86° CONGRESSO NAZIONALE DELLA SOCIETA'ITALIANA DI BIOLOGIA SPERIMENTALE

PALERMO 24-25 OTTOBRE 2013
ORTO BOTANICO-SALA LANZA

ATTI DEL CONVEGNO

La Società Italiana di Biologia Sperimentale (SIBS) costituita a Napoli nel 1925, è una tra le più antiche Società Scientifiche Italiane.

Il Congresso della Società Italiana di Biologia Sperimentale è l'appuntamento annuale di Biologi, Fisiologi, Biochimici, Farmacologi, Medici, Antropologi e di tutte quelle branche multidisciplinari della sperimentazione scientifica della ricerca di base.
I contributi presentati al congresso, dopo aver ricevuto il parere positivo di almeno due revisori esterni, saranno pubblicati per esteso sulla rivista Journal of Biological Research.

L'importanza scientifica dell'evento è dettata certamente dalla presenza di numerosi studiosi che affronteranno le tematiche di ricerca più moderne nei campi della Biologia Sperimentale, Fisiologia, Alimentazione, Biochimica, Biomedicina, Scienze Motorie e di altre discipline affini.

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Programma

Giovedi, 24 Ottobre

8.30-9.30 Registrazione

9.30-10.20 Saluto delle Autorità e cerimonia inaugurale del Congresso

10.20-10.50 Lettura Gaetano Quagliariello

Fernando Goglia – (Sannio-Benevento)

Come la tiroide modula il metabolismo

10.50-11.20 *Coffee break*

Biologia Sperimentale

Moderatori: Giuseppe Crescimanno - Rosa Serio

11.20-11.40 **Proto Pippia** (Sassari)

Recenti progressi nella biologia dei linfociti T umani nello spazio

11.40-12.00 **Flavia Mulè** (Palermo)

GLP-2: peptide intestinale coinvolto nella omeostasi nutrizionale

12.00-12.20 **Herbert Marini** (Messina)

Genisteina e sindrome metabolica nella donna in post-menopausa

12.20-12.40 **Andreina Bruno** (Palermo)

La leptina: molecola pleiotropica nell'apparato respiratorio

12.40-13.00 **Luca Bruni** (Parma)

K:D-Rib on biology of human cancer and not cancer cell line

13.00-14.00 *Lunch*

14.00-15.00 **Sessione Poster**

Ricerca Applicata alle Attività Motorie e Sportive

Moderatori: Antonio Palma – Marcello Traina

15.00-15.20 **Vincenzo Perciavalle** (Catania)
Incidenza della postura nel tiro al volo

15.20-15.40 **Marianna Bellafiore** (Palermo)
Relazione tra l'intensità dell'esercizio fisico, la velocità di ossidazione dei lipidi e lo stato redox nelle allenatrici di ginnastica ritmica

15.40-16.00 **Angelo Cataldo** (Palermo)
I metodi di allenamento ad alta intensità nei soggetti diabetici

16.00-16.20 **Giuseppe Battaglia** (Palermo)
Il ruolo dell'esercizio fisico nel controllo del peso corporeo in soggetti psicotici

16.20-16.50 *Coffee break*

Biologia Sperimentale

Moderatori: Piero Almasio - Maria Grazia Bridelli

16.50-17.10 **Stefania Grimaudo** (Palermo)
Determinanti genetici di fibrosi nei disordini metabolici del fegato

17.10-17.30 **Anna Licata** (Palermo)
I meccanismi molecolari del danno epatico da farmaci

17.30-17.50 **Garden Tabacchi** (Palermo)
Gli strumenti per una corretta sorveglianza nutrizionale degli adolescenti: il Progetto ASSO e la creazione di un sistema web-based

17.50-18.50 **Assemblea dei Soci**

20.30 **Cena Sociale**

Venerdì, 25 Ottobre

Alimentazione e Nutrizione

Moderatori: Massimo Cocchi (Bologna) **- Maria Antonia Livrea** (Palermo)

9.30-10.00 Lettura Filippo Bottazzi

Giorgio Calabrese – (Roma)

La Dieta Mediterranea

10.00-10.20 **Vittorio Calabrese** (Catania)

Invecchiamento, longevità e vitageni redox regolati: ruolo dell'Ormesi

10.20-10.40 **Marcella Renis** (Catania)

Prevenzione primaria, Nutrizione , Stress ossidativo e percorsi individualizzati

10.40-11.10 *Coffee break*

11.10-11.30 **Luisa Tesoriere** (Palermo)

Fitochimici come modulatori dell'espressione genica controllata da variazioni epigenetiche del DNA: il caso Indicaxantina

11.30-11.50 **Giuseppe Venturella** (Palermo)

Il Progetto MYCOT.I.CO.N e l'uso sostenibile dei funghi eduli spontanei nelle aree rurali

11.50-12.10 **Diego Planeta** (Palermo)

Le potenzialità qualitative dell'olio di oliva siciliano

12.10-12.30 **Luciano Lozio** (Burago di Molgora)

Integratori nella sindrome metabolica

13.00-14.00 *Lunch*

14.00-15.00**Sessione Poster**

Biologia Sperimentale

Moderatori: - Caterina Faggio- Agostino Palmeri

15.00-15.20 **Giuseppina La Spada** (Messina)

Fisiologia del nematocita

15.20-15.40 **Antonella Fazio** (Messina)

La risposta della fase acuta nel merluzzo (Gadus morhua L.): Espressione genica della risposta immunitaria dopo infezione con Aeromonas salmonicida subsp.achromogenes.

15.40-16.00 **Daniela Puzzo** (Catania)

Ruolo fisiologico del peptide beta-amiloide e sua implicazione nella malattia di Alzheimer

16.00-16.20 **Rossana Morabito** (Messina)

Effetto dell´estratto crudo da nematocisti di Pelagia noctiluca su vitalità e regolazione del volume cellulare

16.20-16.40 **Simona Armeli Minicante** (Messina)

Le alghe del Mediterraneo quali potenziali farmaci contro la leishmaniosi

16.40-17.00 **Fulvio Santacatterina** (Madrid)

Reverse phase protein microarray (RPPmA) per l'identificazione di marker metabolici nelle malattie neuromuscolari

17.00 **Saluto del Presidente e chiusura dei lavori**

COMUNICAZIONI ORALI

CO1 - HOW THE THYROID MODULATE METABOLISM

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It is worldwide recognized that thyroid hormones (TH) are unique in their capacity to stimulate energy metabolism/thermogenesis (the well-known calorigenic effect of TH.). Basically, their main action consists in both stimulating cellular respiration and reducing metabolic efficiency. But, although this effect has been known since the end of the 19th century the mechanism by which TH exert their effects on energy metabolism is far from being firmly established. Several hypotheses were put forward to try to explain the cellular-molecular mechanisms underlying this effect. Some decades ago (early 1950s-middle 1960s) the most intriguing hypothesis put forward was the uncoupling hypothesis. This suggested that TH stimulate metabolic rate by acting at the mitochondrial level to uncouple the electron transport chain from ATP synthesis. In the early 1960s its validity was questioned on several grounds, amongst others that uncoupling was observed only with pharmacological doses of TH. Since some effects were also observed in vitro (in isolated mitochondria), the theory also implied that TH acted directly at the mitochondrial level. This hypothesis has never been dropped and continues to this day to be investigated in the light of new discoveries (i.e. the presence of uncoupling proteins in the mitochondria of tissues other than brown adipose tissue-BAT).

Moreover, at the beginning of 1960s results obtained by Tata J.R. and coworkers opened a new chapter in the history of our understanding of the action of TH. Those authors showed that administration of T3 to hypothyroid rats induced an increase in basal metabolic rate and, in addition, that injecting T3 in combination with actinomycin D (a well-known inhibitor of transcription) completely abolished the stimulatory effect of T3. These results clearly pointed towards the involvement of transcription in the action of T3 at the cellular level. Subsequently, specific nuclear binding sites for T3 were first described by J. Oppenheimer's group in rat liver and kidney and later in a variety of other tissues and cell cultures. What is now evident from the literature is that a large number of effects attributable to T3 are indubitably initiated via thyroid hormone receptors located within the nucleus, the so-called "genomic" or "nuclear-mediated" effects of TH. T3 receptors are transcription factors: they modulate transcription mainly by binding TREs (thyroid-hormone response elements). In the absence of T3, the TR has an intrinsic transcriptional repressor function. In most cases, the TRs act as heterodimers with a 9-cis retinoic acid receptor (RXR), but there are also multiple TR complexes that bind to TREs. In addition to RXR, many other molecules are directly or indirectly functionally associated with TRs (vitamin D3, peroxisome proliferator-activated receptor (PPAR), co-repressors, co-activators, etc.). The transcriptional activity of TRs is regulated at multiple levels: by T3 itself; by the type of TRE located on the promoters of T3 target genes; by the developmental- and tissue-dependent expressions of TR isoforms; and by a host of nuclear coregulatory factors (coactivators and corepressors) with T3-dependent activity. Because of THs metabolic effects, a most studied target of TH at cellular level is the mitochondrion. Mitochondrial shape and their positioning within cells is crucial and is tightly regulated by processes of fission and fusion, biogenesis and autophagy, thus ensuring a relatively stable mitochondrial population. Recent evidences show that TH affect these processes. Because of the presence of the above-mentioned nuclear receptors and because these receptors have their greatest affinity for T3, the belief developed that T3 is the only active iodothyronine.

