (39 patients), G2 (14 patients) and G3 (2 patients). Our results showed that the tumor grading is the most significant predictor of outcome. The survival curve showed a clear distinction in terms of disease-free interval between the three classes of grading, although statistical significance was not reached between G2 and G3, probably because of the paucity of G3 cases. Our results confirm that the approach to these tumors should be multidisciplinary and show, for the first time in an heterogeneous series, that an accurate histopathological evaluation including tumor grading is crucial for a proper and effective therapeutic choice.

PP243

A PIVOTAL ROLE FOR THE CALCIUM-CALMODULIN DEPENDENT KINASE 2 (CAMKII) IN MEDULLARY THYROID CARCINOMA

E. Russo[1], M. Salzano[1], V. De Falco[1], M. Santoro[1], C. Mian[2], S. Barollo[2], M. Vitale[3]

[1]Endocrinology and Oncology Molecular and Clinical - Napoli, [2]Medicine-DIMED - Padova, [3]Medicine and Surgery - Salerno

Background: We recently demonstrated that the calcium/calmodulin dependent kinase 2 (CaMKII) is involved in the regulation of proliferation and survival of epithelial cells, were it phosphorylates RAF-1 and modulates MAPK pathway. A endogenous CaMKII inhibitor (hCaKIINalpha) is expressed in some cell types. It is down-expressed in colon and in ovarian cancer where it inversely correlates with the disease extension.

Aim of our study is to determine the possible role of CaMKII in medullary thyroid carcinoma (MTC), to determine whether hCaKIINalpha is expressed, and whether its level of expression correlates with the clinicopathological features of MTC.

Design: To this purpose, RET^{C634Y} e RET^{M918T}, two RET mutants most frequently found in MTC, were expressed in NIH-3T3 cells and the activation status of CaMKII was determined. Oncogenic RET expression in serum-starved NIH-3T3 mutants induced maximal CaMKII activation. In two MTC cell lines (TT and MZCRC-1 carrying the same RET mutants) CaMKII was activated. Pharmacological inhibition of RET abrogated CaMKII activation. Inhibition of CaMKII in these cells induced a reduction of Raf-1, MEK and ERK phosphorylation, cyclin D expression and cell proliferation. The expression of hCaKIINalpha RNA was determined by real-time PCR in 24 primary MTCs and was correlated with some clinicopathological parameters. Gender and age at diagnosis did not correlate with hCaKIINalpha RNA expression. Serum calcitonin, ($R^2 = 0.032$, $p = 0.017$ by Spearman rank correlation), tumor volume ($p = 0.0094$ by ANOVA), lymph node metastasis ($p = 0.0297$ by t-test) and staging ($p = 0.0043$ by ANOVA) were negatively correlated with the hCaMKIINalpha mRNA expression.

In conclusion, CaMKII is activated by RET mutants and is activated at baseline in MTC cell lines were it mediates the oncogenic pathway leading to cell proliferation. The mRNA expression of its endogenous inhibitor hCaKIINalpha inversely correlates with the severity of MTC. CaMKII might represent a new target for MTC therapy.

PP244

TRIIODOTHYRONINE (T3) AS A TUMOR SUPPRESSOR IN THYROID CARCINOMA CELL LINES

C. Verga Falzacappa[1], F. Alfei[1], A. Furno[2], M. G. Santaguida[1], C. Virili[1], S. Misiti[2], M. Centanni[1]

[1]Scienze e Biotecnologie Medico-Chirurgiche, "Sapienza" Università di Roma - Roma e Latina, [2]Medicina Sperimentale, "Sapienza" Università di Roma - Roma

Aberrant expression and/or mutations of thyroid hormone receptor (TR) genes seem to be involved in neoplastic transformation in different human cancers. Also, it appears that variation on T3 binding affinity of thyroid hormone receptor-β (TRβ) in mice leads to the development of follicular thyroid carcinoma. It has been also reported that even alterations of thyroid function might lead to cancer development, independently from thyroid receptors status and expression. These observations suggest that thyroid hormone/thyroid receptors interaction may influence the tumor progression and differentiation in thyroid carcinoma. This study assessed the ability of T_3 to affect some steps of neoplastic transformation in a papillary (PTC) and in an anaplastic (FRO) thyroid carcinoma cell line. In these cells the whole thyroid hormone machinery is expressed and, in particular, thyroid hormone receptors. As a first step, the responsiveness of cells to different T_3 amount has been tested and the 100 nM concentration was chosen because it has been proven to be the most effective. The clonogenicity assay revealed that T_3 inhibited the ability of forming new clones of 30% in FRO and of 20% in PTC cells. In addition, by cell growth analysis and cell viability MTT assays, we demonstrated that T_3 also inhibited cell proliferation in both systems, by depleting proliferating cells up to 25-30%. Then the major features of the tumor progression and invasiveness have been analyzed. In particular, as demonstrated by matrigel assay, we observed that the exposure to triiodothyronine treatment deeply reduced the invasiveness in both the cell lines (50% in FRO and 65% in PTC cells). Finally, the activation and expression levels of the main molecules underlying tumorigenic properties (PTEN, p53, MDM2, CXCR4) and cell proliferation or survival regulation (ccnD1, ccnA1, ccnE, cdk6, cdk4, Bax) have been analyzed by RT-PCR. In conclusion, these preliminary data indicates that triiodothyronine, influenced growth and progression of thyroid cancer cells with high thyroid receptor expression, thus highlighting the role of T_3/TRβ interaction in thyroid carcinoma development.

PP245

RELATIONSHIP BETWEEN BMI, PSA AND HISTOPATHOLOGICAL TUMOR GRADE IN A CAUCASIAN POPULATION AFFECTED BY PROSTATE CANCER

F. Turchi[1], R. Manco[2], M. Boscaro[3], R. L. Tenaglia[2], G. Balercia[3], A. Gioia[3]

[1]S.I.T. Diabetologia-Endocrinologia Asur Marche Area Vasta 1 - Urbino, [2]Scienze Umane e dell'invecchiamento, Clinica Urologica - Chieti, [3]Dipartimento di Medicina Interna e Biotecnologie Applicate, Clinica Endocrinologica - Ancona

ABSTRACT

Prostate cancer (CaP) is the most commonly diagnosed malignancy in men from industrialized countries. Lifestyle factors, for example physical activity and eating habits, such a higher intake of dietary fat, play a significant role in the pathogenesis of CaP. The aim of our study is to show the relationship between obesity and aggressivenes of CaP. We conducted a retrospective study of 132 men affected by CaP underwent radical prostatectomy. Gleason Score was abstracted by biopsy specimens and by post-operatory specimens. We evaluated Prostate Specific Antigen (PSA) levels and Body Mass Index (BMI). PSA levels were 8.5-7.9-6.0 ng / ml in normal, overweight and obese respectively ($p < 0.001$). In normal weight the Postoperative Gleason Score (POGS) in the higher tertile of PSA was lower than the POGS in the lower tertile of PSA ($p < 0.05$); in obese subjects we did not find differences in POGS in the function of the tertiles of PSA. The prevalence of POGS$\geq$ 8 among subjects with lowest tertile of PSA was higher in obese (94.4%) vs owerweight subjects (19.2%) ($p < 0.01$); the prevalence of POGS$\geq$8 among subjects with second tertile of PSA was higher in obese (100%) and overweight (70%) vs normal weight subjects (0%) ($p<0.01$ and $p<0.001$ respectively); the prevalence of POGS$\geq$8 among subjects with third tertile of PSA was higher in obese (100%) and overweight (62%) vs normal weight subjects (0%) ($p<0.05$ respectively). We believe that changes in the levels of PSA and Gleason Score, observed as a function of class BMI, are due to separate mechanisms: PSA levels could be influenced by the effect of dilution by increased plasma volume of the subjects obese, while tumor grade could be negatively affected by the hormonal changes induced by adipose tissue.

PP246

THYROGLOBULIN RESPONSE AS PREDICTIVE FACTOR OF RESPONSE TO SORAFENIB IN PATIENTS WITH IODINE-REFRACTORY DIFFERENTIATED THYROID CANCER

V. Marotta[1], M. Del Prete[1], F. Marciello[1], V. Ramundo[1], R. Esposito[1], A. C. Carratù[1], C. De Luca di Roseto[1], R. Fonti[2], L. Camera[2], M. Salvatore[2], A. Colao[1], A. Faggiano[3]

[1]Clinical Medicine and Surgery, Federico II University - Naples, [2]Biomorphological and Functional Sciences, Federico II University - Naples, [3]Endocrinology, National Cancer Institute, "Fondazione G. Pascale" - Naples

Objectives: The tyrosine-kinase inhibitor sorafenib has been demonstrated to be effective in a relevant percentage of patients with progressive iodine-refractory differentiated thyroid cancer (DTC). Aim of the study was to assess if there is a correlation between thyroglobulin (Tg) response and radiological response to sorafenib in these patients. Methods: A retrospective analysis of 15 patients affected with progressive iodine-refractory DTC subjected to off-label treatment with sorafenib was performed. Computed tomography (CT) scans were performed at baseline and every 12 weeks to assess objective response. Radiological response was evaluated by means of RECIST criteria version 1.1. Laboratory assessments including Tg and anti-thyroglobulin antibodies (Ab-Tg) were performed at baseline and every 4 weeks. One-way ANOVA was used to compare changes of Tg levels between responding and non-responding subjects.

Results: Two patients were excluded from our analysis because of Ab-Tg positivity, which prejudices feasibility of Tg assay. Ten patients achieved control of disease progression (7 stable disease and 3 partial response) while 3 subjects showed persisting progressive disease. In all cases, biochemical partial response was achieved with a mean decrease of 75% and a median time of nadir of 2.6 months. The decrease in serum Tg levels was significantly greater in patients who achieved clinical benefit compared with non-responding subjects ($p < 0.01$). Conclusions: Consistency of Tg-response could play a role in decision making and clinical management of patients with aggressive iodine refractory DTC treated with sorafenib. Nevertheless larger trials are needed to set a feasible cut-off in order to perform an early identification of non-responding subjects.

PP247

CORRELATION BETWEEN ULTRASOUND PARAMETERS OF MALIGNANCY AND GALECTIN-3-BASED THYROTEST RESULTS IN CYTOLOGICALLY INDETERMINATE THYROID NODULES

G. Fioroni[1], A. Bartolazzi[2], C. Bellotti[3], G. De Francesco[4], T. Porcelli[1], S. Amendola[1], S. Sciacchitano[1]

[1]Dipartimento Medicina Clinica e Molecolare - Roma, [2]U.O.C. di Istologia ed Anatomia Patologica, Az Osp. Sant'Andrea - Roma, [3]Dipartimento di Scienze Medico Chirurgiche e Medicina Traslazionale - Roma, [4]Az. Ospedaliera Sant'Andrea - Roma

Preoperative identification of malignancy can be extremely challenging in nodules that are classified as indeterminate (Thy3 according to BTA). Any clinical, ultrasound, cytological and molecular parameters useful to guide the decision whether or not to suggest surgery is relevant. This study was undertaken to correlate ultrasound parameters and Galectin-3-based ThyroTest results in a population of thyroid nodule classified as indeterminate at conventional Us-guided FNA cytology. A total of 140 thyroid nodules, classified as Thy3 were studied. Ultrasound parameters were collected and compared to the results of the Galectin-3 ThyroTest, performed by the same pathologist (AB) as previously described (Appl Immunohistochem Mol Morphol. 2012 Jan;20(1):2-7). A positive result at Galectin-3 ThyroTest was given in 16 cases and all of them were confirmed as malignant at final histology. A negative result was rendered in 124 nodules.Thyroid surgery was performed in 84 of them, while the remaining 40 patients are currently under strict clinical and ultrasound follow-up. We found a significant difference between Galectin-3 positive vs negative nodules in the following ultrasound parameters: shape taller than wide (18.7 % vs 0%), margin speculated (68.7% vs 25.8%), marked hypoechogenicity (62.5% vs 20.9%), and microcalcifications (68.7% vs 40.3%). On the other hand, the following ultrasound parameters were more frequently detected in Galectin-3 negative nodules vs positive ones: shape ovoidal to round (60.5% vs 18.7%), isoechogenicity (41.9% vs 12.5%), hyperechogenicity (3.2% vs 0%), echotexture homogeneus (16.9% vs 0%), vascularity peripherical (43.3% vs 25%), and rim calcification (7.2% vs 0%). Therefore, the following ultrasound parameters: taller than wide shape, spiculated margin, marked hypoechogenicity, microcalcifications are associated with a positive Galectin-3 ThyroTest and indicate malignancy, while the following ultrasound features: ovoid to round shape, iso and hyperechogenicity, homogeneous echotexture, peripheral vascularity and rim calcifications are associated with a negative Galectin-3 ThyroTest and indicate benignity. In conclusion, the integration of a careful ultrasound evaluation of specific parameters, a Us-guided FNA cytology report and a Galectin-3 ThyroTest result represent the best strategy to preoperatively identify malignancy in suspicious thyroid nodules.

PP248

PREDICTIVE VALUE OF GENE INSULINE GROWTH FACTOR II (IGF-II) EXPRESSION IN BREAST CANCER

V. Belardi[1], I. Muller[1], E. Fiore[1], D. Campani[2], P. Vitti[1], C. Giani[1]

[1]Endocrinologia e Malattie Metaboliche Università di Pisa - Pisa, [2]Oncologia Università di Pisa - Pisa

OBJECTIVES: IGF-II is an important regulator of neoplastic growth and it is stromal in origin. The aim of the study was to evaluate the impact of IGF-II mRNA expression in clinical outcome of breast cancer (BC).

METHODS: the expression of IGF-II mRNA was compared to several prognostic parameters such as node metastases, oestrogen (ER) and progesterone (PR) receptors, ki-67 protein expression (ki-67) and p53 oncogene protein expression (p53). IGF-II mRNA was examined using in situ hybridization method.

RESULTS: The study group included 68 women (mean age $\pm$ SD = 52.2 $\pm$ 15.7 yrs) submitted to radical mastectomy for ductal infiltrating BC. 37 out of this 68 patients (54.4%) had axillary node metastases (N+), 33 (48.5%) were ER+, 30 (44.1%) PR+, 11 (16.2%) p53+ and 12 (17.6%) Ki-67+. Positive IGF-II mRNA expression (IGF-II +) was detected in 33/68 (51.6%) BC. After a follow-up of 5 years 50/68 BC patients (73.5%) were alive and relapse free. Survival rate was significantly lower in N+ than in N- (20/37, 54% vs 30/31, 96.7%; $p<0.0001$) and in p53+ than in p53- (5/11, 45.4% vs 44/56, 78.6%; $p=0.002$). No relation was found between ER and PR status and survival.

Survival was not significantly different between IGF-II+ and IGF-II- patients (23/33, 69.6% vs 24/31, 77.4%; p=NS). IGF-II mRNA expression did not affect the prognosis in N- and in p53- BC patients, while the majority of p53+/IGF-II+ patients (5/8, 62.5%) died within two year from diagnosis. Ki-67 did not affect survival, but a significant poorer prognosis was observed in IGF-II+/ki-67+ than in IGF II+/Ki-67– BC patients (7/33, 21.2% vs 26/33, 78.8%; $p=0.04$).

CONCLUSIONS: Our data indicate that in BC: 1) node metastases and p53 expression are independent poor predictive factors; 2) ER, PR and ki-67 expression have no impact on survival; 3) IGF-II mRNA expression per se is not connected to five years survival but in association with p53 or ki-67 may contribute to select a group of patients with particular poor clinical outcome.

PP249

SERUM ANTI-THYROID ANTIBODIES (TAB): A NEW PREDICTIVE PARAMETER IN BREAST CANCER (BC)

E. Fiore[1], I. Muller[1], V. Belardi[1], E. Giustarini[1], P. Vitti[1], C. Giani[1]

[1]Endocrinologia e Malattie Metaboliche, Università di Pisa - Pisa

OBJECTIVES: Prognostic value of TAb in high aggressive BC evaluating patients survival.
METHODS: The study group included 47 women (mean age: 53.1±10.1 yrs, mean±SD) submitted to radical mastectomy for high malignancy ductal infiltrating BC with axillary node involvement. All patients were evaluated for thyroid disorders after breast surgery and before any adjuvant therapy. After surgery all patients were submitted to the same protocol of adjuvant chemo-hormonal therapy. Estrogen Receptor (ER) was measured in 43/47 (91.5%) BC specimens.
RESULTS: 31/47 (65.9%) patients were alive (survivors group: SG) and 16/47 (34.1%) were dead (deaths group: DG), five years after BC diagnosis. The overall prevalence of TAb was 15/47 (31.9%): 14/31 (45.1%) in SG and 1/16 (6.2%) in DG (p=0.008). Five years mortality was 15/32 (46.9%) in TAb- and 1/15 (6.7%) in TAb+ patients (p=0.01). 8/47 (17.0%) patients had Hashimoto's thyroiditis and 7 of them (87.5%) were in SG. ER was detected in 19/30 (63.0%) patients in SG and 3/13 (23.1%) in DG (p= 0.01). Five year mortality was 10/21 (47.6%) in ER- and 3/22 (13.6%) in ER+ patients (p=0.008). According to Proportional Hazard Regression Model, the presence of more than 3 axillary lymph node metastasis [odds ratio (OR) 8.53; p=0.006], the absence of ER expression (OR 7.72; p=0.004) and the absence of TAb (OR 18.40; p=0.01) were related to a higher mortality rate.
The worst prognosis was observed in AbT-/ER- patients [5 years mortality rate: 9/13 (69.2%)] and all the 7 patients TAb+/ER+ were alive after 5 years.
TAb were detected in 8/21 (38.1%) ER- and in 7/22 (31.8%) ER+ patients; no relation was found between ER expression and TAb positivity (p=NS).
CONCLUSIONS: Patients with RE+/TAb+ have a better prognosis. The absence of a significant relationship between these two parameters suggests an independent prognostic role of TAb in high malignancy degree BC.

PP250

GROWTH HORMONE PROTECTS ENDOMETRIAL CANCER CELLS FROM CHEMOTHERAPY BY HAMPERING CYTOTOXIC-INDUCED APOPTOSIS IN ESTROGEN RECEPTOR NEGATIVE ENDOMETRIAL

M. Minoia[1], E. Gentilin[1], S. Fochi[1], F. Tagliati[1], M. R. Ambrosio[1], E. degli Uberti[1], M. C. Zatelli[1]

[1]Dept. di Scienze Mediche-Sezione di Endocrinologia-Università degli Studi di Ferrara - Ferrara

Context: (GH) and insulin-like growth factor (IGF-1) are known to promote carcinogenesis and to play important roles in endometrial cancer (EA) development. We previously demonstrated that GH and IGF-I protect ER positive and ER negative breast cancer-derived MCF7 and MDA-MB231 cell lines towards the cytotoxic effects of doxorubicin (D), independently of IGF-I.
Aim of the study: In order to evaluate whether GH/IGF-1 excess might influence EA response to therapy, accounting for the increased mortality correlated with EA, we evaluated the effects of GH on cell proliferation of the ER negative ANC3A cell line and in the ER positive HEC-1A endometrial cancer cell lines, in the presence of D, frequently used in EA chemotherapy.
Results. We found that in serum-free conditions GH induces growth and protects from the cytotoxic effects of D the ANC3A cells, but not HEC-1A cells. Moreover GH significantly reduces basal and D-induced apoptosis in ANC3A cells, an effect blocked by the GH receptor (GHR) antagonist Pegvisomant (Peg). In addition, we observed that in serum condition GH induces HEC-1A cell growth, but does not protect them from the cytotoxic effects of D.
Conclusions: Our preliminary data show that GH induces chemoresistance in a ER negative EA cell line, the ANC-3A cells, an effect completely counteracted by a GHR antagonist. These data suggest that GHR blockade may effectively overcome EA chemoresistance, also in the settings of highly aggressive forms, such as those lacking ER, delineating a possible path of clinical relevance to overcome and/or prevent chemoresistance in ER negative EA.

PP251

METASTATIC THYROID CANCER (TC) UNRESPONSIVE TO CONVENTIONAL THERAPY AND OTHER THYROSINE KINASES INHIBITORS (TKI) TREATED WITH SUNITINIB "OFF-LABEL".

V. Cappagli[1], V. Bottici[1], E. Molinaro[1], L. Agate[1], D. Viola[1], L. Valerio[1], S. Mazzeo[2], V. Battaglia[2], B. Pontillo Contillo[2], L. Dal Canto[3], A. Ribechini[4], P. Vitti[1], R. Elisei[1]

[1]Endocrinologia - Pisa, [2]Radiodiagnostica 1 - Pisa, [3]Farmacia - Pisa, [4]Cardiotoracico - Pisa

Objective: we evaluated the clinical benefits of sunitinib in patients (pts) with metastatic TC, refractory to other conventional therapies and to other TKIs, not eligible for clinical trials and with a progressive disease.
Patients: we enrolled 4 pts (3 females,1 male) with the above mentioned features:1 papillary, 2 follicular and 1 medullary. Pts mean age was 55 years. They were followed from 1 to 18 months after starting the treatment. To evaluate the response to sunitinib we performed a CT scan, a biochemical evaluation (serum thyroglobulin [Tg] or calcitonin [Ct]) and a performance status (PS) evaluation after 1 and every 3 months.
Results: according to RECIST criteria, after one month, 3 pts showed an evident stabilization of the disease (SD) and 1 pt showed a partial response (PR). In all cases a clinical improvement of PS was referred. A stabilization or a decrease of Tg or Ct was also found. During the follow-up, 2/3 SD pts and the pt with PR showed a durable disease stabilization for 4, 9 and 17 months after the first control. Despite the initial good response, the other SD pt required the therapy discontinuation due to severe adverse events (i.e. pulmonary abscess). In all pts, after the starting dose of 50 mg orally/die for 4 weeks followed by 2 weeks off, we adopted an alternative schedule (1 week "off" and 1 week "on") to better control the side effects. With this schedule, 3/4 pts could continue the treatment until now.
Conclusions: we observed an overall clinical benefit in 4/4 pts (100%) which was durable in 3/4 pts for a relatively long term period. The side effects could be managed with a new schedule of administration of the drug. It is desirable that sunitinib could become a "first choice" drug in advanced and progressive TC.

PP252

THERAPEUTIC EFFICACY OF LENVATINIB (E7080) ON BONE METASTASES FROM THYROID CANCER REFRACTORY TO 131-I (RAI)

L. Pieruzzi[1], E. Molinaro[1], A. Biagini[1], C. Giani[1], S. Mazzeo[2], B. Pontillo Contillo[2], M. G. Delle Donne[3], L. Conte[3], P. Vitti[1], R. Elisei[1]

[1]Endocrinologia - Pisa, [2]Radiodiagnostica 1 - Pisa, [3]Cardiotoracico - Pisa

Introduction: Tyrosine Kinase Inhibitors (TKI) are the gold standard for patients with RAI thyroid cancer. International guidelines recommend that these patients are enrolled in experimental protocols with TKI. However bone metastatic disease is still a challenge and the efficacy of TKI in the control of skeletal lesions is debated.
Objectives: to check the therapeutic efficacy of Lenvatinib (E7080) on the metastatic bone localizations of RAI thyroid cancer.
Methods: patients were enrolled at the O.U. of Endocrinology of Pisa (February-April 2012) in the phase 3, multicenter, randomized, placebo-controlled study protocol on the use of E7080 for the treatment of RAI thyroid cancer. Five/17 (29%) patients enrolled in the study had bone metastases at baseline (3 poorly differentiated thyroid cancer, 2 follicular thyroid cancer). They were 2 females and 3 males with age ranging from 49 to 63 years. Patients were regularly followed with radiological controls every 2 months for a median of follow-up of 5.9 months.
Results: In 3/5 (60%) cases we observed a dimensional stability of bone localizations and in two of them stability was accompanied by clear radiological signs of devascularization. Two/5 (40%) cases showed a response in terms of devascularization of the lesions associated with a dimensional reduction of 18% and 30% respectively.
Conclusions: on the basis of this experience, even if the casistisc is limited, it appears that E7080 is effective on bone metastatic lesions.

PP253

SSTR DOMINANT NEGATIVE TRUNCATED VARIANT SST5TMD4 INFLUENCES THE EFFECTS OF SOM-230 ON PROSTATE CELLS IN VITRO

V. Rossi[1], D. Visconti[2], C. De Rosa[3], C. Abbondanza[3], I. Cioffi[2], A. De Masi[2], A. A. Sinisi[2]

[1]Ypsilon Biotechnology s.n.c. - Napoli, [2]Endocrinologia, Dpt Scienze Cardiotoraciche e Polmonari, II Univ Napoli - Napoli, [3]Dpt Patologia Generale, II Univ Napoli - Napoli

Background: truncated variant of somatostatin receptor 5 (sst5TMD4), demonstrated in breast tumors, seems act as dominant negative of SSTR5 inducing the resistance to SST analogue (SSTA) treatment. Aim of present study was to evaluate the presence and role of sst5TMD4 in epithelial prostate cell (EPC) lines. Material and methods: we assayed the expression of ssts and sst5TMD4 transcripts in EPN, derived from a normal surrounding area of a prostate tumor, and CPEC, derived from the core of a prostate cancer tissue. In these cells, we evaluated the effects of SOM230 (Novartis, Basilea, SW), a SSTA pan ligand with strong affinity for SSTR2 and 5. Cell cultures starved in red phenol-free DMEM and 1% charcoal treated FBS for 5d, were treated either with 10-6 SOM230 or 10-8 SOM230. After 24/48h cells were harvested for RT-PCR and for SDS-PAGE/Western blot, or labeled with presidium iodide for cell cycle analysis by flow cytometer. Results: SSTR2 and 5 were equally expressed in both cell lines. SSTR1 and 3, and sst5TMD4 mRNA levels were higher in CEPC than in EPN ($p<0.001$). In EPN SOM 10-6 induced a significant caspase-dependent apoptosis, a reduction of S-phase proliferation together with an increase of bcl2 and a decrease of c-myc expression. In CPEC cells SOM230 treatment resulted in a modest apoptosis induction and a slight inhibition of cell growth, without changes of bcl-2 and c-myc levels. Conclusions: sst5TMD4 variant is differently expressed in the EPC lines studied here. SOM230 is effective in the control of cell growth in EPN cultures, while the reduced apoptotic response and the lack of growth arrest observed in CEPC could be due to presence of high levels of sst5TMD4 interfering with SST signaling.

PP254

THE RS10741657 POLYMORPHISM IN THE CYP2R1 GENE IS ASSOCIATED WITH DIFFERENCES IN VITAMIN D LEVELS AND BONE LOSS IN PATIENTS TAKING AROMATASE INHIBITOR

N. Napoli[1], P. Pozzilli[1], R. Armamento-Villareal[2]

[1]Area di Endocrinologia, Campus Bio-Medico - Roma, [2]University of New Mexico - Albuquerque, USA

Purpose: CYP2R1 converts vitamin D3 to 25-hydroxyvitamin D3 (25OHD). The rs10741657 polymorhpism (A/G at 5'UTR) in this gene is associated with differences in the risk for type 1 diabetes mellitus and other autoimmune diseases; hypothesized to be mediated by varying levels of circulating 25OHD. In this study, we evaluated the effect of rs10741657 polymorphism on bone loss among women taking aromatase inhibitors (AIs) for estrogen receptor positive (ER+) breast cancer. We hypothesize that the rs10741657 polymorphism will result in varying rates of bone loss in women on AIs for breast cancer.

Materials and Methods: Subjects were 99 postmenopausal women age 37-86 years of age participating in a study on bone loss in women with breast cancer on AIs. Bone mineral density (BMD) was measured by DXA at months 0, 6, and 12. 25OHD levels were measured by radioimmunoassay. Genotyping was performed by restriction fragment length polymorphism using Mnl 1.

Results: Women with the AA genotype had significantly lower BMI and higher baseline 25OHD levels than carriers of the G allele. Age-adjusted baseline BMD was significantly lower at both the lumbar spine (LS) and femoral neck (FN) in women with the AA genotype. However, differences in BMD among the genotypes disappeared after adjusting for BMI. More importantly, women with the AA genotype had significantly higher bone loss in the LS after one year of AI therapy relative to those carriers of the G allele.

Conclusion: The rs10741657 polymorphism of the CYP2R1 gene is associated with differences in circulating 25OHD levels, baseline BMD and bone loss in patients on aromatase inhibitors. Age and the rs10741657 polymorphism are important predictors of LS bone loss in these patients. Given the limited sample size, these findings need to be validated in a larger study.

PP255

PROKINETICIN1 IMMUNODETECTION IN TESTICULAR GERM CELL TUMOURS

V. Gigantino[1], R. Franco[3], D. Visconti[1], I. Cioffi[1], V. Rossi[1], P. Chieffi[4], A. A. Sinisi[1]

[1]Endocrinologia, Dpt Scienze Cardiotoraciche e Metaboliche, Seconda Università di Napoli - Napoli, Italy, [2]Endocrinologia, Dpt Scienze Cardiotoraciche e Metaboliche, Seconda Università di Napoli - Napoli,Italy, [3]Dpt Patologia, INT Fondazione G. Pascale - Napoli, Italy, [4]Dpt Psicologia, Seconda Università di Napoli - Napoli, Italy

Prokineticins 1 (Prok1) is a chemokine-like peptide which plays a significant role in tumour development and progression in several tissues, acting through two G-protein-coupled receptors, ProkR1 and ProkR2. There are few data on the involvement of Prok1 in testicular cancer. Aim of this study was to evaluate the expression of Prok1 and ProkR1 in a series of testicular germ cell tumours (GCT). Methods: we evaluated by immunohistochemistry the expression of Prok1 and ProkR1 in 2 Tissue Micro Array (TMA), containing a series of 140 GCT (90 seminoma, S, and 50 mixed non-seminomas, NS) and 30 normal tissues. We selected all representative areas of each component of NS (embryonic carcinoma, EC, yolk sac tumour, YS, chorioncarcinoma, CH, teratoma, T, and carcinoma in situ, CIS). Results and conclusions: Prok1 absent in normal germ cells resulted frequently expressed in GCT (78% S, 58% NS, 83% CIS), with higher level in S than in other types ($p<0.005$). ProkR1 was poorly expressed in GCT, except for EC (50% were positive). No correlation was observed between ligand and its receptor presence. The expression of Prok1-ProkR1 system in GCT suggests a potential role as autocrine pathway in the development and progression of a subset of these tumours.

PP256

ADRENOCORTICAL CANCER AND SECONDARY TUMORS

S. Zovato[1], M. V. Cicala[2], S. Faoro[3], M. Iacobone[4], R. Bertorelle[5], H. Koussis[6], B. Rubin[2], R. Pezzani[2], G. Opocher[1], F. Mantero[2]

[1]Oncoendocrinology-FCC-Veneto Institute og Oncology - Padova, [2]Endocrinology-Dep. Of Medicine –DIMED-University of Padova - Padova, [3]Pharmacy- Veneto Institute of Oncology - Padova, [4]Dep. Of Surgery, Oncology and Gastroenterology-University of Padova - Padova, [5]Molecular Immunology and Diagnostic Oncology-Veneto Institute of Oncology - Padova, [6]Medical Oncology-Veneto Institute of Oncology - Padova

OBJECTIVE Adrenocortical carcinomas (ACC) are rare malignant tumours (incidence1:1000000) whose cause is unknown. Associations with other diseases (i.e. Li-Fraumeni syndrome) have been described as well as the association of other tumours in this disease (Venkatesh 1989, prevalence 11.8%; and Diodolkar 1981, prevalence 22.4%). We will explain our local experience.

DESIGN AND METHODS In the 26 patients with a diagnosis of ACC followed between 2010 and 2012 in Padua, data on family history, the presence of other cancers, the time of their appearance and the treatment performed was recorded.

RESULTS Among the 26 patients, 8 had experienced at least one other tumor. No patient had a family history of other cancers. 4 women had developed breast cancer and were treated with different adjuvant therapies: radiotherapy, hormone therapy and chemotherapy. One patient had a differentiated thyroid tumor and underwent radio-metabolic treatment. Two patients had developed prostate cancer and were treated with hormone therapy. One patient developed colon cancer before and lung cancer later (both treated with chemotherapy). The latency time between onset of the first tumour and adrenal carcinoma ranged from 4 to 16 years (mean 6.4 years). Four out of 8 patients with double neoplasms, so far have undergone genetic investigation for p53 gene mutation. All 4 patients resulted wild type. In a subset of 9000 oncologic patients, with a 3 year follow-up, ACC was discovered in 3 patients (annual incidence 1:3000). CONCLUSIONS The presence of two tumours in the same individual is a rare event and the onset of ACC is also quite rare. In our series, the percentage of patients with double cancer was 30.8%. It is important to underline that, in all these patients, ACC arose as a second tumour (incidence 1:3000). We believe that the coincidence of ACC associated with other tumours is not a random event and requires a strong epidemiological approach in a larger group of patients. It could be important to determine both the impact from oncologic therapies performed for the first tumour and the possibility that this combination hides a germinal mutation (TP53 gene, MEN1 or PRKAR1A)

PP257

NEUROFIBROMATOSIS TYPE 1 AND VITAMIN D DEFICIENCY: ROLE IN TUMOUR PROGRESSION

R. Esposito[1], V. Ramundo[1], V. Marotta[1], M. Del Prete[1], F. Marciello[1], A. C. Carratù[1], C. De Luca di Roseto[1], M. Rubino[1], L. Vuolo[1], C. Di Somma[1], A. Colao[1], A. Faggiano[2]

[1]Department of Clinical Medicine and Surgery, Federico II University of Naples - Italy, [2]Endocrinology, National Cancer Institute, "Fondazione G. Pascale", Naples - Italy

Introduction: Neurofibromatosis type 1 (NF1) is an autosomal dominant inherited disease affecting 1/3500individuals, characterized by multi-organ involvement and cell proliferation as consequence of tumor suppressor gene inactivation. NF1-related neural and neuroendocrine tumorsare common and includecutaneous or subcutaneous neurofibromas, optic glyoma,pheochromocytoma,duodenal neuroendocrine tumors.Vitamin D deficiency seems to be frequent in NF1.

Objective: To evaluate the role of vitamin D deficiency in progression and severity of NF1-related tumours.

Patients and Methods:twenty-eightconsecutive NF1 patientswere enrolled (11 M and 17 F), aged 30 - 80 yrs. In all subjects were evaluated serum levels of 25(OH)-vitamin D(25-OHD), PTH, Ca and P serum and urinary. In all subjects a clinical and biochemical evaluation was performed.A condition of vitamin D deficiency was considered for serum concentrations of 25-OHD<10 ng/ml, while a condition of 25-OHD insufficiency for values between 10-30 ng/ml. The statistical analysis was performed using linear regression and the student's t-test for paired data, with significance for values of $p < 0.05$.

Results: Mean serum concentrations of 25-OHD was18.9?11.2 ng/mL.Among the 28 NF1 pts, 23 had a state of hypovitaminosis-D. This condition was inversely correlated with the number of cutaneous neurofibromas ($p < 0.001$). In particular, 10 pts had vitamin D deficiency (mean: 8.9?1.6ng/mL) and 13 vitamin D insufficiency(mean: 19.5?5.4ng/mL). All the 10 pts with vitamin D deficiency had ≥ 2 cutaneous neurofibromas. Among the13 pts with vitamin D deficiency, 1 had ≥ 2 neurofibromas, 10 had <2 neurofibromas, while 2 pts had no neurofibroma. No neurofibroma occurred in the 5pts but one with normal value of 25-OHD.

Conclusions: Cutaneous neurofibromas progress more frequently in NF1pts with low levels of 25-OHD. The pathogenetic mechanism by which vitamin D deficiency may stimulate the growth of cutaneous neurofibromas is not yet known and requires dedicated studies. Vitamin D status should be evaluated in all patients with NF1. Replacement therapy with vitamin D in pts with deficiency or insufficiency could stop the growth of neurofibromas.

PP258

TORC1/TORC2 INHIBITOR, PALOMID 529, ENHANCHES RADIATION RESPONSE DAYING DNA DAMAGE REPAIR IN ANDROGEN DEPENDENT AND IDEPENDET PROSTATE CANCER MODELS

G. L. Gravina[1], C. Festuccia[1], F. Marampon[1], D. Sherris[2], P. Muzi[3], L. Biordi[1], E. Carosa[1], S. Di Sante[1], E. Ricevuto[4], V. Tombolini[5], A. Lenzi[6], E. A. Jannini[1]

[1]Dipartimento di Scienze cliniche applicate e biotecnologiche - L'Aquila, [2]Paloma Pharmaceuticals, Inc., Jamaica Plain - Massachusetts, [3]Dipartimento di Medicina clinica, sanità pubblica, scienze della vita e dell'ambiente - L'Aquila, [4]Dipartimento di Scienze cliniche applicate e biotecnologiche, Divisione di Oncologia medica - L'Aquila, [5]Divisione di radioterapia, Ospedale Policlinico Umberto I - Roma, [6]Dipartimento di medicina sperimentale - Roma

Purpose: P529, a Torc1/Torc2 inhibitor, has demonstrated its potential as a radiosensitizer. However the molecular mechanism underlying this phenomenon still need to be elucidated Aim of this study is to elucidate mechanisms of radiosensitizing associated with this therapy in prostate cancer models,

Experimental Design: Human in vitro and in vivo models were used to verify this hypothesis.

Results: P529 treatment induced significantly more apoptosis and DNAdouble-strand breaks (DSB) at 24 hours especially when combined with radiotherapy resulting in cellular radiosensitization and growth delay of irradiated tumor xenografts. Upon P529 treatment Rad51 and DNA-PKcs protein expression was downregulated, indicating delayed DNA double-strand damage repair. The radiosensitizing properties of P529 was partially linked to GSK-3? activity modulation with associated inhibition of CRM1-mediated nuclear export of survivin. These phenomena contributed to the induction of caspase-3 dependent apoptotic machinery determining enhancement of radiation-reponse interms of anti-proliferative/anti-survival effects. Importantly, radiation enhancement by P529 coincided with a prominent accelerated senescence phenotype characterized by positive ?-galactosidase staining.

Conclusions: Taken together, increased DNA DSBs, impaired DNA damage repair, inhibition of CRM1-mediated nuclear export of survivin associated with enhanceed of pro-apoptotic events may explain the radiosensitizing properties of P529 in preclinical models of prostate cancer.

PP259

A NEW RET GENE MUTATION IN A PATIENT WITH APPARENTLY SPORADIC PHEOCHROMOCYTOMA AND NO EVIDENCE OF MEDULLARY THYROID CARCINOMA (MTC)

C. Scollo[1], L. Di Gregorio[1], R. Terranova[1], M. Russo[1], M. Tavarelli[1], G. Pellegriti[1], S. Squatrito[1]

[1]Endocrinology, Department of Clinical and Molecular Biomedicine, University of Catania, Garibaldi-Nesima Medical Center - Catania

Introduction: Germline mutations in RET, SDHB, SDHD, and VHL genes have been reported in 28% of patients with pheochromocytoma (Pheo) or paraganglioma (PGL) but also in 13.6 % patients with apparently sporadic disease. Young age at diagnosis, multiple tumors, personal and familiary history should be suspicious of genetic disease. In MEN 2A syndrome, the pheochormocytoma is present in 30-50% of cases, usually either concomitant or subsequent to MTC although being also the first tumor of the syndrome in 13–27% cases. Most frequent RET mutation in MEN 2A are located in exon 11 (C634). Less common are mutations of the cysteine residues at codons 609, 611, 618, and 620 and only rarely at codon 632. **Objective**: This is a case report of an apparently sporadic pheochromocytoma due to a new RET mutation at codon 632. **Case report**: A Caucasian 44 year-old male previously submitted to surgical excision of a skin melanoma was referred to us because of abdominal pain, sweating, chills and hypertension. Abdomen ultrasound examination revealed a 3 cm right adrenal mass, heterogeneous at magnetic resonance with a bright signal on T2-weighted images. Urinary norepinephrine was elevated (100 mcg/24h, n.v. <85). Pre-operatively, calcitonin, PTH , Ca++ blood values and neck ultrasound were normal. After laparoscopic adrenalectomy, histophatological examination confirmed an intra-adrenal pheochromocytoma (3.7 cm). RET gene sequencing revealed a new germline RET mutation in exon 11 c.1895>G (p. Glu 632 Gly). No RET gene mutation was found in his alive first-degree relatives (two brothers and two sons). After 22 months follow-up, the patient has no sign of persistent disease. Since the genotype-phenotype correlation of this new mutation is unknown and therefore the optimal timing for prophylactic surgery for MTC is not defined, our patient is strictly monitored with periodic CT measurement and neck ultrasound. **Conclusion:** This case report suggests systematic genetic screening for identifing a genetic disease in young patients with clinical diagnosis of apparently sporadic Pheo or PGL. In our case, the finding of this new RET mutation allowed the diagnosis of MEN 2 with clinical Pheo as first manifestation of the syndrome. A strict follow-up, for early detection of MTC or hyper-PTH is mandatory in these patients.

PP260

TYPE I GASTRIC CARCINOID IN A YOUNG WOMAN WITH POLYGLANDULAR AUTOIMMUNE SYNDROME TYPE III.

C. Castoro[1], M. L. Arpi[1], L. Sciacca[1], M. Tavarelli[1], G. Pellegriti[1], S. Squatrito[1]

[1] Endocrinology, Department of Clinical and Molecular Biomedicine, Garibaldi Nesima Hospital - Catania

Context: Type III polyglandular autoimmune syndrome (PAS) includes a group of autoimmune disorders: thyroid diseases (Hashimoto thyroiditis and Graves disease) (100%), diabetes mellitus (8%), atrophic gastritis (AG) (15%), pernicious anemia (2%), vitiligo (7%) and alopecia (2%). Young patients affected with autoimmune thyroid disorders have a prevalence of 14-21% of parietal cell antibodies (PCA), the atrophic gastritis markers. Chronic hypergastrinemia, subsequent to hypo/achlorhydria, leads to the dysplasia of the enterochromaffin-like cells (ECL-cells) increasing the risk of type I gastric carcinoid tumors development with a prevalence of 4-10%.

History and findings: A 39 year old female, with hypothyroidism subsequent to Hashimoto thyroiditis and type I diabetes (diagnosed respectively when she was 18 and 19 year old), was found to have autoimmune atrophic gastritis, associated with typical endoscopic features, and a vitamin B12 deficiency pernicious anemia. She often complained dyspeptic symptoms. One year later gastroscopy revealed a 4 mm in size polypoid carcinoid and blood test revealed hypergastrinemia (2793 pg/ml nv13-115) and high chromogranin A level (36 UI/L nv 2-18). Due to the further increase in size (from 4 to 6 mm) associated to erosion of the surface an endoscopic removal was performed. Hystology showed a gastric carcinoid type I, without lamina propria and vascular invasion, low mitotic index and ki67 (<2%).

Conclusions: This case is paradigmatic as shows that in type III PAS: 1) there is a progressive development of autoimmune associated disorders; 2) patients have a high risk of atrophic gastritis and associated hypergastrinemia; 3) hypergastrinemia induces type I gastric carcinoid tumors often clinically silents. A periodic screening is mandatory to early detect both associated autoimmune diseases and pre-cancerous gastric lesions in atrophic gastritis patients.

PP261

DIFFERENTIATED THYROID CARCINOMA IN ACROMEGALY: MOLECULAR AND CLINICAL ASPECTS

F. Ceccato[1], S. Watutantrige Fernando[1], A. Daniele[1], S. Mazzocut[1], S. Barollo[1], G. Occhi[1], G. Pennelli[2], C. Betterle[1], M. Jaffrain-Rea[3], F. Grimaldi[4], F. Vescini[4], D. Nacamulli[1], F. Mantero[1], C. Scaroni[1], C. Mian[1]

[1]Endocrinology Unit, Dept of Medicine DIMED - Padua, [2]Surgical Pathology and Cytopathology Unit, Dept of Medicine DIMED - Padua, [3]Department of Experimental MedicIstituto Neuromed, IRCCS - Pozzilli, [4]Endocrinology and Metabolism Unit, University-Hospital - Udine

Background: Neoplastic risk in acromegaly is due to well-known effect of GH/IGF-1 or to newly genetic/epigenetic factors. Aryl-Hydrocarbon Receptor (AHR) and AHR Interacting Protein (AIP) mutations are related to GH-secreting pituitary adenoma; whereas the mutation V600E in the proto-oncogene BRAF is the most found in Papillary Thyroid Carcinoma (PTC). Differentiated Thyroid Carcinoma (DTC) prevalence is 7% in acromegaly, and PTC is the most one found. **Aim:** to study the prevalence of goiter and DTC in acromegaly and the role of AIP, AHR and BRAF mutations. **Material and Methods:** BRAF evaluation have been performed with sequencing; immunohistochemistry analyses for AIP and AHR expression have been performed from paraffinated sections. **Results:** we analyzed 113 patients (63 female): prevalence of goiter and DTC was 73 % and 11% (12 cases of DTC, 10 PTC and 2 follicular carcinoma). There were no differences in GH/IGF-1 levels and follow-up from acromegaly diagnosis between acromegalic patients with or without DTC, whereas patients with DTC were older (52 vs 44 years). Among DTC, we found higher prevalence of female (11 out of 12) and an association between goiter and DTC (12 out of 12), adrenal incidentaloma and DTC (4 out of 12 in acromegalic with DTC) and familiar neoplastic history. All patients are in remission from DTC, with mean a follow up from 7 to 396 months. Among PTC, we obtained cancer specimens in 7 cases: we found BRAF V600E mutation in 5 (71%). AIP protein expression was similar between neoplastic and normal cells, whereas in PTC patients with BRAF mutations we found higher AHR citoplasmatic and nuclear expression than in normal tissue. **Conclusions**: DTC prevalence in acromegaly is elevated: physicians should pay attention in female with goiter or with adrenal incidentaloma. GH, IGF-1 or insulin levels do not correlate with DTC, but we found that BRAF mutations and AHR expression could be associated: probably genetic or epigenetic mutation are stronger than hormones in promoting thyroid carcinoma.

PP262

SUCCINATE DEHYDROGENASE SUBUNIT B MUTATIONS MODIFY HUMAN NEUROBLASTOMA CELL METABOLISM AND PROLIFERATION

R. Fucci[1], E. Rapizzi[1], T. Ercolino[2], B. Zampetti[1], R. Felici[3], D. Guasti[4], V. Giachè[1], D. Bani[4], A. Chiarugi[3], M. Mannelli[1]

[1]Scienze Biomediche Sperimentali e Cliniche, Università di Firenze - Firenze, [2]Azienda Ospedaliera Universitaria Careggi, Firenze - Firenze, [3]Scienze della Salute, Università di Firenze - Firenze, [4]Medicina Sperimentale e Clinica, Università di Firenze - Firenze

Paragangliomas (PGLs) are rare neuroendocrine tumors. About 30-40% of these tumors are mutated in different susceptibility genes, including those encoding the different subunits of the succinate dehydrogenase, a complex involved both in the tricarboxylic acid cycle and in the oxygen transport chain. The aim of this project was to investigate whether *SDHB* mutations may account for alterations in cell metabolism and functions. Since PGL cell lines are not available, we used the neuroblastoma cell line (SK-N-AS) stably transfected with the wild-type human *SDHB*, or different *SDHB* mutated constructs carrying the most significant mutations found in our patients affected by PGLs.

Interestingly, we found that all the *SDHB* mutated cell clones showed a specific reduction of the SDH enzyme activity, ranging from 27% to 47%, compared to controls. They also showed reduced oxygen consumption (from 2,5±1 nmolO_2/0,3ml to 5±2 nmolO_2/0,3ml, compared to 11,5±2nmolO_2/0,3ml of controls), in agreement with the finding of the decreased SDH activity. Surprisingly and unexpectedly, in all the *SDHB* mutated clones we found a significant decrease in glucose uptake (ranging from 13% to 21%) and in lactate concentration in the culture medium (16% to 30%), while ATP production, was significantly higher (51% to 115% compared to controls), thus suggesting a shift in the utilization of the lactate metabolite towards glucose for energy production. Finally, we found that these metabolic changes are associated to increased potential in cell proliferation and migration (which increase of 40% to 60% in all the mutated clones compared to controls) . Overall, these data demonstrate that *SDHB* mutations deeply affect cellular metabolism and functions.