However, about two decades ago it became evident that some TH effects are undoubtedly non-genomic in origin and that iodothyronines other tha T3 may have biological effects, such as: (T4), reverse T3 (rT3), and 3,5-diiodothyronine (T2). Indeed, surprising results were published showing that (among a large number of iodothyronines tested) T2, like T3, was able at pM concentration to induce a rapid stimulation of oxygen consumption in perfused livers isolated from hypothyroid rats. In the same study, it was shown that the effect of T3 was largely

inhibited by the addition of an inhibitor of D1 deiodinase, whereas the effect of T2 was not. Moreover, in further studies it was shown that, after a single injection, T2, like T3, rapidly stimulated both mitochondrial activity and resting metabolic rate. These findings prompted researchers to try to demonstrate possible effects of T2 on fat accumulation, obesity and the serum lipid profile. Within the last few years, it has been shown that T2, when administered to rats simultaneously receiving a high-fat diet (HFD), can prevent excessive body-weight gain and the development of liver steatosis, while at the same time induces a decrease of visceral fat and significant reductions in the serum triglyceride and cholesterol levels without inducing a thyrotoxic state. Similar results have also been obtained in human. Other studies from different authors have recently shown that T2 is able to ameliorate diabetic nephropathy in streptozotocin-induced diabetic rats . Very recently it has been demonstrated that T2 is able to specifically bind the long form of the receptor β1 and that this binding induce the growth in fishes. However, whether or not the function of T2 is physiological remains to be elucidated. To a deeper information on the aspects described in this manuscript the readers can refer to the following articles.

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CO2 - RECENT ADVANCES IN HUMAN T LYMPOCYTE BIOLOGY IN SPACE

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Immunosuppression during spaceflight is a major barrier to safe long-term human space habitation and travel. Remarkable findings in space have shown that gravity changes affect important cellular mechanisms like proliferation, differentiation, genetic expression, cytoskeletal architecture and motility in lymphocytes, monocytes and other mammalian cells. In particular, several experiments performed in space demonstrated that human T lymphocytes have remarkably reduced mitogenic activation (80-90%), thus implicating gravity as a necessary factor in normal immune function (1, 2). Subsequent space studies using sounding rockets, shuttles and International Space Station (ISS) demonstrated that T cell activation requires tight contacts between each other as well between T cell and monocytes as antigen-presenting cells. We were able to see that cells display autonomous movements and interactions in space (3). Moreover, we investigated the structure of the cytoskeleton and in particular of tubulin and intermediate filaments of vimentin in Jurkat cells by immunofluorescence technique on the sounding rocket MAXUS 1B. We observed, already 30 min after exposure to microgravity, a significant higher formation of large bundles of filaments, showing that the cytoskeleton undergoes important and immediate changes in microgravity (4). Again important differences between the actin pattern between 1xg and 0xg in J-111 cells were observed in an experiment on board ISS (5). Such experiments were accompanied by extensive investigations performed in the ground laboratory by the three-dimensional clinostat, called Random Positioning Machine (RPM). This machine has proven, in the last 15 years, to be a useful tool to simulate low g in the ground laboratory and to prepare space investigations. Next experiments conducted in space and in RPM indicate that there are direct gravitational effects on the genetic expression of IL-2 and its receptor in human T lumphocytes. In our investigation on the IL-2R, we focused our attention on the alfa and beta-chains only, because the gamma-chain is not constitutively expressed. Surprisingly, the expression of the alfa-chain was significantly inhibited whereas the expression of the beta-chain was not influenced by microgravity (6).

Moreover, experiments in RPM using gene arrays and quantitative RT-PCR demonstrated that induction of 91 genes was altered in simulated microgravity conditions. Promoter region analysis found that the majority of genes downregulated in microgravity were controlled by transcription factors NFkB, CREB, ELK, AP-1 and STAT. The fact that phosphorylation of the linker of activation in T cells (LAT) is not down-regulated in simulated microgravity indicating that cholesterol-rich lipid rafts are not involved in the down-regulation of the transcription factors (7).

Our LEUKIN spaceflight experiment on board the ISS allowed the evaluation of the global gene expression pattern of human T cells after 1.5 hours of stimulation by ConA and anti-CD28 in order to identify the immediate early genes whose transcription may be inhibited in microgravity conditions. Importantly, an onboard centrifuge was used to generate a 1xg simultaneous control to isolate the effects of microgravity from other variables of spaceflight. Microarray expression analysis after 1.5 hours of activation demonstrated that 0xg and 1xg-activated T cells had distinct patterns of global gene expression and identified 47 genes that were significantly differentially down-regulated by at least 2 fold in microgravity. Expression of many genes involved in mitogenesis, cytokine production, apoptosis, and signal transduction and several key immediate early genes were inhibited in microgravity. In particular, transactivation of Rel/NFkB, CREB, and SRF gene targets were down-regulated. Expression of cREL gene targets were significantly inhibited and transcription of cREL itself was significantly reduced in microgravity. Analysis of gene connectivity indicated that the tumor necrosis factor (TNF)

pathway is likely a major early downstream effector pathway inhibited in microgravity and may lead to ineffective pro-inflammatory host defenses against infectious pathogens during spaceflight (8).

Recently, we studied the influence of altered gravity on expression and function of cytoskeletal proteins, chemokines, cytokines and their rceptors by the experiment STIM (Signal Transduction In Microgravity) on board the sounding rocket Maser 12. The launch took place the 13th of feruary 2012 at Esrange Space Center and the microgravity lasted 390 sec. During the flight, one automed plunger activation mechanism initiated the confluente between the activators (Concanavalin A, anti-CD28, anti-CD3) and the cells (human T lymphocytes), while a second plunger initiated that between fixative (formalin) and activated cells in a subsequent phase. The hypergravity phase during the launch resulted in a down regulation of the IL-2 and CD3 receptor and reduction of tyrosine phosphorylation, p44/42-MAPK phosphorylation and histone H3 acetylation, whereas LAT phosphorylation was increased. Compared to the baseline situation at the point of entry into the microgravity phase, CD3 and IL-2 receptor expression at the surface of non-activated T cells were reduced after 6 min. of microgravity. Importantly, p44/42-MAPK-phosphorylation was also reduced in low gravity. In activated T cells, the reduced CD3 and IL-2 receptor expression recovered significantly during in-flight 1xg conditions, but not during microgravity conditions. Beta-tubulin increased significantly after onset of microgravity until the end of the microgravity phase, but not in the in-flight 1xg condition. The results of STIM experiment suggest that key proteins of T cell signal modules are not severely altered in microgravity conditions. Instead, it can be supposed that the strong T cell inhibiting signal occurs downstream from membrane proximal signaling, such as at the transcriptional level. However, this study could identify signal molecules, which are sensitive to altered gravity, and indicates that gravity is obviously not only a requirement for transcriptional processes as described before, but also for specific phosphorylation/dephosphorylation of signal molecules and surface receptor dynamics (9).

Future researches in space and in simulated microgravity conditions should focus on delineating the specific mechanisms of how microgravity causes dysregulation of these signal transduction pathways in order to further clarify the molecular basis of spaceflight immunosuppression. Moreover, these findings suggest that the alterations of single cell behaviour observed in the absence of gravity may be exploited for biotechnological and biomedical applications.

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CO3 - GLUCAGON-LIKE PEPTIDE 2: INTESTINAL PEPTIDE INVOLVED IN NUTRITIONAL HOMEOSTASIS.

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Many peptides involved in the eating behaviour are found in the brain, in the enteric nervous system and in the enteroendocrine cells of the gastrointestinal (GI) tract. The gut senses the presence of nutrients and signals it to the brain, *via* neural and endocrine pathways, to regulate short-term appetite and satiety. After food intake, GI tract releases anorexigenic peptides to mediate satiety; on the contrary, during fasting it releases appetite stimulating (orexigenic) factors. The mechanisms by which gut hormones modify feeding are object of ongoing investigations. Peptides can act as circulating hormones with direct effects in the brain or with indirect effects through afferent and efferent vagal fibres. In parallel, peptides can affect different gut functions, such as absorption, secretion and motility, considered additional components in the regulation of the feeding behaviour. The list of gut anorexigenic peptides includes glucagon-like peptide 2 (GLP-2), which was first discovered as an intestinotrofic factor in 1996 (1), but today it is recognized as a pleiotropic hormone.
GLP-2 is a 33-amino acid peptide secreted from L-cells of the small and large intestine following enteral nutrient intake, especially carbohydrate and fats. Its biological actions converge at multiple levels on the regulation of nutrient assimilation and energy homeostasis. The gastrointestinal tract is the principal target for GLP-2 where it affects multiple facets of physiology, including growth, absorption and motility. The GLP-2 biological actions are mediated by the interaction with a specific GLP-2 receptor (GLP-2R), a G protein-coupled receptor, which is expressed mainly in the gut and in the brain.
GLP-2 main biological action is to increase intestinal growth, due to the enhancement of crypt cell proliferation and inhibition of apoptosis, resulting in expansion of villous height (1). Endogenous GLP-2 plays a role in the adaptative intestinal growth that occurs in rodents in response to oral re-feeding after a period of nutrient deprivation (2,3) or in response to high fat diet, as shown by using GLP-2^{3-33}, a GLP-2R antagonist (4).
GLP-2 exerts numerous other actions within the GI tract to promote energy absorption. It increases the uptake of luminal nutrients, including sugars and lipids, by augmenting the activity and the expression of nutrient transporters, the expression of different enzymes involved in the digestion and by increasing the mesenteric blood flow (5). GLP-2 has also been shown to inhibit the intestinal chloride secretion (6).
GLP-2 inhibits the GI motility, thus providing another mechanism to increase digestion and absorption of nutrient. Specifically, GLP-2 reduces the antral motility in pigs (7) and it decreases the mouse gastric fundic tone leading to an increase of the stomach capacity (8). Although it is not clearly established if the GLP-2 effect on mouse gastric stomach is physiological or pharmacological, the GLP-2 action on gastric fundus seems particularly interesting, because GLP_2 could represent a satiety signalling, a role which well fits with the finding that GLP-2 is a chemical mediator inhibiting rodent feeding behaviour (9). In fact, increase in the gastric volume might mean activation of stretch receptors and greater satiety signals to the brain.
GLP-2 inhibits intestinal transit and it reduces spontaneous or electrically-evoked cholinergic contractions of the small and large intestine in vitro (5). The peptide modulation on the gastrointestinal motility may be due to central nervous mechanisms, but involvement of the enteric nervous system has been also clearly shown through the in vitro studies (5). Because GLP-2R is expressed in the subepithelialmyofibroblasts and enteric nervous system as well as

human enteroendocrine cells, it has been proposed that the peptide may exert its actions also indirectly via downstream mediators deriving from GLP-2R-expressing cells. Indeed, modulation of the release of some neurotransmitter, as VIP, nitric oxide and acetylcholine, from enteric nerves have been reported to be involved in the inhibitory motor effects induced by GLP-2 in different regions of the mouse GI tract (5).

Lastly, GLP-2 may be considered an anorexigenic peptide because intracerebroventricular administrations of GLP-2 reduce the food intake in rodents (9). However, up to date studies in humans have not demonstrated decrease in the food intake after peripheral GLP-2 administration, even if our recent data have shown that intraperitoneal injections of GLP-2 reduce food intake in mouse, suggesting a role for GLP-2 in the short-term regulation of the ingestive behaviour (10).

The lecture will emphasize new recent findings about the role of GLP-2 in the control of energy homeostasis.

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CO4 - GENISTEINA E SINDROME METABOLICA NELLA DONNA IN POST-MENOPAUSA

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La donna in menopausa spesso presenta molti segni tipici della sindrome metabolica tra cui obesità addominale, elevati livelli di colesterolo LDL (LDL-C), ridotti livelli di colesterolo HDL (HDL-C), ipertensione arteriosa e ridotta tolleranza al glucosio/diabete mellito. Gli isoflavoni sono frequentemente utilizzati come alternativa terapeutica naturale alla classica terapia ormonale sostitutiva nelle donne in post-menopausa.
Recenti studi sperimentali e clinici hanno suggerito che l' aglicone genisteina, un isoflavone particolarmente abbondante nella soia e nei prodotti da essa derivati, può avere un possibile ruolo preventivo e/o terapeutico nella sindrome metabolica.
Per indagare sugli effetti della genisteina nella donne in post-menopausa con sindrome metabolica è stato effettuato uno studio clinico multicentrico, randomizzato, in doppio cieco e placebo-controllato della durata di 1 anno. Il trial clinico è stato condotto su una popolazione di 120 donne in post-menopausa con sindrome metabolica reclutate in accordo ai criteri modificati NCEP/ATP III (National Cholesterol Education Program/Adult Treatment Panel III).
Dopo un periodo di stabilizzazione di 4 settimane, le pazienti sono state randomizzate a ricevere placebo (n = 60) o 54 mg/die di genisteina (n = 60) per 1 anno. Come *outcome* principale è stato utilizzato l' HOMA-IR (homeostasis model assessment for insulin resistance); *outcomes* secondari dello studio sono stati i livelli ematici di glucosio ed insulina a digiuno, colesterolo totale, LDL-C e HDL-C, trigliceridi, visfatina, adiponectina e omocisteina. Sono stati altresì rilevati i dati sugli effetti collaterali.
Dopo un anno di trattamento la genisteina ha ridotto significativamente l' HOMA-IR, la glicemia e l'insulinemia a digiuno, mentre tali parametri non si sono modificati nel gruppo placebo. Inoltre, la genisteina ha significativamente incrementato i livelli ematici di HDL-C e adiponectina ed ha ridotto LDL-C, trigliceridi, visfatina e omocisteina; anche i valori pressori sistolici e diastolici si sono ridotti in modo significativo nelle pazienti che assumevano genisteina. Infine, nessuna paziente ha presentato effetti collaterali e/o ha abbandonato il trial clinico in seguito al trattamento.
I positivi dati ottenuti, in termini di efficacia e sicurezza, suggeriscono che l' isoflavone genisteina, somministrato in forma pura alla dose di 54 mg/die, si configura come una sostanza ad elevata azione nutraceutica, il cui consumo, integrato nel contesto di una dieta equilibrata nei nutrienti e associato ad una regolare attività fisica, consente di ridurre i fattori di rischio che caratterizzano la sindrome metabolica postmenopausale.

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CO5 - THE ADIPOKINE LEPTIN: A PLEIOTROPIC MOLECULE IN THE HUMAN RESPIRATORY TRACT

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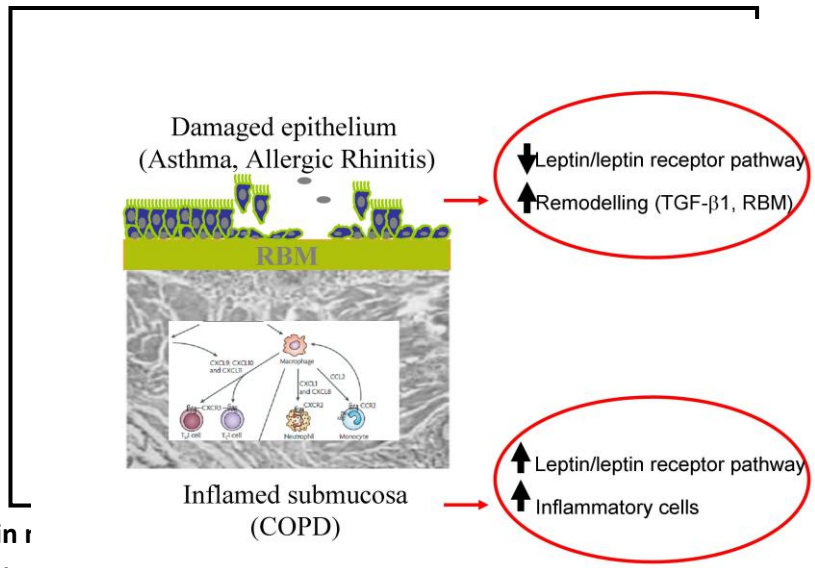
Leptin, a 16-kd adipocyte-derived hormone originally described in metabolism regulation, plays a pleiotropic role in the immune system and inflammation (1). Leptin exerts its action through the leptin receptor (Ob-R), present in several tissues, human respiratory tract included. Leptin is a survival cytokine for human neutrophils and eosinophils (2, 3), other than for other cytotypes, included lung carcinoma cells (4). The following findings highlight the specific role of leptin both in the lung and in the nasal tract. We firstly find that *ex-vivo* leptin expression is increased and co-localized with lymphocytes T inflammatory cells, in bronchial mucosa of chronic obstructive pulmonary disease (COPD) patients and it is associated with COPD severity, airway inflammation and airflow obstruction (5). On the other side, previous our *in vitro* and *ex-vivo* results show that the leptin/leptin receptor pathway is decreased in the bronchial epithelium of subjects with mild, uncontrolled, untreated asthma, whereas RBM thickness and TGF-□1are increased, when compared with healthy volunteers (6). In addition, in another our *in vitro* study, we assess that leptin increases adenocarcinoma cell line proliferation and the pathway with its receptor is increased by the flavonoid apigenin (4,5,7,-trihydroxyflavone) (7). Furthermore, our recent *in vitro* results report that the leptin/leptin receptor pathway is involved in human nasal epithelial homeostasis in allergic rhinitis and its expression is restored by Fluticasone Furoate in presence of the allergens (8). In conclusion: in the submucosa, leptin might act as a cytokine-like mediator capable of playing a role in airway inflammation in chronic obstructive pulmonary disease with a potential impact on the severity of the disease; in the epithelium, the leptin/leptin receptor pathway is involved both in airway and in nasal epithelial homeostasis, in asthma and in allergic rhinitis, promoting also, in a cancer context, epithelial cell proliferation. Its expression decreases in subjects with uncontrolled and severe asthma and in presence of allergen exposure and is inversely correlated with airway remodelling, and cancer cell apoptosis.