PP263

HIGH EXPRESSION OF MAMMALIAN TARGET OF RAPAMYCIN (MTOR) IN NF-1 RELATED PERIAMPULLARY NEUROENDOCRINE TUMOURS: A PILOT CASE OF EVEROLIMUS TREATMENT IN

P. Razzore[1], J. Ramunni, M. Volante[2], E. Gamarra[1], N. Russolillo[3], S. Redana[4], T. Gallo[5], M. Motta[6], B. Torchio[7], L. Capussotti[3], M. Papotti[8], P. Limone[1]

[1]Div. Endocrinologia, AO Mauriziano - Turin , [2]Div. of Pathology, Dept. of Clinical and Biological Sciences, University of Turin at San Luigi Hospital - Orbassano, [3]Div. of Hepato-Biliary-Pancreatic surgery, AO Mauriziano - Turin, [4]Div. of Oncology, AO Mauriziano - Turin, [5]Div. of Radiology, AO Mauriziano - Turin, [6]Div. of Pathology, AO Mauriziano - Turin , [7]Div. of Pathology, AO Ordine Mauriziano - Turin , [8]Div. of Pathology, University of Turin at San Luigi Hospital - Orbassano

Neurofibromatosis type 1 (NF1) is a rare genetic disorder characterized by the presence of distinctive cutaneous lesions and neoplasms of the nervous and gastro-entero-pancreatic systems, including periampullary neuroendocrine tumors. Neurofibromin, the NF-1 coded protein is a tumor suppressor that negatively regulates the mammalian target of rapamycin (mTOR). Preclinical in vitro and in vivo studies demonstrated a constitutive activation of the mTOR pathway associated with significant antineoplastic effects of mTOR inhibitors in NF1-related glial tumor models. However, the activation status of the mTOR pathway and response to its specific inhibition in NF1-related neuroendocrine tumors haven't been described in literature, yet. Here we report on three NF1 patients (all males, aged 47, 54 and 59 yrs respectively) affected by non functioning periampullary neuroendocrine tumors. Tumors features/behavior were the following: case 1 – resectable tumor,G1,N0; case 2– resectable tumor, G2, N1; case 3–unresectable tumor, G2, with extensive abdominal lymph node and liver involvement. All cases showed high expression of p-mTOR (H-scores ranging from 70 to 180) and p70S6K proteins. Patient #3 was started on everolimus (10mg/day) and octreotide LAR (20 mg/month). At 3 and 6 months of treatment the patient had an ECOG steady performance status of 1, a weight gain of 3 Kg and no significant adverse effects; significant reduction in the number of cutaneous neurofibromas was clearly noted. Abdominal CT scan after 3 months of treatment showed a reduction in size of the metastatic lesions with no variation in the size of the ampullary tumor. Our data suggest that NF1-related neuroendocrine tumors are potential candidates for mTOR inhibition therapy due to increased mTOR pathway activation as shown by good clinical response in a pilot patient with a clinically aggressive tumor.

PP264

GLUCAGON-LIKE-PEPTIDE-1 RECEPTOR AGONISTS DON'T STIMULATE THE IN VITRO PROLIFERATION OF MEDULLARY THYROID CARCINOMA CELLS

F. F. Geraci[1], F. Giani[1], M. L. Nicolosi[1], S. Squatrito[1], F. Frasca[1], V. Vella[1]

[1]Department of Clinical and Molecular Biomedicine, University of Catania - Catania

Context: treatment with GLP-1 analogs is associated to an increased levels of plasma calcitonin, incidence of C-cells hyperplasia and medullary thyroid cancer (MTC) in rodents.

Objective: the aim of this study was to evaluate the in vitro effects of GLP-1 and Liraglutide on both human (TT and MZCRC-1) and rat (clone 6-23) medullary thyroid cancer cell lines.

Experimental design: cell lines were implanted in 96-well multiwell and treated with GLP-1 and liraglutide at increasing dosages (from 10^-11 to 10^-7 M), in order to evaluate a possible action on cell proliferation. As a positive control, for the proliferative stimulus, were used FBS (fetal bovine serum) (0.1%-10%) and 17-beta-estradiol (10^-9 - 10^-5 M). After 72 hours of stimulation cell lines were subjected to cell viability MTT assay (thiazolyl Blue Tetrazolium Bromide) and the results expressed as percent on basal (untreated). Each condition was repeated in four wells.

Results: MTT assays showed that, while FBS and 17-beta-estradiol stimulated cell growth by 19,85% ± 1.45% and 25,42% ± 5,67% (SD 2,91 and 5,9) respectively when compared to baseline (untreated). In contrast GLP-1 and liraglutide did not display any significant proliferative effect. Indeed the percentage values deviate from baseline by 3,25% ± 3,65% and 1,75 ± 3,95% (SD 4,93 and 5,39) respectively, showing a growth trend essentially flat.

Conclusion: liraglutide and GLP-1 have no effect on the proliferation of C-cell line MTC TT, MZCRC1 and clone 6-23. The model for in vitro study suggests that GLP-1 and its analogs do not stimulate cancer progression in medullary thyroid carcinoma cells. Further in vitro studies and clinical trials with diabetic patients treated with GLP-1 analogs should be performed in order to exclude an action of initiation or promotion of GLP-1 receptor agonist on the carcinogenesis of thyroid C cells.

PP265

IDENTIFICATION OF AN SDHA MUTATION IN A PATIENT WITH NECK PARAGANGLIOMA

F. Schiavi[1], E. Taschin[1], U. Verga[2], M. Filopanti[2], A. Bozzani[3], V. Arici[3], M. Piazza[4], A. Olivotto[5], P. Beck-Peccoz[2], E. Seregni[6], A. Fassina[5], F. Grego[4], A. Odero[3], G. Opocher[1]

[1]Familial Cancer Clinic and Oncoendocrinology - Veneto Institute of Oncology - Padova, [2]Endocrinology, Ospedale Maggiore - Milano, [3]Vascular Surgery, Policlinico San Matteo - Pavia, [4]Vascular Surgery - University of Padova - Padova, [5]Surgical Pathology and Cytopathology - University of Padova - Padova, [6]Nuclear Medicine - Istituto Nazionale dei Tumori - Milano

SDHA gene is located on chromosome 5p15 and encodes the flavoprotein subunit of succinate dehydrogenase. In 2010, the first heterozygous germ-line mutation associated with a catecholamine-secreting extra-adrenal PGL was identified, and showed that tumor associated with SDHA mutation displays negative staining for SDHA as well as SDHB. More recently, other two different SDHA mutations in five non-related paraganglioma patients were identified. It has been demonstrated that SDHA is a tumor suppressor gene associated to Paraganglioma syndrome type 5.

Case report. In 2009, a 28-years old man presented with an evident mass on the neck; there was no known history of familial syndromes associated to head and neck paraganglioma. The patient underwent computed tomography examination which demonstrated a 2,5 cm right carotid body solid mass. In 2009 a non secreting paraganglioma (2,5x1,2x1,1) was removed. There was evidence of malignancy in the lymphonodes. In 2010 and 2011 the patient underwent additional surgeries for local recurrences. In order to detect alterations related to a heritable disorder, complete genetic testing for the known genetic loci associated with paraganglioma, SDHB, SDHC, SDHD and SDHAF2 genes, has been performed. No pathogenic sequence variant has been identified. Recently, immunohistochemistry was carried out for SDHB and SDHA and both resulted negative for protein expression. Sequence analysis of SDHA identified a pathogenic mutation affecting the splice acceptor site of exon 14.

In case of a patient without familial or clinical indications for a particular type of PGL syndrome, it's possible to combine molecular analysis strategy with SDHA-SDHB staining on paraffin-embedded tumors to identify patients carrying germ-line SDHA mutations. This case report describes the seventh patient affected by PGL5, the fourth SDHA mutation and the first case with evidence of malignancy associated to PGL5.

PP266

IDENTIFICATION OF CHROMOSOME 11 PARENTAL ORIGIN IN A POPULATION WITH ENDEMIC PGL1 SYNDROME

F. Schiavi[1], S. Bobisse[1], B. Macino[1], E. Taschin[1], E. Casagrande[1], D. Di Sarra[2], S. Demattè[2], G. Opocher[1]

[1]Familial Cancer Clinic and Oncoendocrinology - Veneto Institute of Oncology - Padova, [2]Internal Medicine - Santa Chiara General Hospital - Trento

Paraganglioma syndrome type 1 (PGL1) is a rare autosomal dominant disease with maternal imprinting characterized by the development of head-and-neck paragangliomas and pheochromocytoma, associated with germ-line mutations of SDHD gene.

We identified a PGL1 founder effect caused by the SDHD c.341A>G p.Tyr114Cys mutation and we have so far collected more than 100 families resident or native from a restricted area around Trento mountain in North Italy. The genetic evaluation of 4025 resident volunteers allowed to identify 59 carriers of the founder mutation, resulting in a prevalence of 1.5% among the general population.

The identification of a large numbers of carriers with an unknown inheritance highlights the lack of a tool to discriminate the parental origin of the mutated chromosome.

To this aim we isolated the chromosome 11 using the conversion technology and analyzed the methylation pattern of 11p15.5 region.

Hybrids were generated by PEG-mediated fusion of lymphoblastoid cell lines with mouse RAG cell line and were cultured in a selective medium. After hybrid clones were grown, we had determined by genotyping which were haploid for chromosome 11. Each of these clones were characterized for the presence of the founder mutation with an allelic discrimination taqman assay and for the methylation pattern of the 11p15.5 region with the MLPA kit ME030-B2.

We have obtained hybrid clones using lymphoblastoid cell lines from two carriers with known inheritance, and the analysis of the methylation pattern with the MLPA kit ME030-B2 confirmed the parental origin of the chromosome 11.

Using this validated method, we therefore investigated a patient with an unknown inheritance and the analysis of the methylation pattern revealed the maternal origin of the mutation.

Preliminary results indicate that this approach may be useful to demonstrate parental origin of the SDHD mutation, allowing paraganglioma risk estimation in individuals with unknown inheritance.

PP267

A MUTATION IN A REGULATORY ELEMENT OF THE CDKN1B GENE ALTERS MRNA LOAD ONTO POLYSOMES AND CAUSE MEN4 PHENOTYPE

G. Occhi[1], D. Regazzo[1], D. Ciato[1], F. Boaretto[3], S. Bobisse[3], N. Pellegata[4], V. Sidarovich[5], A. Quattrone[5], G. Opocher[3], F. Mantero[1], C. Scaroni[1]

[1]Dipartimento di Medicina - Università di Padova - Dipartimento di Medicina - Università di Padova, [2]Dipartimento di Medicina - Università di Padova - Padova, [3]Unità per i Tumori Ereditari e Endocrinologia Oncologica - IOV - Padova, [4]Istituto di Patologia, Helmholtz Zentrum German Research Center for Environmental Health - Monaco, [5]Laboratorio di Genomica Traduzionale Centro Interdipartimentale di Biologia Integrata Università di Trento - Trento

The *CDKN1B* gene encodes the cyclin-dependent kinase inhibitor $p27^{KIP1}$, an atypical tumor suppressor playing a key role in cell cycle regulation, cell proliferation and differentiation. Impaired $p27^{KIP1}$ expression and/or localization are often observed in tumor cells, further confirming its central role in regulating the cell cycle. Recently, germline mutations in *CDKN1B* have been associated with the inherited multiple endocrine neoplasia syndrome type 4, an autosomal dominant syndrome characterized by varying combinations of tumors affecting at least two endocrine organs. In this study we identified a 4-bp deletion in a highly conserved regulatory upstream ORF (uORF) in the 5'UTR of the *CDKN1B* gene in a patient with a pituitary adenoma and a well-differentiated pancreatic neoplasm. This deletion causes the shift of the uORF termination codon with the consequent lengthening of the uORF-encoded peptide and the drastic shortening of the intercistronic space. Our data on the immunohistochemical analysis of the patient's pancreatic lesion, functional studies based on dual-luciferase assays, site-directed mutagenesis and on polysome profiling show a negative influence of this deletion on the translation reinitiation at the *CDKN1B* starting site, with a consequent reduction in $p27^{KIP1}$ expression.

Our findings demonstrate that, in addition to the previously described mechanisms leading to reduced $p27^{KIP1}$ activity, such as degradation via the ubiquitin/proteasome pathway or non-covalent sequestration, $p27^{KIP1}$ activity can also be modulated by an uORF and mutations affecting uORF could change $p27^{KIP1}$ expression. This study adds the *CDKN1B* gene to the short list of genes for which mutations that either create, delete or severely modify their regulatory uORFs have been associated with human diseases.

PP268

TWO CASES OF HYPERKERATOSIS IN PAPILARY THYROID CANCER (PTC) PATIENTS WITH BRAF MUTATION DURING VEMURAFENIB (PLX4032) TREATMENT

C. Giani[1], E. Molinaro[1], A. Biagini[1], L. Pieruzzi[1], E. Manni[2], A. Marsili[3], S. Mazzeo[4], P. Vitti[1], R. Elisei[1]

[1]Department of Endocrinology and Metabolism - Pisa, [2]Department of Dermatology - Pisa, [3]Department of Rheumatology - Pisa, [4]Department of Radiology - Pisa

Introduction: Vemurafenib is a strong tyrosine-kinase inhibitor mainly directed against BRAF. It is used in unresectable skin melanoma at the third and forth stage, with BRAF-V600E mutation. At the present an open-label, multi-center phase II study with Vemurafenib on patients with metastatic or unresectable PTC positive for the BRAFV600E mutation and resistant to radioactive iodine is ongoing. The skin toxicity is the most important drug-related toxicity that occurs with a progression from hyperkeratosis to keratoacantoma to an end up into a squamous cell carcinoma. These lesions usually develop after 4-17 weeks of treatment with Vemurafenib(1). For this reason the protocol study includes a dermatological examination at the screening time, at the third week, sixteenth week and later every three months. **Results:** we screened 8 PTC-patients but we randomized only 3/8 (37.5%) because of the severity of the inclusion and exclusion criteria. One/3 patients interrupted the drug in the second week for a serious adverse event (acute pancreatitis), so he had not sufficient time to develop skin lesions. Two/2 patients, who are still taking the drug, presented photosensitivity and a lot of hyperkeratotic skin lesions. In particular, the first patient showed a hyperkeratotic skin lesion of the back during the 4th week of Vemurafenib treatment. The second patient showed hyperkeratotic skin lesions of pre-sternal region and dorsal nose region during the 3th week of Vemurafenib treatment. Both patients were submitted to excision of all skin lesions. The final histological examination deposed for viral hyperkeratotic wart in both patients. At the present, they are under the drug for 44 weeks and 28 weeks respectively. They have been developed new skin lesions that are under evaluation but none of them seem to be true squamous cell carcinoma. **Conclusions**: both patients, who are presenting an excellent response to Vemurafenib treatment for the metastatic lesions, should be carefully monitored with dermatological evaluation for the high predisposition to skin tossicity. **1.** Mattei PL, Alora-Palli MB, Kraft S, Lawrence DP, Flaherty KT, Kimball AB 2012 Cutaneous effects of BRAF inhibitor therapy: a case series. Ann Oncol

PP269

SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES: CELLULAR ABSORPTION IN THYROID CANCER AND HUMAN FIBROBLASTIC CELL LINES.

G. Pasqualetti[1], L. Di Bari[2], S. Ursino[1], P. Lenzi[3], G. Vitulli[2], D. Barbaro[4], C. Vantaggiato[1], F. Fornai[3], P. Salvatori[2], F. Monzani[1]

[1]Medicina Clinica e Sperimentale, Università di Pisa - Pisa, [2]Advanced Catalysts S.r.l. - Pisa, [3]Ricerca Traslazionale e delle Nuove Tecnologie in Medicina e Chirurgia, Università di Pisa - Pisa, [4]Sez. Diabetologia, Usl 6 - Livorno

Introduction. Superparamagnetic iron oxide nanoparticles (NPs) hold promise for a variety of biomedical applications. NPs must have a small size (<100 nm) and have to undergo "magnetic functionalization" for biomedical applications. In this study, we evaluated the cellular loading (uptake) mechanism both by chemical analysis and by electronic microscopy of L-glucose- and D-glucose-coated iron oxide NPs in malignant thyroid tumor (FB3) and human fibroblastic cell lines (NHDF). **Materials and methods.** NPs were made by using Metal Vapour Synthesis (MVS) and were stabilized with different chemical compounds as well as L- or D- glucose. Chemical process included several steps: a) Fe vapor condensation with organic compounds in useful reactor to obtain a solid matrix; b) Fusion of solid matrix for obtaining Fe solvated clusters; c) Stabilization of molecules with organic monomeric compounds (L- or D- glucose); d) Isolation of Fe NPs by using drying procedures. Cellular internalization of NPs by using chemical analysis of lysed cells after treatments with 2.5 mg/ml, 5 mg/ml and 10 mg/ml NPs concentration at 1, 2 and 3 hours. Cells were evaluated for NP internalization by using electronic microscopy. **Results.** NPs spectrophotometric method showed a linear concentration curve (R^2>0.96 for both NPs), a precision and accuracy <15% with a compound stability <15%, and the lower limit of detection was 0.01 mg/dl. Preliminary data suggest that the exposition to D-glucose coated NPs resulted in a saturable kinetic, while L-Glucose cell internalization showed a time dependent relationship. Electronic microscopy assays confirmed the NP internalization in both cell lines (cancer and normal cell lines) and showed that D- and L-glucose NPs were mainly internalized by endocytosis process involving macro-vescicular cell structures. **Conclusions.** Our results confirm that in vitro iron NPs interact with cellular structures and suggest that the potential mechanisms of internalization are represented by endocytosis. These findings might characterize the biological activity of the two NPs evaluated.

PP270

MITOTANE EFFECT ON THE GONADS: AN IN VITRO AND IN VIVO STUDY

A. Stigliano[1], L. Cerquetti[1], C. Sampaoli[1], S. Pezzilli[1], F. Innocenti[2], N. Argese[1], P. Lardo[1], R. Canipari[2], V. Toscano[1]

[1]Medicina Clinica e Molecolare - Facoltà di Medicina e Psicologia "Sapienza" Università di Roma - Roma, [2]Istituto di Istologia ed Embriologia - "Sapienza" Università di Roma - Roma

Mitotane acts by inhibiting 11β-hydroxylation and inducing cholesterol side chain cleavage in the mitochondria of steroidogenic cells, therefore blocking cortisol synthesis, decreasing plasma and urine steroid levels. It represents a main drug in the treatment of adrenocortical carcinoma (ACC). In clinical experience one of the most common side effects of mitotane use is gonadal dysfunction. In particular the testis appears more sensitive to damage from treatment than the ovaries that appear less affected. Indeed, a clear hypogonadism was observed only in men. Thus our increasing interest was focused to evaluate toxicities of mitotane treatment.

In this study we investigate the effect of mitotane in an ovarian cancer and Leydig tumor cell lines. The aim of the present study was to clarify the mitotane toxicity on gonadal epithelium and eventually to verify the damage on fertility .

Both cell lines were grown in appropriate culture medium and treated with mitotane at different concentrations. Cell growth proliferation was performed and cell cycle analysis was evaluated by flow cytometry. In the in vivo experiments the mice underwent to mitotane exposure, 152mg/Kg of mitotane were injected for 30 days. Then the animals were put down and testis and ovaries analysis was performed by histological analysis. Our in vitro data evidenced an antiproliferative effect on cell growth after mitotane exposure. In vivo analysis also evidenced an alteration of gonadic morphogenesis after mitotane treatment. Thus mitotane exposure has an antiproliferative effect on both gonadic cell lines and seems that its exposure may interfere with gonadic morphology.

PP271

EVEROLIMUS TREATMENT IN A PATIENT WITH ILEAL NET

F. Rota[1], R. Baldelli[1], A. Barnabei[1], A. Paoloni[1], V. Adinolfi[1], L. Rizza[1], F. Izzo[2], M. Appetecchia[1]

[1]Endocrinology "Regina Elena" Italian National Cancer Institute - Rome, [2]Division of Oncology "Regina Elena" Italian National Cancer Institute - Rome

In ileal neuroendocrine tumors (NETs), the first line treatment is represented by surgery that is always recommended when possible while, in advanced non-resectable stages, treatment options remain limited to somatostatin analogues (SSA). The development of new targeted agents including mTOR inhibitors, in particular everolimus, generated new hope for patients with NETs. Everolimus has been approved in advanced and progressive well differentiated pancreatic NETs. Here we report a case of everolimus treatment in a 51-years old female with an ileal NET (malignant carcinoid). In February 2002, the patient underwent a surgical small bowel resection (35 cm) also involving limph nodes metastases by dissection around the mesentery. The histological examination suggested the presence of an ileal carcinoid tumor, infiltrating the wall up to the subserosa with two omental metastases. The immunohistochemistry was positive for CgA. A post-surgical whole body CT showed the persistence of the left para-aortic nodal disease. It was decide to introduce a long acting somatostatin analog, octreotide LAR. In 2010, on SSA treatment, the patient experienced a clear progression evaluated and confirmed by RECIST criteria (presence of bone and liver metastases). In september 2010, the patient started treatment with everolimus at a dose of 10 mg/day and after 24 months of treatment the patient experienced a stable disease confirmed by RECIST criteria. The therapy with everolimus, after SSA failure, led to a stabilisation of disease and this is the current situation after 24 months of everolimus treatment. In conclusion, everolimus may be a therapeutic option after failure of other treatments also in ileal NETs.

PP272

SMALL NON FUNCTIONING PANCREATIC NEUROENDOCRINE TUMORS: PROGNOSTIC FACTORS AND INDICATION TO SURGICAL TREATMENT

M. Lombardi[1], C. Sardella[1], D. Russo[1], C. Urbani[1], U. Boggi[2], N. De Lio[2], N. Funel[3], D. Campani[3], L. Tomisti[1], P. Vitti[1], E. Martino[1], F. Bogazzi[1]

[1]Department of clinical and experimental medicine, University of Pisa - Pisa, [2]Department of traslational research and new technologies in medicine and surgery, University of Pisa - Pisa, [3]Department of surgical, medical and molecular pathology of critical area, University of Pisa - Pisa

Pancreatic neuroendocrine tumors (pNET) are rare neoplasms with a wide variability in aggressiveness. A staging system based on the tumor-node-metastasis (TNM) assessment and a grading classification have been recently proposed for NET, demonstrating a role in predicting prognosis. Surgery is usually considered the first therapeutic option; however, owing to the indolent outcome of most pNET, a more conservative approach for non functioning (NF) neoplasms ≤ 2cm has been proposed. In the present study 77 patients, surgically treated for a pNET between 2002 and 2009, were retrospectively analyzed. Their histology was revised according to the WHO 2000 and 2010 classification. A pre-surgical staging and a regular radiological follow up were performed in all cases. After a mean follow-up of 62.9±32.5 months, 14 deaths occurred (3 of them in the peri-operative period), leading to a five and nine-years cause specific survival of 93% e 83% with a mean progression free survival (PFS) of 30.0±27.6 months. At univariate analysis: grading, staging, tumoral primitive dimensions, angioinvasion and peri-pancreatic infiltration were significantly associated with survival. Among patients with a pre-surgical NF-pNET ≤2cm, 12 had a grading 1 (G1), 6 a grading 2 (G2) and one a grading 3 (G3) neoplasm. Pre-surgical metastasis were present in 25% of patients with G1, in none of G2, and in 100% of G3 NF tumors ≤ 2cm. The proportion of metastatic neoplasms did not differ in G1 patients with pNET ≤ 2cm or > 2cm (25% vs 17%); on the contrary G2 patients with pNET >2 cm had a higher proportion of metastasis than G2 patients with pNET ≤ 2cm (50% vs 0%). Frequency of long-term surgical complications was significantly lower in patients treated with parenchyma preserving surgery (enucleoresection and intermediate pancreasectomy).

Conclusions: this study confirms the usefulness of the new grading and staging systems, as a tool to predict prognosis. From our data, whether small NF-pNET should avoid a surgical treatment is doubtful.

PP273

O6-METHYLGUANINE DNA METHYLTRANSFERASE IN LUNG NEUROENDOCRINE CANCER AND AGGRESSIVE PITUITARY ADENOMAS

R. Baldelli[1], M. Carosi[2], F. Rota[1], A. Paoloni[1], L. Rizza[1], V. Adinolfi[1], P. Visca[2], R. Covello[2], S. Telera[3], A. Barnabei[1], C. Scaroni[4], G. Aimaretti[5], F. Facciolo[6], A. Pompili[3], E. Pescarmona[2], M. Appetecchia[1]

[1]Endocrinology, "Regina Elena" National Cancer - Rome, [2]Pathology, "Regina Elena" National Cancer Institute - Rome, [3]Neurosurgery, "Regina Elena" National Cancer Institute - Rome, [4]Endocrinology University of Padova - Padova, [5]Endocrinology University of Novara - Novara, [6]Thoracic Surgery, "Regina Elena" National Cancer Institute - Rome

Neuroendocrine carcinomas (NECs) represent relatively rare and heterogeneous malignancies while clinically significant pituitary tumours occur approximately in every 1000 individuals. Temozolomide (TMZ) is converted to the active alkylator that induces DNA methylation at the position of guanine. O-methylguanine-DNA methyltransferase (MGMT) is a DNA repair protein that reverses alkylation at the O6 position of guanine. Low tumour MGMT expression has been shown in some studies to correlate with temozolomide response. We have evaluated the expression of MGMT hypermethylation in aggressive pituitary adenomas (APA) and in a small group of neuroendocrine tumors of the lung (NET-L) (namely methylation-specific PCR). Patients evaluated were 37 with pituitary adenomas namely 17 with recurrances of disease (APA) and 7 with NET-L. In particular 21 out of 37 patients were characterized by the presence of MGMT promoter methylation (57%) (group A) and 16 were not methylated (43%) (group B). In particular 7 out of 17 patients with relapse showed a MGMT methylation. Regarding NMET-L patients previous results seem to indicate that the MGMT methylation of the promoter is present in 4 of 7 samples evaluated (57%). Considering the significant high incidence of MGMT promoter methylation in APA and NET-L patients and the few adverse effects of TMZ treatment, there might be a wider indication for TMZ treatment in these subset of patients.

PP274

METFORMIN AND SUPPRESSIVE THERAPY IN DIFFERENTIATED THYROID CARCINOMA

P. Conton[1], D. Barison[1], F. Presotto[1], C. De Riva[1]

[1]Medicina Interna - Ospedale dell'Angelo - Mestre (VE)

Introduction Suppressive doses of thyroid hormone(s) aimed at blocking the trophic activity of thyroid stimulating hormone (TSH) and preventing disease relapse are commonly used in thyroid cancer. Yet, suppressive therapy can induce thyrotoxicosis that is poorly tolerated. Because metformin may decrease TSH without changes in thyroxine (fT4) levels we used both metformin and l-T4 to maintain TSH suppression.

Patients Among 28 patients followed for differentiated thyroid cancer, 7 revealed signs and symptoms of intolerance to suppressive therapy. Five had papillary and 2 follicular carcinoma. In a patient with paroxysmal atrial fibrillation the suppressive l-T4 doses caused atrial fibrillation recurrencies. All 7 patients had been undergone to thyroidectomy and radiometabolic treatment. TSH levels were initially not suppressed and patients were taking a mean daily l-T4 dose of 114.7 mcg. Patients began metformin (1000-2000 mg/day) while maintaining the previous dosage of l-T4. One patient complained intestinal intolerance to metformin and was therefore excluded.

Results During follow up TSH values showed a progressive decrease (see Table below). Serum fT3 and fT4 levels were always within the normal ranges. The small size of treated patients did not allow to perform appropriate statistical analysis.

TSH levels (reference values 0.3-4.0 mU/L)

Patients	Before metformin	At 3 months	At 6 months	At 9 months	At 12 months
1 M	1.61	1.04	0.21	0.02	<0.01
2 F	1.99	2.04	1.32	0.99	0.19
3 F	2.11	1.66	drop out	-	-
4 F	0.99	1.01	0.54	0.29	0.05
5 F	1.33	0.91	0.73	0.43	0.22
6 M	1.42	1.10	0.96	0.53	0.08
7 F	2.01	1.05	0.33	<0.01	0.04

Conclusions Although in a limited series, we found that subjects intolerant to suppressive l-T4 doses additional metformin administration may act as thyroid hormones "sensitizer" and reduce TSH stimulating effects on residual thyrocytes. This therapeutic strategy may avoid iatrogenic thyrotoxicosis.

PP275

EVALUATION OF THE VARIATIONS OF THE PAN-NEUROENDOCRINE MARKERS IN METASTATIC NET IN COURSE OF TREATMENT WITH NEW DRUGS

G. Scarpelli[1], D. Maiettini[1], L. Crino[2], G. Angeletti[3], P. Ferolla[1]

[1]Gruppo Multidisciplinare NET - Perugia, [2]Oncologia Medica - Perugia, [3]SS Endocrinologia - Perugia

Introduction: Circulating Chromogranin A (CgA) and 24 hour urinary hydroxy-indolacetic (5-HIAA), despite their well know limitations, have been so far used as "pan-neuroendocrine" markers for the diagnosis and clinical management of neuroendocrine tumors and carcinoid syndrome. Their variations in the course of somatostatin analogues octreotide and lanreotide have also evaluated. On the contrary very few data exist at the moment on their variation in the course of the therapeutical approachs with new drugs. **Aim:** The aim of this study was therefore to evaluate the variation of of CgA and 5-HIAA, in metastatic NET in the course of treatment with the new available drugs temozolomide, pasireotide, everolimus alone and in combination with octreotide. Material and Methods: From a total of 850 patient followed in our center we selected 20 patients, with diagnosis of advanced NET treated with temozolomide, pasireotide, everolimus alone and in combination with octreotide at baseline and every 3 months during treatment. **Results:** CgA increased at 3 months in 80% of the patients treated with everolimus alone and in association with octreotide, with only a minimal decrease in 20% of the patients. A further increase was observed at 6 months in 80% of the cases while a small decrease was observed in 20%. An incremental trend in the following months was predictive of a successive progressive disease only in 40% of the cases while an increase lower than 2 fold was correlated with a stable disease in 40% of the cases. Also 5-HIAA at 3 months was increased in 80% of the patients with everolimus and decreased in 20%. In the following months an incremental trend was observed in 60% and a decrease in 40%. Interestingly this incremental trend was observed also in 100% of the patients treated with pasireotide, despite a good impact on the control of carcinoid syndrome. No correlation with CgA and 5HIIA values was observed in patients treted with temozolomide. **Conclusion:** No inhibition in the secretion of pan-neuroendocrine markers was found in NET patients in the course of therapy with new drugs, although an incremental trend in the circulating levels was a precocious predictor of a later progression of the disease. The increase in the values observed at 3 months may be related to the degranulation of the neuroendocrine cells as a consequence of the cytolitic phenomenons induced by the drugs.

PP276

EFFECTS OF TREATMENT FOR ACROMEGALY ON BONE MINERAL DENSITY (BMD): IS PEGVISOMANT PROTECTIVE ON LUMBAR BMD?

G. Brigante[1], D. Santi[1], C. Diazzi[1], S. De Vincetis[1], G. Ferrannini[1], B. Madeo[1], M. Simoni[1], V. Rochira[1], C. Carani[1]

[1]Chair and Unit of Endocrinology & Metabolism, Department of Biomedical, Metabolic and Neural Sciences, University of Modena & Reggio Emilia - Modena

Background: GH-IGF-1 status is important for bone health. Acromegaly affects bone status, but less is known on the role of treatments for acromegaly on bone mineral density (BMD). Pegvisomant (Peg) is effective in treating acromegaly by reducing IGF-1. As serum GH is not influenced by Peg, it is not known if residual, direct GH effects on bone (not IGF-1 mediated) are preserved during treatment. Methods: To evaluate the effects of Peg on BMD, we compared 5 patients treated with Peg (alone or in combination) to 6 patients treated with Somatostatin Analogues (SA) and to 7 patients surgically cured, not under medical therapy. All the patients had normal serum IGF-1. BMD was measured by DEXA (Hologic-QDR-2000 densitometer, Inc., Waltham, MA). A t-score of ≤ 1 and ≤ 2.5 at lumbar spine (L1-L4) and at femoral neck was used for diagnosis of osteopenia and osteoporosis, respectively .

Results: Mean age of subjects (seven males and nine females) was 60.7 ± 9.8 yrs. At lumbar spine, 40% of Peg-patients, 33.3% of SA-patients, and 60% of not-treated patients had osteopenia; none of the Peg-patients, and 16.7% of SA-patients, and none of not-treated patients were osteoporotic. Considering the femoral neck, 60% of Peg-patients, 33% of SA-patients, and 60% of not-treated patients had osteopenia; 20% of Peg-patients and none of the other two groups were osteoporotic.

Conclusions: The percentage of osteoporotic/osteopenic acromegalic patients seems to be lower than that reported in literature. Peg seems to protect bone at lumbar spine, but this protective effect does not seem to be exerted at femoral level where, indeed, patients treated with Peg present lower densitometric values. Patients surgically cured, not under medical therapy, have higher rate of lumbar osteopenia. No data are available on bone quality, a parameter that is usually altered in acromegaly.

PP277

RAPID CLINICAL RESPONSE IN A YOUNG GIRL WITH OSTEOGENESIS IMPERFECTA TYPE 1 AFTER FIRST INFUSION OF NERIDRONATE

L. Vuolo[1], M. Rubino[1], D. Melis[2], V. Brunelli[1], A. Colao[1], C. Di Somma[1]

[1]Dip.to di Medicina Clinica e Chirurgia, Sezione Endocrinologia Università Federico II, Napoli - Napoli, [2]Dip.to di Pediatria Università Federico II, Napoli - Napoli

INTRODUCTION: We present a case report on the rapid clinical response to therapy in a young patient with type I osteogenesis imperfecta (OI). OI is the most common inherited bone disease with an extremely variable clinical presentation: patients are susceptible to fractures from the mildest trauma; reduced bone mass; short stature; skeletal deformities; joint laxity; blue sclerae; dentinogenesis imperfecta; and adult onset deafness.

CASE PRESENTATION: A 14 year old girl was referred at Metabolic Bone Disease outpatients clinic of Department of Endocrinology with newly diagnosed type 1 OI performed at the Department of Pediatrics. On physical examination, young patient presented blue sclerae, short stature (<3SD), walking impairment and pre-pubertal status (B2 PH2); with a previous multiple fractures history to the upper and lower limbs. MOC DEXA showed a lumbar Z-score = - 4.9 SD. The audiometric examination showed a normal hearing in the right ear and a middle-ear transfer function loss at left one. Blood tests showed: serum calcium 9.7 mg/dL (9-11), phosphorus 4.6 m/dL (4 to 5.7), alkaline phosphatase 468 U/L (<187); IGF-1 288 ng/ml (180-780); Parathyroid Hormone 66 pg ml (10-75); 25OH vitamin D 24 ng mL (> 30). Patient was prescribed neridronate intravenous therapy (2 mg/Kg/3 months) according to the Italian reimbursement criteria for OI, and a supplementation of 600 IU/day cholecalciferol. Before and one month after neridronate treatment patient was prescribed two self-administered questionnaires: the Spine Pain Index (SPI) and the SF-12, to investigate the impact of back pain on functional limitation and the perception of Health Related Quality of Life (HRQoL), respectively. One month after first infusion of neridronate we observed a significative decrease at the back disability going from 50 at baseline to 30; in association with an improvement in the perception of HRQoL ranging from 30 to 50.

CONCLUSION: Regardless of well-known objective therapeutic efficacy of neridronate and vitamin D in patients with OI, this case report shows a relevant and rapid impact of treatment on improvement of patients' subjective perception of health status. A correct and multidisciplinary approach must aim to ensure a "normal life" in mild form OI patients (OI type 1).

PP278

SPINAL DEFORMITY INDEX AND VITAMIN D STATUS IN OBESE SUBJECTS.

M. Rubino[1], L. Vuolo[2], S. Savastano[2], V. Brunelli[2], V. Nedi[2], L. Barrea[2], A. Colao[2], C. Di Somma[2]

[1]Med. Clinica e Chirurgia Sez. Endocrinol. Univ."Federico II" Napoli - Napoli, [2]Dip. di Medicina Clinica e Chirurgia Sez. Endocrinol. Univ."Federico II" Napoli - Napoli

Obesity and osteoporosis share many features and recent studies have identified many similarities suggesting common pathophysiological mechanisms. Obesity is associated with a higher risk of non traumatic fractures, despite bone mineral density (BMD) is normal or even increased. Bone strength depends on both BMD and bone quality. Spinal deformity index (SDI) is a semiquantitative method that may be a surrogate index of bone microarchitecture, a component of bone quality. The objective of this study was to assess in obese patients levels of 25 OH vitamin D (25OHD), parathyroid hormone (PTH), serum and urinary calcium (Ca) and phosphorus (P), BMD and SDI evaluated on T4-L4 quantitative morphometric analysis. We analyzed 54 obese subjects (56 ± 11 years, 10 males , 44 females). 22 female obese were postmenopausal and not taking hormone replacement therapy and 22 were pre-menopausal women, and 54 healthy subjects as controls. Among obese subjects, 48% had obesity class I, 32% obesity class II and 20% obesity class III. We assessed lumbar and femoral BMD using DEXA and a spine radiography to assess vertebral fractures and calculate SDI. SDI was calculated by quantifying the severity of fractures, using the classification of Genant, and assigning a value of 0,1,2 or 3 in case of no fracture or mild, moderate and severe fractures respectively. Vitamin D levels were lower in obeses than in controls (21.5 ± 6 vs 30.4 ± 14, $p = 0.002$). The levels of PTH, calcium, phosphorus were similar in obeses and controls. A severe deficiency of vitamin D (<20 ng / dl) was present in 37% vs 4%, a state of deficiency in 40% (20-30 ng / ml) vs. 52%, and normal levels of vitamin D (> 30ng/dl) in 22% vs. 44% respectively in obese subject and normal-weight controls. No significant difference was found in BMD both lumbar spine and femoral neck between obeses and controls. The percentage of radiological vertebral fractures was 75% in obese subjects vs 17% in controls ($\chi2 = 67.96$, $p = 0.000$), and fractures were mild in 28 patients and 12 controls ($\chi2 = 8.934$, $p = 0.003$), moderate in 16 patients and 5 controls ($\chi2 = 5,911$, $p = 0.015$), and no patient and controls had severe fractures. SDI index was higher in patients than in controls (3.62 ± 2.5 vs 0.32 ± 0.2, $p = 0.000$). In 87.5% of patients and 10% of controls we found morphometric vertebral fractures, despite a DEXA T-score not diagnostic of osteoporosis ($\chi2 = 46.55$, $p = 0.000$). In conclusion in obese patients vitamin D is lower than in controls. 87.5% of the obese subjects present non traumatic vertebral fractures and reduced bone quality as measured by SDI.

PP279

GENOMIC ANALYSIS OF A MULTIGENERATIONAL FAMILY WITH EARLY ONSET AND SEVERE FORM OF OSTEOPOROSIS

A. Andreadi[1], S. Zampatti[2], B. Capuani[3], M. Cerilli[1], A. Bellia[4], A. Galli[1], C. Peconi[2], M. P. Caputo[1], E. De Carli[1], M. E. Rinaldi[1], G. Sconocchia[5], E. Giardina[2], F. Pozzi[1], G. Novelli[6], D. Lauro[4]

[1]Center of Type2 Diabetes, Dept of Medicine, Univ. Hospital Policlinic Tor Vergata Found. - Rome, [2]Dept of Biomedicine and Prevention, Medical Genetic Section, Univ. of Rome Tor Vergata - Rome, [3]Dept of System's Medicine, Univ. of Rome Tor Vergata - Rome, [4]Center of Type2 Diabetes, Dept of Medicine, Univ. Hospital Policlinic Tor Vergata Found., Dept of Systems Medicine, Tor Vergata - Rome, [5]Inst. of Translational Pharmacology, National Research Council - Rome, [6]National Agency for the evaluation of Universities and Research ANVUR Fatebenefratelli Hospital S. Pietro - Rome

Osteoporosis is a common complex disease caused by the interplay of genetics and environmental factors. In our clinical unit we described an early and severe form of osteoporosis segregating in an Italian family according autosomal dominant (AD) inheritance pattern. Genes reported in association with osteoporosis are frequently involved in hormonal pathway (ESR1) osteoclast function (RANKL, OPG) or underlie well known syndromic phenotypes as osteogenesis imperfecta (COL1A1, COL1A2). In addition to these, several studies have reported associations in other genes or loci apparently without a direct link with the pathogenetic mechanism of osteoporosis (MHC, ZBTB40, FONG). We analyzed the pedigree using a panel of microsatellite markers located to investigated regions. The presence of linkage was ruled out for candidates genes (LOD<-2) As a consequence, we did not attain the identification of one gene as responsible of this early and severe osteoporosis. These results confirmed the small contribution of numerous genes to the pathogenesis of osteoporosis, leading to a lack in the identification of single responsible genes, every in severe and apparently AD transmitted forms. To our knowledge, this is the second report of a severe and early form of osteoporosis, after Parisi et al. in 2001.Our results underlie the importance of familial evaluation to define BMD, in order to detect family members at high risk of fracture. Moreover, the linkage analysis confirms the genetic heterogeneity of osteoporosis. Further researches are necessary to identify major genes primarily contributing to the phenotype of the disease.

PP280

OSTEOPOROSIS LIFESTYLE, RISK FACTORS AND TYPE OF MEDICAL ASSISTANCE

A. Andreadi[1], M. Cerilli[1], A. Bellia[2], B. Capuani[1], D. Pastore[1], M. Romano[1], R. Fabiano[1], E. De Carli[1], M. P. Caputo[1], M. E. Rinaldi[1], A. Galli[1], F. Pozzi[1], D. Lauro[2]

[1]Reference Center for Type 2 Diabetes, Department of Medicine, University Hospital Policlinic Tor Vergata Foundation - Rome, [2]Reference Center for Type 2 Diabetes, Department of Medicine, University Hospital Policlinic Tor Vergata Foundation - Department of System's Medicine - Rome

The aim of the study has been to evaluate the lifestyle and dietary habits of patients that have done DEXA examination, to calculate how the degree of osteoporosis correlates to the nutrition habit.The questionnaires were administered to 761 individuals and all subjects underwent to the following evaluations: measurement of anthropometric data, bone mineral density with DEXA, Food frequency questionnaire and FRAX questionnaire. Patients had a mean age of 63 years, 734 females and 27 males. The patients have been dived in 3 groups using the T-score: 132 normal, 477 osteopenic subjects and 152 osteoporotic patients. From 761 patients only 263 patients, was assuming medical therapy; in specific in the 43% of the patients, preventive therapy for osteoporosis was prescribed, in particular using bisphosphonate. Body mass index (BMI) was calculated, and patients were divided into categories; the 0.65% of patients were underweight, 34.69% normal weight, 36.6% overweight, and 27.98% were obese. Analyzing the distribution of overweight/obese patients, it is possible to observe that higher levels of BMI are directly linked to increased bone fragility: 11% normal, 65.2% osteopenic and 15% osteoporoticThe FRAX algorithm allows to calculate the risk of fractures, the average probability of major fractures is 3.63% in normal subjects, 7.35% in osteopenic patients and 15.5% in osteoporotic patients. In contrast, the average probability of hip fracture is 0.33% in normal subjects, 1.92% in osteopenic patients and 7.5% in patients with osteoporosis. The specialist for the cure and treatment of osteoporosis; only 12,8% normal, 18,6% osteopenic and 13,1% osteoporotic patients were already followed from an endocrinologist (16,5%). The general practitioner had in care about 54,5% of individuals for all three groups;the remaining 29% were followed from another specialist.We can conclude that osteopenia was widespread and the early identification of high risk population will allow to implement preventive interventions In addition, we consider that FRAX algoritm must be revaluated so it can consider also the dietary habits of the patients.

PP281

EVIDENCE FOR A LINK BETWEEN BONE AND TESTIS IN 25 (OH) VIT D PRODUCTION: ROLE OF OSTEOCALCIN

U. Carraro[1], M. Ferigo[1], G. Strapazzon[2], M. Plebani[3], C. Foresta[1]

[1]Department of Molecular Medicine, Section of Clinical Pathology, Centre for Human Reproduction Pathology, University of Padova, Italy. - Padova, [2]Italy EURAC Institute of Mountain Emergency Medicine, Bozen/Bolzano, Italy. - Bolzano, [3]Department of Laboratory Medicine, University-Hospital, Padova, Italy - Padova

Context: Recent evidence suggests that the skeleton exerts an endocrine regulation of energy metabolism through the release of the undercarboxylated form of osteocalcin (ucOCN), the main non-collagenous bone-related protein. Moreover, it has been demonstrated that ucOCN, acting on its putative receptor GPRC6A, is able to modulate testosterone production by the Leydig cells in the testis. We have previously demonstrated that Leydig cells are also a relevant site of Vitamin D 25 (OH) hydroxylation because they express the key hydroxylating enzyme CYP2R1.
Objective: To evaluate if ucOCN exerts a modulating effect on 25-hydroxyvitamin D production by Leydig cells in the in vitro model of MA-10 cell lines.
Design and setting: MA-10 cells were stimulated with increasing concentrations of ucOCN (1, 3, 10 ng/mL) in specific culture medium containing the 25-hydroxyvitamin D precursor cholecalciferol (10 ng/mL). 25-hydroxyvitamin D and testosterone levels were quantified in the supernatant with immuno-enzymatic methods.
Results: at the lowest concentration ucOCN was able to significantly increase 25-hydroxyvitamin D production. In particular, we have observed a dose-depending kinetics, at the lower concentration of ucOCN we found a 25(OH)Vit D concentration of 14,8+ 0,2 nmol/L compared to 11,4 + 0,3 nmol/L of controls, at the median concentration 15,6 + 0,2 nmol/L and at the higher concentration 15,7 + 0,3 nmol/L (P<0,05).
Conclusions: Our data show that ucOCN is a direct modulator of Leydig cells activity, influencing both testosterone and 25 hydroxyvitamin D production. To the best of our knowledge this is the first evidence of the two-way link between bone and testis in the regulation of bone metabolism.

PP282

SERUM ALDOSTERONE IN PATIENTS WITH PRIMARY HYPERPARATHYROIDISM

L. Vera[1], M. Accornero[1], M. Dolcino[1], S. Oddo[1], F. Minuto[1], M. Giusti[1]

[1]UO Clinica Endocrinologica, Azienda Ospedaliera Universitaria San Martino – IST - Genova

Background: Experimental studies have suggested that aldosterone induces hypercalciuria and might contribute to secondary hyperparathyroidism. Other studies have found that parathyroid hormone (PTH) stimulates aldosterone secretion and cell proliferation in human adrenocortical cells. Indeed, primary hyperparathyroidism (PHPT) is associated with increased cardiovascular (CV) morbidity and mortality. The aim of this study was to ascertain whether there is a mutual interaction between PTH and aldosterone levels and CV comorbidity in patients with PHPT.
Patients and methods: We recruited 81 consecutive PHPT patients, 62 females and 19 males (mean age 65±24 years), 44 of whom had undergone parathyroidectomy (PTX). All patients underwent physical examination, including measurement of blood pressure and body mass index (BMI). In all patients, we measured the plasma levels of aldosterone and renin in orthostatism, PTH, 25OH-VitaminD (VitD), 1.25(OH)2-VitD, serum calcium (S-Ca), phosphorus (P), potassium, sodium and the 24h urinary excretion of Ca and P. All subjects underwent echo-color-Doppler examination of the supra-aortic vessels and measurement of intimal thickness.
Results: Hypertention was documented in 62 of the 81 patients, 49 of whom were already on antihypertensive therapy. Only 4 patients were not receiving any treatment. Mean serum PTH (PTX=70±26pg/ml; no-PTX=172±29pg/ml; P=0.0001) and S-Ca (PTX=9.9±0.2mg/dl; no-PTX=10.5±0.2mg/dl; P=0.005) levels were lower in patients who had undergone PTX; aldosterone levels were also lower, though not to equally significant degrees (PTX=107±15pg/ml; no-PTX=122±22pg/ml). Serum aldosterone and PTH levels were not positively correlated in our population (P=0.07), even when patients who had undergone PTX were distinguished from those who had not. This correlation was not observed in patients who were not on antihypertensive medications. All patients had similary deficient vitD levels (patients in vitD therapy=33.3%) .
Conclusion. In our study, aldosterone was not correlated positively with PTH levels in patients with PHPT. However, aldosterone was reduced after PTX and its correlation with PTH bordered on significance after surgery. Thus, we cannot exclude the possibility that it is a mediator of CV symptoms in patients with PHPT. Further studies will therefore be needed.

PP283

THE RELATIONSHIP BETWEEN METABOLIC SYNDROME AND BONE HEALTH: RESULTS OF A RETROSPECTIVE GENDER SPECIFIC FEMALE STUDY.