Scheme : Leptin r
human respirato

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CO6 - K:D-Rib ON BIOLOGY OF HUMAN CANCER AND NOT CANCER CELL LINE

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This work describes different effects of K:D-Rib solution treatment: from one side the slow down of cell proliferation and the reduction of chemoinvasive potential of human breast cancer cell line (HTB-126) and from the other the maintenance of normal proliferation and normal morphology in mammary human not cancer epithelial cell line (HTB-125). K:D-Rib is a water solution of D-ribose and $KHCO_3$. The role of D-ribose on the energetic metabolism and its involvement into glycogen synthesis [1, 2], as well as the importance of K^+ into the cell physiology, are well known [3, 4]. It has been found that K^+ is essential to fold and to stabilize G-quadruplex [5] with a strong relevance for telomeric structures [2, 6] and for oncogenic promoter regions.
Our results showed that K:D-Rib has a cytostatic effect on canine carcinoma cell line (A72), slows the colony formation ability of the HTB-126 cell line and has an antioxidant behaviour reducing MTT salt to formazan in absence of cells [1]. These results are confirmed by our most recent work, demonstrating that 5mM K:D-Rib increase the cell cycle time of HTB-126 cell line treated with K:D-Rib at the concentration of 5mM, from 44h to 59h. Here it will be show how K:D-Rib interferes both on HTB-126 cell line proliferation and cell morphology. Results on cell morphology using Atomic Force Microscopy (AFM) are presented. K:D-Rib is tested also on human mammary epithelial cell line (HTB-125). HTB-125 cells treated with 5 mM K:D-Rib do not display toxicity or notable cell proliferation decreasing rate compared to the control one. HTB-125 cell morphology is analyzed by AFM. As mentioned before a key point of cancer cells is the capability to invade tissues nearby or far from cancer formation site. Tumour motility is an important step in the intricate process leading to the formation of metastasis. It has been shown that metastatic cells are more motile than non-metastatic tumour cells and most motile of normal cells. Metastatic cells lose growth-inhibitory responses, undergo alterations in adhesiveness and demonstrate enhanced production of enzymes that can degrade extracellular matrix components. Since the development of metastatic disease in breast cancer is one of main responsible of cancer mortality, the stopping and the understanding of the mechanisms that facilitate metastatic tumour progression is of prime importance [7]. We have investigated if K:D-Rib solution within 9 days can modify the migration and the invasion ability. The experiments show HTB-126 cells are able to migrate across the coverslip toward the FBS – agar spot and to invade it within 48, but the relative cell number inside the AGAR-FBS decrease already after five days of treatment. After nine days of treatment with K:D-Rib the relative cell number, inside the AGAR-FBS spot is reduced to 25%, demonstrating that tumorigenic potential is highly decreased with K:D-Rib treatment. These results show that 5mM K:D-Rib causes the change of some aspects like migration, invasion and proliferation of HTB-126 cell line. Despite these evidences K:D-Rib does not interfere neither with the proliferation of HTB-125 cell line nor with cell morphology.

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CO7 - INCIDENZA DELLA POSTURA NEL TIRO AL VOLO

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INTRODUZIONE

Il tiro al volo, disciplina olimpica dalla seconda edizione di Parigi 1900, consiste nel colpire un bersaglio in movimento, detto piattello, che fuoriesce da macchine poste al di sotto del livello del campo. La posizione del tiratore è eretta, con le gambe leggermente divaricate; questi, una volta imbracciato il fucile, ordina la partenza del piattello, con l'obiettivo di colpirlo. Il numero dei bersagli da colpire durate una sessione di tiro è di 25 piattelli.
Lo scopo del presente lavoro di ricerca è stato quello di valutare durante una simulazione di tiro, se esiste una correlazione tra atteggiamento posturale assunto e performance del tiratore [1,2].

MATERIALI E METODI

Il campione che ha preso parte alla ricerca è formato da 14 atleti, suddivisi in due gruppi, in relazione ai risultati ottenuti in gara. Il gruppo 1 è composto da 7 atleti tiratori meno esperti mentre il gruppo 2 da 7 atleti tiratori d'élite. Tutti gli atleti tiratori facenti parte del campione hanno firmato il consenso informato preparato sulla base delle indicazioni del Comitato Etico della nostra Università.
La valutazione posturale è stata effettuata attraverso l'analisi delle seguenti misure relative al Centro di Pressione (COP): medio-laterale (ML) standard deviation del COP, antero-posteriore (AP), COP path length, average sway velocity of COP, area included within the path of the COP (Ao), e l'area dell'ellisse di confidenza al 95% (Ae). Le acquisizioni sono state effettuate utilizzando una piattaforma di forza Advanced Mechanical Technology Incorporated (AMTI, Newton, MA) Model OR-6-7-1000 (3). Si è deciso di valutare solo le due posizioni considerate determinanti per il raggiungimento del successo sportivo (goal) mantenute per 34 secondi. La posizione di preparazione (start) e la posizione di tiro (shoot).
Al fine di evitare influenze sulla stabilità posturale dovute all'orario [3], le acquisizioni sono state effettuate tra le 15.00 e le 18.00.

RISULTATI E CONCLUSIONI

L'ampiezza dell'area dell'ellisse di confidenza dei gruppi 1 e 2 risulta differente. L'area dell'ellisse è risultata significativamente più grande per i componenti del gruppo 1 rispetto a quella osservata nei componenti del gruppo 2, come riportato in figura 1.
La figura 2 riporta l'andamento del COP per due soggetti appartenenti rispettivamente al gruppo 1 e 2 nelle due posizioni prese in considerazione.
Dall'analisi dei risultati ottenuti, l'acquisizione di una corretta posizione al momento del tiro risulta essere una variabile molto importante per questo sport, in quanto appare correlata al raggiungimento di migliori prestazioni.

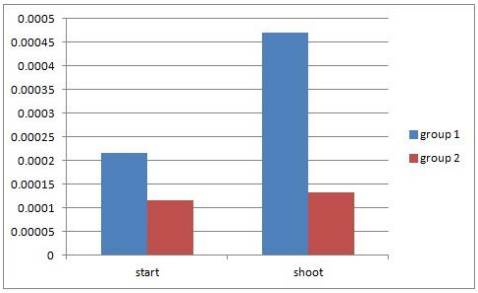

Figura 1 Area dell'ellisse di confidenza, espressa in metri quadri, per i soggetti appartenenti ai due gruppi per le posizioni analizzate