R. Veneziano[1], R. Citarrella[1], G. Smeraglia[2], N. Maltese[3], A. Ciresi[1], M. C. Amato[1], C. Giordano[1]

[1]Policlinico Universitario, P. Giaccone - Palermo, [2]E. Albanese PTA. - Palermo, [3]Policlinico Universitario, P Giaccone - Palermo

Background:Osteoporosis (OP) and Metabolic Syndrome (MS) are prevalent in aging westernized societies and adversely affect the health of the elderly people by causing fractures and vascular complications. It is well known that visceral obesity, the main component of MS, is a source of proinflammatory cytokines that may promote bone resorption. Many studies have pointed out the relationship between MS and bone metabolism, but the results are still contradictory.The aim of our retrospective study was to examine the possible link between classical features of MS and bone metabolism in a cohort of postmenopausal women with type 2 diabetes.
Materials and methods: a total of 125 postmenopausal diabetic women with MS (mean age 66,7± 7,8 yrs) were recruited in our outpatients clinic.We used Pearson correlation analysis to evaluate the associations between MS defined by NCEP-ATP III criteria, with both femoral and lumbar BMD and T-score. Moreover, in each patient HbA1c, Body mass index (BMI) and visceral adiposity index (VAI) were studied.
Results: 74 women (59%) had a BMI ≥ 30 kg/m2 and 103 (86%) had a high waist circumference (mean WC 96,5±10,4 cm). VAI was high in 113 out of 125 women (mean VAI: 4,83±3,03). 47 (40%) subjects had a T-score ≤ -2,5 diagnostic for OP, while 45 (36%) patients showed osteopenia (T-score among -1 e -2,5), and 33 (26%) had a normal T-score. Duration of disease, HbA1c, lipids profile and blood pressure were not related to BMD and T-score. VAI (r: 0,203; p:0,02), BMI (r : 0,311;p<0,001) and WC (r:0,337; p< 0,001) showed a significant positive correlation with both femoral and lumbar BMD and T-score.
Conclusions: In our study MS was not associated with reduced BMD. The low grade inflammation in MS could affect BMD, but the protective effect of adiposity may counteract the negative influence of adipocytokines on bone mass.

PP284

HYPERPARATHYROIDISM IS ASSOCIATED WITH MUSCLE STRENGTH IN PATIENTS WITH MYOTONIC DYSTROPHIES.

E. PASSERI[1], E. BUGIARDINI[2], V. SANSONE[2], R. VALAPERTA[3], E. COSTA[3], B. AMBROSI[1], G. MEOLA[2], S. CORBETTA[1]

[1]Endocrinology Unit, Dept. of Biomedical Sciences for Health, University of Milan,IRCCS Policlinico S. Donato - San Donato M.se, Milano, [2]Neurology Unit, Dept. of Biomedical Sciences for Health, University of Milan,IRCCS Policlinico S. Donato - San Donato M.se, Milano, [3]Clinical Chemistry Laboratory, IRCCS Policlinico San Donato - San Donato M.se, Milano

Parathyroid function in Myotonic Dystrophy (DM) patients has been poorly investigated. Parathyroid and muscle parameters were assessed in 31 male DM1 (44±2 years), 13 male DM2 (56±2 years) and 32 healthy controls. Hyperparathyroidism was diagnosed in 18% of patients without differences between DM types. In all DM patients, hyperparathyroidism was associated with normocalcemia but one with hypercalcemia. DM patient presented significantly higher PTH and lower vitamin D levels (25OHD) compared with controls, also considering seasonality (PTH: 49.7±5.5 vs 22.2±2.0 pg/ml, P< 0.001; 25OHD: 16.0±1.7 vs 26.3±1.8 ng/dl, P<0.001, mean±SE). Furthermore, severe vitamin D deficiency (25OHD<10 ng/ml) was diagnosed in 40% and hypovitaminosis D (25OHD<30 ng/ml) occurred in 88% of DM patients. About one third of DM1 presented hypophosphatemia associated with elevated PTH levels. Serum 25OHD levels negatively correlated with PTH and with body fat mass. Considering DM1 patients, serum PTH levels positively with CTG triplet repeats. Furthermore, PTH levels negatively correlated with total modified Medical Research Council (MRC) and positively with Muscular Impairment Rating Scale (MIRS), parameters of muscle impairment. By contrast, in DM2 patients muscle assessment did not show any correlation with parathyroid function. In conclusion, 1) severe vitamin D deficiency is common in DM patients and it is associated with hyperparathyroidism; 2) primary hyperparathyroidism, though rare, may occur; 3) increased adiposity in DM may be a risk factor for hypovitaminosis D; 4) serum PTH levels may be considered a marker of muscle impairment, at least in DM1.

PP285

TERIPARATIDE MAY HAVE UNFAVORABLE EFFECTS ON VENTRICULAR SHORTENING

E. Passeri[1], F. Bandera[2], S. Corbetta[1]

[1]Endocrinology and Diabetology Unit, Dept. Biomedical Sciences for Health, University of Milan, IRCCS Policlinico San Donato - San Donato Milanese, Milano, [2]Cardiology Unit, IRCCS Policlinico San Donato - San Donato Milanese, Milano

The effect of PTH on cardiac performance is still debated. Patients on treatment with teriparatide may represent a good clinical model to study the effect of PTH1-34 on cardiac function and contractility, insulin sensitivity, lipid profile and fat mass. Fifteen patients (aged 67±2 years, F/M=14/1) affected with osteoporotic fractures according to AIFA advice n.79 were evaluated at baseline and after 6-12-18 months after starting teriparatide 20 mcg/die. All patients received calcium and vitamin D supplementation. Patient receiving steroid therapy or affected by cardiac, liver or renal insufficiency or diabetes mellitus were excluded. Under teriparatide treatment, no sign of hypercalcemia or adverse events were experienced except for myalgia. Total and ionized calcium mildly increased remaining in the upper limit of normal ranges, while PTH levels decreased. Echocardiographic examination was performed: at 6 months of teriparatide treatment, telesistolic left ventricular length and fractional shortening (FS) decreased (FS, 42.3±2.2 vs 36.7±2.3 mm, P= 0.04); at 12 months, the FS was further reduced (33.8±2.6 mm; P=0.02). No changes of left ventricular mass index (LVMI) were detected. We further evaluated the effect of teriparatide on other cardiometabolic parameters: patients experienced a mild progressive weight gain trend (+1.8 Kg at 1 year, +3.5 Kg at 18 months) with consequent BMI increases (27.5±1 vs 28.6±1 kg/m2 at 1 year and 29.1±3 kg/m2 at 18 months, P=0.003), not observed in a control group of 20 osteoporotic women (aged 70±2 years) treated with oral bisphosphonate. Nonetheless, waist circumferences and body fat mass didn't change. Glucose metabolism, including insulin-resistance, were not significantly affected. A mild increase in total cholesterol levels was observed requiring in three patients statin therapy (total cholesterol 211±13 vs 235±12 mg/dl at 1 year, P=0.002). No differences in HDL and triglycerides were detected. These novel data suggest that teriparatide may have unfavorable effects on ventricular shortening.

PP286

TUMOUR-ASSOCIATED FIBROBLASTS (TAF) ARE INVOLVED IN NEOANGIOGENESIS AND INVASIVENESS IN PARATHYROID NEOPLASIA

C. Verdelli[1], L. Avagliano[2], V. Guarnieri[3], A. Scillitani[4], L. Vicentini[5], G. Steffano[6], G. Bulfamante[7], S. Corbetta[8]

[1]Lab.Biol. Molecolare, IRCCS Pol. San Donato - S.Donato M.se (MI), [2]Dip.Ostetricia Ginecologia, DMSD Univ.Milano, Osp. San Paolo - Milano , [3]Genetica Medica, IRCCS Osp. Casa Soll. Sofferenza - S.Giovanni Rot. (FG), [4]U.O.Endocrinologia, IRCCS Osp. Casa Soll. Sofferenza - S.Giovanni Rot. (FG) , [5]Endocrinochirurgia, Fondaz.IRCCS Cà Granda - Milano, [6]Chirurgia I, IRCCS Pol. San Donato - S.Donato M.se (MI), [7]Div.Patologia, DMSD Osp. San Paolo, Univ. Milano - Milano, [8]U.O.Endocrinologia, DSBS Univ. Milano, IRCCS Pol. San Donato - S.Donato M.se (MI)

TAFs are important players in tumour formation, growth and metastasis. We firstly investigated the TAFs component in human parathyroid neoplasia from patients with primary hyperparathyroidism. Alpha-smooth muscle actin (alpha-SMA) has been used to identify activated TAFs (myofibroblasts). Culturing explants from parathyroid adenomas (PAs), large spindle-shaped alpha-SMA+ cells outgrew from explants and the expression of activated fibroblasts markers such as vimentin, stromal derived factor-1 (SDF-1) and fibroblast activated protein were detected. Alpha-SMA+ cells were highly represented in normal parathyroid glands where they lined the acinar structures. In typical PAs, alpha-SMA+ cells were variably reduced. In PAs and in human fetal parathyroids alpha-SMA+ cells surrounded microvessels suggesting a role in neoangiogenesis. In atypical adenomas and carcinomas, the chief cells proliferating in sheets were not sustained by myofibroblasts, which were highly represented in the fibrous bands and capsula stroma, suggesting a role in invasiveness. Coculture of human bone-marrow mesenchymal stem cells (hBM-MSCs) with PA-derived explants induced significant increases of *VEGFa* mRNA in hBM-MSCs. FACS identified 32-63% of PA-derived cells expressing CXCR4, the SDF-1 receptor, whose 47-90% coexpressing PTH. Treatment of cocultures with the CXCR4 antagonist AMD3100 reduced the coculture-stimulated *VEGFa* mRNA in hBM-MSCs, suggesting that the CXCR4/SDF-1 pathway mediates at least in part the proangiogenic effect. Moreover, a subset of alpha-SMA+ cells co-expressed the haematopoietic marker CD34 suggesting they might be perivascular adipose-tissue derived mesenchymal progenitors, while a subset co-expressed the parathyroid marker GCM2 and the transcription factor TBX1, suggesting they might derived from chief cells through epithelial-to-mesenchymal transition. In conclusions, we identified in parathyroid neoplasia, cells showing features of activated TAFs.

PP287

BONE TURNOVER MARKERS VARIATIONS IN GRAVES'OPHTALMOPATHY PATIENTS TREATED WITH INTRAVENOUS GLUCOCORTICOID AND BISPHOSPHONATES

G. Borzì[1], R. Le Moli[1], F. Guardo[1], C. Castoro[1], S. Squatrito[1], G. Padova[1]

[1]Endocrinology, Garibaldi Nesima Hospital, Department of Clinical and Molecular Biomedicine, University of Catania - Catania

INTRODUCTION - Glucocorticoids (GCs) represent an effective treatment for Graves'ophthalmopathy (GO). Long-term use of GCs can lead to osteoporosis; changes in biochemical markers of bone metabolism were reported as short-term effect of GCs therapy, especially if i.v. high doses were used. Presently, recommendations to prevent GCs-induced osteoporosis are intended to be applied to patients (pts) using long term GCs and not to pts receiving short-term i.v. high doses of GCs. Although no randomized clinical trials have specifically addressed this issue, EUGOGO (European Group on GO) suggest that bisphosphonates should be considered in patients receiving i.v. high doses GCs pulse therapy. AIM AND METHODS - In this preliminary study, we evaluated serum calcium, phosphate, PTH, amino-propeptides of type 1 collagene (P1NP, marker of bone formation) and carboxyterminal-propeptides of type 1 collagene (beta-CTX, marker of bone resorption) concentrations and urinary 24-hours calcium and phosphate levels in 7 female pts with GO (medium age 45 years ± SD 13) during the first six weeks of therapy (i.v. methylprednisolone, MPDS, 500 mg once weekly). All biochemical markers were evaluated baseline and weekly for the first six weeks of therapy. Calcium and calcitriol daily supplements and alendronate 70 mg once weekly were prescribed at the start of treatment and for all duration of therapy. RESULTS – One week after the first MPDS infusion, beta-CTX (0.65 ng/ml ± SD 0.17) was higher than baseline (0.55 ng/ml ± SD 0.21) in five pts without reaching statistical significance, and even lower in two pts. Moreover, in subsequent weeks beta-CTX progressively reduced, reaching a significant decrease versus baseline values in sixth week (0.34 ng/ml ± SD 0.23, P <0,01). P1NP instead showed a progressive reduction already after the first infusion (baseline 122 ng/ml ± SD 88,5, in 6th week 80,4 ng/ml ± SD 52,6, P <0,02). No significant differences were observed in other bone biochemical parameters and in thyroid function. CONCLUSION - Decrease of the bone markers variations, beta-CTX and P1NP, during the treatment could express a reduction of bone turnover and therefore a protective effect of bisphosphonates and calcium/calcitriol therapy yet in the first weeks of GCs pulse-therapy.

PP288

QUANTITATIVE ULTRASOUND ASSESSMENT OF BONE QUALITY IN TYPE 1 DIABETIC WOMEN

G. Borzì[1], V. Migliorino[1], F. Guardo[1], D. Ingannè[1], L. Tomaselli[1], M. L. Arpi[1], S. Squatrito[2], G. Padova[2]

[1]Endocrinology, Garibaldi Nesima Hospital, Department of Clinical and Molecular Biomedicine, University of Catania - Catania, [2]ndocrinology, Garibaldi Nesima Hospital, Department of Clinical and Molecular Biomedicine, University of Catania - Catania

Introduction. Numerous studies have demonstrated that type 1 diabetes (DT1) is associated with bone disorders and an increased risk of fractures. This seems to be dependent both on qualitative and quantitative alterations of the bone, probably due to autoimmune disorders that characterize DT1, as well as to extra-skeletal factors that include the neuropathic and microangiopathic complications, altered balance and variations in glycemic control. Bone mineral density can be assessed by DEXA and QUS (Quantitative ultrasound); QUS also studies the quality of the bone and seems to be a valid method in the evaluation of skeleton status. **Methods.** Twenty-two women with DT1 were studied, aged 19-39 years (26,9 ± SD 4,7). We evaluated glycemic control, treatment of diabetes, duration of disease, presence of macro- and microangiopathic complications and other risk factors for bone health. Phalangeal QUS with DBM Sonic Bone Profiler – IGEA was used in all patients to evaluate AD-SoS (amplitude dependent speed-of-sound), T-score and Z- score. The statistical analysis was performed using the SPSS 13.0 for Window with the analysis of variance. **Aim.** Evaluate the quality of bone, through QUS technique in female patients with DT1. **Results.** T-score was >-1 in 63,6% of patients (14/22), while was < -1 in 36,4% (8/22). We observed significant negative correlation between T-score values and insulin requirement per kg of body weight in the 5 years prior to our study (p = 0.002) and in the last year (p = 0.001). In addition, we found a statistically significant correlation between diabetes duration and reduced levels of T-score and Z-score, particularly among patients with diabetes duration of more than 10 years (17/22). Mean HbA1c values of the last 5 years was 7,41±1,8, but we did not find any significant correlation with T-score and Z-score values. None of the patients had macro- or microangiopathic complications. Most of the participants (81.8%) did not report a family history of osteoporosis, while 18.2% had a family history of bone fragility.
Conclusions. A reduced BMD is associated with type 1 diabetes and Phalangeal Quantitative Ultrasound (QUS) assessment may substantially represent a simple and low cost technique for screening bone quality in the aim to prevent fractures in long in type 1 diabetic patients.

PP289

ALTERATIONS OF VERTEBRAL MORFOMETRY IN YOUNG HIV POSITIVE PATIENTS

E. Guerra[1], A. Di Sarno[2], M. Gargiulo[2], C. Di Somma[1], L. Vuolo[1], M. Rubino[1], S. Savastano[1], A. Chirianni[2], A. Colao[1]

[1]Medicina Clinica e Chirurgia, Sezione di Endocrinologia, Università degli Studi di Napoli "Federico II" - Napoli, [2]Azienda dei Colli, Ospedale D. Cotugno - Napoli

Patients with HIV-infection present a risk from 3 to 6 times higher than reduced bone mineral density compared to HIV negative people. The inibitor of proteasis (IP) are related to a significant progression from osteopenia to osteoporosis, in IP treated patients the risk of osteoporosis increases by 1.6 times respect to untreated patients.

From April 2012 to January 2013, we evaluated 20 HIV-positive naïve patients, no HBV-HCV co-infect (19 males, age range 21-42 years, mean 31.8 + 4.8, BMI 23.5 + 3.8).

In baseline, all patients underwent weight, height, waist circumference, BMI, creatinine, albumin, calcium, phosphorus, total ALP, intact PTH (iPTH), 25 OH vitamin D (25 OH D3) evaluations; MOC DEXA and Vertebral Morphometry (VM) absorbimetric were also performed with evaluation of Spine Deformity Index (SDI).

At present 10 (9 males, age range 24-40 years, mean 30.9 + 4.5, BMI 23.7 + 3.6) out of 20 (50%) patients have completed follow up at six months, 8 patients have completed two months follow up , the remaining two were lost during the follow-up.

At six month follow up 10 patients performed: weight, height, waist circumference, BMI, creatinine, albumin, calcium, phosphorus, total ALP, intact PTH, 25 OH D3 evaluations.

In all 10 patients basal 25 OH D3 levels were very low (17.9 + 6.1 ng / ml), whereas creatinine, albumin, calcium, phosphorus, total ALP, iPTH levels were in the normal range. At MOC DEXA, 5 patients were osteopenic and 5 were normal. At VM, in one patient was observed a SDI =7 , that result from 5 fractures ranged from 20%-25% and 1 fracture ranged from 25%-40%. In this patient the MOC was normal.

In all 10 patients colecalciferol replacement therapy was stared at a dose of 100,000 IU/month orally for 3 months, then the dose was reduced to 25,000 IU/month.

At six month of treatment, 25 OH D3 levels increased (28.6 + 16.1 ng / ml), however they persisted under the normal range (p=0,086).

Our data confirm a reduced bone mineral density in HIV positive untreated patient despite young age. We suggest VM as first strumental diagnostic approach in association to MOC DEXA in the routinary bone metabolism screening, because the MOC DEXA alone could misunderstand the real state of the bone in HIV-positive patients. So as to establish appropriate and early treatment.

Finally, the achievement of normal serum 25 OH D3 levels occurs in a longer time than the healthy population, despite high doses.

PP290

CINACALCET IN MILD PRIMARY HYPERPARATHYROIDISM: A MONOCENTRIC STUDY

D. Tolomio[1], M. Battocchio[1], A. Rebellato[1], E. Zanchetta[1], F. Dassie[1], C. Martini[1], P. Maffei[1], R. Vettor[1], E. De Carlo[1]

[1]Clinica Medica III, DIMED - Padova

In primary hyperparathyroidism (PHPT) setting, parathyroidectomy is at present the only curative treatment by removing hyperfunctioning tissue. However, in PHPT patients with contraindications to surgery or previous unsuccessful parathyroidectomy, medical therapy with calcimimetics may be an option to control the effects of parathormone (PTH) excess. As reported in literature, cinacalcet has been suggested to be effective in decreasing hypercalcemia and PTH concentrations in non-operable PHPT patients.

We report herein our experience with cinacalcet in 26 caucasian patients with PHPT (4 males, 22 females; mean age 66) included in this study because of their persistent PHPT associated to elevated surgical risk (7), uncertain tumour localization (8), high probability of surgical failure (1), disease recurrence after surgery (5), PHPT in the context of multiple endocrine neoplasia type 1(4), surgery refusal (1). Biochemical data including PTH and serum calcium levels were collected before treatment with cinacalcet (time 0) and after 3-6-12-24 months. Mean follow-up was 13.7 months. Cinacalcet dosage was adjusted depending on the degree of calcemia reduction and drug tolerance. At time 0, 13/26 patients were symptomatic for hypercalcemia and 8/26 referred previous renal colics. Starting cinacalcet dose was 30 mg per day. After 12 months mean cinacalcet dose was 38.57 mg per day; one patient required the maximal dosage allowed (180 mg per day). At baseline mean PTH and calcium levels were 275.84 pg/mL (ULN 3.75) and 10.62 mg/dL (n.v.< 10.50 mg/dL), respectively. After 6 months: PTH 197.13 pg/mL (ULN 2.60), calcium 10.11 mg/dL. After 12 months: PTH 164.87 pg/mL (ULN 2.71), calcium 9.89 mg/dL. After 24 months: PTH 151.5 pg/mL (ULN 3.00), calcium 9.86 mg/dL. Before starting cinacalcet therapy, all patients had pathological calcium levels and after a 12 months therapy 71% of them normalized serum calcium. As expected, most of our patients maintained abnormal PTH values. Cinacalcet administration was stopped in 8 patients for different adverse effects such as face dermatitis and neck limphoedema, asthenia, dyspepsia, nausea or vomiting, diarrhoea. In 2 patients treatment was reintroduced without disturbance. During the study one patient decided to undergo surgery previously refused.

Our study confirms that cinacalcet could be a valid option in medical management of selected PHPT subjects. In mild hypercalcemia, we observed that cinacalcet dosage of 30 mg/day was generally effective.

PP291

BONE,GLUCOSE METABOLISM AND BONE MARROW ADIPOSE TISSUE IN TWO RAT MODELS OF HYPERCORTISOLISM

V. Camozzi[1], C. Franzin[2], P. Ballanti[3], A. Calcagno[4], C. Pagano, R. Vettor, G. Luisetto

[1]Dipartimento di Medicina, Università di Padova - Padova, [2]Fondazione Città dellaSperanza - Padova, [3]Dipartimento di Medicina Sperimentale, Università la Sapienza - Roma, [4]Dipartimento di Medicina - Padova

Ovariectomy, immobility or glucocorticoid treatment (GC) show an increase of adipose tissue in the bone marrow, due to a dynamic imbalance between adipogenesis and osteoblastogenesi. Recently it was found that osteocalcin is inversely correlated to insulin resistance, suggesting a relationship between bone and glucose metabolism. We aimed to evaluat the effects of endogenous (End-HyC) and exogenous hypercortisolism (Exo-HyC), considering glucose and bone metabolism and the changes in bone marrow adipose tissue (BMAT). We studied 24 Zucker rats: eight fa/fa genetically obese were accounted for End-HyC (OB), eight lean FA/FA were treated with methyprednisolone, 7 mg / kg by s.c. weekly for 6 weeks (LM), and eight lean FA/FA were the controls (LC). At baseline and 6 weeks after, fasting glucose and insulin, osteocalcin (BGP) and serum cross laps (CTX) were evaluated. After sacrifice, on femural sites were calculated the variables of bone remodeling by the histomorphometric analysis and the mRNA expression of leptin by Real Time-PCR; bone marrow adipose tissue was quantified by imaging analysis. Results: BGP and CTX were reduced by 70% in LM compared to OB and to LC ($p < 0.05$) while insulin was significantly increased and comparable in OB and in LM ($p < 0.001$ vs controls). Glucose was similar in the three groups. The histomorphometric parameters showed a reduction of all the remodeling variables only in LM ($p < 0.005$). Leptin was increased in OB and LM ($p < 0.05$ vs LC). The bone marrow adipose tissue was increased by 15% in the OB and 35% in the LM.

Conclusions: The increased BMAT was associated with the decrease of the histomorphometric variables only in LM. Insulin-resistance was similar in both groups, mimicking leptin levels, while osteocalcin was reduced only in LM. It seems that the insulin-resistance is independent of osteocalcin. A possible explanation may be given by leptin resistance in obese rats. Only the activated osteocalcin is able to stimulate the production and release of insulin from the pancreas: an increase of osteoblastic activity due to leptin resistance, could lead to an increase of osteoprotegerin, with reduction of bone resorption, which in turn cause a lower production of activated osteocalcin.

PP292

THE ROLE OF AIP GENE IN FAMILIAL PRIMARY HYPERPARATHYROIDISM

F. Saponaro[1], S. Borsari[1], E. Pardi[1], C. Banti[1], E. Vignali[1], A. Meola[1], A. Picone[1], M. Mastinu[2], S. Mariotti[2], C. Marcocci[1], F. Cetani[1]

[1]Dipartimento di Medicina Clinica e Sperimentale, Università di Pisa - Pisa, [2]Unità di Endocrinologia, Dipartimento di Scienze Mediche, Politecnico di Monserrato, Università di Cagliari - Cagliari

Primary hyperparathyroidism (PHPT) occurs as part of hereditary syndromes in <10% of cases, including multiple endocrine neoplasia types 1 and 2A (MEN1 and MEN2A), hyperparathyroidism-jaw tumor syndrome (HPT-JT) and familial isolated hyperparathyroidism (FIHP).

MEN 1 is an autosomal dominant disorder characterized by tumours in multiple endocrine glands, most commonly parathyroid, enteropancreatic and anterior pituitary glands. To date MEN1 gene germline mutations have been identified in 70-80% of MEN1 kindreds. FIHP has a heterogeneous molecular ethiology, since germline mutations of MEN1, HRPT2 and CASR genes have been reported. Germline mutations of the aryl hydrocabon receptor interacting protein (AIP) gene, responsible for 15-25% of familial isolated pituitary adenoma (FIPA) syndrome, have been recently reported in a MEN1 case.

The aim of this study was to address the role of AIP gene in the etiology of PHPT in 22 MEN1 and 14 FIHP kindreds. All MEN1 kindreds were negative for MEN1 gene mutations and all FIHP families were negative for MEN1, HRPT2, CASR mutations at genetic testing.

Genomic DNA from index cases was analyzed by PCR amplification of the entire coding region and splice sites and direct sequencing by a 16-capillaries automatic sequencer.

Two germline mutations in exon 1 of AIP gene were detected in two MEN1 probands, namely Arg9Gln (c.26G>A) and Arg16His (c.47G>A). Both mutations have already been reported, Arg9Gln in an acromegalic patient, and Arg16His in several FIPA families and patients with apparent sporadic pituitary adenoma. R9Q variant has been described to cause a significant increase in proliferation in cell cultures, while the pathogenetic nature of R16H is still under investigation since it has been identified in few healthy subjects and in some families seems not to segregate with the disease.

Our results suggest that germline AIP mutations may be involved, although rarely, in parathyroid tumorigenesis.

PP293

THE ROLE OF THE CDKN1B GENE IN HEREDITARY PRIMARY HYPERPARATHYROIDISM

E. Pardi[1], S. Borsari[1], F. Saponaro[1], C. Banti[1], N. Pellegata[2], M. Lee[2], E. Vignali[1], A. Meola[1], M. Mastinu[3], S. Mariotti[3], C. Marcocci[1], F. Cetani[1]

[1]Dipartimento di Medicina Clinica e Sperimentale, Università di Pisa - Pisa, [2]Institute of Pathology, Hemholtz Zentrum Munchen-German Research Center for Enviromental Health - Neuherberg, Gemany, [3]Unità di Endocrinologia, Dipartimento di Scienze Mediche, Politecnico di Monserrato, Università di Cagliari - Cagliari

Primary hyperparathyroidism (PHPT) occurs as part of hereditary syndromes in <10% of cases, including multiple endocrine neoplasia types 1 and 2A (MEN1 and MEN2A), hyperparathyroidism-jaw tumor syndrome (HPT-JT) and familial isolated hyperparathyroidism (FIHP). MEN 1 is an autosomal dominant disorder characterized by tumours in multiple endocrine glands, most commonly parathyroid, enteropancreatic and anterior pituitary glands. To date, germline mutations in the *MEN1* gene have been identified in 70-80% of *MEN1* kindreds. FIHP has a heterogeneous molecular ethiology, since germline mutations in *MEN1*, *HRPT2* and *CASR* genes have been reported. Recently, germline mutations of cyclin dependent kinase inhibitor 1B (*CDKN1*B) gene, encoding the p27 protein, have been identified in 8 kindreds with MEN1 syndrome which were negative to the MEN1 genetic screening. The aim of this study was to perform a genetic screening of *CDKN1B* gene in patients with MEN1 syndrome and FIHP (33 and 17, respectively). All MEN1 and FIHP probands were negative for MEN1 gene mutations at genetic testing. Genomic DNA from index cases was analyzed by PCR amplification of the entire coding region and splice sites, and direct sequencing was performed by a 16-capillaries automatic sequencer. A novel frameshift germline mutation in *CDKN1B* gene, c.372_373delCT/p.Asn124AsnfsX2, was identified in a MEN1 proband. A construct expressing p27_c.372_373delCT was generated to assess the functional properties of the mutant protein *in vitro*. Indirect immunofluorescence demonstrated that the mutant protein is mainly retained in the cytoplasm, affecting the cell cycle inhibitory function of p27 in the nucleus. Our results confirm that germline CDKN1B mutations are involved, althought rarely, in parathyroid tumorigenesis

PP294

DOES VITAMIN D DEFICIENCY INFLUENCE THE RATE OF PRESURGICAL LOCALIZATION IN PRIMARY HYPERPARATHYROIDISM?

L. Gianotti[1], F. Tassone[1], C. Baffoni[1], M. Pellegrino[1], S. Cassibba[1], G. Magro[1], F. Cesario[1], G. Borretta[1]

[1]SC Endocrinologia, Diabetologia e Metabolismo, ASO S.Croce e Carle - Cuneo

Objective: In primary hyperparathyroidism (pHPT) vitamin D deficiency (VDD) is reported to be inversely associated with PTH /calcium levels, more severe bone disease and higher weight or volume of parathyroid adenomas. However it is unknown whether VDD may influence the presurgical localization of adenomas.

Methods: 221 consecutive pHPT patients (mean ± S.D.: age = 60.1±13.4 years; male/female = 50/171; PTH = 214.1±204.5 ng/ml, serum calcium = 11.7±1.28 mg/dl; ionized calcium = 1.46±0.19 mmol/l, 25OHD = 29.8±21.1 ng/ml) were studied. In all patients 25-hydroxyvitamin D levels were measured and parathyroid ultrasound (US) and/or sestaMIBI scan (SC) were performed. VDD was defined as 25-hydroxyvitamin D levels < than 20 ng/ml. In 190 patients both US and SC were performed, in the remaining patients US or SC only was available.

Results: VDD was present in 37.1% of pHPT patients. Those with VDD showed higher levels of PTH (p=0.0002), total (p=0.011) and ionized calcium (p=0.002) compared with patients without VDD. No significant difference in the rate of presurgical localization was present between pHPT patients with VDD or without VDD, in particular in 221 patients who underwent US or SC, a positive localization was found in 79.1% of patients without VDD vs 78.1% with VDD (p =0.86); in 190 patients who underwent both US and SC, a positive localization was found in 84.6% without VDD vs 80.8% with VDD (p =0.55); among patients who had US only (n=214), 66.2% without VDD had positive localization vs 63% with VDD (p=0.66), while among those with SC only (n=197 patients) a positive localization was found in 70.7 % without VDD vs 63.5 % with VDD (p = 0.34).

Conclusions: VDD is frequently detected and negatively affects biochemical features in pHPT; however, the recognition of VDD in pHPT does not improve the probability of presurgical localization and, ultimately, does not affect the kind of surgical approach in pPHPT.

PP295

CINACALCET THERAPY IN THE MANAGEMENT OF SPORADIC PRIMARY HYPERPARATHYROIDISM: THE ITALIAN EXPERIENCE.

F. Saponaro[1], A. Faggiano[2], F. Grimaldi[3], G. Borretta[4], M. L. Brandi[5], S. Minisola[6], A. Frasoldati[7], E. Papini[8], A. Scillitani[9], C. Banti[1], M. Del Prete[2], F. Vescini[3], L. Gianotti[4], L. Cavalli[5], E. Romagnoli[6], A. Colao[2], F. Cetani[1], C. Marcocci[1]

[1]Dipartimento di Medicina clinica e sperimentale - Pisa, [2]Dipartimento di Endocrinologia ed Oncologia Molecolare e Clinica, Universit a di Napoli Federico II - Napoli, [3]SOC di Endocrinologia e Malattie del Metabolismo, Azienda Ospedaliero-Universitaria Santa Maria della Misericordia di Udine - Udine, [4]Endocrinologia e Malattie del Ricambio, ASO S. Croce e Carlo - Cuneo, [5]Dipartimento di Medicina Interna, Universit a di Firenze - Firenze, [6]Dipartimento di Medicina Interna, Universit a degli Studi di Roma Sapienza - Roma, [7]Unità Operativa di Endocrinologia, Arcispedale Santa Maria Nuova - Reggio Emilia, [8]Dipartimento di Malattie Endocrine, Metaboliche e Digestive, Ospedale Regina Apostolorum - Albano Laziale, [9]Unità Operativa di Endocrinologia Ospedale Casa Sollievo della Sofferenza - San Giovanni Rotondo, Foggia

The objective of this study was to report the Italian experience on cinacalcet use following its approval by the European Medical Agency (EMA) to control hypercalcemia in patients with sporadic primary hyperparathyroidism (sPHPT). We retrospectively evaluated 100 patients sPHPT followed in 9 Italian centres. The following parameters were recorded in all patients at baseline and during the follow up (1-26 months): albumin-adjusted serum calcium, PTH, 25OHD, daily cinacalcet dose and adverse events. High surgical risk (34%) negative preoperative imaging (19%), control of hypercalcemia before parathyroidectomy (PTx) (24%), and refusal of PTx (19%) accounted for cinacalcet prescription in 96% of patients. Serum calcium decreased from 2.90±0.27 mmol/L to 2.55±0.22 mmol/L at the final observation (p<0.0001) (-12 %) and 65 patients became normocalcemic. Serum PTH significantly declined (-22%) from 18 pmol/L (IQ range 12, 28) to 14 pmol/L (10, 22) (p=0.038). The initial daily cinacalcet dose ranged between 15 and 90 mg. The large majority of patients was given either 30 (n=62) or 60 (n=33) mg cinacalcet daily. The final dose varied between 15 and 120 mg daily. Treatment was well tolerated: upper gastrointestinal side effects were reported by 15 patients and slight symptomatic hypocalcemia occurred in one patient. This study demonstrated that there a wide heterogeneity in the prescription of cinalcalcet in Italy. Cinacalcet is effective in reducing serum calcium in patient with sPHPT and well tolerated

PP296

TREATMENT WITH DENOSUMAB IN PATIENTS WITH SEVERE OSTEOPOROSIS: OUR EXPERIENCE

L. Ceccoli[1], V. Ronconi[1], M. Marcheggiani[1], F. Turchi[1], M. Boscaro[1], G. Giacchetti[1]

[1]CLINICA DI ENDOCRINOLOGIA-UNIVERSITA' POLITECNICA DELLE MARCHE - ANCONA

Denosumab is a human anti-RANKL antibody that inhibits osteoclastogenesis, osteoclast number and function. According to literature, this treatment improves bone mineral density (BMD) at all skeletal sites and decreases fracture risk. Aim of this study was to evaluate the effect of denosumab on BMD and markers of bone turnover in postmenopausal women with osteoporosis eligible for treatment with denosumab according to the AIFA criteria and referred to our specialized centre of Endocrinology in the last year. We studied 31 postmenopausal women (mean age 77,9 yrs) at high risk of fracture. All patients had at least one vertebral fracture, 6,4% had also hip fracture; 13,3 % of patients had a history of parent's hip fracture, 3,2 % took 3 or more units of alcohol daily, 3,2% currently smoked tobacco, 9,6% had secondary osteoporosis. In the 12 months prior to the study, 11 patients had taken bisphosphonates, 8 teriparatide, 5 strontium ranelate and 7 used no medication. Half of the patients assumed calcium supplementation and nearly all of them (96%) used vitamin D. We evaluated biochemical parameters and markers of bone turnover (carboxy terminal telopeptide of collagen type I -CTX and bone alkaline phosphatase- bALP) at baseline, after 3 and 6 months of treatment with denosumab. Risk fracture was evaluated using FRAX® tool. BMD, evaluated at lumbar spine, femoral neck and total hip was performed by dual-energy X-ray absorptiometry at baseline and after 6 months. Morphometric evaluation was made at baseline, while the occurrence of new clinical fractures was established at follow up. CTX levels significantly decreased after treatment (respectively p=0,0007at three months and p=0,002 at 6 months) and bALP tended to be lower at follow up, despite similar and optimal 25-OH vitamin D levels and independently of previous medical therapy. At follow up we observed a significant improvement of BMD and T score values at the three skeletal sites (p<0,05), except for BMD at femoral neck that was not statistically significant (p=0,06). The 10-year probability of hip fracture and the 10-year probability of a major osteoporotic fracture evaluated by FRAX tool was respectively 16,6% and 27,3% at baseline. Although not statistically significant, risk fracture decreased at follow up. No patients developed new clinical fractures. In conclusion, short term treatment with denosumab is associated with inhibition of bone reabsorption and marked improvement of bone mineral density, regardless previous therapy for osteoporosis. In addition, in our experience, the treatment appears to be safe and well tolerated.

PP297

ATYPICAL FEMORAL FRACTURE IN A PATIENT WITH HORTON ARTHERITIS: EARLY CLINICAL FINDINGS AND BONE RADIOGRAPHIC EVIDENCE

P. Franceschetti[1], S. Lupo[1], V. Lorusso[2], L. Massari[2], E. C. degli Uberti[1], M. R. Ambrosio[1]

[1]Section of Endocrinlogy, University of Ferrara - Ferrara, [2]Section of Orthopedy; University of Ferrara - Ferrara

In the last 25 years bisphosphonates (BPs) have became the first-line therapy for osteoporosis. In recent years, femoral atypical fractures (AF) have been described in patients on BP therapy, generating interest and anxiety as a possible clinical manifestation of a "frozen bone" due to bone turnover suppression and bone strength reduction. These fractures have peculiar and striking clinical and radiological features with already identified major and minor diagnostic criteria. Herein we report clinical, biochemical and radiological findings of a patients with Horton arthritis and few risk factors for bone fractures who developed a spontaneous subtrochanteric fracture that could have been avoided 2 months before. The patient is a 82-year-old woman with a history of Horton arthritis with prednisone usage since 2007, anemia due to iron deficiency and arthropathy in multiple joints. She had been treated with weekly oral risedronate, calcium and colecalciferol for 6 yr. Two months before the fracturative event she developed a persistent pain to the back and to the right thigh without any history of trauma. The pain worsened with standing and did not respond to analgesics, so that the patient could hardly walk. She performed a computerized axial tomography of spine and femur that showed herniated discs in L3, L4, L5 , degenerative arthropathy from L3 to L5 and thickening of the right diaphyseal cortex. One month later a diaphyseal fracture occurred without trauma. X-rays showed metadiaphyseal femoral fractures, confirmed by nuclear bone scintigraphy and magnetic resonance imaging. Fracture was stabilized by intramedullary rod fixation. Evaluation for secondary causes of skeletal fragility was undertaken. We found hyperglicemia before and during the hospital stay, normal levels of serum and urinary calcium; serum 25- hydroxyvitamin D levels was 35 ng/ml and PTH was 51 pg/ml. Thyroid function was normal. She had BMD measured by DXA with T score<-2.5. X-rays and bone scintigraphy did not show a bilateral femoral involvement. She had a marked suppression of bone turnover markers. The patient here described displays all the features previously described in patients with subtrochanterc/diaphyseal femur fractures treated with bisphosphonates. Our patient had few risk factors, the most important being Horton arthritis and use of glucocorticoid. Our data support the role of clinical findings and bone radiographic evidence in the early diagnosis of atypical fractures.

PP298

VITAMIN D STATUS IN PATIENTS AFFECTED WITH MULTIPLE SCLEROSIS

M. Filippella[1], C. Musso[2], A. Faggiano[3]

[1]Medicina Fisica e Riabilitativa - Aosta, [2]Neurochirurgia - Ivrea, [3]Medicina e Chirurgia - Napoli

Introduction: Vitamin D has been attributed immunomodulatory, anti-inflammatory effects as well as neuroprotective capacity which implicates a possible role of vitamin D in autoimmune diseases like multiple sclerosis (MS). MS is a chronic inflammatory demyelinating disease of the central nervous system with a disabling progressive course and is complicated by bone loss and fractures. MS is known to be associated to low levels of vitamin D. This study was conducted to determine vitamin D status in MS patients and its correlation with disease severity and duration as well as with patients' characteristics.

Methods: Fifteen consecutive patients affected with MS were enrolled (mean age 43.1±1.9 yrs, 5 F and 9 M). BMI was measured as the ratio between the weight and the square of the height. 25-hydroxy-vitamin D (25-OHD) serum concentrations were measured by direct radioimmunoassay (>30 ng/ml, normal values; 20 - 30 ng/ml, insufficiency; <20 ng/ml deficiency). Duration and severity of disease were also considered.

Results: The mean serum 25-OHD concentration for the total population was 11.7±1.3 ng/ml. All patients had a condition of vitamin D deficiency, independently by age, gender, BMI and disease duration. 25-OHD serum levels were inversely associated with disease activity.

Conclusion: These findings show that vitamin D deficiency is highly prevalent and severe in MS patients. This condition is independent by age, gender, BMI and disease duration, but it strongly related with disease severity. A program of vitamin D supplementation in these patients would be of great benefit to improve neuromuscular function and quality of life.

PP299

OSTEOCALCIN: A POSSIBLE LINK BETWEEN GLUCOSE METABOLISM, CARDIOVASCULAR RISK AND BONE IN CUSHING'S SYNDROME

V. Camozzi[1], N. Albiger[2], M. Barbot[2], M. L. Zilio[2], G. Luisetto[2], F. Mantero, C. Scaroni

[1]Dipartimento di Medicina, Università di Padova - Padova, [2]Dipartimento di Medicina - Padova

Osteocalcin bone-derived protein, a marker of bone formation involved in bone mineralization, has raised much attention as a hormone regulating glucose metabolism. Recent studies has also demonstrated that osteocalcin levels are inversely associated both with glucose impairment and atherosclerosis parameters in patients with type 2 diabetes. Glucocorticoids excess cause insulin resistance, diabetes, dyslipidemia and increased cardiovascular risk. In addition glucocorticoids induced osteoporosis is characterized by low bone formation, which in turn leads to low levels of osteocalcin. Purpose of the study was to evaluate the relationship between osteocalcin, glucose and lipid metabolism, cardiovascular risk and bone mineral density , in a group of 22 women with ACTH-dependent Cushing's Syndrome (mean age 40.73±12.87 y) during the active phase of the disease. Patients were subjected to sampling for fasting blood glucose, insulin, total cholesterol, LDL, HDL and osteocalcin (BGP). The carotid artery intima-media thickness (IMT) was measured by B-mode ultrasound bilaterally at the level of the carotid bulb, the common carotids and the common femoral arteries. Bone mineral density, expressed as Z-score, was assessed at lumbar spine and left femur using a Hologic QDR 4500 C densitometer. At study entry, overweight or obesity were found in 50% of patients (Pz), 72% of Pz had moderate/severe arterial hypertension. Five Pz (25%) had overt diabetes and about 50% had hypercholesterolemia. Wall carotid plaques were detected in 6 patients. One Pz showed a symptomatic atraumatic vertebral fracture and about 30% of Pz showed a Z-Score lower than -1 at any measured site. Osteocalcin levels were inversely correlated only with IMT measured at the carotid bulb, which in turn was inversely correlated with the lumbar spine Z-score.

Since intima-media thickness (IMT) has been shown to be a significant predictor of coronary disease, our data suggest that a low osteocalcin level can be not only an index of reduced bone formation, but also an index of increased cardiovascular risk in patients suffering from endogenous glucocorticoids excess. The lack of correlation between osteocalcin and the parameters of glucose metabolism might be due to the low number of patients and our findings need to be confirmed on a larger series.

PP300

OBESITY YIELDS SITE-SPECIFIC PROTECTIVE EFFECTS ON POSTMENOPAUSAL BONE LOSS: A POTENTIAL ROLE FOR SCLEROSTIN AND LEAN BODY MASS

P. Marzullo[1], G. Guzzaloni[1], C. Ferrari[2], S. Mai[3], M. Rinaldi[1], S. Maestrini[3], M. A. Tagliaferri[1], M. Scacchi[1], G. Aimaretti[4], A. Liuzzi[1]

[1]Division of General Medicine, Ospedale S. Giuseppe, I.R.C.S.S. Istituto Auxologico Italiano - Verbania, [2]Division of Internal Medicine, Università del Piemonte Orientale "A. Avogadro" - Novara, [3]Laboratory of Molecular Biology, Ospedale S. Giuseppe, I.R.C.S.S. Istituto Auxologico Italiano - Verbania, [4]Department of Translational Medicine, Università del Piemonte Orientale "A. Avogadro" - Novara

Opposed to the opinion that obesity protects bone, it is emerging that postmenopausal obese poses a risk for nonvertebral fractures. The role of sclerostin and body composition in this context is unclear.

The effect of obesity and menopause on bone mineral density (BMD) was studied in 28 premenopausal (44.7±3.9 yrs; 46.0±4.2 kg/m2) and 28 postmenopausal women (55.5±3.8 yrs; 46.1±4.8 kg/m2), by dual X-ray absorptiometry (DXA), sclerostin levels, bone turnover markers, glucose metabolism, and a panel of bone-active hormones.

Bone turnover markers CTX and NTX were increased in postmenopausal obese women devoid of changes in osteocalcin, vitamin D, PTH, glucose tolerance, and somatotroph, thyroid and adrenal function compared to premenopause. Sclerostin levels increased nonsignificantly with menopause (18.1±6.3 vs. 21.4±8.6 pmol/L). DXA documented no detriment of menopause on lumbar spine BMD, while menopause significantly reduced BMD at total hip ($p<0.0005$), femoral neck ($p<0.0001$), and total skeleton ($p<0.005$). Total body BMD positively correlated to lean body mass ($p<0.001$) and BMI ($p<0.05$), while being opposed to fat accumulation ($p<0.05$). By multivariate analysis, sclerostin levels were independent predictors of lumbar spine BMD ($p<0.001$), while menopause and lean body mass independently predicted BMD at total hip ($p<0.001$ and $p<0.05$), femoral neck ($p<0.001$ and $p<0.05$) and total body ($p<0.001$ for both).

Our findings suggest that severe obesity is protective on lumbar spine BMD, while being unable to prevent appendicular bone loss associated with menopause. In such setting, sclerostin status may intervene to regulate bone turnover rates in specific sites, in concert with lean body mass and hormone determinants.

PP301

SHORT-TERM BLOOD PRESSURE VARIABILITY IS INCREASED IN CUSHING'S SYNDROME

A. Rebellato[1], A. Grillo[2], F. Dassie[1], N. Sonino[3], C. Martini[1], A. Paoletta[4], B. Fabris[2], R. Carretta[2], P. Maffei[1], F. Fallo[1]

[1]Dept of Medicine - University of Padova, [2]Dept of Medical, Surgical and Health Sciences - University of Trieste, [3]Dept of Statistical Sciences - University of Padova, [4]Endocrine Outpatient Service - Cittadella Civic Hospital

Cushing's syndrome is associated with high cardiovascular morbidity and mortality. Blood pressure (BP) variability within a 24-hour period is increasingly recognized as both a marker and a risk factor for cardiovascular disease, either in hypertensive cohorts or in general populations. The aim of our study was to investigate short-term systolic (SBP) and diastolic (DBP) variability indexes in Cushing's syndrome. Twenty-one newly diagnosed patients with Cushing's syndrome (18F, 3M; mean age 48±13 years; 17 pituitary-dependent Cushing's disease and 4 adrenal adenoma) underwent 24-hour ambulatory BP monitoring (ABPM) and evaluation of cardiovascular risk factors. Based on ABPM, patients were divided into 8 normotensive (NORCUSH) and 13 hypertensive (HYPCUSH) patients, and were compared with 16 normotensive age-, sex-, BMI- and ABPM-matched control subjects (NORCTR). Short-term SBP/DBP variability was derived from ABPM and calculated as the following: (1) Standard Deviation (SD) of 24-hour, daytime, and nighttime SBP/DBP; (2) weighted SD of 24-hour SBP/DBP; (3) average real variability (ARV), i.e., the average of the absolute differences between consecutive SBP/DBP measurements over 24 hours. In comparison with controls, patients with Cushing's syndrome, either normotensive or hypertensive, had higher SD of 24-hour, daytime, and nighttime SBP/DBP, as well as higher weighted SD and ARV of 24-hour SBP/DBP (NORCUSH and HYPCUSH vs NORCTR, P <0.0001 for all indexes). There was no difference between NORCUSH and HYPCUSH in urinary cortisol levels, in absolute BP and variability BP measures, and in the prevalence of diabetes, hyperlipidemia and obesity. Conclusion: Short-term BP variability is increased in patients with Cushing's syndrome, independent of BP elevation. It may represent an additional cardiovascular risk factor in this disease. The role of excess cortisol in BP variability has to be further clarified.

PP302

AUTOANTIBODIES TO INTERFERON-OMEGA IN PATIENTS WITH AUTOIMMUNE POLYENDOCRINE SYNDROME TYPE 1

M. d. P. Larosa[1], R. Mackenzie[1], S. Garelli[2], S. Barollo[2], S. Masiero[2], R. Zanchetta[2], S. Chen[1], J. Furmaniak[1], B. Rees Smith[1], C. Betterle[2]

[1]FIRS Laboratories, RSR Ltd - Cardiff, [2]Department of Medicine, Endocrine Unit, University of Padova - Padova

Objective: To assess the prevalence of autoantibodies (Abs) to interferon omega (IFN-ω) in Italian patients with autoimmune polyendocrine syndrome type 1 (APS type 1) and autoimmune adrenal disease.