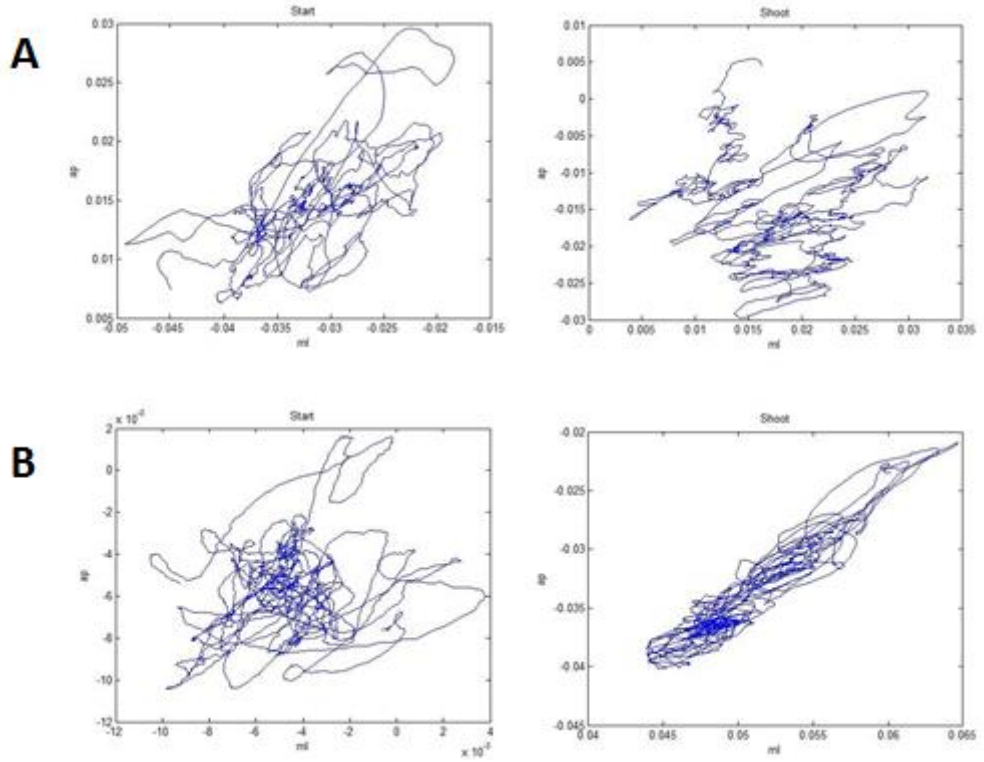

Figura 2. In A il COP in un tiratore del gruppo 1 nella posizione di partenza (a sinistra) e di tiro (a destra); in B COP di un tiratore del gruppo 2 nelle stesse due posizioni.

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CO8 - DOES THE EXERCISE TRAINING INTENSITY AFFECT PLASMATIC REDOX STATUS IN RHYTHMIC GYMNASTICS?

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Introduction
It is known that factors such as exercise intensity (1), length (2) and type (3) can modify the plasmatic oxidant/anti-oxidant production. In rhythmic gymnastics, adolescent female athletes showed a higher lipid peroxidation (4) and an altered antioxidant enzyme profile compared with their untrained peers (5).
The aim of this study was to assess whether the plasmatic H_2O_2 levels and antioxidant capacity were affected by the exercise training intensity in rhythmic gymnastics coaches.
Methods
Ten women (age: 23.8 ± 3.42 y; weight: 52.58 ± 4.57 kg; height: 158.42 ± 2.20 cm; body mass index: 20.88 ± 1.23), with 13.14 ± 5.40 years of practice in rhythmic gymnastics and coaches from at least 4 years at a competitive level, voluntarily participated into this study. One week before training, trainers performed a laboratory graded exercise test on the treadmill in order to determine their maximal heart rate (HR_{max}), maximal oxygen consumption (VO_{2max}) and anaerobic threshold (AT).
Two interval-training sessions, separated by 48 hours of recovery, were performed with different intensities. The first was carried out at a low-moderate intensity; while the second at a high intensity. Both lasted 45 minutes and consisted of exercises aimed to develop anaerobic power, strength, flexibility and body balance. Before cool down, two performances of competition technical skills coordinated to the music were also executed. During training, HR was continuously monitored with 'Polar team' system. Immediately before and after the training session, blood samples were taken from fingertip's coaches and H_2O_2 levels and antioxidant capacity were measured through reactive oxygen metabolites (dROMs) and biological antioxidant potential (BAP) test, respectively. Newman-Keuls multiple comparison test was used for evaluating the significant differences. Alpha level for significance was set to $P<0.05$.
Results
Coaches executed the first training session at an average intensity of 66% HR_{max}, mainly in aerobic condition and only 5.5% of total time in anaerobic condition; while in the second session they spent 42% of total time at an intensity ranging from 80 to 100% HR_{max} and for 25% above the anaerobic threshold.
After low-moderate intensity training, H_2O_2 levels were significantly lower than baseline and they came back to baseline following 48 h of recovery. After high intensity training, H_2O_2 amount slightly decreased compared with baseline ($p>0.05$); while it was significantly higher than after low-moderate intensity training. All these values corresponded to a middle oxidative stress when compared with a standard range (6). Antioxidant capacity did not change following low-moderate intensity training, while it significantly increased after 48 h of recovery. In contrast, it significantly decreased in response to high intensity training reaching the values obtained after low-moderate intensity training.
Conclusions
These results show that training intensity has different effects on ROS production and antioxidant capacity in rhythmic gymnastics. In detail, a low-moderate intensity session induces H_2O_2 production; while a high intensity session negatively affects the antioxidant defences.

Therefore, it would be appropriate to introduce a anti-oxidant diet or supplementation for protecting rhythmic gymnastics trainers by oxidative stress.

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CO9 - HEART RATE RECOVERY AFTER EXERCISE AND MAXIMAL OXYGEN UPTAKE IN SEDENTARY PATIENTS WITH TYPE 2 DIABETES

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AIMS: Heart rate recovery after exercise (HRR) is an estimate of autonomic modulation of the heart, and has been shown to be inversely associated with insulin resistance, metabolic syndrome, and type 2 diabetes [1]. Type 2 diabetes is associated with poor exercise tolerance and maximal aerobic capacity (VO_2max) [2]. Aim of our study was to assess the relationship between HRR and VO_2max in sedentary patients with type 2 diabetes.
METHODS: Maximal treadmill exercise testing using standard or modified Bruce protocol was performed in 16 (8 males and 8 females) sedentary patients with type 2 diabetes (T2D), and in 16 (9 males and 7 females) age-matched sedentary non-diabetic controls (ND). HRR (bpm) was defined as the difference between maximum heart rate during the exercise test and heart rate 2 minutes after cessation of the exercise (Fig. 1). The recovery protocol consisted of walking on treadmill at 2.0 Km/h of speed and 0% of grade. Oxygen uptake was recorded and VO_2max (ml/Kg/min) was defined as the highest 30 seconds average achieved during the test. For the statistical analysis of the data, Student's t-test for independent samples and linear regression analysis were used.
RESULTS: The characteristics of subjects are shown in Table 1. The two groups were similar in age and body weight. BMI was higher in T2D (30.1 ± 3.6 vs. 26.9 ± 4.2, $p = 0.029$). VO_2max was significantly lower in T2D compared to ND (20.6 ± 8.4 vs. 28.2 ± 8.1 ml/Kg/min, $p = 0.002$) and, according to "Normative Table by age and gender" from ACMS, the aerobic capacity was classified very poor in all T2D and in 11/16 of ND. HRR was significantly lower in T2D (28 ± 8.4 vs. 37 ± 8.9 bpm, $p = 0.008$). A significant correlation between HRR and VO_2max has been found in both T2D (Fig. 2) and ND ($r = 0.672$, $p = 0.004$ and $r = 0.620$, $p = 0.010$ respectively).
CONCLUSIONS: The results of our study showed that both HRR and VO_2max were significantly reduced in T2D versus ND. The positive linear correlation between HRR and VO_2max suggests that in T2D the heart rate recovery after exercise, index of autonomic modulation, might improve in response to a training aimed to increase aerobic capacity.

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Legends:
Table 1: Characteristics of subjects.
Figure 1: Example of HRR phase analysis after a maximum reached heart rate.
Figure 2: Correlation between HRR and VO_2max in T2D patients.

Table 1: Characteristics of subjects			
	T2D (*n* =16)	Control (*n* =16)	
	Mean ± *SD*	Mean ± *SD*	*p* -value
Age (years)	57 ± 7.5	53 ± 6.9	0.100
Height (cm)	165 ± 7.7	166 ± 9.8	0.826
Weight (Kg)	82 ± 10.7	74 ± 15.5	0.119
BMI	30.1 ± 3.6	26.9 ± 4.2	0.029
HRmax (bpm)	130 ± 16.1	143 ± 13.5	0.024
VO_2max (ml/Kg/min)	20.6 ± 4.3	28.2 ± 8.1	0.002
HRR (bpm)	28 ± 8.4	37 ± 8.9	0.008

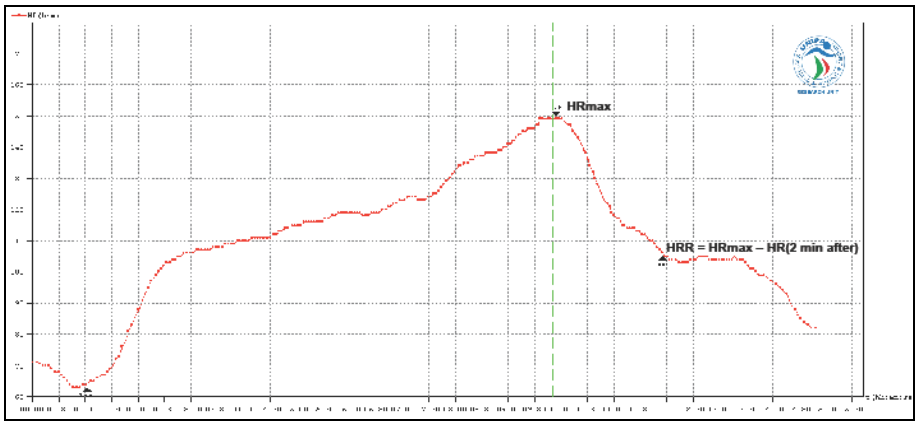

Figure 1.

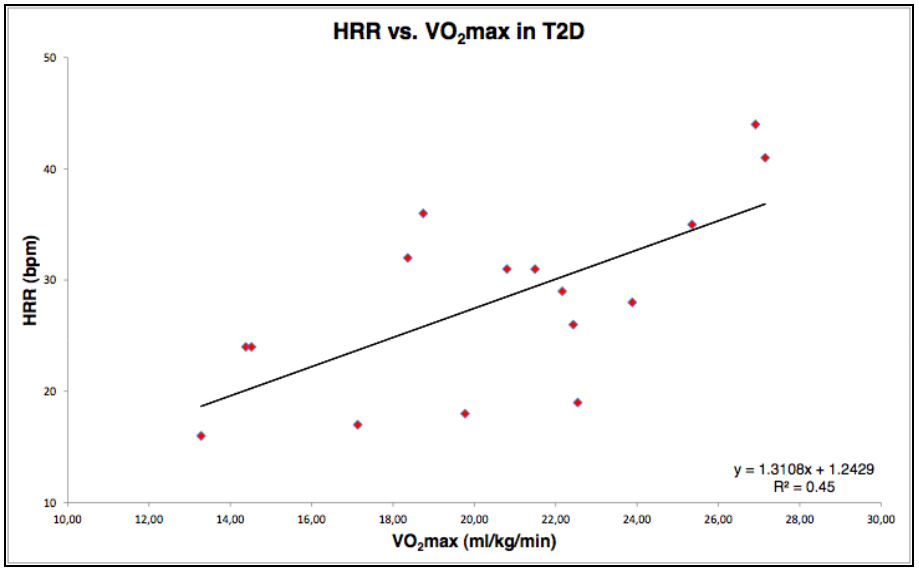

Figure 2.

CO10 - THE ROLE OF PHYSICAL ACTIVITY IN MANAGEMENT OF BODY WEIGHT IN PSYCHOTIC SUBJECTS

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Keywords: schizophrenia, mental illness, exercise, soccer.

Psychotic subjects live an unhealthy lifestyle that tends to reinforce the metabolic syndrome (1). It is known, indeed, that antipsychotic treatments stimulate appetite (2) and induce weight gain (3). Several therapeutic approaches including psycho-educational behavioral interventions and exercise training programs have been elaborated in order to manage antipsychotic-induced weight gain (4,5). Wirshing et al. (2006) elaborated a specific project based on oral presentations in order to educate psychotic subjects about lifestyle changes they can adopt to reduce weight gain. They showed that patients with mental illness are able to benefit from educational presentations about nutrition and a healthy lifestyle (4). Moreover, the prospective naturalistic study of Poulin et al. (2007) investigated the efficiency of a controlled physical activity program and a nutritional counseling in psychotic patients. They found that a weight control program including unstandardized exercise could effectively manage body weight and metabolic syndrome in subjects receiving atypical antipsychotic treatments (5).
The aim of this study was to investigate the effects of soccer practice on the self reported health quality of life (SRHQL), sports performance (SP) and body weight (BW) control in subjects with a diagnosis of schizophrenia.
Twenty-three male subjects were recruited in the Psychiatric Departments of Local Health of Palermo (Sicily, Italy) and randomized into either a control group (CG) and the trained one (TG). All patients participated in the local project "*Calciapensieri*". Two subjects of TG were excluded from study because did not attend 80% of training period; instead 3 patients of CG were not subjected to post-test. For these reasons, eighteen overweight male subjects, of which 10 trained psychotics (age: 36.00±5.00 yrs.; weight: 77.44±13.60 kg; height: 164.44±7.00 cm; BMI: 28.55±4.06) and 8 no-trained psychotics (age: 35.00±4.00 yrs.; weight: 76.71±09.75 kg; height: 163.42±4.99 cm; BMI: 28.65±2.62), were considered in this study. TG was trained for 12 weeks by two soccer training sessions/week. CG did not perform any physical activity during experimental period. Body weight (BW), BMI, SRHQL and SP were evaluated before and after experimental period. SRHQL was assessed using SF-12 questionnaire measuring physical (PCS-12) and mental (MCS-12) component summary scores.
After the training period, TG showed a relevant decrease by 4.6% in body weight (BW) and body mass index (BMI) compared to baseline. On the contrary, CG showed an increased BW and BMI by 1.8% from baseline to post test. Moreover, at 12 weeks we found that control patients increased significantly their BW than trained ones (Δ= 5.4%; $p<0.05$). After the training period, comparing TG's SF-12-scores from baseline to post-test, we found an improvement by 10.5% and 10.8% in PCS and MCS respectively. In addition, TG's SF improved significantly ($p<0.05$) from baseline to post-test compared to CG (6).
Weight gain associated with abdominal obesity and metabolic syndrome are the main collateral effects associated with modern second-generation antipsychotics treatments (7). We showed that regular physical activity could reduce antipsychotic medication-related weight gain and improve

SRHQL and SP in psychotic subjects. However, there are only a few studies that evaluate the effects of exercise training on physical fitness of psychotic subjects. We suggest that it is need to speculate multifaceted interventions aimed at combining traditional pharmacologic treatments and alternative behavioural methods such as physical activity (6).

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CO11 - DETERMINANTI GENETICI DI FIBROSI NEI DISORDINI METABOLICI DEL FEGATO

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La steatosi epatica non alcolica (NAFLD) comprende un ampio spettro di disordini, caratterizzati da un eccessivo accumulo di trigliceridi nel fegato, che vanno dalla steatosi semplice, alla steatoepatite non alcolica (NASH) che spesso può progredire in cirrosi.
Al fine di comprendere i meccanismi per cui l'accumulo di grasso a livello degli epatociti porti alla progressione della fibrosi nella NAFLD, diversi studi hanno identificato l'insulino-resistenza e la risultante iperinsulinemia (associata alla obesità) come responsabili della promozione dell' incremento del traffico di acidi grassi all'interno degli epatociti *(Carter-Kent C. et., al 2008; Schreuder TC., Et al., 2008; Parekh S. et al., 2007; 132:2191-2207; Neuschwander-Tetri BA., Et al 2007).* Nella fibrosi associata alla NASH, le cellule stellate (HSCs) rappresentano le principali responsabili della produzione di matrice extra-cellulare a seguito della loro attivazione e all'acquisizione di un fenotipo miofibroblasto-simile. L'attivazione delle cellule del Kupffer, l'infiltrazione tissutale dei monociti, l' attivazione piastrinica e il danneggiamento degli epatociti sono responsabili del rilascio di molecole, come il fattore di crescita derivato dalle piastrine (PDGF) e il fattore di crescita trasformante β (TGF-β), che portano all'attivazione delle HSCs e all'espressione dei geni della fibrogenesi.

In questo complesso scenario, studi di genome-wide association (GWAS) hanno mostrato come alcuni polimorfismi genetici siano correlati ad una maggiore severità di malattia ed ad una più rapida progressione in pazienti con NAFLD. Tra questi, il polimorfismo a singolo nucleotide rs738409 C/G (Ile148Met) del gene che codifica per l'adiponutrina (PNPLA3) si associa ad una maggiore prevalenza di NAFLD e ad una maggiore progressione e severità sia della steatosi che della fibrosi *(Sookoian S. et al, 2009).*
Negli ultimi anni, il nostro gruppo di ricerca si è occupato di identificare e validare una serie di varianti alleliche associate alla progressione di malattia nella NAFLD. In una coorte di 160 pazienti NAFLD è emersa una correlazione fra la presenza dell'allele C in omozigosi per l'rs 12979860 del gene che codifica per l'IL28B e la severità del danno epatico in pazienti portatori dell'allele sfavorevole per PNPLA3 *(Petta S. et al., 2012*).
Un altro GWAS ha evidenziato il ruolo di varianti di alcuni geni che influenzano differenti profili metabolici nella NAFLD (Speliotes et al., 2011), spingendoci ad analizzare gli SNPs rs 2228603 di NCAN (C/T), rs 780094 di GCKR (C/T), e rs 12137855 di LYPLAL1 (C/T) in una nostra coorte. I risultati ottenuti ci hanno permesso non solo di associare la condizione di omozigosi T per il polimorfismo genico di GCKR alla steatosi e alla progressione della fibrosi nella NAFLD, ma anche di inquadrare la progressione della fibrosi tenuto conto degli alleli sfavorevoli per i genotipi complessi di IL28B e GCKR (*Petta S et Al., submitted*) (fig.1).