Design: IFN-ωAbs was measured in an immunoprecipitation assay (IPA) using 35S-labelled full-length IFN-ω.

Methods: 69 patients with APS type 1 (54 with known AIRE gene mutations on both alleles, 4 with only one mutation detectable, 8 negative for AIRE gene mutations and 3 under evaluation) with a mean duration from the first manifestation of 18 years (range 1-57), 57 patients with APS type 2, 36 patients with isolated autoimmune Addison's disease (AD) or APS type 4, and 28 patients with non-adrenal autoimmune diseases were studied.

Results: 62/69 (90%) of patients with APS type 1 patients had detectable IFN-ωAbs in the IPA. Out of 62 IFN-ω Abs positive patients, 53 had AIRE gene mutation on both alleles, 3had only one mutation detectable, 4 were negative and 2 under evaluation; the majority were point substitution; the mean duration from the first disease was 18 year (range 1-57). In 7/69 IFN-ωAbs negative patients, AIRE gene mutations were found in 4 patients (all point substitution), negative in 2 patients and under evaluation in 1; the mean duration from the first disease was 20 years (range 5-34). 1/57 (1.7%) patients with APS type 2 and 1/36 (2.8%) patients with isolated AD or APS type 4 were positive for IFN-ωAbs and these two patients are under evaluation for AIRE gene mutations. None of 28 patients with non-adrenal autoimmune diseases was positive.

Conclusions: In our study, IFN-ωAbs were highly prevalent in patients with APS type 1 while not detectable in patients with non-adrenal autoimmune diseases. IFN-ωAbs positivity was associated with AIRE gene mutations in APS type 1 patients. Measurement of IFN-ωAbs should be helpful in diagnosis of APS type 1.

PP303

A THREE GENERATION FAMILY WITH LOW CORTISOL, CBG DEFICIENCY, CHRONIC FATIGUE AND PAIN, LIPOMATOSIS AND BEHAVIORAL ALTERATIONS

S. Moia[1], G. E. Walker[1], M. Roccio[1], R. Ricotti[1], E. Giglione[1], S. Bellone[1], F. Fanelli[2], G. Bona[1], F. Prodam[1]

[1]Dip. di Scienze della Salute Università del Piemonte Orientale A.Avogadro - Novara, [2]Dipartimento Medicina Clinica U.O. Endocrinologia Policlinico S.Orsola - Bologna

CBG is the main transport protein for glucocorticoids in blood. CBG gene is a member of the serine protease inhibitor family, located at chromosome 14q32. Inherited CBG deficiency is a rarely reported recessive disorder, and the phenotype associated includes low cortisol levels, presence of normal ACTH levels, hypotension and fatigue, although the exact pathophysiological mechanisms involved remain uncertain. We identified a family with a complex phenotype, that includes low free cortisol levels, chronic fatigue, pain and CBG deficiency, but without mutations in the coding parts of CBG gene.

Methods. We quantified the plasmatic concentration of CBG protein both in our family (n=9) than in a group of healthy controls (n=15) by using a commercial kit. Molecular analysis of four coding exons of CBG gene was performed by direct sequencing. Segregation analysis of parental alleles was performed through linkage analysis.

Results. Salivary and LC-MS/MS analysis identified very low free cortisol levels in two children and in the father, despite normal ACTH levels, with cortisol levels at the end of normal range in the sister and paternal grandfather. The maternal grandfather, the father and the two male children presented low plasmatic CBG levels. Paternal grandfather presented CBG levels at the end of the normal range. No mutations were identified in the CBG gene coding-regions. We identified only five SNPs, all at the heterozygous state. Linkage analysis identified a likely paternal transmission of the CBG alleles.

Conclusion. With the hypothesis that our family is affected by inherited CBG deficiency, molecular analysis of non-coding regions and functional studies of CBG gene will be performed. In addition, the involvement of supplementary gene-disease will be demonstrated by cGH array and exome sequencing analysis.

PP304

ANTIEPILEPTIC DRUG CARBAMAZEPINE CAN CAUSE ADRENAL INSUFFICIENCY IN CONGENITAL ADRENAL HYPERPLASIA ON HORMONAL REPLACEMENT THERAPY: CASE REPORT.

R. Gelsomino[1], I. Marturano[1], D. Restivo[2], C. Pace[1], M. L. Arpi[1], D. Leonardi[1], G. Parrinello[1], F. Calaciura[1], L. Sava[1], D. Gullo[1]

[1]Endocrinology, Department of Clinical and Molecular Biomedicine, Garibaldi-Nesima Hospital - Catania, [2]Neurology, Garibaldi-Nesima Hospital - Catania

Introduction. We first report the case of an adult male with 21-hydroxylase deficiency (21-OH CAH) in which treatment with carbamazepine induced hypoadrenalism despite increased glucocorticoid and mineralocorticoid dosage. Switching to an alternative anticonvulsant, levetiracetam, provided effective hormonal replacement within 5 weeks.

Case report. A 25-year-old man affected from 21-OH CAH was treated with fludrocortisone 0.1 mg/day and dexamethasone (DEX) 0.50 mg/day with good clinical control. After a long period without follow-up visits the patient appeared to us complaining excessive fatigue and inability to concentrate. ACTH, 17-OHPG, androstenedione and renin were raised indicating a state of hypoadrenalism. This condition persisted despite the increase in hormone replacement therapy dose. An effect of carbamazepine, started 3 years earlier because of epilepsy, on metabolism of steroid treatment was hypothesized. The neurologist suggested levetiracetam as an alternative for his different pharmacokinetics characteristics. Hormone levels gradually normalized after five weeks from the time of drug switch. ACTH levels fell from 105 to 24 pg/mL, 17-OHPG from 206.2 to 0.9 pg/mL, androstenedione from 28.2 to 1.6 ng/mL and renin from 120.8 to 53 pg/mL. The patient reported feeling better with symptoms much improved. **Conclusions.** This case highlights the importance of interactions between steroid medications and drugs that increase cytochrome CYP3A4 activity. Carbamazepine, one of the most widely used and effective drugs for epilepsy treatment, is a potent inducer of the activity of CYP3A4 which can accelerate the metabolism of many xenobiotics, such as DEX and fludrocortisone, causing a state of adrenal insufficiency. In this patient with 21-OH CAH five weeks after discontinuation of carbamazepine and introduction of an alternative anticonvulsant therapy with levetiracetam all affected blood parameters were within the desired therapeutic range. Failure to recognize this interaction may result in inappropriate replacement hormonal treatment and lead to serious clinical consequences of adrenal insufficiency in the face of trauma, severe illness, surgery, infectious or neoplastic disease.

PP305

TRYPTOPHAN HYDROXYLASE AUTOANTIBODIES AS MARKERS OF A DISTINCT AUTOIMMUNE GASTROINTESTINAL COMPONENT OF AUTOIMMUNE POLYENDOCRINE SYNDROME TYPE 1

R. Scarpa, R. Alaggio[2], L. Norberto[3], J. Furmaniak[4], S. Chen[4], B. Rees Smith[4], S. Masiero[1], L. Morlin, M. Plebani[1], F. De Luca[6], M. C. Salerno[6], R. Giordano[9], G. Radetti[5], L. Ghizzoni[10], G. Tonini[5], F. Farinati[3], C. Betterle[1]

[1]Dept. of Medicine - Padova, [2]Dept. of Pathology - Padova, [3]Dept. of Surgical and Gastroenterological Sciences - Padova, [4]FIRS Lab - Cardiff, [5]Dept. of Pediatrics - Bolzano, [6]Dept. of Pediatrics - Messina, [7]Dept. of Pediatrics - Napoli, [8]Dept. of Pediatrics - Trieste, [9]Dept. of Clinical and Biological Sciences - Torino, [10]Dept. of Internal Medicine - Torino

Autoantibodies to tryptophan hydroxylase (TPHAbs) directed against enterochromaffin cells (EC) have been reported in Autoimmune-Polyendocrine-Syndrome type 1 (APS-1) patients with gastrointestinal dysfunction (GID). Serotonin plays a critical role in enteric function and its circulating levels reflect serotonin release from the gastrointestinal tract. We test the hypothesis that TPHAbs marked a distinct autoimmune component of APS-1 characterized by an autoimmune attack towards EC, which results in clinical GID. Methods: TPHAbs were measured in 64 APS-1 patients. Endoscopy with gastric (antrum/body) and duodenal biopsy was carried in 16 TPHAbs+ patients (8 with and 8 without GID). Immunohistochemistry of biopsy specimens was carried out using antibodies to serotonin, chromogranin-A, CD3,CD4, CD8,CD20. Serotonin serum levels were measured in TPHAb+ patients who had endoscopy. Results: 37/64 patients were TPHAbs+ (11/12 with GID and 26/52 without GID; $p<0,001$). Gastric and duodenal biopsies in all 8 TPHAb+ patients with GID showed lymphocytic infiltration with increased CD3+/CD8+ intraepithelial lymphocytes and absence of EC. Mean serotonin serum levels were below the normal range in TPHAb+ patients with GID ($p<0,01$). In 8 TPHAb+ patients without GID gastric and duodenal biopsies showed different grades of inflammatory infiltration and reduced number of EC. Mean plasma serotonin levels were near the lower limit of the normal range. In all TPHAbs+ patients the biopsies showed a reduced number of chromogranin-A positive cells consistent with enteroendocrine cells depletion. Conclusions: TPHAbs appear to be markers of a distinct autoimmune component of APS-1. Progressive involvement of the gastrointestinal EC leads to the transition from preclinical to clinical disease, characterized by GID and reduced serotonin serum levels.

*Study supported by EU 7th Framework Progr.: Euradrenal, c.n. 2008 - 201167.

PP306

AUTOIMMUNE DISORDERS IN PATIENTS WITH ENDOGENOUS CUSHING'S SYNDROME: A MULTICENTER ITALIAN STUDY

F. Guaraldi[1], M. De Martin[2], C. Scaroni[3], M. L. Zilio[3], F. Pecori Giraldi[2], I. Karamouzis[1], S. Grottoli[1], E. Ghigo[1], E. Arvat[1]

[1]Div. of Endocrinology, Dept. Medical Sciences - Turin, [2]Dept. of Clinical Sciences and Community Health, University of Milan, Istituto Auxologico Italiano - Milan, [3]Endocrinology Unit, Dept. of Medicine DIMED, University of Padova - Padova

Introduction: new onset/exacerbation of autoimmune diseases (AID) has been reported after remission of Cushing's syndrome (CS), likely due to immune system reactivation after suppression induced by hypercortisolism. We evaluated prevalence and type of AID in pts with: 1) ACTH-omas and active CS vs other pituitary adenomas (PAs); 2) CS (various origins) during active disease vs remission. Materials and Methods: 116 pts (F:M = 95:21; age at diagnosis 41.2 ± 13.6 yr) with CS (23 adrenal, 89 pituitary, 4 ectopic) treated with surgical/medical/RT therapy, with active disease/after remission; 116 pts with other types of PAs (F:M = 95:21; age at diagnosis 42 ± 13.3 yr) from 3 tertiary care Italian Centers. Results: 35 CS pts (28 F) (25 pituitary, 2 ectopic, 8 adrenal) suffered from AID, single in 30 cases (1 fibromyalgia, 3 various types of AI arthritis; 1 Batelman's purpura, 1 vasculitis, 1 nodosum erythema; 2 dermatitis, 4 psoriasis, 1 lichenoid pitiriasis, 1 alopecia aerata, 1 seborrheic eczema; 1 IBD; 11 thyroiditis: 7 Hashimoto, 4 Grave's, 1 Riedel; 1 optic neuromyelitis), multiple in 5 cases (1 acute synovitis + thyroiditis; 1 cutaneous + arthritis psoriasis; 1 cutaneous lymphoma + IBD; 1 cutaneous and rheumatic SLE; 1 cholangitis + IBD + thyroiditis). AID appeared before CS in 12 cases: 3 resolved before CS, 5 persisted during CS' active phase and remission, 2 improved during active phase but worsened after CS' remission, 2 resolved after CS' remission. In 10 cases AID appeared in the active phase of CS: 9 persisted, 1 resolved after CS' remission. In 17 cases AID appeared only after CS' partial/complete remission. 40 PA pts (26 F) developed AID (3 rheumatoid arthritis, 1 spondylitis anchylosans; 1 eczematoid dermatitis, 1 vitiligo, 6 psoriasis; 1 celiac disease, 4 IBD; 1 vasculitis; 19 thyroiditis, 2 type I diabetes; 1 IgA glomerulonephritis). AID activity did not correlate with hormonal status. Conclusions: 1st comparative, largest study on AID in CS. AID's prevalence was similar in ACTH-dependent (33%) and independent (30.4%) CS. Frequency of AID was similar in pituitary CS and other PA. In both CS and PA,males were significantly more affected than females (number of affected/total: 21/42 for M, 54/190 for F); thyroiditis and psoriasis were the most prevalent. Hypercortisolism influenced AID's evolution as the great majority of new cases/exacerbation of AID happened after CS's remission (confirming previous data); hormonal status in other PAs did not affect AID's evolution.

PP307

AN UNUSUAL CASE OF CLINICAL HYPERCORTICISM

E. Zanchetta[1], M. Battocchio[1], G. Giordano[2], M. Ferrata[1], F. Fallo[1], E. De Carlo[1], R. Vettor[1], C. Martini[1]

[1]Clinica Medica III, DIMED - Padova, [2]Endocrinologia, Ospedale S. Giacomo - Castelfranco V.to

A 55 years-old woman referred to our clinic for a recent increase in the cervical fat pad. Her clinical history was significant for arterial hypertension, moderate aortic stenosis, vascular atherosclerotic disease, type 2 diabetes mellitus, autoimmune thyroiditis. The clinical examination revealed the typical somatic features of Cushing's syndrome: facial rubeosis, increased cervical fat pad, buffalo hump, purple abdominal striae, abdominal obesity. Laboratory tests showed: significantly reduced levels of ACTH, serum cortisol and UFC, mild fasting hyperglycaemia, normal Na^+ and K^+ serum concentrations. The patient denied any previous steroid consumption, but she reported a chronic application of a phytotherapic cream for the treatment of a sub-mammary intertrigo since 3 years. The active principles of the cream were 10% ethanol extract of Cardiospermum Halicacabum plant, 0.5% bisabolol, 0.3% 18beta-glycyrrhetinic acid. The patient developed deep asthenia and hypotension after the cream application was stopped. An ACTH stimulation test was thereafter performed showing no response of cortisol and DHEAS. Moreover normal PRL levels and gonadotropin elevation according to the menopause state were found. Determination of anti-adrenal gland antibodies resulted negative; abdominal CT and sellar RM didn't describe any adrenal gland or pituitary mass. Cosyntropin therapy (with Cortisone acetate for the first 4 months) allowed a progressive resolution of Cushing features, restoring a normal ACTH secretion but only a partial adrenal function. The presence of synthetic steroids in the cream was ruled out by HPLC. Cardiospermum halicacabum L. (family Sapindaceae) is an herbaceous climber widely distributed in tropical and subtropical Asia and Africa, largely used in the traditional medicine also because of its anti-inflammatory properties. It has been recently shown that C. halicacabum ethanol extract in mouse macrophage cell lines inhibits LPS induced COX-2, TNF-alfa and iNOS expression, mediated by NF-kB regulation, mimicking glucocorticoid action. Since the presence of phytosterols has been already documented in Cardiospermum plant extract, it is possible that their prolonged systemic absorption could have induced a pituitary-adrenal axis suppression.

PP308

HIGH FREQUENCY OF MORPHOLOGICAL ADRENAL ABNORMALITIES IN ADULT ITALIAN PATIENTS WITH CONGENITAL ADRENAL HYPERPLASIA

O. Prontera[1], A. Gambineri[1], V. Vicennati[1], C. Mosconi[2], E. R. Cosentino[3], E. Rinaldi[3], A. Balsamo[4], D. Rinaldini[4], R. Pasquali[5]

[1]Endocrinology Unit, Dept. of Clinical Medicine, S. Orsola-Malpighi Hospital, University of Bologna - Bologna, Italy, [2]Division of Radiology, S. Orsola-Malpighi Hospital - Bologna, Italy, [3]Department of Internal Medicine, Aging and Kidney Diseases, Internal Medicine Unit, S. Orsola-Malpighi Hospital - Bologna, Italy, [4]Department of Gynaecologic, Obstetric and Paediatric Sciences, S. Orsola-Malpighi Hospital of Bologna, University of Bologna - Bologna, Italy, [5]Endocrinology Unit, Dept. of Clinical Medicine, S. Orsola-Malpighi Hospital, University of Bologna, - Bologna, Italy

Congenital adrenal hyperplasia (CAH), most often due to 21-hydroxylase deficiency, results in inadequate cortisol production and subsequent possible adrenal structural compensatory alterations. However, no data are available on the prevalence of morphological adrenal abnormalities in patients with CAH. This study was therefore performed with the aim of estimating the prevalence of morphological adrenal abnormalities in a population of adults with CAH received from Paediatrics as part of a structured transitional program. Thirty-four subjects with CAH, 9 males (mean age: 29.6, rage: 19-46 yrs) and 24 females (mean age: 34.2, rage: 18-55 yrs) were enrolled. In 33 subjects CAH was due to 21-hydroxylase deficiency (13 salt-wasting; 6 simple-virilisation; 14 non-classic) and in 1 subject CAH was due to 11 ß -hydroxylase deficiency (A306V-/-). Most (29/33) subjects had been treated from infancy with glucocorticoidis and, eventually, mineralcorticoids. A CT scan of the abdomen was proposed to all subjects and an ambulatory 24-hour blood pressure measurement was performed.T he CT scan was not performed in 8 subjects for different reasons (epilepsy, pregnancy, etc.). Of the remaining 25 subjects who underwent the CT scan, 3 (12%, mean age 44.3 yrs) proved to be affected by a single adenoma, 8 (32%, mean age 26.8 yrs) by unilateral hyperplasia, and 5 (20%, mean age 30.2 yrs) by bilateral hyperplasia (overall frequency 64%). 24-h blood pressure was increased in 12.5% of subjects with unilateral hyperplasia, in 40% of subjects with bilateral hyperplasia, and in 22.2% of subjects with a normal adrenal morphology. No hypertension was found in the subjects with adrenal adenoma. The number of classic and non classic forms of CAH did not differ between subjects with and without morphological adrenal abnormalities. In conclusion, morphological adrenal abnormalities are frequently observed in classic and non classic forms of CAH, regardless of the time of initiation of steroid therapy. A CT scan of the abdomen should therefore be included in the screening of all subjects with CAH in adulthood.

PP309

VISCERAL ADIPOSITY INDEX AND METABOLIC PROFILE IN ADULT PATIENTS WITH CONGENITAL ADRENAL HYPERPLASIA: HYDROCORTISONE VERSUS PREDNISONE TREATMENT

C. Simeoli[1], A. Cozzolino[1], D. Iacuaniello[1], P. Vitale[1], M. Galdiero[1], M. C. Amato[2], C. Giordano[2], A. Colao[1], R. Pivonello[1]

[1]Department of Clinical Medicine and Surgery - Section of Endocrinology, Federico II University - Naples, Italy, [2]Section of Endocrinology and Metabolic Disease, DIBIMIS, University of Palermo - Palermo, Italy

Background: Patients with Congenital Adrenal Hypeplasia (CAH) due to 21-hydroxylase deficiency need a life-long therapy with glucocorticoids (GCs) and tend to have a cluster of metabolic risk factors, which are consistent with metabolic syndrome (MS). Most frequently used GCs are Hydrocortisone (HC) and Prednisone (P), different for both pharmacodynamic and pharmacokynetic characteristics.

Aim: The aim of this study was to evaluate the impact of HC and P on VAI, a new indicator of visceral fat function, and on metabolic profile in CAH patients long term treated with GC.

Materials and Methods: Thirty-two patients (22 F, 10 M, 18-46 yrs), among which 16 (11 F, 5 M) treated with HC (dose 10-45 mg/die) and 16 (11 F, 5 M) treated with P (dose 5-15 mg/die), were retrospectively enrolled in the study. VAI was calculated according to Amato and colleagues. Metabolic profile was evaluated measuring each component of MS, in line with IDF criteria, as well as the area under the curve (AUC) of glucose and insulin during 120 min oral glucose tolerance test (OGTT), the homeostasis model assessment of the insulin resistance index (HOMA-IR) and the insulin sensitivity index (ISI).

Results: Patients treated with P showed higher VAI ($p< 0.001$), waist circumference ($p=0,03$), triglycerides ($P<0,001$), fasting insulin ($p= 0,047$), AUC for insulin ($p=0,001$), HOMA-IR ($p=0,038$) and lower ISI ($p=0,038$) than patients treated with HC, whereas no significant difference was found in total cholesterol, LDL- and HDL-cholesterol, blood pressure, fasting glucose and AUC for glucose as well as in the prevalence of MS.

Conclusion: The results of the current study demonstrated that among CAH patients, long-term treatment with P is strongly associated with an higher visceral adiposity dysfunction and insulin resistance compared to treatment with HC.

PP310

ENDOTHELIAL PROGENITOR CELLS IN CUSHING'S SYNDROME

M. I. Maiorino[1], G. Bellastella[1], L. Olita[1], A. Cozzolino[2], D. Iacuaniello[2], R. Pivonello[2], A. Colao[2], D. Giugliano[1], K. Esposito[3]

[1]Dipartimento di Scienze Mediche, Chirurgiche, Neurologiche, Metaboliche e dell'Invecchiamento, Seconda Università di Napoli - Napoli, [2]Dipartimento di Endocrinologia ed Oncologia Molecolare e Clinica, Università di Napoli Federico II - Napoli, [3]Dipartimento di Scienze Cardio-Toraciche e Respiratorie, Seconda Università di Napoli - Napoli

Background: Endogenous hypercortisolism is associated with premature atherosclerosis. Bone marrow-derived endothelial progenitor cells (EPCs) are involved in endothelial homeostasis and repair. There are no data on circulating EPCs in endogenous hypercortisolism.

Aim: the aim of this study was to assess the circulating levels of different EPCs phenotypes in subjects affected by Cushing's syndrome (CS).

Subjects and Methods: we studied 21 CS patients and 21 age and sex matched healthy controls; eleven were newly-diagnosed (ND) and the other 10 were treated. Seven subpopulations of EPCs were determined by flow cytometry on the basis of the surface expression of CD34, CD133, and KDR antigens.

Results: compared with subjects of the control group, CS patients showed a significantly decreased number of six EPCs phenotype (CD 34+, cells per 106 events, median and interquartile range, 292[173-314] vs 380[326-414] $P <0.00$, KDR+ 20[15-25] vs 46[36-69] $P <0.001$, CD133+ 175[117-250] vs 271[226-344] P 0.004, CD34+CD133+ 138[87-233] vs 244[170-281] $P = 0.01$, CD34+KDR+ 17[12-26] vs 25[16-32] $P = 0.07$, CD133+KDR+ 8[4-12] vs 19[13-34] $P <0.001$, CD34+KDR+CD133+ 6[3-8] vs 16[11-20] $P <0.001$). No correlation between any EPCs phenotypes and plasmatic and urinary cortisol or ACTH levels was found in all CS patients.

Conclusions: CS is associated with a decreased number of EPCs, which may contribute to the elevated cardiovascular risk of these patients.

PP311

RIB CRACKS REVEAL CUSHING DISEASE, WHICH COMPLICATES WITH TRANSIENT PRIMARY POST-SURGICAL HYPOTHYROIDISM

M. Cappagli[1], A. Montepagani[2], P. Mortini[3], M. Losa[4]

[1]Endocrinologia Osp. S.Andrea - La Spezia, [2]Endocrinologia Osp.S.Andrea - La Spezia, [3]Neurochirurgia HSR - Milano, [4]Endocrinologia HSR - Milano

A 39-year-old woman comes to our observation due to recent multiple rib cracks (VII, VIII left; VIII, IX, X right) for minimal trauma. She complains of asthenia and muscle weakness. In anamnesis: uterine polyp and ovarian cysts. No therapy. DEXA: osteopenia unexpected to his young age (Tscore_L =:-1.9; Zscore_L =-1.6; Tscore_F =-1.5; Zscore_F =-1.3). 25OH vit D = 10.8 nmol/l (30-100); PTH = 55.72 pg/ml (13-54) Calcium: 8.2 mg/dl (8.4-10). Milk and cheese in her diet. Regular physical activity. Diagnostic hypothesis: osteomalacia. At the physical examination: mild acne and hirsutism, yellow skin; B.P.: 150/100 mm Hg. PRL, TSH GH, IGF1, Renin, Aldosterone are normal; Cortisol: 760 nmol/L (266-720). Cortisoluria = 507 nmol/L (38-208) and ACTH = 102 pg/ml (5-77). Liddle cortisol test: 638 nmol/L. Abdomen CT scan: increase of volume of left adrenal gland with micronodule of 8 mm; Thyroid US: regular size and shape; micronodules of 7.5 and 8.5 mm in the left lobe. The Patient (Pt) performs a pituitary MRI: "increased volume of the pituitary gland; intra-pituitary cystic nodule of 12 mm; the lesion presents contrast-enhancement ".Radiological diagnosis is: Pituitary Cystic Adenoma (PCA)".Pt undergoes adenomectomia through trans-sphenoid access. Histologic result confirms the diagnosis of " PCA secreting ACTH ". She starts therapy with cortone acetate 37.5 mg a day. Early, persist asthenia, muscle weakness, weight gain. Hormonal evaluation excludes a recurrence: ACTH = 40.67 pg/ml; cortisol = 35 nmol/l; menstrual cycles and sexual behavior are normal; also FSH, LH and estradiol are normal; but a monitoring of thyroid function shows hypothyroidism: TSH = 33.96 uUI/ml (0.20-4.0); T4: 0.53 ng/ml (0.93-1.86); Abtpo and AbHtg negative. Thyroid US: unchanged. Pt starts thyroxin: 100 mcg a day and euthyroidism is restored. Therapy with thyroxin and cortone are continued for 6 months with clinical improvement and normalization of thyroid parameters. After about a year the cortone acetate therapy is gradually reduced until interruption, without changes in the clinical picture and restoring a normal cortisolemia: 310 nmol/l). She also discontinues thyroxin and thyroid function just remains normal (TSH= 0.82 uUI/ml).Our Pt has resumed her normal activities and does not present clinical and bio-chemical evidence of adrenal or thyroid dysfunction.

PP312

MITOTANE INHIBITS CELL GROWTH PROLIFERATION IN DIFFERENT HUMAN CANCER CELL LINES: ROLE OF MRP1 PROTEIN

L. Cerquetti[1], C. Sampaoli[1], P. Fiore[1], B. Bucci[2], D. Amendola[2], A. De Venanzi[1], P. Lardo[1], V. Toscano[1], S. Antonio[1]

[1]Medicina Clinica e Molecolare - Facoltà di Medicina e Psicologia "Sapienza" Università di Roma - Roma, [2]Centro Ricerca Ospedale San Pietro Fatebenefratelli - Roma

Mitotane is an adrenal-specific agent employed in the treatment of adrenocortical carcinoma (ACC). Although it has been used for several decades, its exact mechanism of action, remain to be fully elucidated. Some authors reported that mitotane affected the multidrug resistance protein. MRP1, belonging a multidrug resistance protein family, exerts a chemo-resistance activity in many cancer leading to the failure of chemotherapy. In this study we investigated the effect of mitotane in different human cancer cell lines in order to determine the hypothetical link between MRP1 protein expression level and response to mitotane treatment. ACC, glioblastoma, medulloblastoma, ovarian cancer and breast cancer cell lines were treated with mitotane. Cell cycle analysis was analyzed by flow cytometry (FCM). Instead MRP1 protein was evaluated by immunofluorescence FCM. Mitotane treatment induced in all cell lines examined a cell growth inhibition characterized by an alteration of cell cycle. In some cell lines MRP1 expression levels was modulated after mitotane exposure, indicating its possible role in response to treatment. Moreover in other cancer cells the MRP1 pathway was only partially involved. In these cells the use of MRP1 inihibitor improves the antiproliferative effect of mitotane. We also observed a different expression of cell cycle molecules and apoptotic cell death protein affected by mitotane treatment in human cancer cell lines examined. In conclusion mitotane exposure shows an antiproliferative effect in several cancer cell lines and supporting an hypothetical role in therapeutic approach of different type of human cancers.

PP313

EVALUATION OF THYROID AUTOIMMUNE DISORDERS IN PRIMARY ALDOSTERONISM.

C. Sabbadin[1], P. Zanon[1], G. Donà[2], L. Bordin[2], S. Garelli[1], G. Clari[2], C. Betterle[1], D. Armanini[1]

[1]Medicina-Endocrinologia - Padova, [2]Medicina Molecolare-Chimica Biologica - Padova

Several studies evidenced a significant incidence of thyroid dysfunction in patients affected by primary aldosteronism (PA), suggesting a certain role of aldosterone in the development and/or progression of autoimmune disorders. In an earlier retrospective study, Armanini et al. found a high prevalence of thyroid morphological alterations in PA compared to general population (60% vs 27.5%). Another Italian work (Turchi et al.) confirmed a significant increased of thyroid ultrasonographic alterations in PA than in patients affected by essential hypertension (66% vs 46%), while the prevalence of anti-thyroid antibodies and thyroid dysfunction were similar in the two groups.

The aim of our study was to evaluate thyroid autoimmune disorders in PA. We enrolled 38 patients with proven PA, 21 affected by adenoma producing aldosterone (APA) and 17 affected by idiopathic hyperaldosteronism (HIA). We also enrolled 38 healthy normotensive subjects as controls, comparable for age, sex, geographical area and iodine intake (about 100 µg/d). Thyroid function, anti-thyroid antibodies and thyroid ultrasonography were performed in both groups.

The prevalence of morphological alterations was significantly higher in PA than in controls (57% vs 13%), but similar among two groups of patients with PA (57% in APA and 58% in IHA). The multinodular nontoxic goiter was the most frequent abnormality in PA (42.1%) and its prevalence was higher in female than in male patients (45% vs 38%). Thyroid function was not statistically different in the patients and controls (only 3 patients had hypothyroidism). The prevalence of anti-thyroid antibodies was significantly higher in PA than in controls (31% vs 7%) and greater in APA than IHA (33% vs 29%); no significant difference was found among female and male patients (30% vs 33%).

Our data about prevalence of thyroid ultrasonographic abnormalities are consistent with Armanini's and Turchi's data. The mechanism underlying the association between PA and multinodular goiter could be due to an imbalance between several common growth factors or cytokines. The elevated aldosterone concentrations might exacerbate immune responses but not the clinical course of autoimmune disease. Further studies are in progress to better evaluate the relationship between aldosterone, inflammation and autoimmune disorders.

PP314

ALDOSTERONE EFFECTS ON ERYTHROCYTE MEMBRANE: IN VITRO AND IN VIVO STUDY.

C. Sabbadin[1], L. Bordin[2], G. Donà[2], P. Zanon[1], E. Ragazzi[3], G. Clari[2], D. Armanini[1]

[1]Medicina-Endocrinologia - Padova, [2]Medicina Molecolare-Chimica Biologica - Padova, [3]Farmacologia e Anestesiologia - Padova

Aldosterone induces rapid non genomic effects by rising intracellular calcium and cAMP contents, triggering Na+/K+ channel exchangers and increasing the activities of enzymes such as protein kinases, MAPK and PKC, or NADPH-oxidase.

Erythrocytes are particularly sensitive to oxidative assault generated by reactive oxygen species (ROS), and both the association in high molecular weight aggregates (HMWA) and the Tyr-phosphorylation (Tyr-P) level of erythrocyte membrane band 3 can be used as parameters to monitor the redox status of these cells.

We studied 12 patients affected by hyperaldosteronism (HA) and 6 healthy controls (HC), evaluating band 3 HMWA formation and Tyr-P levels, both in baseline conditions and after treatment with diamide, a bland oxidant efficaciously utilized to evidence pre-existent membrane alterations induced by oxidative stress.

In affected patients diamide-induced band 3 Tyr-P was higher than controls, with an increase of 132±33%. Also formation of band 3 HMWA induced by diamide was much higher in patients compared to HC (321±8 compared to 130±2%). This was expected due to diamide-induced disulfide bond formation between cysteine residues of band 3. More interestingly, in basal conditions, i.e. in absence of diamide, HA patients showed a net increase in the HMWA content (183±8%) compared to HC, thus confirming that membranes from patients were altered. Evaluation of healthy volunteers erythrocyte pre-incubated with increasing aldosterone concentrations for 1 or 24 h in plasma previously depleted of steroids by charcoal treatment, confirmed that aldosterone induced membrane alterations (HMWA) leading to increased autologous IgG binding in dose and time-dependent manner. In addition, these effects were prevented by co-incubation with 1µM canrenone.

In conclusion, our study demonstrates that aldosterone induces oxidative-like stress in human erythrocytes, by altering membrane structure. This modification leads to an increase of the IgG binding, which may be responsible for a premature removal of the cell from circulation. It would be of great interest to investigate this mechanism, through which this hormone can regulate this non-genomic effect.

PP315

AN AUDIT ABOUT THE MANAGEMENT OF PATIENTS WITH ADRENAL INCIDENTALOMA IN INTERNAL MEDICINE: FROM GUIDELINES TO PRACTICE

V. Nuzzo[1], T. Attardo[2], D. Brancato[3], C. Canale[4], A. Franco[5], M. Gambacorta[6], M. Loreno[7], A. Maffettone[8]

[1]PO San Gennaro - Napoli, [2]PO Barone Lombardo - Canicattì, [3]PO Partinico - Palermo, [4]AO Bianchi-Melacrino-Morelli - Reggio Calabria, [5]PO Parodi-Delfino - Colleferro, [6]PO Media Valle del Tevere - Todi, [7]PO Avola-Noto - Siracusa, [8]AO Monaldi - Napoli

Audit is a Clinical Governance tool performed by a structured peer review process aiming at improving clinicians' behaviors in daily clinical practice. Adrenal masses are some of the most prevalent human tumors and are frequently detected unexpectedly during an imaging study (usually performed for systemic diseases). In Italy every year Internal Medicine units hospitalize about 1,450,000 patients, so it can be assumed that at least 58,000 of them could have adrenal lesions that might be identified with CT or MRI imaging. Through a research in the radiological archives, we examined all abdominal CT scans performed in year 2012 in 8 Internal Medicine Operative Units of 8 Italian public hospitals. We chose those reporting adrenal incidentaloma (excluding adrenal hyperplasia). Furthermore, we examined the medical records of these patients in order to evaluate both patients' phenotype and their clinical features, as well as the real incidence of adrenal incidentaloma (AI) and its subsequent management. The distribution of our pathologic findings shows a significative incidence of adrenal incidentaloma in Italian patients, most of them secondary to cancer. Although AI is accounted as a rare disease, its clinical and economic burden are significant. Several questions still remain open; first is the association between the duration and severity of the underlying disease, the morbidity and how dimensions affect the morbidity itself . Furthermore, there is a growing body of evidence suggesting a high morbidity of AI, including the associated global cardiovascular risks, which, to a large extent, lead to death. These findings could be corroborated by larger studies with longer follow-up, increased awareness, and earlier diagnosis. In conclusion, we will prosecute our Audit setting- as Clinical Guidelines state- the standards, the objectives for improvement and the implementation of behavioral changes in order to significantly affect the diagnostic-therapeutic management of AI in Internal Medicine patients.

PP316

RESOLUTION OF HYPERTENSION AND SECONDARY ALDOSTERONISM AFTER SURGICAL TREATMENT OF PRIMARY HYPERPARATHYROIDISM

C. Sabbadin[1], E. Cavedon[1], P. Zanon[1], M. Iacobone[2], D. Armanini[1]

[1]Medicina-Endocrinologia - Padova, [2]Scienze Chirurgiche e Gastroenterologiche - Padova

We report the case of a 50-yr-old woman, admitted to our Endocrine Unit because of weakness, arthralgias, polyuria and polydipsia. Her personal history was characterized by resistant hypertension for 3-yr, associated with secondary aldosteronism evidenced after pharmacologically wash-out, without evidence of any alteration at abdominal CT and renal ultrasound. As therapy she was taking diltiazem 60 mg twice a day, nebivolol 5 mg and potassium canreonate 100 mg daily. On examination she showed a generalized obesity, dry skin and mucosa, mild hepatomegaly and altered blood pressure values (190/100 mmHg). Biochemical evaluation revealed severe hypercalcemia (4.5 mmol/L), hypophosphatemia (0.5 mmol/L), elevated levels of PTH (855 ng/L) and a deficiency of vitamin D (19 mmol/L); CBC, liver, renal and thyroid function values were all normal. The ECG showed a sinus rate of 80 bpm, without remarkable signs of hypercalcemia. Neck ultrasound showed a hypoechoic vascularized mass (2.5x1.6x2.2 mm) at the lower pole of the right thyroid lobe, consistent with enlarged parathyroid. The patient was treated with isotonic saline hydration, furosemide, an injection of zoledronate 4 mg, vitamin D and Cinacalcet sequentially increased to 60 mg daily. 99mTc-MIBI scintigraphy demonstrated a focus of altered activity in the lower right thyroid lobe. Further investigations were performed to exclude a familial origin (MEN syndromes), resulting negative. An echocardiogram showed a hypertensive cardiomyopathy with normal ejection fraction. DEXA revealed an osteoporosis at lumbar spine and total femoral. The patient underwent to the surgical removal of the enlarged parathyroid, with rapid decrease of intraoperative PTH and normalization of calcium-phosphate equilibrium; the histological diagnosis was consistent with parathyroid adenoma. The following days, all the symptoms and hypertension disappeared, therefore antihypertensive therapy was gradually reduced and the patient was discharged with only calcitriol 0.5 mcg twice a day and carbonate calcium 3 g daily. After one month, calcium-phosphate balance and PTH were normal; since blood pressure was well controlled without any drugs, the evaluation of upright aldosterone and plasma renin activity was repeated and resulted normal. We conclude that both hyperaldosteronism and hypertension were secondary to the PHPT being resolved after the surgery. Since it is known that both these clinical situations are involved in the development and progression of CVD, diagnosis and treatment should be done as soon as possible.

PP317

ROLE OF ADRENAL VEIN SAMPLING IN PRIMARY ALDOSTERONISM. IMPACT OF DIFFERENT DIAGNOSTIC CRITERIA ON SUBTYPE DIAGNOSIS.

M. Cicala[1], A. Patalano[1], M. Salvà[1], B. Rubin[1], R. Pezzani[1], D. Miotto[2], F. Mantero[1]

[1]Dipartimento di Medicina, Endocrinologia - Padova, [2]Dipartimento di Medicina, Radiologia - Padova

In patients with primary aldosteronism (PA), adrenal vein sampling (AVS) is considered the gold standard to distinguish between unilateral and bilateral autonomous production of aldosterone, while diagnostic imaging tests by CT scan or MRI are often inconclusive for the diagnosis. To date agreement is lacking on the best criteria indicating successful cannulation and lateralization.

The aim of the study was to evaluate the impact of different diagnostic criteria for the successful cannulation and lateralization on subtype diagnosis and to compare the difference of the findings between adrenal CT scan and AVS.

Seventy-four patients with confirmed PA underwent AVS. The different diagnosis of PA subtypes reached using AVS data assessed by more permissive (type 1) and strict (type 2) criteria were compared. Al patients performed CT scan before AVS and imaging results were compared with results of AVSs (using both criteria).

Using Type 1 criteria AVSs were successful in 86% of patients, and in only 64,5% using type 2 criteria. Type 1 criteria led to a higher rate of diagnosis of unilateral PA (85% of successful procedures) than type 2 (75%). There was considerable disparity in the diagnosis reached, with a concordance in only 45% of patients. In conclusion more permissive criteria for successful cannulation and lateralization on AVS can lead to incorrect diagnosis and accordingly to inappropriate treatment options. In the selected group of patients with successful AVS, CT findings correlated with AVSs findings in 58,5% of patients using type 1 criteria and in 47,5% using type 2 criteria. Finale diagnosis was based on histological results in 36 patients (49%) which underwent adrenalectomy based on AVSs findings using the more permissive criteria. On the basis of CT findings alone 17% of patients from the first group and 32,5% of patients of the second group probably would have been incorrectly bypassed as candidates for adrenalectomy. CT scanning lacks sensitivity and specificity and should be followed by AVS, which is the only reliable means of differentiating unilateral from bilateral PA and lateralizing APAs preoperatively. However, there are still controversies to be solved by large prospective studies on the criteria to adopt for defining the most appropriate cut off for both correct cannulation and lateralization.

PP318

IMMUNOHISTOCHEMICAL ANALYSIS OF ADRENAL CORTICAL TUMORS.

B. Altieri[1], G. Fadda[2], A. Capozzi[1], A. Pontecorvi[1], S. Della Casa[1]

[1]UOC di Endocrinologia e Malattie del Metabolismo, Università Cattolica del Sacro Cuore - Roma, [2]Dipartimento di Anatomia Patologica e Istopatologia, Università Cattolica del Sacro Cuore - Roma

Introduction: Adrenocortical tumors (ACTs) are frequent neoplasias recurring in 2% of population and usually divided in adenoma (ACA) or carcinoma (ACC) according to histopathologic methods based on Weiss score. Some lesions are occasionally difficult to classify according to classical criteria. Recent studies proposed many immunohistochemical markers to identify the most aggressive masses. We studied the use of some particular markers to try to recognise the difference between malignant and benign tumors. **Materials and methods:** We studied 12 patients affected by ACC and 10 by ACA. Clinical evaluation and hormone analysis were performed in all patients who underwent to adrenalectomy. Immunohistochemisty (IHC) was performed on formalin-fixed paraffin tissue of adrenal tumours except for a singular case in which our materials come from lymph node metastasis because patient presented a stage IV disease since diagnosis. We analysed in particular Ki-67, IGF2, Ghrelin, PPARγ and ACTH expression. **Results:** All 10 ACAs showed a low proliferation index Ki-67 (<5% cell stained), while 4 out of 12 (33%) ACCs showed a high proliferative index (Ki-67>5%). The Wilcoxon-Mann-Whitney test demonstrated a statistically significant difference between ACAs and ACCs for Ki-67 (p 0,025). We didn't find statistically significant differences between IGF2 (p<0,0462), Ghrelin (p 0,738), PPARγ (p 0,403) and ACTH (p 0,369). Even if there were not differences for IGF2 between the two groups, we observed an overexpression of this marker in 50% of patient with ACC (IGF2>60%). Analysis based on Spearman correlation coefficient didn't find correlation between stage disease, tumor dimension and immunohistochemical markers in ACCs. **Conclusion**: According to the current literature, we found the use of Ki-67 could be able to distinguish between ACAs and ACCs. Although many studies considered IGF2 as a malignant parameter, our results didn't confirm its use alone could be helpful to identify malignant lesions. Besides, we showed the other different immunohistochemical markers, less commonly investigated in these tumors, should not be useful to discriminate adenoma from carcinoma. None immunohistochemical marker considered in our study showed a correlation with the characteristics of size or local extension of lesions. Therefore, we need more studies about a higher number of patients to obtain much more significant data about IHC utility.

PP319

LONG TERM FOLLOW-UP OF PATIENTS WITH ACTH-INDEPENDENT MACRONODULAR ADRENAL HYPERPLASIA AFTER UNILATERAL ADRENALECTOMY

N. Albiger[1], M. Iacobone[2], S. Rizzati[1], F. Ceccato[1], M. Barbot[1], M. L. Zilio[1], M. Salvà[1], F. Mantero[1], C. Scaroni[1]

[1]Dipartment of Medicine, Endocrinology, University of Padua - Padua, [2]Dipartment of Surgery, University of Padua - Padua

Introduction: ACTH-independent macronodular adrenal hyperplasia (AIMAH) is a rare form of Cushing's syndrome (CS) with bilateral adrenal involvement and clinical heterogeneity. Unilateral adrenalectomy has been proposed as an effective and safe treatment in asymmetric enlargements. We aimed to evaluate clinical presentation and long-term results of unilateral adrenalectomy in patients with AIMAH seen in our center from 2000 to 2012.

Methods: We evaluated 17 patients (12 female; 5 men, 53±12 yrs) with diagnosis of AIMAH. Clinical, biochemical and morphological data were evaluated at diagnosis and after a long-term follow-up in particular in patients who underwent unilateral adrenalectomy. An in vivo protocol in search of aberrant cortisol responses was also performed.

Results: Hypercortisolism at presentation was overt in 10, subclinical in 6 and cyclical in one patient. All patients showed a partial or positive response to one or several stimulation tests. The most frequent responses were recorded for vasopressin/ terlipressin (55% of patients) and the postural test (58% of patients). Bilateral adrenalectomy was performed in one patient and unilateral adrenalectomy in 11 patients. Five patients are in follow-up. After unilateral adrenalectomy, persistent remission was observed in 4 patients (at 80±55 months; range: 6-135), recurrence in 6 patients (at 67±58 months; range:30-180) and persistent disease in one patient. Recurrence presented as overt in 3, subclinical in 2 and cyclical in 1 patient. Adrenal morphology evaluation during follow-up showed an asynchronous enlargement of adrenal glands in 2 patients and a slight enlargement of the controlateral gland in one patient. No changes were evidenced in the other patients.

Conclusion: our observations confirm that AIMAH is a clinical heterogeneous disorder with hypercortisolism that usually develops slowly over several years or can progress in an asynchronous or cyclical manner. Unilateral adrenalectomy of the largest gland can be a valid option in selected patients but a close follow-up is indicated due to possible recurrence.

PP320

A CLINICAL-RADIOLOGICAL SCORE TO DIAGNOSE HASHIMOTO'S THYROIDITIS: A PROPOSAL

G. Grani[1], G. Carbotta[1], A. Nesca[1], M. D'Alessandri[1], M. Vitale[1], M. Del Sordo[1], A. Fumarola[1]

[1]Dipartimento di Medicina Sperimentale - Sapienza Università di Roma - Roma

Introduction. Early diagnosis of Hashimoto's thyroiditis (HT) may be difficult. The heterogeneity of criteria used to diagnose HT may prevent strong conclusions from being drawn in studies focusing on clinical aspects of HT. The aim of this study is to design a simple score to diagnose chronic autoimmune thyroiditis.

Methods. 1021 consecutive patients that were advised to undergo total thyroidectomy at an University thyroid referral center. Given a dichotomous outcome, a set of 2 demographic (sex and age), 3 biochemical (overt or subclinical hypothyroidism, positive anti-Tg Ab, and positive anti-TPO Ab) and 4 imaging covariates (hypoechogenic parenchyma, heterogeneous thyroid echopattern, color-Doppler pattern and estimated thyroid volume) was analyzed.

Results. Analysis showed that anti-TPO Ab [area under the curve (AUC) under the ROC curve, 0.67], and anti-Tg Ab (0.63) were univariate predictors of the diagnosis of HT, which is largely recognized. Combined covariates were then tested using stepwise logistic regression. A model to predict the final diagnosis was calculated by using multivariate logistic regression analysis. The final model included anti-TPO Ab, anti-Tg Ab and thyroid vascularity (AUC 0.72). A second scoring system was developed to diagnose HT, with the addition of heterogeneous echopattern and goiter (AUC 0.76).

Conclusions. A simple scoring system for the early diagnosis of HT could easily be applied in clinical practice and research. The better proposed score has been shown to have an overall low degree of sensitivity and speci?city, but higher than the single predictors alone (sensitivity 45.5% and specificity 89.0%, with a cutoff value of 1.7). International multicenter studies can recruit a higher number of patients and provide a sufficient amount of data to integrate all features of HT into a consensus diagnostic score.