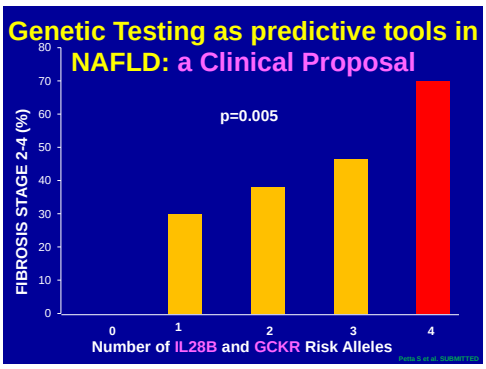

Fig. 1: Percentuale di fibrosi severa in associazione al numero di alleli sfavorevoli IL28B e GCKR in 216 pazienti NAFLD.

Attualmente abbiamo in corso uno studio sull'impatto sulla progressione della NAFLD della variante genica di MERTK (G/A rs4374383) associata al rischio di sviluppare fibrosi in pazienti con infezione cronica da virus C (*Patin E. et al.,2012*). Questo gene codifica per una tirosin-kinasi della superfamiglia dei recettori TAM coinvolta nella fagocitosi delle cellule apoptotiche (efferocitosi). I risultati preliminari mostrano che pazienti con genotipo AA hanno una prevalenza significativamente più bassa di steatosi severa, di infiammazione lobulare e di fibrosi severa (Fig.2) rispetto a soggetti con genotipo GG e GA.

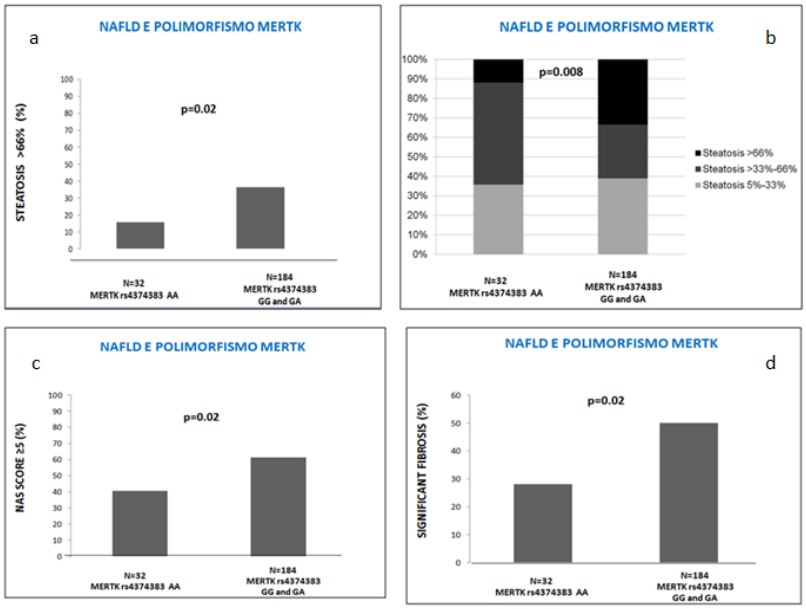

Fig.2: Influenza del genotipo MERTK rs4374383 sulla steatosi (a,b) l'infiammazione lobulare (c) e sulla fibrosi (d) in 216 pazienti NAFLD.

I risultati finora ottenuti dal nostro e da altri gruppi di ricerca ci confermano l'importanza del profilo genico nel condizionare l'insorgenza e la progressione della NAFLD e ci suggeriscono la possibilità di approntare uno studio di profilo dei genotipi al fine di stratificare i pazienti per classe di rischio.

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CO12- MECCANISMI IMMUNOLOGICI E MOLECOLARI DEL DANNO EPATICO DA FARMACI

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Il fegato è la centrale metabolica del nostro organismo. I farmaci, in seguito alle reazioni di fase I e II vengono trasformati in metaboliti attivi meno tossici, ma idrofili pronti per essere eliminati. Il sistema responsabile di questa trasformazione è il citocromo P450 presente nel reticolo endoplasmatico liscio dell'epatocita. In seguito, intervengono le glucoronosil-tranferasi, le sulfotransferasi e le GSH-transferasi che idrolizzano definitivamente il composto. Il trasporto del metabolita del farmaco fuori dall'epatocita avviene attraverso il polo biliare ed è mediato da un sistema di trasportatori di membrana, l'ATP Binding Cassette (ABC) Transporter Superfamily. L'alterazione dell'attività dei trasportatori, a diversi livelli, molecolare e trascrizionale, è uno dei meccanismi responsabile di epatotossicità.
I polimorfismi genetici e/o i fattori ambientali, ad esempio l'alcol e/o i farmaci concomitanti, contribuiscono alla suscettibilità individuale nel determinare il danno epatico da farmaci. L'HLA si è dimostrato essere uno dei più importanti predittore di suscettibilità individuale anche per quei farmaci per i quali questa non era stata mai sospettata. A questo proposito, è stato dimostrato che c'è una forte associazione tra il danno epatico indotto da flucloxacillina e l'allele HLA B* 5701, e tra aplotipi HLA di classe II e danno epatico da amoxicillina-clavulanico e ximelagatran.
I farmaci, in generale, possono avere un effetto diretto sugli epatociti o suscitare una reazione immune che può essere di due tipi: innata o adattativa. Nella maggior parte dei casi, la bioattivazione di un farmaco porta ad un metabolita reattivo che determina una disfunzione mitocondriale con conseguente riduzione dei livelli di ATP, disaggregazione del citoscheletro e quindi rottura della membrana cellulare epatocitaria. I metaboliti attivi influenzano il trasporto delle proteine (MDR-3) attraverso il polo biliare della membrana eritrocitaria determinando l'interruzione del flusso biliare, il blocco di escrezione della bilirubina e infine la colestasi.
In alternativa ad una azione diretta sulla membrana cellulare, lo stress epatocitario determina l'attivazione del sistema immune innato attraverso le cellule natural killer (NK) del fegato che secernono interferone-gamma (IFNγ) ed interleuchina (IL)-4, e sono in grado di uccidere direttamente le cellule tramite il sistema Fas/FasLigand. Cellule di Kupffer ed NK contribuiscono alla progressione del danno epatico producendo mediatori pro-infiammatori (citochine, chemochine, ROS); questi possono avere azione citotossica diretta (perossido d'idrogeno, ossido nitrico) degradando la matrice extracellulare, oppure promuovendo l'adesione e l'infiltrazione cellulare dei leucociti polimorfonucleati.
Nella patogenesi del danno epatico è coinvolto anche il sistema immune adattativo. Il metabolita reattivo può infatti legarsi in modo covalente ed alterare le proteine del fegato, promuovendo l'attivazione delle cellule T citotossiche e la produzione di citochine (reazione immuno-mediato). Il meccanismo del danno da farmaci immuno mediato non è ben chiaro, e comporta un'azione "apten-like". Generalmente infatti le sostanze chimiche a basso PM non sono immunogeni ma possono diventare tali quando sono legati ad una macromolecola, come una proteina. Se un metabolita attivo di un farmaco prodotto dal citocromo P450 è in grado di agire come un aptene, e si lega covalentemente ad una proteine del fegato, il sistema immunitario la percepirà come "non-self" causando una reazione autoimmune. Il risultato di questi eventi, sia attraverso una reazione diretta sulla membrana cellulare, sia attraverso l'induzione di una risposta immunitaria, è la morte cellulare: necrosi o apoptosi.

L'induzione dell'apoptosi piuttosto che la necrosi dipende da diversi fattori, tra cui lo stato energetico (ATP). Una lesione grave per i mitocondri determina deplezione energetica della cellula, che perde la regolazione osmotica e va in necrosi. Una lesione meno grave senza importante deplezione di ATP è in grado di mantenere la regolazione osmotica e porta all'apoptosi. La necrosi epatocellulare è l'evento principale di cui è responsabile il danno epatico da farmaci; ne possono essere bersaglio sia cellule endoteliali che quelle dei dotti biliari. In fatti distinguiamo il danno epatico da farmaci di tipo epatocellulare (nimesulide), di tipo colestatico (amoxicillina clavulanico) e misto.