PP321

TSH-DEFICIENCY IS ASSOCIATED WITH A LOWER THYROID GLAND VOLUME IN HYPOPITUITARIC PATIENTS COMPARED TO HEALTHY VOLUNTEERS: A CROSS-SECTIONAL STUDY

D. Santi[1], G. Brigante[1], V. Gnarini[1], B. Madeo[1], S. De Vincentis[1], C. Carani[1], M. Faustini-Fustini[2], A. Balestrieri[3], V. Rochira[1]

[1]Chair and Unit of Endocrinology & Metabolism, Department of Biomedical, Metabolic and Neural Sciences, University of Modena & Reggio Emilia - Modena, [2]Department of Medicine, Bellaria Hospital - Bologna, [3]Department of medicine, endocrine and metabolism unit, Ospedale "M. Bufalini " - Cesena

Introduction: Thyroid Volume (TV) depends on age, gender, anthropometry, smoking and iodine status. IGF-1 plays a role on thyroid growth, as demonstrated in acromegaly and GH-deficiency. TSH is a well recognised permissive factor for thyroid tissue growth. Aim of the study is to evaluate the long-term effect of TSH-deficiency on TV in hypopituitaric patients compared with healthy volunteers. **Methods**: We performed a cross-sectional, controlled study on 58 hypopituitaric patients (36male, 22female) with multiple hormonal deficiency (confirmed diagnosis of central hypothyroidism was the main inclusion criteria) (60.0±13.9years), and 244 volunteers (73male, 171female) (47.7±11.63years). All subjects underwent thyroid ultrasonography (US) (Siemens Acuson Antares®, Philadelphia, USA) performed by the same operator. TV was calculated as the sum of TV of the two lobes, each estimated as: length(cm) x width(cm) x depth(cm) x 0.52. **Results**: Age, weight, BMI and body surface area (BSA) were greater in hypopipuitaric patients than healthy volunteers. Thyroid nodules were incidentally discovered at US in 17 hypopituitaric (29.3%) and 93 volunteers (38.1%). TV was lower in hypopituitaric patients than in volunteers (6,066±5,079mL; 9,695±3,702mL,p<0,001). This difference was confirmed also in the subgroup without nodules (mean:4,719±3,230mL;9,430±3,497mL, p<0,001), but not when comparing hypopituitaric patients and volunteers with goiter. Finally, TV was lower in hypopituitaric patients without nodules (4,73±3,27mL) than in those with goiter (9,62±7,18mL) (p=0.003). These differences were held even after correction of TV for BSA, BMI and age. **Discussion**: TV is significantly lower in hypopituitaric patients than in healthy subjects, but the prevalence of thyroid nodules seems to be similar. The reduction of TV in hypopituitaric patients seems to occur only in thyroid glands without nodules. The chronic lack of TSH, as in hypopituitarism, seems to be responsible in vivo for a reduction of TV, but this effect seems to involve mainly the normal thyroid tissue rather than the hyperplastic nodular tissue.

PP322

PKCD PLAYS AN IMPORTANT ROLE IN REGULATING HUMAN MEDULLARY THYROID CARCINOMA CELL VIABILITY

D. Molè[1], E. Gentilin[1], T. Gagliano[1], F. Tagliati[1], M. R. Ambrosio[1], E. degli Uberti[1], M. C. Zatelli[1]

[1]Scienze Mediche - Ferrara

Protein kinase C (PKC) is a family of serine-threonine kinases that regulate many cellular processes including proliferation and survival. Previous evidence has shown that PKC is involved in the control of human medullary thyroid carcinoma (MTC) proliferation and survival by modulating apoptosis, with a mechanism that implicates PKCβII isoform and translocation in different subcellular compartments.

In this study, we investigated the role of PKC-delta (PKCδ) signaling in the proliferation of a human MTC cell line, the TT cells. We found that pharmacological inhibition of the PKCδ pathway with Rottlerin reduces caspase 3/7 activity. Using a shRNA vector system, which provides more than 90% gene expression inhibition, we found that cell proliferation is greater in PKCδ-defective-TT cells than in mock-transfected cells, this difference being significant after three days. In addition, we found that PKCδ silencing reduces STAT5(Y694-699) phosphorylation but not AKT(Ser473) and p70S6K(T389) phosphorylation, all downstream targets of PKC pathway involved in cell growth, cell cycle and proliferation.

Moreover, we demonstrated that PKCδ silencing increased human VEGF secretion after four days.

These observations indicate for the first time that PKCδ pathway plays an important role in the growth control and VEGF secretion of human MTC cells.

PP323

INVOLVEMENT OF PKCß AND PKCD ISOFORMS IN TSH SIGNALING PATHWAY IN THYROID CANCER CELL LINES

D. Molè[1], E. Gentilin[1], T. Gagliano[1], F. Tagliati[1], E. degli Uberti[1], M. C. Zatelli[1]

[1]Scienze Mediche - Ferrara

It is well established that most TSH effects on the thyroid gland, including stimulation of proliferation, thyroid hormone synthesis and expression of thyroid-specific genes, are transmitted mainly by the adenylate cyclise pathway. However, in human follicular cells and in rat FRTL-5 cells, TSH can also stimulate the β-isoforms of PLC that catalyzes the hydrolysis of phosphatidyl-inositol (4,5) phosphate, yielding the second messengers DAG and Inositol (1,4,5) phosphate facilitating an increase in intracellular Ca2+. In FRTL-5 cells TSH has been suggested to increase DAG via phospholipase D, which produces DAG from phosphatidylcholine hydrolysis, suggesting an alternative mechanism for TSH-dependent activation through protein kinase C (PKC).

In the present study, we characterize the PKCβ and PKCδ isoforms expression and function in human follicular carcinoma cells, FTC133, and in human transformed thyrocytes, Nthy-ori cells, in order to understand whether these PKC isoforms are involved in TSH-mediated follicular cell proliferation and apoptosis. We mainly focus on PKCβ and PKCδ isoenzymes which are the most abundantly expressed isoforms in several tissues, are the most extensively studied and have two opposing roles in regulating cell proliferation.

In the Nthy-ori cells TSH stimulated cell proliferation and protected from apoptosis with a PKC-mediated mechanism. At the contrary, TSH did not increase FTC-133 cell viability nor protected the cells from PKC-inhibitor induced apoptosis. However, in FTC-133 cells TSH induced PKC expression, as well as downstream PKC targets GSK3? and AKT phosphorylation through a PKC-mediated mechanism. Moreover, immunofluorescence showed PKCβ and PKCδ perinuclear and citosolic location. These data suggest that TSH plays different roles in normal vs. neoplastic thyrocytes. Further studies are needed to clarify the role of PKCβ and PKCδ in the TSH signaling pathway in thyroid cells.

PP324

REFRACTORINESS OF TWO NOVEL FORMULATIONS OF L-T4 (ORAL SOLUTION AND SOFT GEL CAPSULE) TO THE COFFEE-INDUCED INTESTINAL MALABSORPTION OF TABLET L-T4.

R. Vita[1], G. Saraceno[1], F. Trimarchi[1], S. Benvenga[1]

[1]Dipart. di Medicina Clinica e Sperim., Università di Messina - Messina

Coffee was reported to impair the intestinal absorption of tablet L-T4 when it is swallowed concomitantly or with water but followed by coffee within the next few minutes (Thyroid, 2008). Recently, a novel formulation of L-T4 (soft gel capsule, Tirosint™, IBSA) (SGC) was proven to be refractory to such coffee interference (Endocrine, 2013). In a liquid formulation (OLF), sodium L-T4 is solubilized in ethanol and glycerol. Pharmacokinetics studies have shown that the profile of intestinal absorption is faster (particularly Tmax) for OLF than tablet. This more favorable absorption is anticipated because L-T4 is a hydrophobic hormone that, in the OLF, is solubilized in an organic solvent. Accordingly, solubilized L-T4 is ready to reach the absorption sites in the upper intestine, having skipped the dissolution phase that is unavoidable for solid formulations. As a continuation of a previous study in which we had switched 3 women with impaired absorption of tablet to SGC, we switched them from SGC to OLF at the same daily dose, continuing to have coffee concomitantly or within 5 minutes after L-T4. In addition, another woman with coffee-induced tablet L-T4 malabsorption was switched directly to OLF. All 4 patients took L-T4 for TSH-suppressive purposes. Blood was sampled for TSH assay at least two months after each switch. Data are expressed as m±SD. We used Fisher's exact test to compare proportions and Wilcoxon test to compare means. Serum TSH levels were higher under tablet (0.78±0.72 mU/L) compared to OLF (0.30±0.32) or SGC (0.36±0.36) (tablet vs. OLF, P=0.009; tablet vs. SGC, P=0.04). Serum TSH did not differ under OLF or SGC (P=0.62). The number of serum TSH values <0.10 was higher under OLF (5/13 samples, 38.5%) or SGC (5/13, 38.5%) compared to tablet (1/20, 5%) (tablet vs. OLF or SGC, P=0.02). In one patient, the daily dose of L-T4 had to be decreased after the switch from tablet to SGC, and again after the switch from SGC to OLF because of hyperthyroidism symptoms. Patients often complain that it is difficult for them to change their habits of having coffee soon after L-T4. For this reason, physicians have to adjust frequently L-T4 daily dose to reach target serum TSH. This strategy exposes patients to the risk of iatrogenic hyperthyroidism. Not only the soft gel capsule formulation, but also the oral liquid formulation is refractory to the coffee-induced L-T4 malabsoprtion. Either formulation is attractive for patients who elect not to change their habits.

PP325

PROSPECTIVE STUDY ON THE TRIGGERING ROLE OF STRESS IN GRAVES' DISEASE.

R. Vita[1], D. Lapa[1], F. Trimarchi[1], S. Benvenga[1]

[1]Dipart. di Medicina Clinica e Sperim., Università di Messina. - Messina

The role of stress in triggering the onset and the relapses of Graves' disease (GD) remains controversial. To corroborate the role of stress as an environmental trigger of GD-related hyperthyroidism, in this prospective study we have evaluated the effects of stressful events (SE) on the onset and outcome of GD patients. We enrolled 58 patients in whom at least one SE had occurred within 12 months before the onset of GD. We excluded patients with ophtalmopathy, smokers, patients who underwent radioactive iodine treatment or thyroidectomy. Data are presented as m±SD, with statistics based on Wilcoxon test, chi-square or Fisher's exact test and linear regression. To identify SE, we administered patients a specific questionnaire, in which SE were divided in three groups: psychological (PSE), infectious, physical or of other kind. We treated patients with antithyroid drugs (ATD) for ≥12 months and the follow-up after their withdrawal lasted ≥5 years. Depending on the outcome of GD, we divided patients in 3 groups: REM (who reached remission; n=15, 26%); EXA (who experienced ≥1 exacerbation during ATD; n=6, 10.3%); REL (who experienced ≥1 relapse after ATD withdrawal; n=37, 64%). Age at onset (35.3±15.5 years) was more frequently ≤30 years in REM patients (9/13=69%), compared to EXA (1/6=17%) and REL (12/32=37%) (REM vs. EXA vs. REL, df=2, P=0.057). The time lag between SE and onset (19.3±11.9 weeks) was linearly correlated with age at onset (r=0.585, P<0.001), particularly in REL (r=0.643, P<0.001). PSE were the most frequent SE (51/58 patients, 87.9%). REL had experienced more SE than REM (2.7±1.7 per patient vs. 1.2±0.6, P<0.001) and more PSE (2.3±1.4 vs. 1.2±0.6, P=0.001). In REL, patients with ≥ 2 relapses had more PSE than patients with one relapse (3.3±1.2 vs. 1.5±1.0, P<0.001). They also had PSE preceding the onset and PSE preceding the first relapse more frequently than patients with one relapse (before the onset, 14/14=100% vs. 15/22= 68.2%, P=0.029; before the first relapse, 12/14=85.7% vs. 9/23=39.1%, P=0.007). The overall number of SE was linearly correlated with the number of relapses (r=0.697, P<0.001). In conclusion, there exist patients with GD who are prone to develop hyperthyroidism after SE, so that every occurrence of hyperthyroidism is systematically preceded by ≥1 SE. They are relatively young; the younger they are, the shorter is the time lag between SE and the occurrence of hyperthyroidism, and the higher the odds of GD recurrence.

PP326

CLUSTERIN ISOFORMS EXPRESSION IN THYROID TISSUE : A POTENTIAL MARKER OF MALIGNANCY?

A. Ciampolillo[1], P. Fuzio[2], E. Perlino[3], A. Pezzolla[1], S. Lattarulo[1], E. Maiorano[1], A. Napoli[1], F. Giorgino[1]

[1]Dipartimento dell'Emergenza e dei Trapianti di Organi - Bari, [2]Istituto di Biomembrane e Bioenergetica IBBE-CNR - Bari, [3]Istituto di Tecnologia Biomedica ITB_CNR - Bari

Clusterin (CLU) is a ubiquitous multifunctional protein implicated in neoplastic transformation. The sCLU and nCLU isoforms play a crucial role in the balance between cell proliferation and death.

We studied the expression of CLU isoforms in normal and neoplastic thyroid tissues to assess the potential role of these proteins as biomarkers for thyroid cancer. CLU expression was investigated in 40 patients affected by follicular adenoma and 10 patients affected by papillary carcinoma by a semi-quantitative immunohistochemical assay; moreover, the differential expression of sCLU and nCLU isoforms was determined by RT-qPCR in the thyroid tissues from 10 patients undergoing thyroidectomy for a suspected malignancy on the basis of FNAB results.

Immunohistochemical analyses showed that CLU protein is up-regulated in papillary carcinoma in comparison to follicular adenoma (>50% immunoreactive cells in 62% of thyroid carcinomas in comparison to 0-25% immunoreactive cells in 90% of thyroid adenomas; p<0.01). RT-qPCR analysis showed a significant increase in nCLU (215±5%) and sCLU (1585±15%) mRNA levels in neoplastic compared to normal thyroid tissue (p<0.05). The sCLU/nCLU ratio was also significantly augmented in thyroid cancer (288±5%; p<0.05). Moreover, there was a trend for a correlation between sCLU mRNA levels and age, sex, tumour variant and tumour stage, respectively. Finally, in patients with a TIR3 FNAB, there was a specific increase of sCLU expression (501±15%) in comparison to nCLU (100±3%) in those with histologically confirmed thyroid cancer and not in those with benign lesions, which showed a decrease in sCLU mRNA expression (58±3%; p<0.05).

In conclusion, albeit preliminary, these results suggest the existence of a differential CLU mRNA expression during the progression from normal to malignant cell phenotype and a specific alteration of the sCLU/nCLU ratio towards sCLU in thyroid cancer, thus providing the first circumstantial evidence for the potential use of CLU isoforms as effective biomarkers for indeterminate thyroid nodules.

PP327

IMPACT OF PREGNANCY ON PROGNOSIS OF DIFFERENTIATED THYROID CANCER: CLINICAL AND MOLECULAR FEATURES

I. Messuti[1], B. Puligheddu[1], R. Pellerito[2], M. Volante[1], F. Orlandi[1], S. Corvisieri[1]

[1]Oncologia - Università di Torino, [2]Medicina Nucleare AO Mauriziano - Torino

Differentiated Thyroid Cancer (DTC) is commonly found during pregnancy or in the early postpartum period. There are only a few studies about outcome of DTC related to pregnancy. Most of wich show that there was no impact of pregnancy on DTC outcome, even if they evaluate overall survival. Such findings, however, are not in agreement with the study published by Vannucchi et al (2010), who reported that DTC in pregnant women showed a increase of persistent/recurrent disease than in non pregnant. Moreover, the expression of the estrogen receptor α (ERα) by immunohistochemistry was significantly higher in pregnant women compared with the control groups. The aim of our study was to verify these conflicting results on a very large population. **Methods**: 340 female patients with DTC <45 years old were retrospectively studied. In order to evaluate the impact of pregnancy on DTC outcome, patients were divided into three groups according to the time of tumor diagnosis. Group1: women with DTC diagnosis at least 2 years after delivery, Group2: women with DTC diagnosed during pregnancy or within the second year after delivery, Group 3: nulliparous patients, or diagnosed and treated for DTC before pregnancy. We evaluate clinical outcome and immunohistochemical expression of estrogen receptor α (ERα), β, progesterone receptor (PGR) and aromatase. **Results**: Persistence/recurrence of disease was significantly higher in group 2 patients than control groups (p: 0,02). No significant differences were observed when other clinical parameters were considered. ERα, PGR and aromatase were poorly expressed in all hystological samples. By contrast, ERβ showed high degree of expression in a part of tumor samples without any significant difference among the 3 groups. **Conclusions**: Our data confirm that persistence/recurrence of DTC is significantly higher in pregnant patients, in agreement with the data by Vannucchi et al. The lack of significant differences in tumor staging or hystology among the groups considered, suggests that the worse outcome of group 2 could be related to a long-term effect of pregnancy (growth factors, oncogenes, etc) on DTC progression or on a different response to treatment. By contrast we can't confirm the data of Vannucchi et al. when estrogen receptor expression is considered. We're not able to explain the conflicting results, but a difference in methodological approach could be hypothesized (e.g. antibody diluition). In conclusion our results, obtained in a large population seems to confirm that pregnancy could really have a prognostic role on patients with differentiated thyroid cancer. Further perspective studies are strongly needed to clarify this important topic.

PP328

CIRCULATING THYROID TUMOR CELL DETECTION: POTENTIAL USE IN THE FOLLOW-UP OF PATIENT WITH FOLLICULAR CELL-DERIVED THYROID CARCINOMA

A. Moretti[1], E. Aroasio[1], S. De Francia[1], M. Pautasso[1], M. Volante[1], F. Orlandi[1], S. Enrico[1]

[1]Dipartimento di Oncologia - Università di Torino

The follow-up program of patients with Differentiated Thyroid Cancer (DTC) is based on evaluation of serum thyroglobulin (Tg) and neck ultrasonography. Nevertheless, in a part of patients, in particular those with poorly differentiated cancers, Tg evaluation has lower sensitivity. In these cases, the availability of alternative diagnostic methods would be important. In the last years a new diagnostic tool has been proposed, based on the identification of Circulating Tumor Cells (CTCs) through immunomagnetic assay (CellSearch System®), cytofluorimetric methods and molecular biology analysis. The aim of our study was to identify thyroid CTCs in patients with DTC underwent both thyroidectomy and radioiodine (131-I) remnant ablation (RAI) and to assess the possible role of CTCs in the follow-up. METHODS: A total of 22 patients with DTC treated by thyroidectomy + RAI were retrospectively studied. Seventeen out of 22 had detectable serum Tg concentrations (persistent/reccurrent disease), 5 out of 22 had undetectable stimulated Tg levels (cured), whereas 6 out of 22 were healthy volunteers. In all patients CTCs were assessed by both CellSearch® System and multichannel cytofluorimetric assay. Thyroid CTCs were confirmed by RT-PCR for Tg-mRNA. As CellSearch® System is concerned, only CTCs with immunophenotype EpCAM(+)/Cytokeratin(+)/CD45(-)/DAPI(+) were counted, whereas the pattern TSHR-PE+/CD45- was employed to identify thyroid circulating cells by cytofluorimetric method. RESULTS: CTCs were observed in 65% of DTC patients with persistent disease, and in 0% of cured patients. Thyroid phenotype of CTCs was confirmed in 90% of samples by RT-PCR for Tg-mRNA. Levels of circulating thyroid cells detected by the cytofluorimetric method were higher in DTC patients with persistent disease than in cured patients, as well as in the healthy volunteer subjects. CONCLUSION: Thyroid CTC identification by CellSearch® System is a very specific method to identify thyroid cancer recurrence, but the low sensitivity doesn't yet allow the clinical use. With regards to the cytofluorimetric method, the number of events TSHR+/CD45- is higher in patients with persistent disease than in the other groups, althought with a significant overlap of the data. The lack of statistical correlation between CTCs and histotype, TNM, basal serum Tg levels, RAI dose and metastatic localizations, may suggest that CTCs could represent a different cell population, with a possible precursor role. Our data suggest that CTCs detection can't represent an alternative tool to the serum Tg level evaluation in the follow up of DTC.

PP329

THYROID MYOPERICYTOMA WITH BRAFV600E: A NOVEL IN VITRO AND IN VIVO MODEL OF THYROID TUMOR MICROENVIRONMENT USING VEMURAFENIB THERAPY

C. Nucera[1], S. Nanni[2], C. Priolo[3], M. Duquette[4], F. A. Karreth[5], R. Martinelli[3], C. Carman[3], A. Farsetti[2], L. Macconaill[6], G. Fadda[2], C. P. Lombardi[2], C. Fletcher[4], P. P. Pandolfi[5], P. Sadow[4], A. Pontecorvi[7], J. Lawler[4]

[1]Translational Thyroid Cancer Program, BIDMC, Harvard Medical School - Boston, [2]Cattolica - Roma, [3]Medicine - Boston, [4]Pathology - Boston, [5]Cancer Genetics - Boston, [6]Oncology - Boston, [7]Medicine, Univ. Cattolica - Roma

Myopericytoma is a mesenchymal tumor characterized by perivascular proliferation of pericytes (fundamental for vessels stability and angiogenesis). We studied a 45 yrs old woman with a thyroid mass (~4cm). DNA genotyping (over 500 genes analyzed) by Mass Spectrometry, immunohistochemistry, mRNA knock-down by sh-RNA, gene expression profile, and anchorage (Matrigel) assays were performed. Cell viability was determined with vemurafenib (a novel oral selective inhibitor of BRAFV600E). Angiogenesis in vitro assays and patient-TM derived xenograft mouse model GFP+/+ were developed. Thyroid imaging revealed a goiter associated with an irregular, hypoechoic, hypervascularized, and hypofunctional mass in the left thyroid lobe. The mass was diagnosed as thyroid myoperytoma (TM) and harbored the heterozygous BRAFWT/V600E mutation, and showed mediastinal extension. Both TM specimen and established primary TM cells in vitro harbored BRAFWT/V600E and were positive for PDGFRB, pERK1/2, and stem cell markers. A higher vascular density (CD31+) was also found in the TM vs normal thyroid. Calcitonin, PTH, and thyrooid markers were negative in the TM. TM cells expressed high levels of extracellular matrix (ECM) proteins (e.g. integrins) and grew as ellipsoids in Matrigel. Knock-down of BRAFV600E mRNA significantly reduced TM cell adhesion/migration vs controls. Vemurafenib significantly down-regulated pERK1/2 and ECM protein levels, and suppressed viability and migration of TM cells vs controls. TM cells triggered angiogenesis in vitro in a 3-Dimensional cell system; treatment with vemurafenib significantly disrupted angiogenesis vs controls. Furthermore, TM-derived and xenografted mice showed a significant therapeutic response to vemurafenib vs controls. We found the first BRAFV600E-TM and -isolated primary TM cells. We show that BRAFV600E-ECM pathway is crucial for TM development and angiogenesis. Vemurafenib might have a potential therapeutic efficacy in patients with BRAFV600E-TM.

PP330

NEW INSIGHTS ON FOLLICULAR THYROID CARCINOMA: THE ROLE OF PRE-MIR-146A

E. Pignatti[1], E. Vighi[1], L. Roncati[2], E. Magnani[1], E. Kara[1], B. Madeo[3], V. Rochira[1], A. Maiorana[2], C. Carani[1], M. Simoni[1]

[1]Dip.to di Scienze Biomediche, Metaboliche e Neuroscienze, Unimore - Modena, [2]Dip.to di Medicina Diagnostica, Clinica e di Sanità Pubblica, Unimore - Modena, [3]Azienda AUSL di Modena-NOCSAE - Modena

INTRODUCTION. Cancer of the thyroid is the most common endocrine malignancy. Follicular thyroid carcinomas (FTC) make up about 15 % of all cases. MicroRNAs, small non-coding RNAs, are a class of post-transcriptional regulators with several functions, including apoptosis. They display different expression profiles from tissue to tissue, reflecting the diversity in cellular phenotypes and, as such, suggest a role in tissue differentiation and maintenance. Pre-miR-146a represents one of the most up-regulated miRNAs in papillary thyroid carcinomas (PTC) and a single-nucleotide polymorphism (SNP: rs2910164), identified in pre-miR-146a, contributes to genetic predisposition to PTC, but data on FTC are still lacking. In this study the expression of pre-miR-146a was evaluated in FTC, both in neoplastic and non-neoplastic tissues, and the possible correlation between rs2910164 SNP and pre-miR-146a expression profiling was assessed. DESIGN. The study included 35 male and female patients with FTC, aged 58±34 years. RNA was extracted from neoplastic and non-neoplastic FFPE samples obtained by surgically removed thyroids. A cDNA synthesis was carried out using the stem-loop RT method. A semi-quantitative PCR was implemented starting from a standard TaqMan PCR protocol, according to the manufacturer's instructions. The small nuclear RNA U6 was selected as normalization control. The pre-miRNA expression level was quantified using the CFX96 Real-Time System (Bio-Rad). The pre-miR-146a common G/C polymorphism, designated rs2910164, was genotyped by sequencing. Wilcoxon signed-rank test and Friedman test were used for statistical analysis. RESULTS. The expression of pre-miR-146a is significantly down regulated in tumor compared to non-neoplastic tissues in patients with FTC (p=0.043). rs2910164 genotype is related neither to the level of expression of pre-miR-146a nor to the type of tissue analyzed. Finally, no correlations between the expression of pre-miR-146a and the genotype of the SNP in the transition from non-neoplastic to neoplastic tissue in patients with FTC were found. CONCLUSIONS. Our findings reveal, for the first time, the pre-miR-146a down regulation in FTC tissues. The expression variation is not related to the rs2910164 SNP.

PP331

EFFECTS OF B-RAF INHIBITOR, RAF265, IN TUMOR CELL LINES OF THYROID PAPILLARY CARCINOMA.

S. Barollo[1], L. Bertazza[1], R. Pezzani[1], B. Rubin[1], C. Betterle[1], C. Mian[1]

[1]Department of Medicine - Padua

INTRODUCTION: The most common genetic event in papillary thyroid carcinoma (PTC) is a tranversion in exon 15 of the tyrosine kinase B-Raf which leads to a V600E aminoacid substitution with frequency of 38%-60%. New anticancer drugs targeting mutant B-Raf could represent a valid therapeutic approach for the treatment of the thyroid cancer harbouring *B-Raf* V600E mutation.

AIM: The aim of this research project is to test RAF265 (kindly granted by the Novartis Farma Spa), directed specifically against the product of *B-Raf* oncogene.

MATERIALS AND METHODS: RAF265 was tested on K1 cell line (*B-Raf* V600E heterozygous mutation), BCPAP cell line (*B-Raf* V600E homozygous mutation) and TT cell line (wt). Cell viability was evaluated by MTT test, while cell cycle alterations and apoptosis were assessed by flow cytometry. The effects of RAF265 was also investigated by western blot.

RESULTS: Cell viability was evaluated at 24, 48 and 72 hours from 75 nM to 10000 nM. For BCPAP cells, IC50 at 48h was abaut 930 nM, for K1 cells was about 1000 nM, while for TT cells was about 160 nM. RAF265 induced a G2-M and S-phase reduction in BCPAP and K1 cells. No appreciable differences were observed on apoptosis using Annexin V-FITC method. Western blot analysis revealed that RAF265 reduced p-Erk1/2 in PTC cell lines, while reduced p-Akt in TT cells.

CONCLUSION: These preliminary results indicate that RAF265 appears to be of potential interest in thyroid cancer. This therapy is promising for further development both in differentiated and medullary thyroid cancer, but additional studies are needed to support this hypothesis.

PP332

THYROID CANCER IN THYROGLOSSAL DUCT CYSTS REQUIRES A SPECIFIC APPROACH DUE TO ITS UNPREDICTABLE EXTENSION

G. Sapuppo[1], G. Pellegriti[1], G. Lumera[1], P. Malandrino[1], A. Latina[1], R. Masucci[2], C. Scollo[1], A. Spadaro[1], C. Regalbuto[1], V. Pezzino[3], S. Squatrito[1]

[1]Department of Clinical and Molecular Bio-Medicine, Garibaldi-Nesima Hospital, University of Catania - Catania, Italy, [2]Department of Surgical Oncology, Garibaldi-Nesima Hospital - Catania, Italy, [3]Endocrinology and Diabetes Center, Cannizzaro Hospital - Catania, Italy

Context: Differentiated thyroid cancer (DTC) in thyroglossal duct cysts is uncommon. The requirement of total thyroidectomy and lymph node dissection is still controversial.

Setting: The study was performed in a referral thyroid cancer center at an academic hospital.

Patients: We conducted a single center retrospective study of a consecutive series of 26 patients with DTC in thyroglossal duct cyst, all having undergone cyst resection and total thyroidectomy.

Main Outcome Measures: Diagnostic modalities, surgical treatment, histopathological features, and clinical outcome were included in the study.

Results: Thyroglossal duct cyst cancer histotype was papillary in 23 of 26 patients (88.5%) and follicular-Hurthle in 3 of 26 cases (11.5%). A concomitant papillary DTC in the thyroid gland was found in 16 of 26 cases (61.5%), and it was multifocal in 8 of 16 cases (50%). At presentation, the patients with cancer in both the thyroglossal duct cyst and the thyroid were older than the patients who only had cancer in the thyroglossal duct cyst (44.9±7.6 vs 32.0±12.7; P=0.006). Lymph node dissection, performed in 17 of 26 patients (65.4%), indicated that the central compartment was involved in 6 patients (35.3%, all having cancer also in the thyroid), the laterocervical compartments in 10 patients (58.8%), and the submental in 4 (23.5%). Six patients (23.1%) had persistent disease at 6-year median follow-up.

Conclusions: DTC in thyroglossal duct cysts occurs at a younger age and with more aggressive features at presentation. Concomitant cancer in the thyroid and lymph node metastases is present in most cases. Lymph node compartment involvement is different from that of cancers in the thyroid gland. Therefore, surgical treatment should include both thyroglossal duct cyst resection and total thyroidectomy, with individualized surgical nodal dissection. Subsequent management should follow current DTC guidelines.

PP333

Abstract withdrawn

PP334

THE SONOGRAPHIC PATTERN OF NECK LYMPH NODES IN CHRONIC AUTOIMMUNE THYROIDITIS

D. Brancato[1], R. Citarrella[2], P. Richiusa[2], M. C. Amato[2], N. Maltese[2], C. Vetro[2], C. Giordano[2]

[1]UOC Medicina Interna e Diabetologia - Ospedale di Partinico, ASP 6 Palermo, [2]UOC Endocrinologia - Università di Palermo

Background: Neck lymph nodes may be involved in the pathogenesis of chronic autoimmune thyroiditis (CAT).We hypothesized that the involvement of cervical lymph nodes in CAT may be associated with a different sonographic pattern of neck nodes in comparison to subjects without CAT.
Methods: We included 106 patients (92 females and 14 males) with CAT and 70 control subjects (53 females andm17 males) without clinical, biochemical, and ultrasonographic evidence of thyroid and neck diseases. We performed laboratory tests (thyrotropin, antithyroperoxidase antibodies, antithyroglobulin antibodies, and ultrasonography) to evaluate in each group: (i) thyroid function, autoimmunity, and morphology; (ii) number, topographic distribution (levels I–VI), and morphology of neck nodes (long-axis diameter; short-axis diameter; short-axis/long-axis ratio; absence or presence of hilus).
Results: Total number of neck nodes with long-axis diameter > 10 mm was significantly higher in the CAT group than in the control group (mean ± standard deviation [SD]: 3.7 ± 2.4 vs. 0.8 ± 1.3; $p < 0.001$) and we found also an increased number of neck nodes in levels II (1.4 ± 0.8 vs. 0.3 ± 0.5; $p < 0.001$), III (2 ± 1.2 vs. 0.3 ± 0.7; $p < 0.001$), and IV (0.7 ± 0.7 vs. 0.07 ± 0.2; $p < 0.001$). Moreover, we found more nodes with a hilus in the CAT group than in the control group (mean number of nodes ± SD: 2.8 ± 1.9 vs. 0.7 ± 1.1; $p < 0.001$). Short-axis diameter of level III (4.4 ± 1 vs. 3.7 ± 1.2 mm; $p = 0.002$) and level IV nodes (3.9 ± 1 vs. 3.1 ± 0.5 mm; $p = 0.030$) was increased in CAT patients when compared with healthy controls.
Conclusions: The present study is the first one aiming at a systematic description of the sonographic pattern of cervical lymph nodes in CAT. An increased number of benign hyperplastic neck nodes, especially in levels II–IV, appears to be a characteristic sonographic finding associated with CAT.

PP335

ULTRAVIOLET RADIATION EFFECTS ON FRTL-5 CELL GROWTH AND THYROID SPECIFIC GENE EXPRESSION

E. Baldini[1], S. Sorrenti[2], N. Prinzi[1], C. Coccaro[1], G. Grani[1], R. Mocini[2], S. Morrone[1], L. Gnessi[1], F. Curcio[3], M. D'Armiento[1], S. Ulisse[1]

[1]Dipartimento di Medicina Sperimentale, "Sapienza" Università di Roma - Roma, [2]Dipartimento di Scienze Chirurgiche, "Sapienza" Università di Roma - Roma, [3]Dipartimento di Scienze Biologiche e Mediche, Università di Udine - Udine

Radiation exposure during space missions represents a major threat for human health, affecting all body organs and tissues. Regarding thyroid function, it has been shown that ultraviolet radiation (UV-C) has dose-dependent apoptotic effects on FRTL-5 cells, a normal strain of rat thyrocytes. The present study examined the effects of a sub-lethal dose of UV-C on FRTL-5 cell growth and gene expression. Cells exposed to 10 J/m2 UV-C showed no differences in viability compared to control cells after 24 h, but the BrdU incorporation was reduced, indicating a cytostatic effect. Quantitative RT-PCR carried out at 24 h and 48 h after irradiation demonstrated that the mRNA levels of thyroglobulin (Tg), thyroperoxidase (Tpo) and sodium/iodide symporter (Nis) were transiently decreased at 24 h in treated cells, while the mRNAs of the thyroid transcription factors, TTF1, Foxe1 and Pax-8, were not affected. In cells cultured with TSH-free medium, the basal transcription of Tg, Tpo and Nis genes was equally impaired by radiation, and no longer stimulated by TSH.
In conclusion, the results demonstrated that a sub-apoptotic dose of UV-C compromises not only thyrocyte proliferation, but also the expression of genes involved in thyroid hormone production. These findings might contribute to explaining the histological, biochemical and clinical features of hypothyroidism observed in both animals and humans during space flight.

PP336

SNP RS2910164 IN PRE-MIR146A UNDERGOES SOMATIC CHANGES IN FOLLICULAR THYROID CARCINOMA

E. Vighi[1], E. Pignatti[1], L. Roncati[2], V. Rochira[3], E. Kara[3], B. Madeo[4], E. Magnani[1], A. Maiorana[2], C. Carani[3], M. Simoni[3]

[1]Dipartimento di Scienze Biomediche, Metaboliche e Neuroscienze; UNIMORE - Modena, [2]Dipartimento di Medicina Diagnostica, Clinica e di Sanità Pubblica - Modena, [3]Dipartimento di Scienze Biomediche, Metaboliche e Neuroscienze; UNIMORE; Azienda AUSL di Modeana-NOCSAE - Modena, [4]Azienda AUSL di Modeana-NOCSAE - Modena

INTRODUCTION: Follicular Thyroid Carcinoma (FTC) is the second most common thyroid tumor, but the mechanism underlying its development is still partially unknown. Changes in the expression of multiple regulatory RNA (miR) genes seem to be involved in thyroid carcinogenesis: for example, several studies suggested that SNP rs2910164 in pre-miR146a, a precursor of miR146a, might be correlated to papillary carcinoma. In this study we also evaluated the possible role of SNP rs2910164 in FTC tumorigenesis and, for the first time, we genotyped by sequencing the pre-miR146a common G/C polymorphism (SNP rs2910164), both in genomic and somatic DNA of patients affected by FTC (n=39).
MATERIALS AND METHODS: Somatic DNA was extracted from formalin-fixed paraffin-embedded tissue, while genomic DNA was obtained from peripheral blood. We compared the SNP distribution and the SNP genotype frequencies between patients' genomic and somatic DNA (both unaffected and tumor tissue). In addition, patients' genomic DNA was compared to 208 controls with negative thyroid sonography. SNP distribution was correlated to the clinical data.
RESULTS: 37% of patients present SNP changes in the transition from genomic to somatic DNA and 31% of cases from unaffected to tumor tissue, although SNP distribution in the patients' genomic DNA was resulted the same as compared to negative controls ($p=0.9106$; $\chi 2$ test). Moreover, we observed an increase of allele G frequency in tumor tissue ($p<0,05$, $\chi 2$ test), in which CC genotype was completely absent. SNP seems not to be correlated with clinical features.
CONCLUSION: Our data suggest that somatic GG and GC genotypes are associated with FTC, while CC homozygous state might have a protective role. We can conclude that the SNP status could influence the expression of either mature miR-146a or related target genes.

PP337

RADIOIODINE THERAPY OF MULTINODULAR TOXIC GOITER USING AN IMPLEMENTED DOSE CALCULATION ALGORITHM ALLOWING REDUCTION OF RADIOIODINE AMOUNT

L. Camerieri[1], M. C. Bagnara[2], E. Pomposelli[1], M. Bevegni[2], I. Calamia[3], P. Antola[1], G. Pesce[1], M. Caputo[3], G. Sambuceti[3], M. Giusti[4], M. Bagnasco[1]

[1]Medicina Int. Laboratorio Autoimmunità Univ. degli Studi di Genova IRCCS S. Martino IST - Genova, [2]UO Fisica Sanitaria IRCCS S. Martino IST - Genova, [3]UO Medicina Nucleare Univ. degli Studi di Genova IRCCS S. Martino IST - Genova, [4]UO Endocrinologia Univ. degli Studi di Genova IRCCS S. Martino IST - Genova

Aim: radioiodine is a common option for treatment of hyperfunctioning nodular goiter. Relatively high "fixed" activities are often used, alternatively, the activity is individually calculated upon the prescription of a fixed value of target absorbed dose. We evaluated the use of an algorithm for personalized radioiodine activity calculation, which allows as a rule the administration of lower radioiodine activities. Patients and methods: Ninety-three (28M, 65F; 43-84 aa) patients with mutinodular toxic goiter eligible for 131I treatment were studied.The activities of 131I to be administered were estimated by the method described by Traino et al. for Graves' disease that we successfully adopted in single hyperfunctioning thyroid nodule (Schiavo et al QJ Nucl Med in press). The method takes into account 131I uptake and its effective half-life, thyroid volume and its expected reduction during treatment. A comparison with the activities calculated by other dosimetric protocols, and the "fixed" activity method was performed. 131I uptake was measured by external counting, thyroid volume by ultrasonography (US), thyroid hormones and TSH by ELISA. Results: After a follow-up of 8-108 months, remission of hyperthyroidism after a single administration of radioiodine was observed in 77/93 patients, among them 65 are euthyroid, while 12 developed hypothyroidism. We observed a median -12% (-7%/-51%) goiter volume reduction in patients achieving euthyroid, -25% (-22/-50%) in patients developing hypothyroidism and -21% (-19%/-41%) in patients with persistent hyperthyroidism. Thyroid volume reduction observed by US after the treatment fairly correlated with what predicted by our model despite a inhomogeneous radioiodine distribution within the gland. Effective half-life was highly variable in different patients, and critically affected dose calculation. The administered activities (156-600 MBq, median 526 MBq) were clearly lower with respect to "fixed" activities (600 MBq) and other protocols' prescription (-38% than Marinelly-Quimby, -15% than Snyder). Conclusion: The proposed algorithm proved to be effective also for multinodular toxic goiter treatment and allowed a significant reduction of administered 131I activities, without loss of clinical efficacy.

PP338

PROTECTIVE EFFECTS OF SELENIUM ON CELL VITALITY IN ORBITAL FIBROBLASTS FROM PATIENTS WITH GRAVES' OPHTHALMOPATHY

M. De Gregorio[1], R. Chiarini[1], G. Rotondo Dottore[1], M. Leo[1], F. Menconi[1], M. A. Altea[1], E. Sisti[1], M. A. Profilo[1], B. Mazzi[1], R. Rocchi[1], F. Latrofa[1], C. Marcocci[1], M. Marinò[1]

[1]Unità Operativa di Endocrinologia 1 - Pisa

Oxidative stress is accounted as one of the inflammatory mechanisms involved in the perpetration of the autoimmune reaction responsible for Graves' Ophthalmopathy (GO). In a recent study is was shown that the antioxidant agent selenium has some beneficial effects in patients with mild GO. However, little is known on the actions of selenium in vitro. Here we investigated the effects of selenium in primary cultures of orbital fibroblasts from six patients with GO and from five control subjects. To induce oxidative stress, fibroblasts were challenged with 100 mcMol H2O2, which resulted in a mean reduction of cell vitality of 38% in GO fibroblasts and 19% in control fibroblasts. When cells when co-incubated with 10 mcMol selenium (selenium-methylcysteine), cell vitality increased by approximately by 19% in both GO and control fibroblasts, an effect that was not observed when cells were co-incubated with 10 mcMol methylcysteine, used as a control. Based on these findings, we concluded that oxidative stress reduces cell vitality in orbital fibroblasts, an effect that is less pronounced in control than in GO fibroblasts, which therefore seem to be more sensitive to oxidative stress. Selenium seems to protect from the effects of oxidative stress on cell vitality, regardless of the origin of orbital fibroblasts. A possible explanation for our observations is that oxidative stress may reduce cell vitality either by necrosis or apoptosis, with subsequent liberation of autoantigens contributing the perpetration of the autoimmune reaction primarily responsible for GO. Selenium may prevent or reduce this phenomenon by protecting cells from oxidative stress, therefore resulting in a lesser liberation of autoantigens. Further studies are required to investigate how oxidative stress affects orbital fibroblasts and the mechanisms involved in the action of selenium.

PP339

SPONTANEOUS IMPROVEMENT OF UNTREATED GRAVES' OPHTHALMOPATHY: THE RUNDLE CURVE REVISITED

E. Sisti[1], M. Leo[1], M. A. Altea[1], M. A. Profilo[1], F. Menconi[1], E. Albano[1], B. Mazzi[1], R. Rocchi[1], F. Latrofa[1], C. Marcocci[1], M. Marinò[1]

[1]Unità Operativa di Endocrinologia 1 - Pisa

Graves' Ophthalmopathy (GO) is though to ameliorate spontaneously with time, according to the so called Rundle curve. However, this remains almost an anecdotal postulate, as very few studies have investigated the issue, considering that this should be analyzed in patients who are untreated concerning their eye syndrome. Here we studied retrospectively 92 patients (32 males, 60 females; age 42.8±14.4 yr.) with untreated GO, except for local measures and low dose glucocorticoids given to those who had undergone radioiodine treatment for Graves' hyperthyroidism. These 92 patients were identified out of a total of 740 consecutive GO patients seen in our GO Clinic from September 2010 to December 2012. They had been observed for the first time 12.5±37.5 mo. after the diagnosis of GO. Eighty-five of them had Graves' disease (GD), 4 had autoimmune thyroiditis and 3 euthyroid GO. Ten were treated only with anti-thyroid drugs, 44 had been treated with radioiodine, 27 with thyroidectomy, 4 were treated with LT4 for primary hypothyroidism, and 3 had not received any thyroid treatment. They had a mild GO at our first observation, as determined using a modification of the NOSPECS score (Profilo et al, Thyroid 2013, 23:97-102), which was 2.3±1.5 points. After a period of 57.8±49.5 mo. from the first observation, the modified NOSPECS score decreased significantly ($p<0.0001$ by paired t-test), namely to 1.8±1.3 points. By simple, linear regression, the eyelid aperture ($p=0.04$), the clinical activity score ($p=0.05$) and the degree of diplopia ($p=0.0002$) at our first observation were positively correlated with the change of the modified NOSPECS score, which was confirmed by multiple regression only for diplopia ($p=0.0002$). In contrast, the change of the modified NOSPECS score was not correlated with age, gender, thyroid volume before thyroid treatment, low dose glucocorticoids, overall GO and thyroid disease duration, type of thyroid disease, thyroid treatment and exophthalmometry at our first observation. Thus, in accordance to the Rundle postulate, our findings demonstrate a spontaneous improvement of untreated GO, which seems to be more pronounced in patients with a more severe GO at the beginning, especially concerning diplopia.

PP340

INDIVIDUALLY TAILORED DOSE OF THYROXINE: A NOVEL TOOL TO DETECT OCCULT AUTONOMOUS THYROID FUNCTIONING NODULES (ATFN)

M. G. Santaguida[1], L. Bianchi[1], C. Virili[1], S. C. Del Duca[1], I. Gatto[1], L. Gargano[2], M. Centanni[1]

[1]Dip. Scienze e Biotecnologie Medico-Chirurgiche Sapienza Univ. di Roma - Latina, [2]UOC Endocrinologia, AUSL Latina - Latina

The individually tailored dose (ITD) of thyroxine (T4) is a new approach to achieve the proper T4 requirement, in relation with age and weight of each patient, to reach the target TSH. We have previously shown that an increased need for thyroxine (T4) help to detect occult forms of gastrointestinal malabsorption. Here, we assume that even the reduced need for T4 may be a tool to discover occult disorders, e.g. ATFN or autonomous functioning areas in the thyroid. Development of functional autonomy is part of the natural history of nodular thyroid diseases. Nowadays, the autonomous areas may be subclinical and even associated to TSH value in the low-normal range and, therefore, may remain a hidden problem. This study provides a new tool to unveil occult autonomous areas. In a cohort of about 3000 consecutively examined outpatients, we found 94 patients (84F and 10M; median age=56 years) with multinodular goitre in treatment with ITD, who showed an inappropriate serum TSH as compared to the one observed in a gold standard reference group (n= 123; 109F and 14M; median age=54 years) in whom iodine and drug interferences, as well as ATFN, were positively excluded. Patients of the study group showed significantly lower median TSH (0.052 vs 0.20 mU/l, $p<0.0001$), despite a slightly lower dose administered (1.19 vs 1.49 µg/Kg/day, $p<0.0001$). When separately analyzed according to age (<o> 60 years), these differences were confirmed both in adult patients (median TSH: 0.06 vs 0.2 mU/l, $p<0.0001$; median T4 dose: 1.29 vs 1.56 µg/Kg/day, $p<0.0001$) and in older patients (median TSH: 0.05 vs 0.22 mU/l, $p<0.0025$; median T4 dose: 0.94 vs 1.33 µg/Kg/day, $p<0.0005$). All these patients underwent thyroid scintiscan and radioactive iodine uptake test (RAIU- 4° and 24° hour). Interestingly, when treatment was withdrawn, 86/94 patients (91.5%) had serum TSH in the normal range and the median TSH of the whole study group was 1.09 mU/l. The presence of autonomous functioning areas has been detected in 65 out of 94 patients (69%); the differences in RAIU at 4° hour (17 vs 13%, $p<0.0199$) and 24°hour (33.5 vs 28%, $p<0.001$) were both highly significant. These data show that more than 2/3 of patients hyperresponding to an individually tailored dose of T4 have an occult ATFN or autonomous functioning areas, thus exposing them to possible heart arrhythmias. ITD has been proven as a valuable tool to suspect and detect occult autonomous functioning areas in the thyroid.

PP341

PRIMARY THIROID LYMPHOMA: A CASE REPORT.

E. Marchesi[1], M. Marina[1], S. Haddoub[2], N. Venturi[2], F. Spagnoli[2], M. Zardo[2], M. C. Baroni[2], G. Ceresini[2], R. Minelli[2]

[1]Dipartimento di medicina clinica e sperimentale - Parma, [2]Dipertimento di medicina clinica e sperimentale - Parma

Primary thyroid lymphoma is rare, representing approximately 1-5% of thyroid malignancies and less than 2% of extranodal lymphomas; most are non-Hodgkin's lymphoma of B-cell origin. Hashimoto's thyroiditis (HT) is the only well known risk factor. In this report, we discuss the case of a 39-years-old man; he was referred to our clinic complaining a rapidly enlarging thyroid swelling since one month. He had no personal history of thyroid disease, but reported a family history of HT. Laboratory examination indicated a subclinical hypothyroidism with positive AbTPO. Thyroid ultrasonography showed a diffusely non-homogeneous and hypoechoic structure with fibrous striae, consistent with a chronic autoimmune thyroiditis; it revealed also a not well circumscribed hypoechoic nodule in the left lobe of the thyroid measuring 19x10 mm. No other lymph nodes and organomegaly were present. Subsequent fine-needle aspiration from the left lobe was performed, showing a rich cellularity with primary component lymphoid, medium to large in size, and secondary component of histiocytes with tingibles bodies. On FDG –PET examination, a FDG hyper-accumulation was detected in the left thyroid lobe, no other sites of pathological accumulation were detected. Bone marrow aspiration was normal. An open biopsy was performed and histologic examination revealed a neoplastic lymphoid proliferation composed of elements of large size with relatively large and clear cytoplasm and widespread growth. Immunohistochemistry showed positivity for CD20 +, Bcl2 +, BCL6 +, CD10 +, with negativity for CD3-, CD23- and Ki67 proliferation index of 50%. No residual thyroid tissue were seen. Final diagnosis of primary diffuse large B-cell lymphoma of the thyroid gland was made. According with literature, the patient was treated with CHOP regimen plus rituximab; after 4 cycles of therapy, the lesion size decreased, the thyroid function recovered and the control FDG-PET didn't show any hyper-accumulation areas. The patient received other 2 cycles of treatment to consolidation. A FDG-PET was performed six month after therapy conclusion without any pathological findings. Although rare, a primary thyroid lymphoma has to be ruled out in a preexisting case of HT if there is a sudden increase in size associated or not to other local symptoms. In our case, the disease was suspected by FNAC and later confirmed by biopsy; the patients received chemotherapy instead of surgery and showed a good response.

PP342

COURSE OF GRAVES' DISEASE MANIFESTED DURING THERAPY WITH IFN IN INDIVIDUALS WITH HCV HEPATITIS COMPARED TO GD IN CONTROLS

F. Spagnoli[1], E. Marchesi[1], N. Venturi[1], M. Marina[1], A. Orlandini[2], M. Zardo[1], C. Ferrari[2], R. Minelli[1]

[1]Dipartimento di medicina clinica e sperimentale - Parma, [2]Dipartimento Onco-ematologico-internistico - Parma

The production of anti-thyroid antibodies and the alteration of the thyroid function are the most common disorders associated with interferon alpha (IFN-α) and ribavirin (RIBA) therapy in individuals with chronic hepatitis HCV-related (C-HC).The incidence of thyroid disorders induced by IFN-α in patients with C-HC varies from 3, 9% to 27, 2% with an average of 11, 02%. The aim of our study was to evaluate the alteration of the thyroid function in patients treated with IFN-α for C-HC, in particular the course of Graves' Disease (GD) induced by IFN compared to GD arising in healthy control individuals. This study is of a retrospective type, it refers to the period from 1999 to 2011 and includes 175 patients, 7.3% of these patients were subjected to an antiviral therapy for C-HC in the above mentioned years, 111 were women (63,43% of the total) and 64 were men (36,57%). Of these patients 84 (48%) developed Hypothyroidism (hypo), 39 (22,29%) Hyperthyroidism from GD and 52 (29,71%) Destructive Thyroiditis (DT). The course of GD was compared in both sexes, in particular we evaluated the number of remissions, the duration of the therapy, the dose/day of methimazole in individuals with C-HC in therapy with IFN compared to GD arising in HCV-negative individuals. From our data, GD from IFN presents a significantly less serious course compared to the same pathology arising in healthy patients. No such data exists in literature up until now, our hypothesis is that the thyroid diseases manifested during the therapy with IFN are less serious compared to the natural course of this disease in healthy individuals.
Further studies are necessary to confirm our hypothesis.

PP343

DISTINCTIVE ROLES OF THYROID HORMONES ON ZEBRAFISH TISSUES DEVELOPMENT

F. Marelli[1], L. Persani[2]

[1]IRCCS Istituto Auxologico Italiano - Milano, [2]IRCCS Istituto Auxologico Italiano, Università degli Studi di Milano - Milano

Introduction: Zebrafish is a useful model to study the endocrine system. Both in zebrafish and humans, thyroid hormones (TH) act by binding with their specific nuclear receptors (TRα and TRβ), promoting the embryonic development. The tissue-specific action of TH is closely linked on the type of TR expressed. For that reason, patients carrying mutations on TRα or TRβ genes, display several morphological anomalies, which depend on the preferential expression of TRs in the different organs.

Methods: By the injection of morpholinos, we silenced the expression of endogenous TRα and TRβ, creating two defective-zebrafish lines (MO_A and MO_B). We than evaluate: 1) the embryonic growth through the measurement of different parameters (e.g. body length, head curvature and eyes inter-distance) and 2) morphological and functional alterations using several analytical methods (histology, whole mount in situ hybridization, immunohistochemistry and confocal microscopy).

Results: Both of defective-fish lines display a severe growth delay with small head and short body length. In addition, morphants exhibit characteristic patterns of anomalies due to the absence of one or other receptor. MO_A shows severe defects on brain, heart and liver development while MO_B displays retina and otoliths malformations and hypothyroidism results by the lost of the negative feedback of the IIT axis.

Conclusions: Zebrafish is an excellent model to study the role of THs and TRs during embryonic development, representing an interesting and new biotool to test human TRα and TRβ mutations.

PP344

RADIOFREQUENCY ABLATION (RFA) OF PREDOMINANT THYROID NODULES: SAFETY AND CHANGES IN QUALITY OF LIFE (QOL).

S. Oddo[1], L. Mortara[1], E. Monti[1], V. Caorsi[1], E. Felix[1], G. Turtulici[2], F. Minuto[1], M. Giusti[1]

[1]U.O Clinica endocrinologica DiMI, Ospadale universitario San Martino - Genova, [2]U.O. Radiologia Ospedale Internazionale Evangelico - Genova

This relatively new application of RFA was started in our center in 2012 to manage large benign thyroid nodules. Literature data report significant nodule reduction after 1-2 RFA sessions and a very low incidence of adverse events. We report our preliminary experience of RFA in thyroid nodules with confirmed benign cytology. In addition to nodule reduction, we evaluated the safety of the procedure and changes in neck problems and QoL. To date, 12 subjects (8 females, 4 males; age range: 37-81 yrs) have been treated on an outpatient basis. RFA was performed by means of the moving shot technique with a cooled electrode (StarMed G18 x7 cm; 1 cm active tip) via a trans-isthmic approach. After RFA, efficacy (nodule reduction on sonography), changes in nodule features (on contrast-enhanced sonography) and adverse events were monitored for 6 months. Subjective neck discomfort due to thyroid nodules was scored on a visual analogic scale (VAS) ranging from 0 (no complaints) to 10 (maximum degree of neck discomfort). QoL was evaluated by means of ThyPRO, which is a new 13-scale questionnaire that has been validated for QoL assessment in thyroid diseases. RFA was performed without sedatives. Mean active shot time was 7 min; 2 patients reported slight pain and 1 suffered bleeding from the site of needle insertion. One week after RFA, 40% of patients reported slight neck discomfort, but the VAS score significantly decreased (by 50% on average) from the baseline (3.1±0.8) to the 6-month examination (1.5±0.5; P<0.05). QoL improved slightly from the baseline to the last examination. However, a decrease in ThyPRO scores (i.e. fewer symptoms or lower impact of thyroid disease) was noted on the goiter scale (baseline 14.8±4.8 vs. last examination 10.5±2.6, P=0.06) and some other scales concerning the impact of thyroid disease on social and daily life. The physical scale "tiredness" displayed the highest score both at the baseline (33.8±5.0) and at the last examination (37.1±5.4). The average thyroid nodule reduction in the 6-month period was 42% (range 0-56%); this was statistically significant (baseline 33±4 ml vs. 6-month examination 22±5 ml; P<0.05). No change in thyroid function was noted. After RFA, contrast-enhanced sonography revealed avascularized areas corresponding to treated parts of nodules. Our preliminary data seem to confirm the safety and acceptable efficacy of RFA. The cost/benefit ratio of a single RFA session is very acceptable). To our knowledge, this is the first use of the ThyPRO questionnaire to evaluate QoL in patients undergoing RFA. RFA does not impair QoL. However, further studies in larger populations are needed in order to validate the present data.

PP345

HALF DOSE L-THYROXINE PROTOCOL IS NOT ADEQUATE TO PREPARE THYROIDECTOMIZED THYROID CANCER PATIENTS FOR DIAGNOSTIC EVALUATION AND 131-I TREATMENT.

I. Marturano[1], P. Malandrino[1], M. Russo[1], A. Spadaro[1], A. Latina[1], S. Squatrito[1], C. Regalbuto[1]

[1]Endocrinology, Dept of Clinical and Molecular Biomedicine, Univ. of Catania - Catania

INTRODUCTION After total thyroidectomy for differentiated thyroid cancer (DTC), patients often have to undergo L-T4 withdrawal in preparation for diagnostic or therapeutic purposes. L-T4 withdrawal produces symptoms of hypothyroidism and signs of organ dysfunction. To reduce these inconveniences it has been proposed to induce a slighter hypothyroidism, reducing replacement therapy to one half, without L-T4 withdrawal. **AIM** To evaluate half-dose L-T4 protocol, in comparison to conventional L-T4 withdrawal, in terms of effectiveness to achieve the TSH target value of 25 μU/ml and in terms of improvement of clinical and biochemical disorders. **MATERIALS AND METHODS** We randomized 55 patients thyroidectomized for DTC and having no residual thyroid tissue and negative serum Tg into two groups: (A) 29 pts were prescribed 5 weeks of half-dose of previous L-T4 treatment (HALF-LT4) and (B) 26 pts received replacement of previous L-T4 with L-T3 for 3 weeks followed by 2 weeks of withdrawal (SUSP). Clinical features (sex, age, BMI), LT4 dosage, the Zulewsky clinical score to evaluate hypothyroidism (Z) and biochemical parameters (total cholesterol, triglycerides and the enzymes GOT, GPT, CPK and LDH) were evaluated in all patients at baseline and after 5 weeks. TSH, FT3 and FT4 were measured at baseline and 3, 4 and 5 weeks later. **RESULTS** At baseline clinical and biochemical features were similar in the two groups. Biochemical parameters and Z increased at 5 weeks in both groups, but significantly more in SUSP, except for triglycerides and Z. Patients who achieved the serum TSH target value were 24/26 (92.3%) in the SUSP group and 14/29 (48.3%) in the HALF-LT4 group ($p < 0.001$). In the HALF-LT4 group, only basal (before L-T4 reduction) serum TSH statistically correlated with the achievement of the TSH target. ROC curves indicated that a basal TSH $\geq$ 0.52 uU/ml is required to reach an adequate TSH level (sensitivity 66.7% and specificity 100%). **CONCLUSIONS** Half-dose L-T4 protocol, compared to conventional thyroxine withdrawal, is associated with less biochemical disorders but no significant clinical advantage. The half-dose protocol reaches an adequate TSH target in only one half of patients. This protocol, therefore, is not effective for preparing DTC patients for diagnostic or therapeutic purposes, unless basal serum TSH is $\geq$ 0.52 uU/ml.

PP346

P53 AND CELL CYCLE TARGETING AS SELECTIVE THERAPY FOR POORLY DIFFERENTIATED THYROID CANCERS.

E. S. Grassi[1], V. Vezzoli[2], I. Negri[3], L. Persani[4]

[1]Dipartimento di Scienze Cliniche e di Comunità, Università degli Studi di Milano - Milano, [2]Laboratorio Ricerche Endocrinologiche, Istituto Auxologico Italiano - Milano, [3]Università degli Studi di Milano - Milano, [4]Laboratorio di Ricerche Endocrinologiche, Istituto Auxologico Italiano; Dipartimento di Scienze Cliniche e di Comunità, Università di Milano - Milano

Poorly differentiated thyroid cancers are highly aggressive neoplasia that frequently harbour p53 point mutations; the high lethality of this tumours is mainly due to the lack of effective therapies. SP600125 is a compound commonly used as JNK inhibitor which also suppresses different serine/threonine kinases. In this study we analized the effects of SP600125 on four different thyroid cancer cell lines, three with p53 point mutations and one with a p53 null state. Our results show that SP600125 exerted antiproliferative effects only on the p53-mutated cell lines with more than 80% growth inhibition after 96 hours of incubation and EC50s range less than 10 μM, concentration lower than the one required for JNK inhibition. Further analysis showed a significant increase in p53 phosphorilation at serine 15, subsequently causing a sustained increase in p21 levels; these modifications are consistent with alterations in cell cycle progression and cell replication. Microscopy analysis demonstrated significant dose and time dependent alterations with enlarged, polylobated or multiple nuclei in treatment-responsive cell; nuclear area quantification demonstrated a significant (2.5 fold) increase. On the other hand only slight variations in apoptosis marker were observed, with lack of p53 phosphorilation at serine 46 and only a transient caspase 3 and PARP activation. Taken together these results demonstrated that SP600125 act in a p53 selective way with mainly cytostatic effects. It is able to alter cell cycle progression with probable induction of endoreduplication, a process consisting in subsequent cycle of DNA replication without nuclear and cell division, which finally culminates in cell death. In conclusion SP600125 is able to inhibit poorly differentiated thyroid cancers derived cell lines and the p53-selective mechanism of action make it a reliable candidate for scarcely differentiated cancers therapy, such as PDTCs.

PP347

BASAL THYROGLOBULIN (BTG) AND RISK OF RECURRENCE IN DIFFERENTIATED THYROID CANCER (DTC)

A. Taccaliti[1], F. Silvetti[1], M. Boscaro[1]

[1]Dept of clinical and molecular sciences Division of Endocrinology - Polytechnic University of Marche Ancona

DTCs comprise papillary and follicular thyroid cancers (PTCs and FTCs). European Consensus Report (ECR) and American Thyroid Association (ATA) distinguish 3 and 4 risk categories of recurrence respectively. According to ECR, Very Low Risk (VLR) patients undergo only surgery as therapy, on the contrary Low Risk (LR) and High Risk (HR) patients need total thyroidectomy plus radioidine (RAI) ablation. Aim of our study is to evaluate the role of BTg in recurrences of disease. BTg was performed at 6-12 months after total thyroidectomy and RAI ablation during L-tiroxine suppressive therapy. Relapsed of cancer is evaluated by thyroglobulin value after recombinant TSH (LTg) at the last follow-up. We retrospectively considered a total of 437 patients, mean age at diagnosis was 47.5 ± 15.6years; 150 LR (34.4%) with mean age at diagnosis of 47.9 ± 14.3 years and 287 HR (65.6%) with mean age of 47.3 ± 16.3 years. The mean follow-up was 6.6 ± 6.2 years, in particular 4.9 ± 3.7 years in LR and 6.1 ± 5.7 years in HR. Our results showed that the mean value of BTg, in all 437 patients was directly correlated with recurrence of cancer ($p=0.0002$). Particularly, patients with recurrence showed mean BTg 5.9 ± 14.3 ng/ml while patients without recurrence had lower mean BTg value, amounted to 0.3 ± 0.7 ng/ml ($p < 0.0001$). As literature data suggest, we considered as positive predictor factor of recurrence BTg value $\geq$ 0.5 ng/ml. In our series, recurrence was demonstrated by LTg $\geq$ 2.0 ng/ml in 8.4% of patients with BTg < 0.5 ng/ml and in 50 % of patients with BTg $\geq$ 0.5 ng/ml. This difference was statistically significant ($p < 0.0001$). In LR group positive LTg was demonstrated in 3.5% of BTg < 0.5 ng/ml, and no patients had positive LTg if BTg was $\geq$ 0.5ng/ml. On the other hand in HR group LTg $\geq$ 2ng/ml was identified in 13.4% of BTg < 0.5ng/ml, and in 52.4% of BTg $\geq$ 0.5ng/ml ($p = 0.0002$). Conclusion: our results show a role of BTg in the follow-up of DTCs. Independently of risk category, patients without recurrence had mean value of BTg significantly lower than patients with recurrence. Moreover, we have demonstrated a low positive predictive value of BTg to identify recurrence in LR group. Instead in the HR group BTg $\geq$ 0.5ng/ml appears as indicator of higher risk of recurrence. These data suggest that HR patients with BTg $\geq$ 0.5ng/ml require more frequent follow-up respect to patients with BTg < 0.5ng/ml. In LR group, independently by BTg values, the low prevalence of recurrence suggests a most gap in follow-up. Other data are required to confirm our hypothesis.

PP348

BIOINFORMATICS CERNA ANALYSIS FOR THE STUDY OF STEM FACTOR SOX2 IN ANAPLASTIC THYROID CANCER.

W. Arancio[1], G. Pizzolanti[1], P. Richiusa[1], M. Pitrone[1], C. Baiamonte[1], L. Tomasello[1], V. Carina[1], C. Giordano[1]

[1]Di.Bi.M.I.S. - Palermo

Cancer stem cells (CSC) are believed to play a central role in oncogenesis, but until today their isolation and characterization is still particularly complex. Anaplastic thyroid cancer (ATC) presents several characteristics suggestive of a tumour highly enriched in CSC (high mitotic rate, poor prognosis, high aggressiveness, resistance to treatments, etc). For these reasons ATC represents a good candidate to study CSCs. *SOX2* is a key stem transcriptional factor, usually only transiently expressed, that plays a fundamental role in stem cell identity. *SOX2* proved to be constitutively expressed in SW1736 cell line, a well established and recognized ATC cell line. The bioinformatics ceRNA analysis permits to discover gene transcripts that may be post-transcriptionally positively co-regulated with the 3'UTR probe.

We aimed to perform ceRNA analysis in SW1736 to identify candidate genes that might be involved in *SOX2* functional network and in turn in stemness and CSC biogenesis. A competing endogenous RNA (ceRNA) *in silico* analysis was performed on 3'UTR of *SOX2* mRNA. Our analysis harvested several genes involved in the RNA interference mechanism (*DICER1, DROSHA, AGO2*) and in cell cycle control (*TP53, CCND1*). To further validate the *in silico* analysis, *in vitro* analysis via RT-PCR was performed. Knocking down *SOX2* in SW1736 (0.417 expression rate), the interacting ceRNA transcripts were coherently downregulated with significant differences (expression rates from 0.355 to 0.705, $p < 0.05$). Moreover, a statistical analysis of the ceRNAs in other cell lines and clinical specimens revealed a positive correlation with the expression of *TP53, DICER1, DROSHA* and *AGO2*, suggesting the existence of a fine regulatory network.

PP349

ENDOCRINE DISRUPTORS: REPRODUCTIVE TOXICITY AND THYROID EFFECTS IN SPRAGUE DAWLEY RATS EXPOSED TO LOW DOSES OF ETHYLENETHIOUREA

S. De Angelis[1], F. Maranghi[2], R. Tassinari[2], F. Chiarotti[1], S. Lorenzetti[2], G. Moracci[2], D. Marcoccia[2], E. Giladi[1], D. Rotondi[1], A. di Virgilio[3], A. Eusepi[3], A. Mantovani[2], A. Olivieri[1]

[1]Dpt. Cell Biology and Neurosciences-Istituto Superiore di Sanità - Rome, [2]Dpt. Food Safety and Veterinary Public Health-Istituto Superiore di Sanità - Rome, [3]Service Biotechnology and Animal Welfare-Istituto Superiore di Sanità - Rome

Ethylenethiourea (ETU) is the common metabolite of ethylenebisdithiocarbamates, a group of fungicides widely used in agriculture and with a well known anti-thyroid activity. Although ETU does not show a significant bioaccumulation, biomonitoring studies indicate a diffused diet-related intake in the general population. No studies have been performed to assess potential effects of ETU exposure at low dose levels during critical phases of development. The aim of the study was to verify the short- and long-term effects on thyroid function, reproduction and development of oral exposure to low doses of ETU in rats. Dams were treated daily by gavage during pregnancy and lactation with 0 (CTRL), 0.1 (ETU0.1), 0.3 (ETU0.3), 1.0 (ETU1.0) mg/kg bw per day of ETU. F1 generation was similarly treated from weaning until sexual maturity. Serum triiodothyronine (T3), thyroxine (T4) and thyroid stimulating hormone (TSH) were analyzed in dams and offspring. Pregnancy adverse outcomes (PAC: litter resorptions and dystocia) were observed in all treated groups of dams. However, the highest frequency of PAC was found in ETU1.0 dams showing a serum TSH mean value significantly higher than CTRL (8.3±3.7 vs 4.7±1.6 ng/ml, P<0.01). As regards offspring, short- and long-term hypothyroidism was observed in both sexes as supported by the dose-dependent histological and histomorphometrical alterations found either at weaning or in adult rats. Concerning thyroid function tests, the variance analysis showed a global thyreostatic effect of treatment on serum T4 (P<0.001) and TSH (P=0.001) levels with the most evident effects observed in both sexes of ETU0.3 group. Concerning reproductive effects, estrous cycle length was significantly altered in all treated females showing increased mean cycle length. These results may indicate that females exposed to low doses of ETU during development until sexual maturity may show signs of reproductive senescence at an earlier stage of life compared to control group. For the first time this study has demonstrated reproductive toxicity and ETU-induced hypothyroidism at low doses of exposure in pregnant dams and F1 generation. Moreover, ETU exposure during critical period of development and maturation can alter the reproductive programming in females, with important implications for human reproductive health.

PP350

HASHIMOTO'S THYROIDITIS MAY PRESENT DIFFERENT CHARACTERISTICS IN NEIGHBORING GEOGRAPHIC AREAS.

D. Gullo[1], A. Latina[1], F. Trimarchi[2], S. Benvenga[2]

[1]Endocrinology, Department of Clinical and Molecular Biomedicine, Garibaldi-Nesima Hospital - Catania, [2]Department of Clinical and Experimental Medicine, Section of Endocrinology, University Hospital - Messina

Context. *Hashimoto's thyroiditis* (HT) is one of the most common autoimmune endocrine disorders that, like other autoimmune diseases, involves interactions between environmental and genetic factors. In Sicily data on HT are available for the province of Messina (1975-2005) with an increasing frequency over the years. The aim of the present study is to assess the frequency and clinical characteristics of thyroid autoimmunity in two adjacent sicilian areas that differ in their natural geological setting, i.e. Messina and the volcanic territory of Catania.

Objective. To replicate in Catania, on comparable years, the HT data of Messina.

Methods. Review of the clinical records of thyroid patients in the years 1995-2005 to compare presentation and yearly changes of HT at tertiary endocrine centers in Catania and Messina. Total thyroid patients seen at the centers were approximately 28,000 in Catania and 11,000 in Messina; population was approximately 1,100,000 (Catania province) and 660,000 (Messina province).

Results. Catania is outnumbered by Messina (742 vs. 3,409 HT patients). Similar were: the linear increase in the yearly number of HT patients (r = 0.953 *vs*. 0.979), rates of thyroid dysfunctions (e.g., hypothyroidism = 43% *vs*. 46%), but with different proportions of subclinical and overt hypothyroidism (53% *vs*. 83% and 47% *vs*. 17%, P< 0.0001), and rates of positiveness for TgAb (52% *vs*. 55%) or TPOAb (64% *vs*. 67%), with TPOAb sharing the linear yearly decrease (r= - 0.656 *vs*. -0.874). Different were: age (42.3±14.5 *vs*. 41.6±2.4, P = 0.01) and its yearly trend; gender distribution (females and males = 92.6 and 7.4% *vs*. 89.5 and 10.5%, P=0.10); rates of the sonography variants, though yearly trends were similar (e.g., nodular variant = 24% *vs*. 56, P< 0.0001; r = 0.16 *vs*. 0.35).

Conclusions. The HT epidemics is smaller in Catania, with changes in presentation overlapping partially those in Messina. The reasons for this difference remain unclear, although different environmental factors might be involved in each area. A possible protective effect on autoimmune processes in the thyroid gland exerted by volcanic trace minerals, such as selenium, can be hypothesized.

PP351

LATE PRIMARY AUTOIMMUNE HYPOTHYROIDISM IN A PATIENT WITH POST-DELIVERY HYPOPITUITARISM ASSOCIATED WITH ANTIBODIES TO GH AND PRL SECRETING CELLS

A. De Bellis[1], C. Colella[1], G. Bellastella[1], E. Lucci[1], T. Turino[1], A. Bizzarro[2], I. Holdaway[3]

[1]Department of Cardiothoracic and Respiratory Sciences - Napoli, [2]Department of Clinical and Experimental Medicine and Surgery "F. Magrassi, A. Lanzara" - Napoli, [3]Greenlane Clinical Centre and Auckland City Hospital Auckland - New Zealand

Pituitary and thyroid autoimmunity can be triggered by pregnancy. We report the first association of combined growth hormone (GH) and prolactin secretion deficiency due to autoimmune damage to GH- and prolactin- secreting cells in a patient with post delivery lactational failure, presenting subsequently with primary autoimmune hypothyroidism. Patient Findings: A 34 year old woman presented with failure of lactation following delivery of her first child. She had a family history of hypothyroidism without a history of pituitary dysfunction. Physical examination did not show any abnormal findings. Laboratory investigations showed normal gonadotropin levels after the restoration of normal menstrual cycles following pregnancy, normal basal and stimulated cortisol levels, but an impaired GH response to insulin-induced hypoglycemia, and low basal prolactin and insulin-like growth factor-1 concentrations. Thyroid function was normal when initially investigated 3 months after delivery, but 5 months later marked primary hypothyroidism (TSH levels >100 mIU/L) occurred. Immunological investigation revealed the presence of antipituitary antibodies, identified by double immunofluorescence and targeting GH- and prolactin- secreting cells. Antithyroid antibodies, in the normal range 3 months post-partum, became significantly elevated when the hypothyroidism appeared.

Conclusions: Autoimmune hypophysitis is responsible for selective or multiple pituitary-hormone deficiencies, sometimes involving TSH secretion and causing secondary hypothyroidism, but usually associated with hyperprolactinemia. To our knowledge this is the first observation of autoimmune hypopituitarism involving deficient growth hormone and prolactin secretion in a patient with lactation failure after delivery, subsequently followed by severe primary autoimmune hypothyroidism, thus falling into an unusual constellation of autoimmune polyendocrine syndrome type 3. Considering the well known relationship between pregnancy and autoimmunity, an early post-delivery immunological and functional investigation in women presenting with disorders of lactation may be useful to detect potential pituitary and thyroid dysfunction even at a subclinical stage.

PP352

INHIBITION OF THYROID FUNCTION BY POLYPHENOLS

C. Giuliani[1], S. Di Santo[1], I. Bucci[1], M. Piantelli[2], A. De Remigis[1], F. Monaco[1], G. Napolitano[1]

[1]Medicina e Scienze dell'Invecchiamento, Università di Chieti-Pescara - Chieti, [2]Oncologia e Neuroscienze, Università di Chieti-Pescara - Chieti

Polyphenols such as Quercetin and Resveratrol are dietary compounds present in fruits and vegetables. They are available also as dietary supplemental and ingestion of 1 g/day or even more, has been reported. Several studies have shown that both Quercetin and Resveratrol possess many therapeutically relevant properties as induction of apoptosis in tumor cells, antiviral, antioxidant, anti-inflammatory and antiproliferative activities. Beside these positive health effects, potential side effects should be considered in case of excessive intake. Indeed, several polyphenols have potent antithyroid properties. In a previous report we have shown that the treatment with Quercetin of the rat thyroid cell lines FRTL-5, inhibited cell growth, iodide uptake and sodium-iodide symporter (NIS) gene expression. Preliminary data showed a similar effect of Resveratrol in the FRTL-5 cells. In the present study, we further investigated the effect of Quercetin and Resveratrol on thyroid gene expression performig Northern blotting, Western blotting and ELISA and we showed that both compounds downregulated the expression of others thyroid-restricted genes, as TSH receptor (TSHR), thyroid peroxidase (TPO) and thyroglobulin (TG). We further studied the antithyroid effect of Quercetin and Resveratrol evaluating the radioiodine uptake (RAIU) in Sprague-Dawley rats. Twelve rats were treated for 14 days with Quercetin or Resveratrol 50 mg/Kg i.p. After treatment, 125-I was administerd i.p. and RAIU was evaluated after 24 hours. A significant decrease of RAIU was observed after treatment. These data indicate a potential role of Quercetin and Resveratrol as a thyroid disruptor and suggest further studies to evaluate their use in hyperthyroidism.

PP353

ZEBRAFISH AS A NEW IN VIVO MODEL TO STUDY ANGIOGENESIS IN HUMAN MEDULLARY THYROID CANCER.

G. Gaudenzi[1], F. Cotelli[2], A. Dicitore[3], L. Persani[1], G. Vitale[1]

[1]Department of Clinical Sciences and Community Health, University of Milan - Milano, [2]Department of Biosciences, University of Milan - Milano, [3]Laboratory of Experimental Endocrinology, Istituto Auxologico Italiano IRCCS - Milano

In the last years, the teleost Zebrafish (*Danio rerio*) has emerged as a powerful human disease model system and a promising alternative platform in cancer research. Zebrafish has a relatively complex circulatory system similar to that of mammals. This feature, together with the optical transparency and ability to survive for 3–4 days without functioning blood circulation, makes the zebrafish embryo amenable for vascular biology studies.

In this study, we developed a new *in vivo* model to study angiogenesis in human medullary thyroid cancer (MTC). We have taken advantage of the *Tg(fli1:EGFP)y1* zebrafish line that expresses enhanced green fluorescent protein (EGFP) under the control of the *Fli1* promoter, thereby labeling all blood vessels and providing a live visual marker for vascular development. Stained TT cells, a human MTC cell line, diluted in PBS were injected between the periderm and the yolk syncytial layer of 48hpf (hours post fertilization) *Tg(fli1:EGFP)y1* embryos in the proximity of the developing sub intestinal vein (SIV) plexus. A proper control group, represented by zebrafish injected with only PBS without TT cells, was included in the experimental protocol. At least 20 embryos were used in each group. While injection of PBS without tumor cells did not modify the morphology of the SIV plexus, the injection of TT cells stimulated the migration and growth of sprouting vessels from the SIV toward the MTC implant. The vasoproliferative sprouting response triggered by the tumor xenograft has been detected by both fluorescence microscopy and alkaline phosphatase staining at 24–48 hours post injection.

In conclusion, the easy and rapid visualization of the tumor induced vascularization makes our zebrafish/tumor xenograft model a powerful tool to investigate molecular events involved in tumor angiogenesis and to assess the effects of new antiangiogenic compounds in MTC.

PP354

LOW ELASTICITY SCORE AT ELASTOSONOGRAPHY IN THYROID NODULE IS CORRELATED WITH MALIGNANCY,DEGREE OF FIBROSIS, HIGH EXPRESSION OF GALECTIN-3 AND FIBRONE

°. Scutari[1], °. Rago[1], °. Loaicono[2], *. Torregrossa[3], *. Giannini[3], *. Borrelli[3], *. Proietti[3], *. Basolo[3], °. Vitti[1]

[1]Dpt. of Clinical and Experimental Medicine - Pisa, [2]loiaconovaleria@hotmail.it - Pisa, [3]department of surgical medical and molecular pathology - Pisa

Introduction: Ultrasound elastography (USE) provides an estimation of tissue stiffness and has been already applied to differentiate malignant from benign lesions in several tissues. Galectin-3 (Gal-3) and Fibronectin-1 (FN-1) are involved in multiple functions, including cell-cell and cell-matrix adhesion, tissue fibrosis, and tumor progression.

Methods: This study included 78 consecutive patients with a single thyroid solid nodule who underwent surgery for a cytology result indeterminate (n=39) or indicative /suspicious of malignancy (n= 39) at fine needle aspiration. USE was performed using a real-time instrument (Hitachi, Logos EUB 8500 with a 10 MHz linear transducer). Tissue stiffness was scored from 1 (high elasticity) to 2-3 (low elasticity). Gal-3 and FN-1 mRNA quantification was performed using the two-step Real-Time Quantitative RT-PCR Rotor Gene Sybr Green PCR Kit (Qiagen) on a Rotor Gene 6000 (Qiagen) instrument. Beta-Actin mRNA was used as an internal control. Pooled normal thyroid tissue was used as external control. The comparative threshold cycle (Ct) method, defined as $2\text{-}\Delta\Delta Ct$, were used for the calculation of fold amplification of each sample versus external control. Gal-3 and FN-1 mRNA expression was normalized, positive and negative z scores indicating a value above or below the mean, respectively. The converted z scores were then aggregated into one large set, identifying two different categories: 'low expression' (negative z score), 'high expression' (positive z score). Tumor fibrosis, evaluated using haematoxylin and eosin staining by both optical and digital microscope analysis, was scored as the percentage of fibrosis with respect to tumor cell-occupied area.

Results: Score 1 at USE was found in 33 cases, 32 benign lesions and 1 carcinoma at histology; score 2-3 in 45 cases, all carcinomas. The low elasticity was highly predictive of malignancy ($p<0,0001$). High expression of Gal-3 was found in 33/45 nodules with score 2-3 at USE and only in 2/32 nodules with score 1 ($p<0.0001$). High expression of FN-1 was found in 26/45 nodules with score 2-3 at USE and in none with score 1 ($p<0.0001$). The degree of fibrosis was 10,46% + 17,15 in nodules with score 1 and 22,5% + 25,73 in those with score 2-3 ($p=0,025$). In conclusion these data: i) confirm that low elasticity at USE is highly correlated with malignancy; ii) suggest that low elasticity is linked with fibrosis and higher expression of Gal-3, FN-1, involved in tumor progression.

PP355

LEVOTHYROXINE ORAL SOLUTION REPLACEMENT THERAPY. AN OBSERVATIONAL STUDY.

D. Brancato[1], A. Scorsone[1], L. Ferrara[1], G. Saura[1], A. Di Noto[1], V. Provenzano[1]

[1]UOC Medicina e Diabetologia, ASP 6 Palermo - Ospedale di Partinico

Aim: to evaluate if any change in TSH level occurs from baseline when hypothyroid patients switch from LT4 tablets to LT4 oral solution (LT4OS).

Subjects and methods: from 121 consecutive patients who switched from LT4 tablets to LT4OS and unable or unwilling to take LT4 tablets with a lag time before breakfast (LTime) ≤ 60', we selected 44 patients (42 females and 2 males; age: 44.8 SD ± 15.2 years; BMI = 27.6 SD ± 5.2 kg/mt2;): 1) who did not change the daily dose nor the Ltime at the switching time; 2) for whom were available TSH values at the switch (TSH1) and after > 3 months (TSH2). We compared TSH1 and TSH2, and evaluated if TSH2/TSH1 ratio was related to age, BMI, daily dose/weight, LTime and absence/presence of conditions/drugs interfering with LT4 absorption.

Results: TSH2 was reduced in 28/44 (63.6%) patients and increased in 15/44 (34.1%) patients; 1/44 (2.3%) patient did not show any TSH change. TSH2 (2.21, 95% CI 1.78 - 2.48 microUI/mL) was lower than TSH1 (3.01, 95% CI 2.29 - 4.54 microUI/mL), and the difference was significant (P = 0.0405; Wilcoxon test for paired data); the TSH2/TSH1 ratio was not related to age, BMI, daily dose/weight, LTime. Fourteen patients with conditions/drugs interfering with LT4 absorption showed a TSH2/TSH1 ratio lower than 28 patients without interfering conditions/drugs (0.47 95% CI 0.19 - 0.68 versus 0.88 95% CI 0.65 to 1.25), and the difference was significant (P = 0.0211; Wilcoxon test for unpaired data).

Conclusion: our study shows that TSH is reduced after switching from LT4 tablets to LT4OS; we hypothesize that this reduction could be due to a faster and more complete absorption of LT4OS, especially when a condition or a drug may interfere with LT4 absorption.

PP356

BREAST CANCER AND OTHER TISSUES THAN THE THYROID EXPRESS THYROPEROXIDASE AND SEVERAL NEW ISOFORMS HAVE BEEN IDENTIFIED

I. Muller[1], C. Giani[1], E. Fiore[1], V. Belardi[1], V. Rosellini[1], E. Giustarini[1], N. Funel[2], D. Campani[2], F. Grennan-Jones[3], L. Zhang[3], M. Lewis[3], A. Bakhsh[3], M. Roncella[4], M. Ghilli[4], A. Pinchera[1], P. Vitti[1], C. Dayan[3], M. Ludgate[3]

[1]Endocrinology - Pisa, [2]Pathology - Pisa, [3]Institute of Molecular and Experimental Medicine - Cardiff (UK), [4]Senology - Pisa

OBJECTIVES: Serum anti-thyroperoxidase(TPO)-autoantibodies (TPOAb) are common and protective in breast cancer (BC) patients. We hypothesized T-lymphocyte immunoreactivity against a shared antigen expressed in thyroid and BC cells: TPO is a logical candidate and we analyzed its expression in tissues/cells samples.

MATERIALS AND METHODS: Ex vivo frozen tissues included 8 BC, 8 peri-tumoral breast tissues (PT), 3 pancreatic adenocarcinoma (P), 2 kidney cancers (K), 17 adipose tissues (AD) and thyroid tissue (Thy) as positive control. In vitro study included 3 BC cell-lines (C): MCF-7, T47-D and MDAMB-231. Total RNA and proteins were extracted from samples. TPO mRNA expression was evaluated by Reverse Transcriptase (RT) – Polymerase Chain Reaction (PCR), LongRange RT-PCR and Quantitative-PCR. TPO protein expression was studied by Western-Blot (WB), indirect immunohistochemistry (IHC) and indirect immunofluorescence (IF).

RESULTS: Known TPO mRNA variants were expressed highly in Thy, 103 times less in AD, 104 times less in BC/PT and at the limit of detection in P, K and C. Furthermore we found many new TPO variant mRNAs in Thy and/or other tissues. In WB TPO protein was found at the expected level (105-110 kDa) in Thy, C and many BC, PT, AD, P, K; the signal was reduced after pre-absorption of the monoclonal antibody to TPO with recombinant TPO (but not lactoperoxidase) fragments, indicating specific binding. Moreover lower molecular weight bands were present: they could represent corresponding proteins of smaller TPO isoforms. IHC and IF were performed on Thy and BC and confirmed the presence of positive signal in BC, even if weaker than Thy.

CONCLUSIONS: TPO no longer seems to be thyroid specific: mRNAs and proteins for known TPO isoforms are weakly but clearly expressed in BC and other tissues. This could explain at least in part the high frequency and protective role of TPOAb in BC patients, hypothesizing an enhancement of specific T-lymphocyte immunoreactivity. Further studies are needed to investigate tissue specificity, function and immunogenicity of several novel TPO variant mRNAs identified in this study.

PP357

LAT-1 AND GLUT-1 EXPRESSION IN SPORADIC MEDULLARY THYROID CARCINOMA: PRELIMINARY DATA.

E. Cavedon[1], S. Barollo[2], R. Pezzani[2], L. Zambonin[2], S. Sensi[2], G. Pennelli[3], F. Galuppini[3], M. R. Pelizzo[4], F. Torresan[4], F. Mantero[2], D. Nacamulli[2], C. Mian[2]

[1] Endocrinology Unit; Department of Medicine-DIMED - Padua, [2]Endocrinology Unit; Department of Medicine-DIMED - Padua, [3]II Pathology Unit, Department of Medicine-DIMED - Padua, [4]II General Surgery; Department of Surgery Oncology and Gastroenterology - Padua

Purpose: Medullary Thyroid Carcinoma (MTC) is a neuroendocrine tumor producing calcitonin. In consideration of its neuroendocrine origin, recent studies demonstrated that 18F-DOPA has a higher sensitivity in cancer staging than 18F-FDG. Aim of this study was to analyse gene and protein expression of LAT-1 (L-type Na-independent neutral amino acids transporter 1, involved in the transport of 18F-DOPA) and GLUT-1 correlating their expressions to clinical outcomes. **Materials and methods**: In 35 patients with sporadic MTC (18 females and 17 males), LAT-1 and GLUT-1 gene expression was evaluated by Real-time PCR and their protein expression by immunofluorescence; moreover Ki-67 index was analyzed by immunohistochemistry. **Results**: preoperative calcitonin levels correlated significantly with disease stage, and patient's outcome. GLUT-1 gene expression was similar to that of normal thyroid tissue, while LAT-1 gene expression was higher. By immunofluorescence, LAT-1 protein was principally located in cytoplasmatic membrane and the staining degree reflected gene expression level. We did not observe a statistically significant correlation between LAT-1 and GLUT-1 gene expression and clinical, biochemical, and immunohistochemical characteristics of disease (plasma calcitonin levels presence of somatic RET mutation, stage, recovery and proliferation degree). **Conclusions**: calcitonin levels have been confirmed to be the most important prognostic parameter in predicting MTC disease course. In contrast to what reported in other neuroendocrine tumors and not, LAT-1 does not appear to play a fundamental role neither as a marker of well-differentiated tumor nor as a prognostic marker of more aggressive phenotype. In MTC, GLUT-1 does not represent a parameter of de-differentiation and aggressiveness, as it is described in other tumors.

PP358

PRESURGICAL BRAF ANALYSIS IN PTC: CORRELATION WITH CLINICO-PATHOLOGICAL FEATURES AND PROGNOSIS IN A SINGLE MONOINSTITUTION PROSPECTIVE EXPERIENCE.

F. Vianello[1], S. Barollo[2], S. Sensi[2], E. Cavedon[2], O. Servodio[1], S. Watutantrige-Fernando[2], D. Nacamulli[2], L. Zambonin[2], E. Casal Ide[3], M. R. Pelizzo[3], O. Lora[1], G. Sotti[1], F. Mantero[2], G. Pennelli[4], C. Mian[2]

[1]Radiotherapy Department; Istituto Oncologico Veneto – IRCCS - Padua, [2]Endocrinology Unit; Department of Medicine-DIMED - Padua, [3]II General Surgery; Department of Surgery Oncology and Gastroenterology - Padua, [4]II Pathology Unit, Department of Medicine-DIMED - Padua

Background: the risk stratification in PTC is based on postoperative parameters. Preoperative BRAF-mutation testing could provide a novel tool to preoperatively identify patients at higher risk for extensive disease. Aims of the present study were 1) to correlate the BRAF status in FNAB specimens of a large series of consecutive PTC followed in a single institution with classical indicators of poor prognosis and 2) to evaluate prospectively the BRAF status impact per se in patient's prognosis.
Patients and methods: 186 consecutive patients (38 males and 148 females, mean age 48 , median 49, range 22–81 years, mean follow-up was 36± 8 months) with a histological diagnosis of PTC and a BRAF analysis performed on the thyroid FNAB were included.
Results: On a whole, 116 of 186 (62%) PTC carried a BRAF mutation. The univariate analysis showed that BRAF status was correlated with the histological variant of PTC (p=0.001), the cancer size (p=0.02), the extrathyroid extension (p = 0.04) and advanced stages (p=0.03) at diagnosis, whereas gender, multifocality, lymph node metastases were not . At the end of the follow-up 14 patients (7%) showed persistent disease and 3 died for disease progression. Age higher than 60 years, extrathyroidal extension, lymph nodes involvement and an advanced stage are all significantly correlated factors with the risk of persistent disease or disease-related death. 11 out of 14 persistent patients carried BRAF mutations and 3 did not: compressively, 9% of BRAF mutated cases versus 4 % of BRAF wild-type showed a persistent disease at the end of follow-up; this difference was not statistically significant.
In conclusion, our study confirmed the association of BRAF mutation with some clinical and pathological features of PTC classically associated with high risk PTC patients but its presence per se does not seem to influence patient's prognosis.

PP359

AFTER 20 RET GENETIC SCREENING YEARS STILL INDENTIFIES NEW GERMILINE AND SOMATIC MUTATIONS

A. Tacito[1], C. Romei[1], A. Vivaldi[1], R. Ciampi[1], A. Matrone[1], V. Bottici[1], V. Cappagli[1], R. Elisei[1]

[1]sezione di endocrinologia, dipartimento di medicina clinica e sperimentale - Pisa

Germline RET proto-oncogene point mutations are causative of 98% of hereditary medullary thyroid carcinomas (MTC) and somatic RET point mutations have been described in about 40% of sporadic MTC.

In the last 20 years we performed RET genetic screening in about 1000 MTC patients either hereditary or sporadic. RET genetic screening was performed in DNA extracted from blood to detect germline mutations and in the tumoral thyroid tissue to detect somatic mutations.

In the last year, we have identified 3 different RET somatic mutations and 1 germline mutation that had never been reported before. In particular we found the simultaneous presence of a somatic E616Q mutation in exon 10 and a somatic C630G mutation in exon 11. Furthermore the tumoral DNA of an MTC patient was characterized by the presence of a 7bp deletion in exon 11 encompassing codon 629-631. The new germline mutation was a E632K RET mutation in exon 11. The transforming ability of this RET mutations was investigated by an "in silico" approach using Align GVGD. The system predicts the degree of structural difference of the mutated protein with respect to the normal which is expressed with a numerical score ranging from 0 (no difference) to 65 (maximum difference). The assumption is that the higher is the score the higher is the transforming activity. The E616Q and the E632K RET mutations had a 0 score. Conversely the score of the C630G RET mutation was 65.

Further studies are ongoing to evaluate "in vitro" the transforming ability of these mutations and we are also planning cloning experiments to better define the 7 bp deletion.

Our data indicate that: 1) RET genetic screening should be performed in MTC patients, at least in the exons most frequently involved, by sequence analysis to detect new RET mutations that would be missed when looking only for the "hot spot" mutations; 2) all new mutations must be evaluated by in silico and/or in vitro analysis to define their transforming ability.

PP360

THYROGLOSSAL DUCT CARCINOMA: A DESCRIPTION OF A CASE

A. Pezzolla[1], D. Paradies[1], S. Lattarulo[1], V. F. D. Borraccia[1], S. Spinelli[1], A. Ciampolillo[1]

[1]Dipartimento dell' Emergenza e Trapianti d'Organo Università degli Studi "A. Moro" - Bari

The thyroglossal duct cyst (TDC) is the most common developmental anomaly of the thyroid gland and one of the commonest congenital childhood midline neck masses. Thyroglossal duct carcinoma (TDCa) is malignant tumor arising within a thyroglossal duct remnant (TDR) or a thyroglossal duct cyst. Its development in a TDC is extremely rare. Approximately 250 cases of TDCa have been published in the literature. Controversies exist regarding exact origin of TDCa in a TDC as to whether it represents a metastatic lesion of a primary thyroid cancer versus its "de novo" origin. Ultrasound scan, fine-needle aspiration cytology and CT imaging of the neck could have beneficial in the preoperative diagnosis of carcinoma in TDC. Surgery is an adequate treatment for TDC and the prognosis for TDCa arising in a TDC is excellent. We report a case of a 20-year-old male with a visible and palpable subhyoid mass compatible with a TDC. Preoperative ultrasound scan and US-guided fine-needle aspiration cytology revealed suspicious papillary carcinoma (TIR4) in the TDC and benign thyroid nodules. Surgery consisted in removal of the hyoid bone, which we associated with removal of the TDC and total thyroidectomy. Histopathologic examination revealed papillary TDCa and a focus of papillary var. follicular microcarcinoma in the right lobe of the thyroid gland. In our patient, adjuvant radioactive iodine and post-operative L-thiroxine suppressive therapy, as appropriate to their disease status, were performed (to keep TSH levels between 0.2 and 0.5 mU/l). Annual measurement of serum thyroglobulin and semestral ultrasound scan of the neck are recommended in the follow-up for these patients.

PP361

CLINICOPATHOLOGICAL FEATURES OF PAPILLARY THYROID CARCINOMA WITH CONCURRENT BRAF MUTATION AND HASHIMOTO'S THYROIDITIS LYMPHOCYTIC INFILTRATION

A. Guerra[1], V. Marotta[2], V. Di Stasi[1], M. C. Zatelli[3], E. Degli Uberti[3]

[1]Medicine and Surgery - Salerno, [2]Clinical and Molecular Endocrinology and Oncology - Napoli, [3]Biomedical Sciences and Advanced Therapies and LTTA - Ferrara

Background: Concomitant papillary thyroid cancer (PTC) and Hashimoto's thyroiditis (HT) is a frequent occurrence. Whether these two conditions are linked and whether PTC with concurrent HT has distinct clinicopathological characteristics are still debated issues. Lymphocytic infiltration is abundant in HT and might be relevant in the pathogenesis of PTC. *BRAF* mutation is associated with a more advanced PTC at diagnosis, however its role in the clinicopathological characteristics of PTC with concurrent HT is unknown.

Aim of this study was to determine the impact of lymphocytic infiltration of HT on PTC progression and its correlation with *BRAF* mutation.

Design: We analyzed 146 PTC cytology samples for the presence of $BRAF^{V600E}$ and lymphocytic infiltration and results were correlated with gender, age at diagnosis, tumor size, extrathyroidal extension, TNM and staging.

Results: Concurrent HT lymphocytic infiltration was associated with the female gender (p= 0.003), a smaller tumor size (p= 0.003), a less frequent extracapsular extension (p= 0.001) and a lower grade of TNM classification (p= 0.028). $BRAF^{V600E}$ was more frequent *in* PTC with concomitant lymphocytic infiltration. In PTC harboring $BRAF^{V600E}$, concurrent lymphocytic infiltration was still associated with the female gender (p=0.007), a less frequent extracapsular extension (p= 0.013) and a lower TNM grade (p= 0.026).

Conclusions: These results suggest that lymphocytic infiltration of HT is a protective factor against PTC progression, independent by *BRAF* mutational status.

PP362

FREQUENCY OF POLYMORPHISMS IN THE VEGF, VEGFR AND HIF GENES IN NORMAL SUBJECTS AND PATIENTS WITH NODULAR GOITER FROM AN AREA WITH MILD IODINE DEFICIEN

A. Molinaro[1], F. Niccolai[1], G. De Marco[1], P. Agretti[1], C. Di Cosmo[1], P. Piaggi[2], P. Vitti[1], G. Bocci[3], M. Tonacchera[1]

[1]Department of Endocrinology and Metabolism - Pisa, [2]Department of Energy and Systems Engineering - Pisa, [3]Department of Internal Medicine - Pisa

Introduction

Nodular goiter is due to the growth of follicular and endothelial cells. Numerous factors modulate angiogenesis: a potential role of different vascular-related growth factors in the pathogenesis of the thyroid nodular goiter have been proposed. The aim of our study was to examine the relationship of known polymorphisms in the VEGF, VEGF receptor (VEGFR) and HIF (hypoxia inducible factor) and the risk of nodular goiter development.

Patients and Methods

We selected 116 normal subjects (41 males, mean age 48.1 years; 75 females, mean age 51 years; subjects without any thyroid disease) and 108 subjects with nodular goiter (41 males, mean age 49.6 years; 67 females, mean age 50.3 years; subjects with goiter and at least one thyroid nodule of > 1 cm of maximum size and in absence of signs of autoimmunity) from a homogeneous population living in a mild iodine deficiency geographic area of southern Italy. Genomic DNA was extracted from blood with standard methods. Genotyping was carried out by the TaqMan technology. We studied the following polymorphisms: VEGF+936C/T, VEGFR-604A/G and HIF-1ALFA C/T. As statistical test we used the Chi-squared test.

Results

In normal subjects the frequency of the polymorphisms was: VEGF+936 CC 78.4%, CT 19.8%, TT 1.7%; VEGFR-604 AG 44%, GG 25.8%, AA 30.2%; HIF-1ALFA CT 97.4%, TT 2.6%. In patients with nodular goiter the frequency was: VEGF+936 CC 75%, CT 21.3%, TT 3.7%; VEGFR-604 AG 55.6%, GG 16.7%, AA 27.8%; HIF-1ALFA CT 99.1%, TT 0.9%. The frequency of the studied polymorphisms was not statistically different between normal subjects and patients with nodular goiter.

Conclusions

Our study did not prove the role of VEGF, its receptor and an other vascular growth factors in the development of the nodular goiter in patients coming from an area with mild iodine deficiency.

PP363

CHARCOAL TATTOO LOCALIZATION FOR DIFFERENTIATED THYROID CANCER RECURRENCE IN THE NECK

M. Puccetti[1], M. G. Sama[2], C. Cappi[2], F. Soprani[3], V. Armaroli[3], F. Bondi[2]

[1]Anatomia Patologica - Ravenna, [2]Endocrinologia - Ravenna, [3]Otorinolaringoiatria - Ravenna

Differentiated thyroid cancer recurrence can often require further surgical options.

During reoperations risk of surgical complications is significantly higher; the surgeons may encounter meaningful problems in the identification and preservation of recurrent laryngeal nerves and parathyroid glands due to fibrosis and scar tissue formation in the surgical field with consequent alteration of the normal anatomic relationships; for the same reason, there is the possibility of leaving residual neoplasm.

In order to obviate, at least partially, these problems during reoperations for differentiated thyroid cancer recurrence, we have introduced the technique of preoperative ultrasound-guided tattooing localization of the nodes to be removed with a 4% solution of active charcoal.

Using ultrasound guidance, the lesion is identified and 0.5-2 ml of colloidal charcoal is injected near the lesion. The extraction of the needle is accompanied by an injection at constant pressure of other charcoal in such a way as to leave a trace of colouring along the path of the needle up to the skin.

The preoperative injection was well tolerated in all cases.

In the last 7 years, we have reoperated 16 patients with suspected node recurrence in the neck (all resulting from papillary carcinomas) using the technique of US-tattoo localization.

Post operative US and histological examination confirmed the removal of the suspected lesions in all patients; in one case the lesion proved to be a parathyroid cyst.

We observed complications in two cases out of 16, in one case this was a transitory hypoparathyroidism and in the other one it was a transitory vocal cord paresis.

Considering our experience we can affirm that charcoal tattoo localization is a safe technique which is of low cost and is extremely useful for facilitating surgical procedures, allowing a reduction of the risk of iatrogenic damages.

PP364

CLINICAL UTILITY OF THYROTEST BASED ON GALECTIN-3 IN THE SURGICAL SELECTION OF THYROID NODULES CLASSIFIED AS INDETERMINATE AT CONVENTIONAL CYTOLOGY

S. Amendola[1], G. De Francesco[2], C. Bellotti[3], T. Porcelli[1], G. Fioroni[1], A. Bartolazzi[4], S. Sciacchitano[1]

[1]Dipartimento Medicina Clinica e Molecolare - Roma, [2]Azienda Ospedaliera Sant'Andrea - Roma, [3]Dipartimento di Scienze Medico Chirurgiche e Medicina Traslazionale - Roma, [4]U.O.C. di Istologia ed Anatomia Patologica, Az. Osp. Sant'Andrea - Roma

ThyroTest based on Galectin-3 immunodetection directly performed on FNA cytological samples is considered useful in the management of cytologically indeterminate lesions, classified as Thy3 according to BTA. Despite its inclusion in ATA guidelines, its application in clinical practice is not performed on large-scale yet. The present study was undertaken to assess the clinical utility of ThyroTest in the selection of Thy3 nodules for surgery and in order to promote its use in the routine evaluation of these nodules. A total of 358 Thy3 nodules were analyzed by Galectin-3-based ThyroTest, performed as described (Appl Immunohistochem. Mol. Morphol. 2012;20:2-7). Among them, 269 (75%) were considered negative because no Galectin-3 immunostaining was detected in the cytoplasm of thyrocytes. Total thyroidectomy was suggested in 89 of them (31%), despite negative result, because of enlargement of nodule, oncocytic lesion and/or other risk factors. Malignancy was detected in 5 of them, with a false negative rate of 1.8%. The remaining 185 patients (69%) are currently under strict clinical and sonographic follow-up and nodules volume is either stable or reduced. ThyroTest was positive in 89 patients (25%), all of them subjected to surgery. A malignant lesion was found in all of them except 8 adenomas and 3 hyperplasia, for a false positive rate of 12.3%. Our approach enabled us to accurately select nodules very likely to be malignant among the group of indeterminate lesions that would otherwise had been treated by surgery, thus avoiding unnecessary operations for the rest of them, benign, with a very low rate of false negative results. Considering that the reimbursement for each total thyroidectomy performed in our institution is 3,300.00 euro, our low-cost method allowed a total saving of 610,500.00 euro, with an annual saving of 122,100.00 euro. In conclusion, our data strongly indicate the clinical utility of the galectin-3-based ThyroTest, when routinely applied to select cytologically indeterminate nodules for surgery. This study has been approved by our Ethical Committee (RS: 125/2012).

PP365

INTRANUCLEAR CYTOPLASMIC INCLUSIONS IN CYTOLOGICALLY SUSPICIOUS OR MALIGNANT THYROID NODULES: CORRELATION WITH ECHO TEXTURE AND SIZE OF THE NODULES

S. Arena[1], A. Latina[2], M. Stornello[1], G. Saraceno[3], S. Benvenga[3]

[1]Dept. of Internal Medicine, Ospedale Umberto I - Siracusa, Italy, [2]Endocrinology Division, Dept. of Clinical and Molecular Biomedicine, University of Catania, Garibaldi-Nesima Hospital - Catania, Italy, [3]Dept. of Clinical and Experimental Medicine, University of Messina - Messina, Italy

Background- Intranuclear inclusions (ICI) represent one cytological feature suggestive of malignancy, particularly papillary thyroid cancer, though they are detectable rarely in adenomatous nodules. Rate of malignancy is 50-75% or close to 100% in thyroid nodules that, at fine needle aspiration cytology (FNAC), are suspiciously malignant (THY 4) or malignant (THY 5). Aim of the present study was to correlate ICI with size and echogenicity of the THY4 and THY5) nodules, and to ascertain whether ICI alone or combined with some ultrasonography (US) characteristics would help predicting malignancy. ***Materials & Methods***- We studied 90 consecutive thyroid nodules from 90 patients that were examined by US-guided FNAC and that fell in the THY4 (n= 60) or THY5 (n=30) categories (British Thyroid Association classification). All 90 patients were thyroidectomized, so that a cytology/histology correlation was possible. ***Results***- Seventy nodules were cancerous (82.2%; THY4=73.3%, THY5=100%). ICI positive (ICI+) were 53/90 nodules (THY4=48.3%, THY5=80.0%), of which only 3 (all THY4) were benign. Of the 53, two (3.8%) were adenomatous and one (1.9%) was a trabecular hyalinizing adenoma. Maximum diameter was smaller in the 53 ICI+ than in the 37 ICI- nodules (14.2±5.4 *vs.* 20.0±9.4 mm, P= 0.0001; median volume 1.32 *vs.* 4.03 ml). Malignancy rate was 18/19 (95%) in the THY4 hypoechoic nodules with a diameter of ≤20 mm. Especially when coupled with the two US characteristics, performance (sensitivity, specificity, positive predictive value and overall diagnostic accuracy) in THY4 nodules compared fairly with performance reported for expensive genetic tests. ***Conclusions***– In THY4 nodules, ICI positivity is associated with a relatively smaller size and a hypoechoic texture. When coupled with these two US characteristics, ICI positivity selects THY4 nodules that have very high chances of being malignant (95%), leaving only the very few (5%) ICI negative and non hypoechoic nodules for molecular analysis.

PP366

THYROID NODULES: CORRELATION BETWEEN ULTRASOUND CHARACTERISTICS, CYTOLOGICAL AND HISTOLOGICAL EXAMINATION

R. Fabiano[1], A. Andreadi[1], A. Cioci[1], M. E. Rinaldi[1], A. Bellia[2], A. Galli[1], M. Romano[1], F. Pozzi[1], M. Cerilli[1], D. Lauro[2]

[1]Center of Type 2 Diabetes, Department of Medicine, University Hospital Policlinic Tor Vergata Foundation - Rome, [2]Center of Type 2 Diabetes, Department of Medicine, University Hospital Policlinic Tor Vergata Foundation,Department of Systems Medicine, Tor Vergata - Rome

The thyroid nodule is the one of the most common's disease regarding thyroid and predominantly affects the women. The aim of this study was to evaluate a correlation between ultrasound nodule characteristics with the cytological and histological examination of the thyroid nodule. We analyzed thyroid nodules of 1578 patients, 329 males and 1249 females, with "fine needle aspiration biopsy" (FNAB) with ultrasound-guided technique. Of the 1578 patients observed in 944 cases there was a nodular solid, 218 patients had mixed nodules and in 39 anechoic. The cytological evaluation, showed in the great majority of cases, a negative report for malignant cells TIR2, 52 cases diagnosed TIR3 which means an indefinite score sheet. Rest of the results was 14 cases of suspected malignant "TiR4" and 18 cases for positive malignant cells, "TIR5." The "TIR 5" have shown, a relational significance with the pattern of vascularization of type III. Of the 18 "TIR 5" 10 showed this pattern III and in all cases it was regarding hypo echoic formations. The detection of these lesions histologically, confirmed the diagnosis for papillary or follicular carcinoma. The study confirms the important contribution of FNAB in the diagnostic characterization in the presence of a nodular mass, that it becomes an indispensable criterion, since no characteristic ultrasound among those analyzed directs us safely to a definitive diagnosis. In particular, a pattern of vascularization of type III is significantly correlated only with formations that in the cytological were found to be of type "TIR5." In all other cases, even in nodules "TiR4" the blood flow distribution was not a reliable parameter . Plus from our preliminary data, we prooved that exists a significant correlation through elastosonography and cytological findings, considering it as an additional information for the characterization of the thyroid nodule. Precisely from the 25 nodules that was performed, 64% had a diagnosis for carcinoma and 36% a negative or insufficient report from FNAB. In the mean time we continue to increase the number of the thyroid nodules examined with elastosonography and the data obtained will be presented in the future.

PP367

UNUSUAL METASTASES FROM TALL CELL VARIANT (TCV) OF PAPILLARY THYROID CANCER (PTC)

M. Caputo[1], M. Zavattaro[1], L. Pagano[1], M. T. Samà[1], F. Prodam[1], M. G. Mauri[2], F. Pia[3], A. Alonzo[4], G. Valente[5], G. Aimaretti[1]

[1]Endocrinologia, Dipartimento di Medicina Traslazionale, Università del Piemonte Orientale "A. Avogadro" - Novara, [2]SCDO Malattie Metaboliche e Diabetologia, AOU "Maggiore della Carità" - Novara, [3]Otorinolaringoiatria, Dipartimento di Scienze Mediche, Università del Piemonte Orientale "A. Avogadro" - Novara, [4]Chirurgia Generale, AOU "Maggiore della Carità" - Novara, [5]Laboratorio di Patologia, Dipartimento di Medicina Traslazionale, Università del Piemonte Orientale "A. Avogadro" - Novara

Background: Tall Cell Variant (TCV) is considered more aggressive than classical variant of Papillary Thyroid Cancer (PTC). Distant metastases are more common among this variant and affect survival. Little is known about the molecular pattern of this histotype.
Methods: We reported 2 cases of unusual metastases from TCV, BRAF V600E positive.
Results: The first case, a 38-years-old-woman, developed subcutaneous metastases during short term follow-up; at medium term follow-up, the patient showed detectable stimulated serum Thyroglobulin without disease evidence at imaging. The second case, a 33 years-old-man, presented incidental thymic metastases at the time of surgical treatment; this is the first case of not-ectopic thymic metastases from PTC.
Conclusions: TCV may present with unusual metastases already during early follow-up. The more aggressive behavior could be linked to the higher prevalence of BRAF point mutations, but only a long term follow-up might clarify if this association could worsen the prognosis. Moreover, skin metastases have been predictive factors of worse outcome in our patient, while not thymic metastates.

PP368

GENETIC ANALYSIS IN PATIENTS WITH THYROID HEMIAGENESIS

I. C. Nettore[1], V. Cacace[1], R. Gelsomino[2], M. L. Nicolosi[2], A. M. Ferrara[1], D. Leonardi[2], P. E. Macchia[1], F. Calaciura[2]

[1]Department of Molecular and Clinical Endocrinology and Oncology, University of Naples "Federico II" - Naples, [2]Endocrinology Unit, Department of Clinical and Molecular Bio-Medicine, University of Catania Medical School, Garibaldi Hospital - Catania

INTRODUCTION. Thyroid hemiagenesis is a rare congenital abnormality, in which one of the thyroidal lobes fails to develop. The causes of thyroid hemiagenesis are still unclear and it is unknown whether the disturbance of the lobulation process is due to interference of environmental factors or to genetic abnormality.
Several genes have been found to control thyroid migration, development and morphogenesis and mutations in some thyroid transcription factors (NKX2-1, FOXE1, PAX8, NKX2-5) have been associated with thyroid development defects.
PATIENTS: We selected 10 patients with thyroid hemiagenesis identified at the screening for congenital hypothyroidism (CH). Patients (4 males and 6 females) presented a wide spectrum of neonatal thyroid function alterations. Thyroid hemiagenesis was due to absence of the left thyroid lobe in 6/10 (60%) and agenesis of the right lobe in the remaining 4/10 subjects. One child was a discordant monozygotic twin for CH. In one family a brother's patient presented a hypoplasic left lobe and mild hyperthyreotropinemia in childhood and sister's patient had CH with thyroid gland in situ.
METHODS: DNA was extracted from peripheral blood lymphocytes and the entire coding sequence of NKX2-1, PAX8, NKX2-5 was studied by single stranded conformational polymorphism (SSCP) and direct sequencing.
RESULTS. No mutations or polymorphisms were detected in the coding sequences of the studied genes.
CONCLUSIONS. Alterations in the coding sequence of NKX2-1, NKX2-5 and PAX8 seems not to be the cause of hemiagenesis. A role of these genes in the pathogenesis of the disease, can not be definitively ruled out, since both regulatory region and introns have not been investigated in this study. In addition, stochastic events interfering with the activity of thyroid transcription factors during thyroid development will not be detected with the used techniques.

PP369

SUNITINIB-INDUCED THYROID AND ADRENAL DYSFUNCTION

P. Conton[1], D. Barison[1], M. Schiesaro[2], C. De Riva[1], F. Presotto[1]

[1]Medicina Interna Ospedale dell'Angelo - Mestre (Ve), [2]Medicina Interna - Bussolengo (Vr)

Introduction: Sunitinib is a multitargeted tyrosine kinase inhibitor with antiangiogenic and antineoplastic activities approved for the treatment of advanced or metastatic renal cell carcinoma (RCC) and gastrointestinal stromal tumors. Retrospective studies indicate that sunitinib may induce thyroid dysfunction. No data are available on sunitinib effects on adrenal function.
Materials and methods: Since 2008 we followed 9 patients with metastatic RCC (7 males and 2 females, mean age at diagnosis 59 yrs) who were receiving 4-wk daily treatment with sunitinib at the dose of 50 mg orally and 2-wk withdrawal. Thyroid function tests (fT3, fT4, TSH) were performed in all, and thyroid autoantibodies in 8 patients. Adrenal function was also evaluated in 2 hypothyroid patients with persistent asthenia unresponsive to thyroid replacement therapy.
Results: Eight patients (89%) developed hypothyroidism after a mean period of 7.5 months of sunitinib therapy (range 1-25 months). Six developed overt hypothyroidism (67%) requiring replacement therapy with l-T4, while the remaining 2 had mild hypothyroidism. Only one patient satisfied the diagnostic criteria for autoimmune thyroiditis, while another patient developed a destructive thyrotoxicosis after one month therapy. One patient who had been operated by unilateral nephrectomy and adrenalectomy developed also overt adrenal failure during sunitinib treatment, while another patient revealed low-normal plasma and urine cortisol levels.
Conclusions: The great majority of our cases with RCC receiving sunitinib developed various degree of thyroid dysfunction, usually from mild to severe hypothyroidism. One patient also developed complete adrenal failure, while another one revealed borderline adrenal hypofunction. We strongly recommend to perform thyroid function tests at baseline and during sunitinib therapy in order to recognize and treat early thyroid dysfunction. Clinicians should also be aware of possible impairement of adrenocortical function in these patients.

PP370

EFFECTS OF SELENIUM SUPPLEMENTATION ON THYROID FUNCTION IN OVERWEIGHT-OBESE WOMEN AFTER A BALANCED MILD HYPOCALORIC DIET.

L. Barrea[1], G. Pizza[1], V. Nedi[1], E. Guerra[1], A. De Rosa[2], C. Di Somma[3], A. Colao[1], S. Savastano[1]

[1]Department of Clinical Medicine and Surgery, division Endocrinology, "Federico II" University, - Naples, [2]Department of Clinical Medicine and Surgery, division Clinical Nutrition, "Federico II" University, - Naples, [3]SDN Foundation Naples, - Naples

Background: The selenium content in foods varies widely, with foods high in protein and low in fat, such as fish, lean meats, legumes, cereals and dairy products being its best nutritional sources. Selenium is a component of type 1 deiodinase, the enzyme converting T4 to T3 peripherally. Treatment of obesity with hypocaloric diets causes changes in thyroid function consisting of a decrease in total T4 and total and free T3 with a corresponding increase in reverse (r)T3. Aim: To evaluate the effect of selenium in combination with a balanced mild hypocaloric diet on thyroid function and body composition in euthyroid overweight/obese women. Patients and methods: Twenty-one euthyroid overweight/obese women (BMI median value 31(26-42) kg/m2), with age range 19-49 yrs, were consecutively recruited. Fifteen age and BMI-matched women served as control group. Anthropometric parameters, TSH, FT3, and FT4 levels and FT3/FT4 ratio , as an index of deiodination, were evaluated. The intake of selenium was evaluated with a 3- Days Food Diary and then calculated with software WinFood Light 1.0. A balanced mild hypocaloric diet (57% CHO, 28% fat, 15% protein) with Kcal median values 1435(1280-1580) was associated with a supplementation of 400 mg/die of L-selenomethionine, (Syrel® 400 mg) for 6 months. Body composition was evaluated by bioimpedance analysis (single-frequency 50 kHz BIA 101 RJL, Akern) and biavector analysis (BIVA software [Piccoli A, Pastori G (2002)]. Results: After 6 months BMI and waist circumference were reduced in both patients (BMI: 31(26-42) vs 28(24-41) kg/m2, p<0.001; waist circumference: 95(79-126) vs 91(72-122) cm, p<0.001) and controls (BMI: 33(28-44) vs 29(26-42) kg/m2, p=0.02; waist circumference: 99(84-133) vs 96(81-123) cm, p=0.02). Percentage of fat-free mass increased in patients (56%(27-69) vs 62%(50-71), p=0.05), while decreased in controls (58%(39-76) vs 53%(31-69), p=0.04). In patients, TSH, FT3 and FT4 levels did not change significantly, but FT3/FT4 ratio increased significantly (2.2(0.9-3.2) vs 2.6(1.1-3.3), p=0.05), whereas in control group FT3 levels (1.4(0.8-2) vs 1.0(0.7-1.5) pg/ml, p<0.001) and FT3/FT4 ratio (2.3(1.9-2.6) vs 1.8(1.7-2.3), p=0.05) were significantly reduced. Conclusions: Our data suggest that the supplementation with selenium associated with a balanced mild hypocaloric diet in euthyroid overweight/obese women might contribute to preserve thyroid function and to counteract some of the adaptive mechanisms implicated in weight regain after weight loss.

PP371

RECURRENCE OF HYPERTYROIDISM AFTER TOTAL THYROIDECTOMY

L. Proietti Pannunzi[1], M. G. Deiana[1], S. N. Morgante[1], S. Monti[1], V. Toscano[1]

[1]UOC Endocrinologia Ospedale Sant'Andrea II Facoltà di Medicina e Chirurgia Università Sapienza - Roma

INTRODUCTION. Thyroid gland derive in the embryo as a mesodermal outpouching in the pharyngeal floor at the foramen cecum, from which it descends anterior to the trachea. Ectopic thyroid tissue can be found anywhere along or beyond this thyroglossal duct, fro the basis of the tongue to the thyroid gland.
CASE REPORT. A 54 years old women presented with decreased weight, insomnia, anxiety and a solid mass in the midline of the anterior neck. In her anamnesis Hodgkin's lymphoma treated with surgery and radiotherapy in the neck region 8 years ago and total thyroidectomy (TT) 6 years before for a Grave's disease. After TT patient started therapy with L-Thyroxine (1.5 mcg/kg) with normal levels of TSH, FT3 and FT4. The ultrasound of the neck performed one year after the surgery showed little residual tissue in the left of the lodge (maximum diameter 0.5 cm). Thyroid hormone dosage were performed every sixth month for the first two years and then every year. Five years later thyroidectomy, the patient anticipated the follow up visit for the appearance of anxiety, tremble, loss of weight and thyroid function test showed an hyperthyroidism (TSH< 0.01 mUI/ml, FT3 = 6.5 pg/ml [n.v 2.4-5.2], FT4 = 2.2 ng/ml [n.v. 0.9-1.8]). The patient discontinued L-Tiroxine for 45 days and repeated dosage of thyroid hormones and thyroid antibody that showed a subclinical hyperthyroidism (TSH = 0.013 mUI/ml, FT3 3.97 pg/ml, FT4 1.35 ng/ml, TRAb = 38.2 U/L [n.v. 0-1.5], TPOAb = slightly positive; TGAb = negative; thyroglobulin = 78.6 ng/ml) and functional imaging with 131-I thyroid scan demonstrated active thyroid tissue within the thyroglossal duct remnant, no functional tissue was demonstrated in the lodge; Iodine uptake at 6 hour was 13% (n.v. 10 - 40%), at 24 h was 33% (n.v. 14 - 60%). Neck ultrasound showed a hypoechoic, inhomogeneous, well vascularized, solid mass with dimensions of 4 x 1 x 0.7 cm (LLxAPxT), without nodule lesion. Patient has started anti-thyroid drugs waiting for radioiodine treatment (patient refused surgery).
CONCLUSION: thyroid tissue remnants on the mid cervical neck line are not so rare; the tissue in thyroglossal duct may be subject to the same pathological processes of thyroid cells, such as cancer and, less frequently, hyperfunction.

PP372

RISK OF RECURRENCE IN DIFFERENTIATED THYROID CARCINOMA ONE YEAR AFTER TOTAL THYROIDECTOMY AND RADIOIODINE TREATMENT

S. Melanzi[1], I. Bucci[1], F. Febo[1], C. Giuliani[1], A. Carpenito[1], G. Napolitano[1], F. Monaco[1], V. Di Francesco[2], A. Di Nicola[2]

[1]Medicina e Scienze dell'Invecchiamento, Università di Chieti - Chieti, [2]U.O. Medicina Nucleare, Ospedale S. Spirito - Pescara

The management of differentiated thyroid cancer (DTC) requires risk estimates which are set after the primary treatment (surgery and I131) and have to be reassessed over time. According to ATA guidelines, the AJCC/UICC staging system is recommended for all patients with DTC because of its utility in predicting disease mortality. Aim of our study was therefore to compare the evaluation of risk, based on AJCC/UICC staging system after radioiodine ablation, with the evaluation of TSH-stimulated Thyroglobulin (Tg) levels and whole-body scintigraphy 1 year afterwards. 385 patients (289 F, 96 M, age 14-85 years) have been I131-treated from 2007 to 2011; according to the staging system they were classified as follows: stage I 275 pts, stage II 27 pts, stage III 29 pts, stage IVa 48 pts, stage IVc 6 pts. 205 patients (144 stage I, 20 stage II, 11 stage III, 28 stage IVa and 6 stage IVc9) were again evaluated after 1 year with whole-body scintigraphy and Tg levels (plus Tg-Ab) after stimulation with LT4 suspension or Thyrogen. Tg was < 1 ng/ml in 173 patients and ≥ 1ng/ml in 32; Tg-Ab levels were positive in 9 patients with Tg <1 ng/ml, 3 of them having a positive whole body scan. Patients were considered "not free" from disease if their stimulated-Tg levels were ≥1 ng/ml or if scan showed I131 uptake in those patients with positive Tg-Ab. 23 (15.9%) patients in the stage I group, 1 (5%) in the stage II group, 3 (27.2%) in the stage III group, 7 (25%) in the stage IVa group and 1 (50%) in the IVc group were "not free" from disease. Because of the unforeseen high percentage of patients "not free" from disease in stage I group, we evaluated the age of patients. Amongst the 76 patients younger than 45 years old 15 (19.7%) were "not free" from disease while the remaining 8 (out of 68 patients, 11.7%) were 45 or older. Our data show that a significant percentage of patients in the stage I group cannot be considered "free" from disease 1 year after I131ablation and that the majority of them are younger than 45 years old. These data recommend caution in the follow up of stage I patients too, and might suggest the possibility that not all patients (without metastasis) younger than 45 years old have to be considered at low risk, as inclusion in the stage I group would imply.

PP373

MODULATION OF CLAUDIN-1 AND -7 EXPRESSION DURING THE EPITHELIAL-MESENCHYMAL TRANSITION IN THYROID CANCER: THE ROLE OF THE SLUG TRANSCRIPTION FACTOR.

C. Colato[1], S. Pedron[1], F. Monzani[2], P. Brazzarola[3], M. Chilosi[1], M. Ferdeghini[1]

[1]Patologia e Diagnostica - Verona, [2]Medicina Interna - Pisa, [3]Chirurgia e Oncologia - Verona

Objectives: Acquisition of epithelial-mesenchymal transition (EMT) by cancer cells is associated with disrupted epithelial integrity, local invasion, and metastasis.

The tight junction (TJ) is a key structure that determines epithelial cell polarity and disappears during EMT. The Snail superfamily transcription factors, already firmly established as repressors of E-Cadherin, have been recently recognized as an important regulators of EMT. Actually, Snail has also been shown to be able to downregulate the TJ proteins Claudins/Occludin whereas Slug appears to act as repressor of Claudin-1 expression. Tubulin is a multifunctional cytoskeletal protein involved in cell movement, intracellular transport, and mitosis.

Few knowledge is available about the expression of EMT regulators in thyroid cancer.

In this study, we examined the expression pattern of Slug (Cell Signaling), Claudin-1/7 (Zymed) and βIII-Tubulin (Covance) in a panel of well-differentiated and anaplastic thyroid carcinomas by immunohistochemistry.

Methods: 5 anaplastic thyroid carcinomas (ATC), 12 papillary thyroid carcinomas (PTC) including 3 tall cell variants, and 12 normal thyroids (NT) were analyzed. Unequivocal nuclear staining for Slug, membranous staining for Claudins and cytoplasmic staining for βIII-Tubulin were considered for interpretation.

Results: All cases of ATCs showed strong nuclear immunoreactivity for Slug (4 cases diffuse, 1 case focal). Slug expression was associated with absence of Claudin-1/7 and ectopic expression of βIII-Tubulin. None of the NTs and PTCs were immunoreactive for Slug ($p<0.0001$). As expected, all PTCs were positive for Claudin-1/7 with heterogeneous and variable staining for βIII-Tubulin.

Conclusions: The EMT regulator Slug is expressed in ATC and is associated with absence of Claudin-1 and -7 and up-regulation of βIII-Tubulin, suggesting the role of EMT in this cancer. These observations support the recent insights into the relationship between alterations in cell polarity proteins and EMT in cancer, opening new avenues for their potential use as therapeutic targets to prevent tumour progression.

PP374

C-KIT/CD117 TYROSIN-KINASE RECEPTOR IN PAPILLARY THYROID CARCINOMA: AN IMMUNOHISTOCHEMICAL EVALUATION.

S. Pedron[1], C. Colato[1], M. Ivo[2], G. Di Coscio[2], C. Parolini[1], P. Brazzarola[3], M. Chilosi[1], M. Ferdeghini[1]

[1]Patologia e Diagnostica - Verona, [2]Sezione di Citopatologia - Pisa, [3]Chirurgia e Oncologia - Verona

The proto-oncogene c-Kit encodes a type III trans-membrane receptor tyrosin-kinase (c-Kit/CD117), involved in the morphogenesis and in the regulation of proliferation, apoptosis and cell differentiation through the activation of multiple signaling pathways. Altered expression of c-Kit has been reported in a wide spectrum of cell lines and human tumors and associated with the malignant transformation and tumor progression.

Few studies investigated c-Kit expression in thyroid and the results are contradictory. Although molecular studies, with the use of different methods, detected the loss or down-regulation of the proto-oncogene c-Kit in papillary thyroid carcinomas compared to benign lesions, a high staining rate of positivity has been reported by immunohistochemical studies.

The aim of this study was to evaluate the protein expression of c-Kit in various thyroid lesions and to explore the putative diagnostic role of this marker.

The analysis was performed on 50 papillary carcinomas, the corresponding healthy tissue and 30 follicular adenomas and nodular hyperplasias, using the CD117 monoclonal antibody (Dako, Denmark).

CD117 was negative in all the papillary carcinomas tested, but showed variable and heterogeneous expression, as regard staining intensity and percentage of positive cells, both in the normal tissue and in the benign lesions. Positivity for CD117 was mainly detected at cell-membrane level.

Our results are in full accordance with the previous papers of Natale et al. and Tomei et al. mentioning a loss of c-Kit in papillary carcinomas compared to benign lesions. These data suggest an involvement of the proto-oncogene c-Kit in the neoplastic transformation of the follicular epithelium and its negative regulation would indicate a role in the differentiation rather than in the proliferation of thyrocytes.

The use of this marker, in combination with other markers such as galectin-3, CK19, HBME-1 and Claudin-1, could be a useful tool for assisting in the diagnosis of follicular-derived neoplasms/lesions.

PP375

IMMUNOHISTOCHEMICAL DETECTION OF THE BRAF V600E - MUTATED PROTEIN IN PAPILLARY THYROID CARCINOMA AND COMPARISON WITH DIRECT SEQUENCING ANALYSIS.

C. Colato[1], M. Ivo[2], P. Piccoli[1], L. Montagna[1], G. Di Coscio[2], P. Brazzarola[3], M. Chilosi[1], M. Ferdeghini[1]

[1]Patologia e Diagnostica - Verona, [2]Sezione di Citopatologia - Pisa, [3]Chirurgia e Oncologia - Verona

Background: The mutation of the B-type Raf kinase gene (BRAF V600E) is a common event in papillary thyroid carcinoma (PTC) and seems to play an important role in the development and progression of this disease. Sequencing analysis is the 'gold standard' for mutation detection but requires resources beyond many diagnostic pathology laboratories. Recently, the development of new antibodies directed against the V600E protein has opened the door for an easier method for identifying this mutation.

Aim: To determine the efficacy of a novel mutation-specific antibody in a well-characterized cohort of PTC patients and correlate the immunohistochemical results with that obtained by direct sequencing analysis.

Materials: We evaluated the expression of the mutated BRAF V600E protein in archival formalin-fixed, paraffin-embedded tissue specimens and corresponding cytological smears of 50 PTCs, using the monoclonal antibody mAbVE1 (UCS Diagnostic). The BRAF V600E sequencing analisys was performed on cytological samples.

Results: 52.2% of PTCs showed unequivocal cytoplasmic expression of the mutated BRAF protein. BRAF V600E protein expression was significantly more common in tumors with tall cell or oncocytic features but was less common in tumors with follicular growth pattern. Solid and follicular variants did not show the mutated BRAF protein. Comparing the immunohistochemical data to that of sequencing analysis, we found a correlation in 48/50 cases (96%).

Conclusions: We confirm high specificity and sensitivity of the mAbVE1 for the detection of BRAF V600E protein in tumor formalin-fixed and paraffin-embedded archival tissue samples and in cytological preoperative smears.

The present findings support the potential use of immunohistochemistry as an ancillary screening tool to assess the BRAF V600E mutation status in PTC. Moreover, immunohistochemical detection of the mutated BRAF V600E protein in PTC could facilitate mutational analysis in the clinical setting.

PP376

OVERWEIGHT/OBESE WOMEN WITH PRIMARY ACQUIRED HYPOTHYROIDISM IN OPTIMAL LT4 REPLACEMENT THERAPY HAVE IMPAIRED WHOLE BODY ENERGY METABOLISM

F. Martucci[1], G. Manzoni[1], G. Lattuada[1], G. Perseghin[2]

[1]Endocrinologia e Malattie Metaboliche, Policlinico di Monza - Monza, [2]Scienze Biomediche per la Salute, Università degli Studi di Milano - Milano

INTRODUCTION: appropriately titrated LT4 replacement therapy may not be able to fully correct the entire set of metabolic defects afflicting individuals with primary hypothyroidism. Based on this hypothesis, the present study was undertaken to establish whether these patients have impaired whole body energy metabolism.

METHODS: we recruited 30 hypothyroid women with duration of the disease > 2 years, BMI > 25 kg/m2 and serum TSH < 3.5 µU/mL under replacement therapy with LT4 (mean dose: 73±34 µg/die) and compared them to 18 eu-thyroid women matched for age (53±13 vs 48±10 years), BMI (32.5±7.0 vs 33.7±8.3 kg/m2), menopausal state and life-style habits ($p>0.3$ for all). They underwent bioelectrical-impedentiometry (BIA) and indirect calorimetry to assess body composition and resting energy expenditure (REE) respectively.

RESULTS: TSH (1.92±1.06 vs. 1.87±0.89 µU/mL; $p=0.91$) and body composition (body fat: 41.4±7.4 vs 42.1±8.3%; LBM 58.6±7.4 vs. 57.8±8.3%; $p > 0.7$ for all) were not different between groups. REE was reduced in hypothyroid women when compared to the control group in absolute terms (1347±171 vs. 1447±154 Kcal/die; $p < 0.05$), when adjusted for LBM (28.3±2.6 vs. 30.5±3.0 Kcal/kg LBM die; $p<0.02$) and when expressed as the ratio between the measured REE and the expected REE based on the Harris-Benedict equation (91±7 vs. 95±7%; $p<0.05$). The respiratory quotient was also different between groups (0.92±0.07 vs. 0.86±0.06; $p<0.01$), suggesting for impaired fasting lipid oxidation in hypothyroid women.

CONCLUSIONS: this study demonstrates that middle-aged, overweight/obese hypothyroid women in LT4 replacement therapy, in spite of achieving an optimal serum TSH level, are characterized by altered whole body energy metabolism and substrate disposal supporting the view that additional interventions may be necessary to fully revert the entire set of hypothyroidism-related metabolic alterations.

PP377

Δ133P53 EXPRESSION AND FUNCTION IN THYROID CANCER

F. Giani[1], M. L. Nicolosi[1], V. Vella[1], F. Geraci[1], R. Vigneri[1], S. Squatrito[1], F. Frasca[1]

[1]Department of Clinical and Molecular Biomedicine, University of Catania - Catania

Background. The human protein Δ133p53 is an N-terminally truncated p53 isoform and has been shown to inhibit p53-induced apoptosis and cell-cycle arrest.
Objective. Aim of the present study was to evaluate the expression and function of Δ133p53 in thyroid tumor.
Methods and results. Expression pattern of wt-p53 and Δ133p53 was first evaluated in normal and cancer thyroid tissues and in cell lines by qRT-PCR. These data were then confirmed at the protein level by western blot. We found that all thyroid cancer cell lines (n=10) express Δ133p53 isoform although at different level, but we didn't observe any correlation with hystotype. At mRNA level, a higher expression of TAp53 compared to Δ133p53 (3 orders of magnitude) was observed. In human thyroid tissue (n=14) qRT-PCR revealed that, compared to normal counterpart, the expression of Δ133p53 was 10-fold higher thyroid carcinomas. To study the effect of Δ133p53 on p53 transcriptional activity in thyroid cancer cells, luciferase assays were done. TPC-1 cells were transiently transfected with either wt-p53, Δ133p53 or p53 mutant, along with p21Luc, BaxLuc and Mdm2Luc promoters. We observed that Δ133p53 was a poor activator of p21, Bax and Mdm2 promoters compared to wt-p53 and its activity was comparable to that of p53 mutant. Moreover, Δ133p53 behaved as a dominant negative toward TAp53. The role of Δ133p53 in resistance to chemotherapy was analyzed in ecdysone-inducible Δ133p53 TPC-1 clones exposed to DNA damaging agents (doxorubicin, cisplatin or taxol) for different time points. After PI staining, FACS analysis revealed an increase in G2/M population in cells transfected with Δ133p53 together with a decrease in SubG1 (apoptotic) population in response to DNA damage.
Conclusions. Taken together, these results indicate that Δ133p53 is overexpressed in thyroid cancer cells and may be involved in thyroid cancer progression as it is able to reduce p53 activity thereby interfering with its tumor suppressor activity and increasing thyroid cancer cell chemo-resistance.

PP378

DIFFUSE INCREASE OF 99TECHNETIUM UPTAKE IN THE THYROID GLAND AFTER FAILURE OF RADIOACTIVE IODINE THERAPY FOR HOT THYROID NODULE. A CASE REPORT.

M. Marina[1], E. Usberti[1], A. Versari[2], R. Minelli[1], G. P. Ceda[1], G. Ceresini[1]

[1]Medicina Clinica e Sperimentale Università di Parma - Parma, [2]Med. Nucleare-Arcispedale S. Maria Nuova - Reggio Emilia

Introduction. Radioactive iodine therapy (RAIT) represents an important tool for the treatment of toxic thyroid nodule. However, recurrence of hyperthyroidism occurs in a moderate percentage of patients. Here, we describe a case of toxic thyroid nodule with RAIT failure and a subsequent development of a diffuse increase of thyroid uptake at the second scintiscan.
Clinical Case. A 68-year old man was admitted to the Endocrine Unit because of palpitations and heat intolerance. Thyrotoxicosis was diagnosed with serum TSH of 0.01 mIU/L, increased FT4 (1.88 ng/dL) and slightly elevated FT3. A thyroid nodule was palpable in the left thyroid lobe. Ultrasonography (US) confirmed a 2.5-cm mixed solid/cystic nodule in the left thyroid lobe with no other nodular lesion. Serum anti-thyroperoxidase (TPOAb) and anti-thyroid receptor (TRAb) antibodies were negative. Thyroid scintiscan with technetium-99m pertechnetate (99Tc) revealed a hot area in the left thyroid lobe, corresponding to the nodule which was found at the US evaluation, and an increased uptake in two small areas, one in the right lobe and one in the upper portion of the left lobe, which however did not correspond to any nodular lesion at the US. Uptake was very weak in the remaining parenchyma. The nodule had peri- and (to some extent) intranodular vascularization. Vascularization was normal/weak in the remaining thyroid bed. Nodule cytology was benign. Methimazole and beta-blockers were started with subsequent RAIT with 15 mCi radioactive iodine. Six months after RAIT, the patient still needed 10 mg/day of methimazole. Nine months later, TSH was 0.9 mIU/L with methimazole 5 mg/day. A further thyroid US confirmed the unmodified nodule in the left thyroid lobe without any other nodules. A further scan with 99Tc showed a diffuse, homogeneously increased uptake of the whole thyroid gland without focal areas. TPO-Ab and TRAb were still negative. Therefore, the initial scan image of a hot thyroid nodule turned to be characterized by a diffuse increase of 99Tc uptake at the 15-month subsequent scan, after RAIT failure.This phenomenon was accompanied by the negativity of anticorpal status, thus leaving uncertainty on the underlying mechanism.
Conclusion. A second scan, after RAIT failure for hot thyroid nodules, may reveal a diffuse homogeneous uptake. In this case, patients should be monitored for the potential occurrence of a second RAIT-induced hypothyroidism.

PP379

TSHR AND GALFAS GENE POLYMORPHISMS IN AUTONOMOUSLY FUNCTIONING THYROID NODULES (AFTN).

T. Vicchio[1], A. Campenni[2], S. Giovinazzo[1], R. Certo[1], G. Giuffrida[1], C. Micali[3], S. Baldari[4], S. Benvenga[1], F. Trimarchi[1], R. M. Ruggeri[1]

[1]Medicina clinica e sperimentale, Endocrinologia, Università di Messina - Messina, [2]Scienze Biomediche e delle Immagini, Medicina Nucleare, Università di Messina - Messina, [3]Medicina Trasfusionale, Università di Messina - Messina, [4]Scienze Biomediche e delle Immagini, Medicina Nucleare, Universtà di Messina - Messina

Background and Aim. Somatic mutations of the thyrotropin receptor (TSHR) and/or Gαs gene have been found in a number – but not in all – AFTNs. Recently, we have found in a 15-year-old girl with a papillary thyroid carcinoma, which was functionally autonomous, two combined non-functioning mutations: a SNP of the TSHR gene, in exon 7, at codon 187 (AAT/AAC, both encoding asparagine), and a SNP within exon 8 of Gαs gene at codon 185 (ATC/ATT, both encoding isoleucine) 1. The same silent SNP in exon 7 of the TSHR gene (codon 187 Asn) had been reported in patients affected by both AFTN and familial non-autoimmune hyperthyroidism. No further data about the prevalence of each of the two SNPs in AFTNs as well as in the general population are available in the literature. In order to clarify the possible role of these SNPs in predisposing to/causing the disease, we evaluated the genotype distribution of such SNPs in a cohort of patients with AFTNs, in comparison with a large control group of healthy individuals from the same marginally iodine deficient geographic area.
Methods. Germline DNA extraction was performed on blood leukocytes of 100 patients with AFTNs (37 males and 63 females, aged 63 ± 13 [(mean ± SD)], range 31-85 years) and 100 age- and sex-matched healthy individuals as controls, with the Eurogold Blood DNA Mini Kit (Euroclone, Italy) according to the manufacturer's instructions. The genotype distribution of the two SNPs was investigated by restriction-fragment length polymorphism (RFLP)-PCR, as elsewhere specified1.
Results. We have found that the two SNPs are quite rare in healthy individuals from our geographic area: 9% of controls were heterozygous for the TSHR SNP, and 7% were heterozygous for the Gαs SNP. None harbored both SNPs. The prevalence of the two SNP in our study population was not different to that found in healthy individuals: 7 % were heterozygous for the TSHR SNP, and 4 % were heterozygous for the Gαs SNP.
Conclusions. These results suggest that these two SNPs do not confer susceptibility for the development of AFTN. (1)Ruggeri RM et Al. Follicular variant of papillary thyroid carcinoma presenting as toxic nodule in an adolescent. Co-existent polymorphism of the thyrotropin receptor and gαs genes. Thyroid. 2012 Sep 17

PP380

FIRST DESCRIPTION OF A PATIENT WITH CONGENITAL GOITROUS HYPOTHYROIDISM DUE TO NIS DEFECT AND THYROID CANCER

B. Bagattini[1], P. Agretti[1], G. De Marco[1], C. Di Cosmo[1], L. Montanelli[1], P. Vitti[1], M. Tonacchera[1]

[1]Department of Clinical and Experimental Medicine, Endocrinology Section - Pisa

Introduction: Iodide transport defect (ITD) is a rare cause of congenital hypothyroidism (CH). In some cases this defect is determined by anomalies of the sodium/iodide symporter (NIS), that can also be responsible of the presence of goiter.
Proband: We describe the case of a 40 years old woman affected by CH, treated with levothyroxine since she was one year old. She developed a large goiter which underwent total thyroidectomy at the age of 32.
After 8 years, the patient came to our attention because of recurrence of multinodular goiter. Cytological examination of the two larger nodules deposed for "indeterminate nodules". The patient reported the consanguinity of her paternal grandparents and reported that her younger brother had a congenital hypothyroidism treated with levothyroxine from the first days of life. None of her three children had a thyroid disease.
Results: suspecting an ITD we performed a thyroid uptake and a neck scan with 123-I, after discontinuation of therapy with levothyroxine for a month. At the time of the study the patient had an overt hypothyroidism and radionuclide uptake was absent in the anterior cervical area. She was therefore subjected to a total body scan which showed an absent tracer uptake at the level of salivary glands and stomach. An ITD by a lack of NIS was so diagnosed and the patient underwent surgical resection of recurrent goiter. The genetic analysis (cDNA) showed a large deletion of 5 exons (from VIII to XII), Δ (324 - 509), in homozygosity of NIS gene, which determined the production of a truncated protein. Histological examination of the recurrence showed a papillary carcinoma follicular variant, multifocal, infiltrating fibro-muscular tissues.
Conclusions: We describe the first case of dyshormonogenetic goiter due to NIS defect associated with papillary thyroid carcinoma. This finding raises the problem of the completion of treatment and ablation of residual thyroid, which cannot occur by 131-I. In particular, the infiltration of fibro-muscular tissues suggests the possibility of a more aggressive treatment of the residue.

PP381

IMMUNOHISTOCHEMICAL EXPRESSION OF HER2 IN THYROID CANCER .

R. M. Ruggeri[1], A. Campenni[2], A. Simone[3], R. R. de Krijger[4], S. Benvenga[1], G. Tuccari[3], G. Giuffrè[3], F. Trimarchi[1]

[1]Medicina clinica e sperimentale, Endocrinologia, Università di Messina - Messina, [2]Scienze Biomediche e delle Immagini, Medicina Nucleare, Università di Messina - Messina, [3]Patologia Umana, Università di Messina - Messina, [4]Department of Pathology, University Medical Center Rotterdam - Rotterman, The Netherlands

The Epidermal Growth Factor Receptor (EGFR) family, including EGFR, HER2, HER3, and HER4, is expressed in many human epithelial malignancies, including thyroid cancer. Several molecules were synthesized to inhibit the extracellular domain of EGFR (cetuximab), the extracellular domain of HER2 (trastuzumab) or the EGFR tyrosine kinase domain (gefitinib and erlotinib). Levels of EGFR expression and the presence of EGFR mutations correlate with responsiveness to targeted therapy. Recent findings in gefitinib treated patients support HER2 analysis as a complementary test for selection of patient candidate for EGFR targeted therapies. Thus, we focused our attention on HER2 expression in thyroid cancer. Materials and Methods. We studied HER2 expression in a series of 20 papillary thyroid cancers (PTC), 20 follicular thyroid cancers (FTC) and 20 medullary thyroid cancers (MTC, of which 6 sporadic, 12 MEN2A and 2 MEN2B) by immunohistochemistry. Results. HER2 was expressed in 90% of differentiated thyroid cancers (18/20 PTC and 18/20 FTC) and 50% of MTC (10/20, 3 sporadic, 6 MEN2A, 1MEN2B), with an intensity ranging from 1+ to 3+. The intensity of the reaction was higher in differentiated thyroid cancer [FTC, median value: 3+ (mean ± SD, 2.5 ± 0.5); PTC, median value: 2+ (2 ± 0.9); median value if considered as a whole: 2.5+] than in MTC [median value: 1+; mean ± SD, 1.3 ± 0.6), p <0.001]. In one sporadic MTC, the primary tumour was negative for HER2, while liver metastatic tissue stained positively (1+). In both FTC and PTC, the positivity for HER2 was diffuse throughout the section, and displayed a diffuse cytoplasmic and membranous pattern. In MTC the positivity presented an intra-tumour focal distribution, and displayed a granular cytoplasmic pattern. HER2 was not expressed in the adjacent normal thyroid tissue, except a case of MEN2A, where a focal positivity was observed in peri-tumoral tissue. No significant correlation was found between HER2 immunoexpression and the clinical stage of the disease.Conclusions. We found that HER2 was consistently expressed in differentiated thyroid cancer and, to a lesser extent, in MTC, suggesting a role of this receptor in thyroid tumorigenesis. There might be practical implications: HER2 expression may be validated for potential use in the stratification of patients for targeted therapy.

PP382

EFFECTS OF SELENIOMETHIONE IN PATIENTS WITH HASHIMOTO'S THYROIDITIS IN EUTHYROIDISM

A. Renzullo[1], G. Accardo[1], D. Esposito[1], F. Barbato[1], C. Ambrosio[2], M. G. Caprio[2], G. Vallone[2], D. Pasquali[1]

[1]Dip di Scienze Cardio-Toraciche e Respiratorie, Sezione di Endocrinologia, Seconda Università di Napoli - Napoli, [2]Dip di Scienze Biomorfologiche e Funzionali, Università Federico II - Napoli

The thyroid expresses several specific selenoproteins of which some are implicated in thyroid hormone metabolism and others play an antioxidant defence role. The principal selenoproteins include deiodinases that are expressed in the thyroid gland in large quantities. The three deiodinase isoforms (D1, D2, D3) catalyze conversion of T4 into T3. Recent studies underlined the importance of selenium supplementation in thyroid autoimmune disease, nevertheless the clinical application in Hashimoto's thyroiditis (HT) need further clarification. The aim of this study was to evaluate the effect of selenomethionine administration on TSH, FT4, FT3, AbTPO, AbTG levels and ultrasound structure of the thyroid gland in HT in euthyroidism, furthermore with the use of the developed algorithm, a discriminant analysis was performed to analyze specificity and sensitivity as well as the impact of sensitivity of ROI shift, repositioning and rotation on the measured features. Methods: In a perspective study we enrolled 40 HT patients with euthyroidism. We administered seleniomethionine (Syrel) 166 ug/die for 3 and 6 months (Se, n20) or placebo (controls, n 20). TSH, FT3, FT4 , TPO Ab, TG Ab serum levels were assayed at time 0, and after 3 and 6 months of treatment. For each person, an ultrasound examination of the left and right thyroid lobe in transverse and longitudinal sections was performed. Each ultrasound image was analyzed in great detail and, then, an expert physician selected for analysis a rectangular region (ROI) which covered the thyroid lobe in individual sections. Each time, the ROI included the greatest possible and most representative area of the patient's thyroid lobe. Results: TSH level were significantly reduced in SE compared to controls after 3 months of seleniomethionine treatment, while no significant differences in TPOAb and TGAb were found. Actually, only 10 subject have been evaluated after 6 months of treatment and FT4 level appeared to be statistically reduced compared to time 0, while TSH was not significantly different. We did not find any significant modification in thyroid echogenicity using the ROI before and after 3 and 6 months of treatment. Conclusions: Our preliminary data indicate that seleniomethionine could have a role in modulating thyroid function in HT with euthyroidism but more data are needed to draw any conclusion.

PP383

ANTITUMOR EFFECTS OF SULPHORAPHANE ON THYROID CARCINOMA CELL LINES

M. Pitrone[1], G. Arcoleo[1], W. Arancio[1], C. Baiamonte[1], L. Tomasello[1], V. Carina[1], C. Giordano[1], G. Pizzolanti[1]

[1]Di.Bi.M.I.S. - Palermo

Thyroid cancer is the most frequent endocrine malignancy with a global increasing incidence. Many evidences show that sulphoraphane (SF), a natural isothiocyante found in cruciferous vegetables has a wide range of chemopreventive as well as apoptosis inducing properties. The ability of SF in inducing apoptosis and cell cycle arrest is associated with the regulation of many proteins including Bcl-2 family proteins, caspases, p21, and cyclin dependent kinases.

In the present work, we investigated *in vitro* the activity of SF in three human thyroid cancer cell lines. For this purpose we studied SW1736 (ATC), BC-PAP (PTC) and TT (MTC) cell line by MTT assay, after addition of SF ranging from 0 to 20μM. Cell lines were treated with SF at different concentration (1μM, 5μM, 10μM, 20μM) for 72 hours and after treatment we observed a SF- induced toxicity in all thyroid cancer cell lines, whereas SF had not significant effects on nonmalignant cells. In order to demonstrate the role of SF in the apoptotic pathway, we analyzed its effect on Bcl-2 and BAX protein expression by western blot. The increase of SF concentration induce an upregulation of BAX followed by down-regulation of Bcl-2, confirming its pro-apoptotic role in all three cell lines. So, our preliminary data, suggest its possible use in prevention trial in high-risk areas and as enhancer in anti-neoplastic therapy of thyroid cancer.

PP384

ANTIOXIDANT AND ANTIPROLIFERATIVE EFFECTS OF OLIVE-LEAF EXTRACT OLEUROPEIN AND ITS PERACETYLATED DERIVATE ON HUMAN THYROID CANCER CELLS.

D. Russo[1], S. Bulotta[1], R. Corradino[1], J. Maiuolo[1], M. Celano[1], M. D'Agostino[1], M. Oliverio[1], M. Maranghi[2], A. Procopio[1]

[1]Dipartimento di Scienze della Salute - Catanzaro, [2]Dipartimento di Medicina Interna e Specialità Mediche - Roma

The olive tree phenolic component oleuropein and its semisynthetic derivatives have shown many biological properties, and are currently under investigation for the treatment of several diseases, including neoplasia. Aim of this study was to evaluate the activities of oleuropein (OLE) and its peracetylated derivative (peracetylated oleuropein, Ac-OLE), against two thyroid tumour cell lines, namely TPC-1 and BCPAP cells, which host genotypic alterations detected in human papillary thyroid cancer.

Cells were treated with OLE and Ac-OLE, and the effects on viability evaluated by cell counting and MTT assay. Antioxidant effects were analyzed by measuring the accumulation of intracellular radical oxygen species (ROS) after treatment with hydrogen peroxide (H2O2). The levels of phosphorylated ERK and Akt, as markers of activation of MAP kinase and PI3k-Akt signalling pathways, were evaluated by western blot.

We found that OLE at micromolar concentration inhibited significantly the proliferation of both cell lines. This effect was paralleled by a reduction of phospho-Akt and phospho-ERK levels. Also the ROS production induced by H2O2 resulted markedly inhibited in both cell lines. A stronger effect was elicited by Ac-OLE either in inhibiting cell growth or as antioxidant, in particular on BCPAP cells.

Our results demonstrate that OLE and especially Ac-OLE inhibit in vitro thyroid cancer cell proliferation acting on growth promoting signal pathways, as well as protecting against ROS production. Further studies will reveal the potential application as novel targeted therapeutics in thyroid cancer.

PP385

AN UNUSUAL CASE OF MARINE-LENHART SYNDROME.

S. Giovinazzo[1], R. Certo[1], G. Giuffrida[1], T. Vicchio[1], F. Trimarchi[1], A. Campenni[2], R. M. Ruggeri[1]

[1]Medicina Clinica e Sperimentale, Endocrinologia, Università di Messina - Messina, [2]Scienze Biomediche e delle Immagini, Medicina Nucleare, Università di Messina - Messina

Graves' disease and autonomously functioning thyroid nodules (AFTN) both cause thyrotoxicosis by different pathophysiological mechanisms. The coexistence of both diseases has been termed "Marine-Lenhart syndrome". During the last years, several papers have been published on the development of Graves' disease shortly after radioiodine therapy of AFTNs, especially in patients with elevated TPOAb. Herein we report an unusual case of Marine-Lenhart syndrome. A 42-year-old woman was seen in our outpatient clinic in December 2008 because of fatigue, palpitations, tremors, nervousness, insomnia, oligo-amenorrhea, sweating, and weight loss of three-months durations. GD was diagnosed on the basis of clinical symptoms/signs, a TSH level of <0.001 mIU/L (normal values, 0.27-4.2) with elevated free triiodothyronine (FT3, 17.39 pg/ml, v.n 2-4.4) and free thyroxine (FT4, 38.3 pmol/L, n.v. 12–22), and positivity of anti-TSH-receptor antibodies (TRAb, 19 IU/L, n.v.<1.5), as well as of TPOAb (158 U/L; n.v. <35). Ultrasound (US) examination showed a diffuse enlargement of the thyroid gland, associated with hypoechogenicity and increased vascularity. The 131I thyroid scan revealed an enlarged gland with diffuse increased uptake of radio-iodine at 6 and 24 hours. Therapy with methimazole (MMI, 30 mg/day) was started, and the patient was referred for radioactive iodine treatment (RIT) in March 2009. Within three months from RIT, her thyroid function test normalized with a TSH of 1.4 and a FT4 of 16 pm/L. Ab-TPO were 364 UI/L. Six months later, thyroid US examination showed the appearance of a 7 mm ipoechoic nodule, with regular margins and an increased intra-nodular blood flow by color-doppler, in the upper portion of the right lobe. Over the next 18 months, the nodule increased in size up to a maximum diameter of 12 mm. Biochemical evaluation showed low TSH (0.347 mIU/L) with normal levels of FT3 and FT4. TRAb were negative. 131I thyroid scintigraphy revealed an avid uptake of the tracer in the right lobe, corresponding to the nodular lesion demonstrated at US, consistently with an AFTN. In conclusion, our patient developed an AFTN three years after the onset of a hyperthyroidism due to Graves' disease, treated successfully with radioiodine. Unlike most cases reported in the literature, in which Graves'-like hyperthyroidism develops after RIT, this particular case of Marine-Lenhart syndrome shows the appearance of an AFTN as a consequence of Graves' disease treatment with radioiodine.

PP386

EFFECTS OF SELENIUM ON TSH LEVELS IN AUTOIMMUNE THYROIDITIS

D. Tumino[1], S. Squatrito[1], F. Frasca[1]

[1]Department of Clinical and Molecular Biomedicine, University of Catania - Città

Background: it has been reported that the beneficial effect of selenium in Autoimmune thyroiditis (AIT) is related to the reduced ROS damage via enhanced expression of the glutathione peroxidases (Gpxs) and improvement of redox status in the thyrocyte through increasing activity of thioredoxin reductases (TRxs).

Rationale: indeed, in patients with Hashimoto's disease, selenium supplementation decreases anti-thyroid antibody levels and improves the ultrasound structure of the thyroid gland. However it is unknown whether long-term selenium supplementation has relevant clinical benefits in patients with Hashimoto's thyroiditis.

Aim: we evaluate whether Selenium supplementation prevents the evolution into hypothyroidism thereby obviating the need for L-T4 treatment. Sixty patients aged 17-71 years (median age 42 years, 55F/5M) with AIT were retrospectively evaluated. Group I (n=21): patients treated with selenomethionine (Seme) 200 µg/daily. Group II (n=39): patients treated with sodium selenite (80 µg/daily). Group III (n=60) untreated patients were used as a control group. All patients were not treated with L-T4. Serum thyroid hormone status (TSH, FT3, FT4, TPOAb, TgAb) was measured.

Results: after an average of six months of tratment serum TSH was reduced only in the group I (n=21, P=0,18), although difference was not statistically signifcant for the low number of cases.

Conclusion: the available data on selenium supplementation in patients with autoimmune thyroid disease suggest a slight improvement in those patients treated with Seme in terms of lowering TSH levels.

PP387

A SEQUENTIAL EXPRESSION OF D2 AND D3 DEIODINASES IS REQUIRED FOR REGULATION OF THYROID HORMONE SIGNALING DURING SKELETAL MUSCLE REGENERATION.

M. Dentice[1], R. Ambrosio[1], A. Sibilio[1], C. Bonelli[1], M. A. De Stefano[1], V. Torre[1], M. Sorrentino[1], S. Manganiello[1], L. Cristinsiano[1], F. Alfano[1], F. L. Trivisano[1], G. Fenzi[1], D. Salvatore[1]

[1]Endocrinologia ed Oncologia Molecolare e Clinica - Napoli

The primary product of the thyroid gland, thyroxine (T4), must be converted to T3 in order to be active. This metabolic process is an essential mechanism that regulates thyroid hormone (TH) action at cellular level. TH action is regulated by the pre-receptoral activity of the deiodinases. D1 and D2 activate the thyroxine (T4), whereas D3, by inactivating T3, terminates TH action. Although a central role for TH and skeletal muscle function is well recognized, the molecular mechanism(s) by which TH regulates muscle development and regeneration are poorly understood. D2 provides a mechanism for tissue-specific stimulation of T3-dependent genes. Among these genes, *myoD* and its downstream regulatory factors are required for the proper myogenic program. We demonstrated that D2 plays a key role in skeletal muscle regeneration. Indeed, blockade of D2 action inhibits the expression of myoD and the programmed differentiation of muscle stem cells (mpc), an effect rescued by elevated T3 concentrations. Conversely, D3 is highly expressed in proliferating mpc and in the early phases of the regeneration. Our preliminary data clearly indicate that D2 and D3 are expressed in a defined spatio-temporal sequence and are essential components of the myogenic program in muscle regeneration. In conclusion, our results suggest that during regeneration, a fine-tuned, sequential expression of deiodinases D2 and D3 is strictly required allowing muscle stem cells amplification and differentiation. In addition it set the stage to use deiodinase's regulation as a tool to manipulate at will the physiology of muscle stem cells, modulating their expansion and differentiation in a therapeutic context.

PP388

IODINE SUPPLEMENTATION AND THYROID FUNCTION IN A COHORT OF TUSCAN PREGNANT WOMEN

E. Gianetti[1], L. Russo[1], L. Montanelli[1], S. Bottai[1], S. Licari[1], P. Vitti[1], M. Tonacchera[1]

[1]UO Endocrinologia 1 - Università di Pisa - Pisa

Background: Thyroid function is essential during pregnancy.

Aim: Aim of this study was to evaluate the effects of iodine status on thyroid function in pregnant women in an area with moderate iodine deficiency.

Patients/Methods: 397 Tuscan women were evaluated for thyroid hormones concentrations, thyroid autoimmunity, urinary iodine (UI, mcg/l) and use of iodine supplementation (iodized salt, multivitamins drugs containing iodine or both [IS], or none [NO]) at 10, 15, 20, 25 and 35 weeks of pregnancy.

Results: 320/397 women did not present any thyroid disease (group A), 38 were affected by chronic autoimmune thyroiditis [group B] and 39 presented with thyroid nodule(s) [group C]). UI was consistent with iodine supplementation: at 10 weeks of pregnancy, considering all patients together, UI was 163.5±136.8 in IS (n=165) and 100.3±90.7 in NO (n=232) (p value=0.002). Dividing groups, in A, IS=182.4±147.9 (n=126) and NO =106.2±97.0 (n=193); in B, IS =103.4±63.6 (n=17) and NO=64.3±54.0 (n=21); in C, IS =98.2±72.7 (n=21) and NO =85.5±47.1 (n=18). Statistical significance was reached only in group A (p value=0.002). At 10 weeks, adequate UI (150 <UI<250) was found in 25% of women in A-IS, 14% in A-NO, 13% in B-IS, 13% in B-NO, 40% in C-IS and 13% in C-NO. Lower UI was found in 53% in A-IS, 75% in A-NO, 76% in B-IS, 89% in B-NO, 60% in C-IS and 89% in C-NO. Higher UI was found in 22% in A-IS, 10% in A-NO, 13% in B-IS and none in the other groups. In each group and at each time point, correlations were evaluated between UI and FT4, TSH, FT3/FT4 ratio, AbTg and AbTPO, but no significances were found. In group B, no significant correlations were found between TSH and AbTg or AbTPO levels at none of the time points.

Conclusions: Although UI measurement method was validated by the positive correlation with the use of iodine supplementation, no significant correlations were found between UI and thyroid function or autoimmunity during pregnancy. More patients are currently being evaluated to increase the statistical power of the study and further verify these data.

PP389

IMMUNOLOGICAL ASPECTS AND EFFECT OF MMI IN VITRO TREATMENT OF T-LYMPHOCYTES FROM PATIENTS WITH GRAVES'S DISEASE

M. Caputo[1], A. Coppola[2], M. Romano[3], A. Bellia[1], D. Pastore[2], B. Capuani[2], A. Andreadi[2], E. De Carli[3], M. E. Rinaldi[3], M. Cerilli[3], F. Pozzi[3], G. Donadel[2], P. Sbraccia[2], M. Federici[2], A. Bertoli[2], G. Sconocchia[4], D. Lauro[1]

[1]Dip.to di medicina dei Sistemi Università di Tor vergata e Centro per il diabete di tipo 2 Policlinico Tor vergata - Roma, [2]Medicina dei Sistemi Università Tor Vergata - Roma, [3]Centro per il Diabete tipo 2, Policlinico Tor Vergata - Roma, [4]Istituto di Farmacologia Traslazionale, CNR - Roma

Background. Graves's disease (GD) results from many pathogenetic factors leading to an immune self-response against thyroid antigens. This process involves a dysregulation of a subset of regulatory CD4 cells (Tregs), important in maintaining self tolerance. Based on literature data and our preliminary results, GD immunological dysfunction may not be limited to Tregs alteration but could involve other T cell subpopulations. In this study we evaluated some immunological aspects and the effect of anti-thyroid treatment (MMI) of patients with GD. **Methods.** In this study were included 10 GD patients; peripheral blood sample T lymphocytes counts and CD4/CD8 ratio were studied by a direct staining of whole blood utilizing flow cytometry. For functional studies, peripheral blood mononuclear cells were isolated from the patients and were cultured, in vitro, in presence or absence of MMI and Interleukin2 (IL2). Apoptosis was assessed by annexin V and Fas while Tregs rate was evaluated by intracellular staining for FoxP3. **Results.** An inversion of CD4/CD8 ratio was detected in 5 out of 10 patients; we asked if inverted CD4/CD8 ratio could involve directly CD4 or was a result of increased CD8 number. Since GD patients with inverted CD4/CD8 ratio did not show evidence of HIV related manifestation, we found that CD4 number were not affected in these patients. In vitro studies showed significantly higher apoptosis rate in CD4 of patients GD with inverted CD4/CD8 ratio, in the presence of MMI and IL2, than GD patients without CD4/CD8 inversion (10±1.9 *vs* 6.5±3.2 $p<0.01$). We didn't find significant reduction of Tregs, whether the absolute number of CD8 in GD patients with inverted CD4/CD8 ratio was higher (787 cells) compared to patients with normal CD4/CD8 ratio (414 cells), suggesting a CD8 T cell deregulation. **Conclusions.** We found two different phenotype in GD population: normal or inverted CD4/CD8 ratio; GD patients with inverted CD4/CD8 have increased number of CD8 and higher apoptosis of CD4 in response to MMI treatment. However, these are preliminary results and a higher number of people with GD should be investigated to confirm the data.

PP390

ADVANCED MEDULLARY THYROID CANCER (MTC) CAN BE ASSOCIATED WITH HIGH LEVELS OF SERUM CARBOHYDRATE ANTIGEN 19.9 (CA 19.9)

L. Lorusso[1], C. Romei[1], L. Agate[1], V. Bottici[1], E. Molinaro[1], L. Torregrossa[2], F. Basolo[2], G. Materazzi[2], P. Vitti[1], R. Elisei[1]

[1]Department of Endocrinology and Metabolism - Pisa, [2]Department of Surgery - Pisa

Background: both calcitonin (CT) and carcinoembryonic antigen (CEA) are serum diagnostic markers of MTC and their increase is related to the progression of the metastatic disease. We observed a peculiar case of an aggressive MTC in a young patient, who rapidly died for the disease and who had high levels of serum Ca19.9 without any gastrointestinal malignancy. This case arose the question of whether Ca19.9 can be a marker for MTC.

Objective: to evaluate the serum levels of Ca19.9 in a series of advanced MTC pt and their correlation with CT and CEA levels. To this purpose we measured the Ca19.9, CT and CEA in 54 advanced MTC pt. We also evaluated the mean survival of pt with high levels of Ca19.9 (>37 U/ml) compared to those with normal levels of Ca19.9 (<37 U/ml).

Results: eight/54 (14,8%) MTC pt showed high levels of Ca19.9 (mean: 140 U/ml, range: 43-276 U/ml); the mean serum CT and CEA values were 11,401 pg/ml and 2,112 U/ml, respectively. Five/8 pt (62,5%) died after a mean follow-up of 9 years (survival range:1-29 years). In the group of 46 pt with normal values of Ca19.9 the mean serum CT and CEA values were 2,340 pg/ml and 235 U/ml, respectively: both this mean values were significantly lower than the values of pt with high Ca 19.9 ($p<0,0001$ for Ct; $p=0,0003$ for CEA). In this group, the died pt (n=10, 21%) were significantly less than the died pt in the group with high level of Ca 19.9 ($p=0,01$). Another statistically significant difference ($p=0,007$) between the group with high level of Ca 19.9 and the group with normal level of the marker was found regarding the gender: 7/8 pt with high Ca 19.9 level were female. Conversely, between the two group there was no difference regarding the presence of limph nodes metastasis and/or distance metastasis nor the age at the diagnosis.

Conclusions: 1) high levels of Ca 19.9 are related to higher Ct e CEA levels; 2) in MTC pt with high level of Ca 19.9 the mortality rate is higher; 3) among the female pt there is a higher rate of Ca 19.9 positivity; 4) the presence of lymph nodes metastasis and/or distance metastasis and/or the age at diagnosis are not related to Ca 19.9 positivity; 5) on the basis of these results, Ca19.9 appears to be a poor prognostic factor in pt with MTC. It is still unclear whether the measurement of this marker is useful in the early stage of MTC or only in advanced cases.

PP391

NEONATAL THYROID FUNCTION AND ANTHROPOMETRIC PARAMETERS OF NEWBORNS TO MOTHERS AFFECTED BY THYROID DISEASES.

E. Gianetti[1], L. Russo[1], M. Ciampi[1], F. Santini[1], D. Gazzarrini[2], S. Pierini[2], G. Gelato[2], T. Donato[2], A. Celandroni[2], A. Pinchera[1], P. Vitti[1], M. Tonacchera[1]

[1]UO Endocrinologia 1 - Università di Pisa - Pisa, [2]UO Pediatria - Ospedale F. Lotti - Pontedera

Background: Thyroid function is essential during pregnancy.

Aim: To verify the effects of maternal thyroid diseases and treatments on neonatal thyroid function and anthropometric parameters (AP).

Patients/Methods: 93 newborns to thyropathic mothers (78 autoimmune thyroid diseases [ATD] and 15 thyroid nodule(s) [TN]) were enrolled in the study. Gestational age, mode of delivery, Apgar index (at the 1st and 5th minute) and neonatal weight, length, cranial and thoracic circumferences at the time of birth were evaluated. Serum FT4, FT3, TSH, AbTg, AbTPO and TRAb at the 3rd day of life were also measured.

Results: When newborns to ATD women (n=78) were compared to newborns to TN women (n=15), no significant differences in neonatal thyroid function nor in AP were found. No significant differences were found in AP and neonatal thyroid function between newborns to LT4-treated (n=58) and non-treated ATD mothers (n=16). Since no differences were found in newborns data on the basis of the thyroid diseases nor on their treatments, patients were considered as a whole group for the following analysis. Significant correlations were found between gestational age and weight, length, cranial and thoracic circumferences of the newborns. Significant direct correlations between gestational age and serum FT4 and FT3 were found (p-value = 0,0126 and 0,0054 respectively).

Conclusions: The presence of ATD or TN and LT4 therapy do not interfere with neonatal thyroid function and AP. Serum FT4 and FT3 levels at the third day of life directly correlate with gestational age.

PP392

TOXIC ADENOMA MASKING FOLLICULAR VARIANT OF PAPILLARY THYROID CARCINOMA

M. Cappagli[1], A. Montepagani[1], P. Magistrelli[2], M. Moroni[3], N. Gorji[3]

[1]Endocrinologia Osp.S.Andrea - La Spezia, [2]Chirurgia Osp.S.Andrea - La Spezia, [3]Anatomia Patologica Osp.S.Andrea - La Spezia

Mr. A.P. 56 years old, in 2003 came to our observation with hyperthyroidism (Hy): T4 = 1.98 ng/dl (0.89-1.79); T3 = 0.61 ng/dl (0.16-0.38); TSH = 0.05 uUI/ml (0.20-4.0); AbTg AbPO negative; Trab = 8.5 U/l. (< 1.50). In anamnesis: TBC with moderate respiratory failure (RF). 99mTc Scan (SC) showed a large "hot nodule" in the left lobe, solid, 4 cm long at US, with functional inhibition of extranodular parenchyma. A Toxic Adenoma (TA) was diagnosed and Patient (Pt) began treatment with methimazole, 15 mg a day, then reduced to 5 mg, with fine functional and clinical outcome (T4 = 1.28 ng/dl; T3 = 0.38 ng/dl; TSH = 0.10 uUI/ml). Since Pt refused surgery, in 2004 we opted for radioiodine therapy, 131I : 13.6 mCi = 503 MBq (Guidelines: SIE-AIMN-AIFM). After about 14 months TSH increased from 4 uUI/ml to 8 uUI/ml) so we started therapy with thyroxin: 75 mcg a day. In 2008, owing to the development of severe RF, oxygen chronic therapy was required. In 2010 Pt accused dysphonia, without any evidence of larynx pathology, which receded after corticosteroids. In 2011 the dysphonia represented permanently, with evidence of left vocal cord paralysis. Pt was submitted to a new evaluation and the US confirmed the known left hypo-echoic nodule which was very irregular and 5.3 x 3.2 x 3.2 cm; SC showed regular morphology of both thyroid lobes and a "cold" area corresponding to the large nodule. We performed a first cytological specimen (CY) with a Thy1 feedback; a second CY, after extensive discussion of the pathologists, was judged Thy4. The Pt underwent surgery; intervention was proved more complex than expected for adhesions, contiguous tissue infiltration and intra-thoracic extension of the goiter, which forced the surgeon to a proximal sternotomy. The histological diagnosis was "papillary carcinoma (PC), follicular variant of 6.5 x 5 cm, with endovascular neoplastic, infiltrative emboli and extension to the adipose tissue and to a parathyroid gland". Comment: detection of thyroid carcinoma in TA is a very rare event, so the international guidelines don't suggest cytology specimen of hot nodules. Our Pt, at onset, was clearly affected from a TA, as demonstrated by the associated Hy and by the SC evolution of the nodule after 131I therapy, but in 7 years the diameter of the lesion significantly increased from 4 to 6.5 cm, assuming the characters of a carcinoma. The final diagnosis is therefore: follicular variant of thyroid papillary carcinoma in previous toxic adenoma.

PP393

CALCITONIN AND PTH LEVELS IN FINE-NEEDLE ASPIRATION WASHOUT OF PATIENTS WITH THYROID NODULES

A. M. Isidori[1], L. Rizza[1], R. Lauretta[1], C. Graziadio[1], A. Anzuini[1], D. Gianfrilli[1], C. Pozza[1], A. Radicioni[1], E. Giannetta[1], A. Lenzi[1]

[1]Dept of Experimental Medicine, Sapienza University of Rome - Rome

Objective: To establish the role of calcitonin (Ct) and PTH measurements, and their cut-off value, in the washout fluid from fine needle aspiration of thyroid nodules (FNA-Ct; FNA-PTH).

Methods: 145 patients with thyroid nodules referred to our Unit for FNA were consecutively enrolled. According to the cytological classification SIAPEC-IAP 2007, we obtained 52 indeterminate aspirates (Tir1), 55 benign lesions (Tir2), 14 suspicious lesions (Tir3) and 24 malignant lesions (Tir4 and Tir5). After setting the cytological slides with all the material present in the hub, the needle was washed with 1 ml of 0.9% NaCl solution and the solution centrifuged at 13,000 rpm for 10 min at 4°C to remove any eventual residual cells debris. The supernatants were stored at -80°C and subsequently assayed for Ct (RIA) and PTH (IRMA) using validated in-house assays.

Results: FNA-Ct washout yield to values between 4 and 30 pg/ml in all FNAs, except in two cases of medullary thyroid carcinoma, reading respectively 920 pg/ml and 716 pg/ml. FNA-PTH washout was undetectable in all the samples, except in six cases of primary hyperparathyroidism in which the readings were between 46 pg/ml and 1920 pg/ml.

Conclusions: Our study shows that in FNA-washout Ct values below 30 pg/ml exclude parafollicular malignancies, while detectable PTH values (greater than 1 pg/ml) are consistent with an intrathyroidal parathyroid gland. These cut-off values could be used in clinical practice as an additional tool for the work-out of non-diagnostic thyroid cytology.

PP394

THYROID VOLUME CHANGES IN PATIENTS UNDERGOING CONTROLLED OVARIAN HYPERSTIMULATION DURING ASSISTED REPRODUCTION TECHNOLOGY

F. Zerbini[1], F. Magri[1], V. Capelli[1], S. Curti[1], M. Gaiti[1], E. Brambilla[2], R. E. Nappi[2], L. Chiovato[1]

[1]Medicina Interna ed Endocrinologia, Fondazione Salvatore Maugeri - Pavia, [2]Centro per la procreazione Medicalmente Assistita, IRCCS Policlinico San Matteo - Pavia

Introduction: Thyroid volume is known to increase by 10-15% during pregnancy. After controlled ovarian hyperstimulation (COH), serum estrogen levels are similar to ones of a second-trimester pregnant woman, and thyroid function modifies as in a physiologic pregnancy. To date, no data exist about thyroid morphology variations during COH.

Aim: To assess thyroid volume and thyroid nodules changes during COH in healthy women and in women affected by chronic autoimmune Hashimoto thyroiditis (TH)

Subjects and methods: Thirty-five women refering for the first time to our assisted reproduction technology Center in 2012, 12 of which affected (TH+) and 23 not affected (TH-) by TH were enrolled. Before the procedure, serum values of FT3, FT4 e TSH, anti-TG antibodies, anti-TPO antibodies, TRAb, E2, FSH, LH, PRL, AMH and progesterone were assessed in all patients. Thyroid ultrasonography was performed at basal time and on the day of embryo transfer. FT3, FT4 e TSH, Ab anti-TG, Ab anti-TPO, TRAb, E2, FSH, LH, PRL, AMH and progesterone were measured with immunochemiluminescence and commercial kits.

Results: Clinical and demographic features, thyroid volume, TSH, FSH, LH, E2, PRL and AMH serum values were similar in both TH+ and TH- subgroups. This data were confirmed also when patients were divided according to their thyroid echoic pattern. The prevalence of subcentimetric nodules was 33% in the whole group and similar in the two subgroups. TH+ women showed a mean nodular volume significantly higher than TH- women ($p=0.025$). After COH, thyroid volume in TH- women increased by 7.11%, whether in TH+ women it reduced by 3.87% (n.s.). This difference becomes significant when comparing women with normoechoic pattern, showing a 6.64% increase, and the ones with a markedly hypoechoic pattern, showing a 10.9% reduction ($p=0.04$). Thyroid nodules did not vary in any subgruoup.

Conclusions: Our data suggest that in healthy women undergoing COH thyroid gland undergoes functional adjustments involving early parenchymal enlargement. This volumetric increase is not reported in TH+ women, probably due to the inflammatory process. COH does not influence, at least in this early phase, thyroid nodules volume or ultrasonographic features.

PP395

CLINICAL OUTCOME OF PATIENTS AFFECTED BY DIFFERENTIATED THYROID CARCINOMA IN PRESENCE OF ANTITHYROGLOBULIN ANTIBODIES

G. A. Lupoli[1], A. Panico[1], F. Fonderico[1], A. Marinelli[1], M. R. Poggiano[1], L. Barba[1], L. Coviello[1], F. Papa[1], G. Neri[1], I. Vetrani[1], G. Lupoli[1]

[1]Dipartimento di Medicina Clinica e Chirurgia, Università degli Studi Federico II - Napoli

INTRODUCTION: About 15-20% of patients affected by differentiated thyroid carcinoma (DTC) presents circulating thyroglobulin autoantibodies (TgAbs). This condition may interfere with Tg assessment, also depending on the detection method used. The evaluation of post-thyroidectomy TgAbs/Tg profiles represents a decisive and useful tool for early recurrence detection.

AIM: To study the relationship between TgAbs and clinical outcome of patients treated for DTC.

METHODS: 120 DTC patients, 60 with serum TgAbs positivity (Group A) and 60 without (Group B) were retrospectively enrolled in this study. All pts underwent total thyroidectomy and complementary radioiodine therapy. Clinical, laboratory (Tg baseline and/or after stimulation, FT3, FT4, TSH) and instrumental (ultrasound of the neck, and, if needed, total-body scintigraphy, CT, PET/CT) data were evaluated over time. TgAbs and Tg serum levels were assessed by a chemioluminescent immunoassay.

RESULTS: 4 pts of Group A and 7 of Group B showed high Tg levels and underwent further surgical/radioiodine therapy with subsequent normalization of Tg levels (2 Group A and 6 Group B pts). 7 Group A pts with Tg levels <2.0 ng/ml showed metastasis at imaging assessment (laterocervical lymphnodes [4 pts], lung [2 pts] and bone [1pt]) while no relapse was found in Group B pts with <2.0 ng/ml Tg levels.

CONCLUSIONS: The finding of a high frequency of metastasis in Group A suggests that DTC seems to have a more aggressive course in presence of TgAbs, probably also because of difficulties in Tg assessment when circulating autoantibodies are present. Our data highlight the importance of a tighter follow-up in these cases, including an accurate clinical examination, a more frequent evaluation of Tg and TgAbs (using different kits) and the use of imaging techniques, in order to obtain an early detection of possible recurrence or thyroidal metastases.

PP396

A RARE CASE OF ECTOPIC SUBMANDIBULAR THYROID

R. M. Paragliola[1], R. M. Lovicu[1], A. Pontecorvi[1], S. M. Corsello[1]

[1]Endocrinologia, Università Cattolica - Roma

Ectopic thyroid tissue is a rare abnormality (1 of 100.000-300.000 people) caused by aberrant thyroid gland embryogenesis during its passage from the floor of the primitive foregut to its final position. In most cases ectopic thyroid tissue is the only thyroid tissue present. The most frequent place of ectopic thyroid tissue is the base of tongue, accounting for about 90% of the reported cases while lateral thyroid gland is a very rare finding. A 61 years old woman came to our attention for asthenia and dizziness. She complained defluvium and psoriasis and she had hypertension and osteoporosis treated respectively with ACE-inhibitors and bisphosphonates. Until then she had never performed thyroid function tests or thyroid ultrasound. Family history was negative for endocrine disease while biochemical evaluation revealed subclinical hypothyroidism (TSH 8.5 µU/ml, FT4 9.7 pg/ml) without other pathological findings. Anti thyroglobulin and anti thyroperoxidase autoantibodes were negative, while thyroid ultrasound showed athyreosis in anterior neck. Therefore, thyroid scintigraphy was performed showing a focal uptake near the left submandibular gland. Ultrasound focused study confirmed, next to left submandibular gland caudal margin, an ectopic thyroid tissue of about 3 cm. The ectopic thyroid had normal echo pattern except a hypoechoic micronodule of about 1 cm. Fine needle aspiration biopsy was performed showing benign findings (Thy 2). Thus, patient started levothyroxine therapy with periodic biochemical and clinical assessment.

This case represents a rare condition of thyroid dysgenesis. Ectopic thyroid tissue has been described in several sites, as foramen caecum, base of the tongue, mediastinum or distant subdiaphragmatic areas. A lateral ectopic thyroid gland, in particular in submandibular region, could occur very rarely when the cells of the lateral anlage do not join those of the median. Patients usually present with a lateral, palpable, mobile, painless mass in the carotid triangle or the submandibular area. In our case, patient was asymptomatic and diagnosis was performed only on the basis of subclinical hypothyroidism.

PP397

A VERY UNUSUAL CASE OF SUBACUTE THYROIDITIS

R. M. Paragliola[1], M. P. Ricciato[1], V. Di Donna[1], L. Castellino[1], P. Locantore[1], A. Pontecorvi[1], S. M. Corsello[1]

[1]Endocrinologia, Università Cattolica - Roma

A 73 years old man came to our observation for severe dysphagia and loss of weight (10 kg in one month). About 30 years before he had myocardial infarction and he underwent coronary artery bypass graft. One week before the first medical evaluation, patient suspended all drugs per os because he could not swallow pills and food. Thyroid function test revealed a severe hyperthyroidism (FT3 11.9 pg/ml; FT4 40 pg/ml; TSH <0.01 mcU/ml). Anti TSH receptor and anti TPO autoantibodies were negative while thyroid ultrasound showed an increased gland with inhomogeneous pattern, without nodules or abnormal vascularization. He did not take amiodarone. He started methimazole 30 mg/die, without any benefit. Patient then came to our evaluation about one month after the onset of symptoms: clinical examination showed tachycardia, enlarged and tender thyroid gland at neck palpation without relevant pain. Biochemical evaluation showed increased VES and C-reactive protein. Thyroid scintigraphy was not performed because of the interference caused by iodinate contrast medium (coronary angiography performed few days before). Neverthless subacute thyroiditis appeared strongly probable. Therefore methimazole was stopped and steroid therapy was started with i.v. methylprednisolone 40 mg for one week, 20 mg for one week and then prednisone 25 mg/day, which was tapered and continued for 30 days. Clinical symptoms, and in particular dysphagia, improved after few days of i.v. methylprednisolone while biochemical evaluation performed after two months showed a normalization of thyroid function test and inflammatory parameters. In conclusion, we described an unusual case of subacute thyroidits in which only dysphagia and thyrotoxicosis, without anterior neck pain, suggested an inflammatory condition. Diagnosis was made on the basis of clinical and laboratory features, because thyroid scintigraphy with RAIU, which is crucial for differential diagnosis in uncertain condition, was not possible to be performed for iodine overload.

PP398

RESULTS OF THE APPLICATION OF A NEW TECHNIQUE FOR THE CYTOREDUCTIVE TREATMENT OF THYROID NODULES USING RADIOFREQUENCY THERMAL ABLATION

M. Deandrea[1], A. Mormile[1], F. Ragazzoni[1], F. Garino[1], E. Gamarra[1], G. Magliona[1], J. Ramunni[1], L. Germano[1], P. Limone[1]

[1]Azienda Ospedaliera Ordine Mauriziano di Torino - Torino

Ultrasound-guided radiofrequency thermal ablation (RFA) has proven to be a safe and effective technique for the cytoreductive treatment of thyroid nodules that cause compressive symptoms or aesthetic discomfort. Our previous experience using expandable needles (14G) on large nodules (mean volume 25 ml) had shown a mean volume reduction of 44.5% six months after a single treatment. Recently Korean authors have reported results with a new, smaller needle (18G), to be used with the "moving shot" technique. Aim of this study was to verify the efficacy of this new RFA needle and to evaluate the learning curve of two experienced operators

We analized 73 consecutive patient (60F and 13M), all with large thyroid nodules (mean volume 37.9 ml, range 7-310 ml, median volume 21.9), over a period of 12 months, with random assignment of patients to each operator. Baseline volume was compared with the volume 6 months after treatmen. Mean volume reduction vs baseline was 51.6 ± 17.1% ($p<0.001$), with a mean volume reduction of 46.5% for the first 10 nodules treated, improving in time and reaching 57.6% for the last 10 patients (though p value ns). Mean volume reduction differed on the basis of baseline volume, respectively 60% for nodules with baseline volume < 10 ml, 58% for nodules with volume 11-20 ml, 50% for nodules with volume 21-30 ml and 45% for nodules with volume > 31 ml. Taking into consideration the nodules for which a volume reduction > 60% was obtained, the mean energy delivered normalised for nodule volume was 26.5 W/ml, while for those showing a volume reduction < 45% the mean energy delivered was 20 W/ml ($p < 0.005$).

To conclude, RFA has been confirmed as effective in the volumetric reduction of thyroid nodules, even using the "moving shot" technique. The learning curve for experienced operators was very rapid, and it appears to improve as experience with this technique increases. These preliminary results reveal that the degree of response varies depending on the volumethe of the nodule, but also that there might be an optimal ratio between nodule volume and energy delivered. Further studies are necessary to confirm this aspect, which might have crucial repercussions in the optimisation of this technique

PP399

COMPARISON BETWEEN SPORADIC AND FAMILIAL PAPILLARY THYROID MICROCARCINOMA

M. Capezzone[1], G. Busonero[1], R. Forleo[1], C. Durante[2], S. Filetti[2], K. Pazaitou-Panayiotou[3], F. Pacini[1]

[1]Section of Endocrinology and Metabolism, Department of Internal Medicine, Endocrinology and Metabolism and Biochemistry, University of Siena - Siena, Italy, [2]Department of Clinical Sciences University of Rome "Sapienza," - Rome, Italy, [3]Department of Endocrinology-Endocrine Oncology, Theaghenion Cancer Hospital - Thessaloniki, Greece

Introduction: Papillary thyroid microcarcinomas (PTM) generally have an excellent prognosis but cervical lymph adenopathy is discovered in 25 to 43 % of cases, the prevalence of multifocality appears to be present in 30-40% of cases and distant metastases may be occur, although infrequently, at a rate of 1.0-2.8%. However, the characteristics and prognostic outcomes for familial PTM are not well established.

Aim: To perform a retrospective study on a series of patients with familial and sporadic PTM, to address the question if familial PTM have a different clinical presentation and outcome compared to sporadic PTM and if it requires a different therapeutic approach.

Patients: The study was conducted using the clinical records of 271 patients affected by PTM with a mean follow-up of 77.3 months (range 12-289 months). There were 217 females and 54 males, ranging 14-84 years. Initial treatment consisted in near total thyroidectomy for the large majority of them (98.9%), followed by 131-I remnant ablation in 74% of the cases. The familial form of PTM was defined as the presence of the tumour in two or more first-degree relatives. We analyzed the clinical and pathological features of familial and sporadic PTM patients.

Results: Familial PTM is more multicentric (p=0.02) than sporadic PTM. No difference was found regarding gender, age at diagnosis, incidental or not incidental discovery, tumor diameter, distant metastases, lymph node metastases, I-131 remnant ablation, length of follow-up, recurrence and final status at the end of follow up. At the end of follow up, 121/124 (97.6%) patients with familial PTM and 140/147 (95.2 %) with sporadic PTM were disease free instead 3/124 (2.4%) familial PTM and 7/147 sporadic PTM (4.8%), shown persistence disease.

Conclusions: Our study doesn't show statistically significant differences in the biological and clinical behavior between familial and sporadic PTM. Familial PTM displays a more frequent multicentricity than sporadic cases.

PP400

LONG TERM EFFECT OF PARENTERAL STEROIDS PULSE THERAPY ON HYPERTHYROIDISM RELAPSES IN PATIENTS WITH GRAVES' AND ORBITOPATHY

R. Le Moli[1], V. Muscia, M. T. Furneri, C. Regalbuto, S. Squatrito

[1]BIOMEDICINA CLINICA E MOLECOLARE - CATANIA

Background: thionamides (TH) are first choise treatment of hyperthyroid Graves' disease (GD). Relapses of hyperthyroidism (H) are frequently observed after TH have been withdrawn. A variegate grading of thyroid related orbitopathy (GO) is diagnosed in about 50% of GD patients. **Aim**: to test effect of parenteral steroids (metilprednisolone pulse therapy (MPDS) that is usually employed as immunosuppressant therapy in patients with GO) on natural course of GD. **Methods**: n 207 patients with GD and moderate or moderate-severe GO, evaluated according to the European Group on GO (EUGOGO) recommendations, received in our institution (from 2002 to 2009) MPDS (n 133) or retrobulbar irradiation (RI) (n 74). We selected 33 patients that received TH for almost 18 months, median of TH treatment was 25 months (range 18-34), n 23 (G1) received MPDS (cumulative dose was 4500 grams in 12 weeks) and n 10 (G2) RI (cumulative dose was 20 Gy for boths eyes) respectively within 12 months from H onset, median was 7 months (range 1-15). The median follow–up after discontinuation of TH was 17 months (range 3-50) (G1) and 21 months (range 1-66) (G2) respectively. **Results**: age, smoking habit, thyroid function, autoimmunity markers, thyroid volume, time of H onset and GO severity and activity were not different between the groups. All patients of G1 were female, 16 patients of G2 were females and 7 were males. The median of relapses free interval of H was 7.5 months (range 3-15) (G1) and 7 months (range 1-18) (G2), p= ns. The relapses rate of H was 13% (G1) and 20% (G2) after 12 month, 17.4% (G1) and 30% (G2) after 24 months (p=0.2, G1 vs G2, Log Rank Test). H relapses were detected at 3, 15 months (G1), at 1, 7,18 months (G2) and were inside 18 months after TH were withdrawn. Smoking abit and FT3/range ratio ≥ 3 were determinants of H relapses (Odds ratio = 5.6 C.I. 95% (1-35.4) and 2.5 C.I. 95% (1.1-5.3), smoking and FT3/range ratio respectively). **Conclusions**: MPDS pulse therapy do not prevent GD relapses, remission rate of H was considerable in both groups. Smoke and elevated FT3 plasmatic levels at presentation are confirmed determinants of GD relapses.

PP401

GRAVES' OPHTHALMOPATHY AND TYPE 2 DIABETES

R. LE MOLI[1], V. MUSCIA, C. CASTORO, C. REGALBUTO[2], S. SQUATRITO, R. VIGNERI

[1]BIOMEDICINA CLINICA E MOLECOLARE - CATANIA, [2]BIOMEDICNA CLINICA E MOLECOLARE - CATANIA

Aim: Graves' ophthalmopathy (GO) is an autoimmune disease. Oxidative reactive species (ROSs) are increased in patients with Graves' disease (GD) and GO in respect to patients with only GD. Hyperglycemia and ROSs are linked and ROSs are one of the major causes of chronic diabetic complications. We studied the clinical features of GO in DT2 patients. Subjects and methods: 90 consecutive GO patients, 30 with DT2 (group 1, G-1) and 60 without (G-2) were mached according to age, sex and smoking abit, at ratio one G-1 vs two G-2. In all patients we evaluated: the clinical activity score (CAS), soft tissue (ST), eyelid aperture (E), proptosis (P), diplopia and oftalmological examination. Total extra ocular muscle (EOM) surface and total orbit area (TOA) were measured using three different contiguous CT slices A, B and C chosen at the globe pole tangent (A) and 2 and 4 mm backward and the respective EOM/TOA ratio was calculated. GO was defined severe in case of optic nerve damage (DON). Results:thyroid function, autoimmunity markers, CAS, duration of hyperthyroidism and eyes simptoms were not different between G-1 and G-2 patients. Data were as follows:

	n	DON	MGO	Mild GO	Asimmetrical GO	Before Thyro Toxic GO	Motility Impair.	Diplopia	EOM/TOA ratio
G-1	30	36.6	36.7	26.7	30	15.4	53.3	50.0	0.280±0.5
G-2	60	1.7*	40.0	58.3*	5*	0*	10.0*	17.0*	0.205±0.5*

*(data are depicted as percentage, media and SD, n=number, *p≤0.05)* DT2 and smoking were determinants of severe GO (OR:14, 95% CI 4.1-28; 5, 95% CI 1.7-18),DT2was determinant of diplopia, GO asimmetry and GO onset before iperthyroidism OR:1.8(95% CI=1.1-2.9); 6.4(1.9-22); 9(9-27). Conclusions: DT2 is an important determinant of GO severity, EOM/TAO ratio is significantly increased in DT2.

PP402

THYROID DYSFUNCTION IN PREGNANT HOSPITALIZED PATIENTS FOR MATERNAL AND FETAL COMPLICATIONS

D. Arpaia[1], C. Peirce[1], S. Ippolito[1], F. Improta[2], M. V. Locci[2], B. Biondi[1]

[1]Department of Molecular and Clinical Endocrinology and Oncology - Naples, [2]Department of Obstetrics and Gynecology, University "Federico II" - Naples

Thyroid dysfunctions are particularly frequent during pregnancy; they can produce maternal and fetal complications when untreated. Subclinical and overt hypothyroidism are common disorders during pregnancy. The aim of our study was to evaluate thyroid function in a group of pregnant hospitalized patients with maternal and fetal complications. The assessment of thyroid function was performed in 115 patients admitted to the Department of Gynecology and Obstetrics of the University of Naples from January 2010 to May 2012.

At the time of the recruitment, we assessed the history of previous thyroid dysfunction , the week of pregnancy and how it was obtained, previous miscarriage and the treatment of thyroid dysfunction. We evaluated the risk of obstetric and fetal diseases through standard US monitoring and cardiotocography. In all of the patients thyroid function was evaluated by means of TSH, FT4, FT3, AbTg, AbTPO assays and a thyroid US scan. The diagnosis of overt or subclinical hypothyroidism during pregnancy was performed, according to the recent ATA and Endocrine Society guidelines.

Hypothyroidism was found in 40% of hospitalized patients; 69% of them had Hashimoto thyroiditis and 31% reported a previous total thyroidectomy. 98% of patients had subclinical hypothyroidism and the remaining 2% presented the overt form. 50% of hypothyroid patients had been receiving replacement therapy with L-tiroxine; however, euthyroidism was not observed in 47% of cases. Maternal and fetal complications were found in 59% of hypothyroid patients. Oligohydramnios was observed in 19% of cases, polihydramnios in 4%, pre-eclampsia in 26%, miscarriage in 11% and premature rupture of membranes in 22%. 7% of hypothyroid hospitalized patients reported a history of recurrent miscarriage. Intrauterine growth retardation was found in 4% of the newborns. Our data highlight the high risk of maternal and fetal complications in hospitalized hypothyroid women during pregnancy and the high rate of inadequate treatment with L-thyroxine during pregnancy. These results could suggest the necessity of a screening program for thyroid function in all hospitalized pregnant women to avoid maternal and fetal complications.