CO13 - GLI STRUMENTI PER UNA CORRETTA SORVEGLIANZA NUTRIZIONALE DEGLI ADOLESCENTI: IL PROGETTO ASSO E LA CREAZIONE DI UN SISTEMA WEB-BASED

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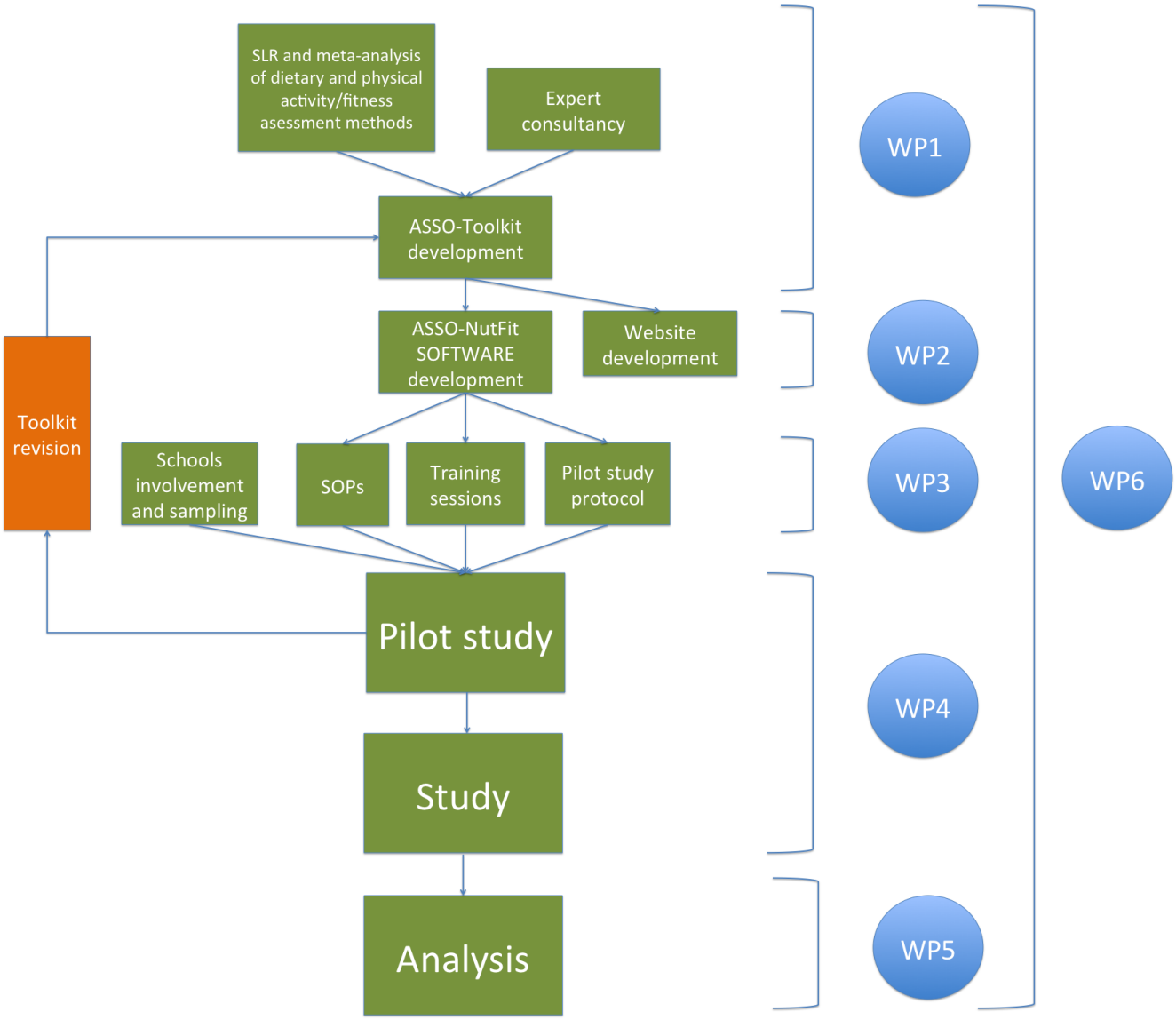
Introduzione: L'adolescenza è un periodo critico, caratterizzato da molti cambiamenti che includono anche le abitudini alimentari e gli stili di vita. Le statistiche relative a queste informazioni sono spesso frammentarie, in quanto gli interventi effettuati sono generalmente brevi, locali, non continui e non standardizzati, e un sistema di sorveglianza strutturato in Italia non è ancora stato sviluppato.
Obiettivi**:** Il Progetto ASSO (Adolescenti e Sistema di Sorveglianza per la prevenzione dell'Obesità), finanziato dal Ministero della Salute per un periodo di tre anni, si svolge presso l'Università di Palermo e coinvolge diversi partners a livello nazionale e internazionale, quali l'INRAN, l'Università di Greenwich, l'OMS. Il principale obiettivo è quello di sviluppare un sistema basato sul web per una continua e standardizzata raccolta di dati nelle scuole sui consumi alimentari, gli stili di vita e i parametri antropometrici nell'intera popolazione di adolescenti.
Disegno dello studio: Il Progetto è strutturato in 6 Work Packages (WPs): revisione sistematica della letteratura e preparazione dei questionari; programmazione dell'applicativo web e del portale informativo; campionamento scuole e attività di formazione; raccolta dati e analisi; valutazione; attività di disseminazione (Figura 1).
Risultati: Sulla base della revisione della letteratura, sono stati creati e inclusi nel software ASSO-NutFit i seguenti questionari per la raccolta dati: ASSO-PIQ (Personal Information Questionnaire), ASSO-PASAQ (Physical Activity, Smoke and Alcohol Questionnaire), ASSO-FHQ (Food Habits Questionnaire), ASSO-FFQ (Food Frequency Questionnaire). E' stata, inoltre, creata una batteria di test per valutare la fitness dei ragazzi, la ASSO-FTB (Fitness Tests Battery), e sono state standardizzate le procedure per la raccolta dei dati antropometrici, da riportare nella scheda ASSO-BFMF. Tutto il materiale è accompagnato dalle relative Procedure Operative Standard (POS). E' stato effettuato un campionamento delle scuole, all'interno delle quali sono stati identificati due docenti referenti, che dopo essere stati adeguatamente formati hanno supportato la raccolta dei dati. La fase di raccolta ha previsto un primo studio pilota su un piccolo campione di popolazione e successivamente lo studio allargato (Figura 1), sul quale è stata eseguita una validazione. Ci si trova in questo momento nella fase di analisi dei dati, effettuata attraverso il software statistico STATA 12.0.
Conclusione**:** I dati preliminari mostrano che gli strumenti creati da ASSO possono fornire una stima prossima al reale stile di vita di ciascun allievo. Lo sviluppo di un sistema di sorveglianza web-based ben definito è un esempio di buona pratica, permettendo una raccolta di dati semplice, user-friendly, economica ed efficace. In tal modo è possibile ottenere un quadro dei problemi di salute sugli adolescenti e pianificare azioni preventive adeguate.

Figura 1**.** Strutturazione del Progetto ASSO


SLR and meta-analysis of dietary and physical activity/fitness asessment methods
Expert consultancy
ASSO-Toolkit development
ASSO-NutFit SOFTWARE development
Website development
Toolkit revision
Schools involvement and sampling
SOPs
Training sessions
Pilot study protocol
Pilot study
Study
Analysis
WP1
WP2
WP3
WP4
WP5
WP6

CO14 - CHE COS'E' LA DIETA MEDITERRANEA?

G. Calabrese

La Dieta Mediterranea è sempre sinonimo in tutto il mondo di alimentazione sana, ed è capace di mantenere l'organismo in salute, prevenendo molte patologie e regalare una lunga aspettativa di vita.
Ancel Keys aveva scoperto che chi consumava una dieta ipocalorica, ma ricca di nutrienti, poiché a base di alimenti vegetali, aveva una mortalità per malattie cardiovascolari molto bassa e di conseguenza, nel 2010 la dieta mediterranea è stata premiata come patrimonio culturale immateriale dell'umanità dall'UNESCO, ed è stata valutata come la migliore proprio grazie alla presenza nel menù giornaliero dei consumatori di generose porzioni di spaghetti fumanti, pane, olio, vino e quant'altro.
La dieta mediterranea in effetti deve essere ipocalorica, ma non eccessivamente, con nutrienti derivanti soprattutto da prodotti vegetali come: cereali, tuberi, legumi, oli vegetali, associati a prodotti di prodotti animali, che restano essenziali per vivere bene e a lungo.
Oltre alla scelta dei gruppi di alimenti, anche la ripartizione dei macronutrienti è molto importante, con una maggiore presenza di proteine, a discapito dei grassi (55% carboidrati, 27% grassi e 18% proteine). Ciò permette di avere un equilibrio nell'introito di alcuni micronutrienti come potassio (4,7 gr), fibra (30 gr) e di sodio (meno di 2,3 gr) ; quindi di calcio (1,250 gr) e di colesterolo di derivazione alimentare (150 mg).
Nel documento prefinale di sintesi della revisione 2012, infatti, non vengono fornite raccomandazioni fisse per il contributo energetico dei tre nutrienti energetici (contrariamente a quanto riportato nelle precedenti versioni (che definivano un contributo percentuale all'energia di 55-30-15, rispettivamente per carboidrati, grassi e proteine). Come avviene per gli altri nutrienti (vitamine e minerali), sono invece definiti degli intervalli di riferimento per ognuno dei tre macronutrienti entro i quali è possibile un'adeguata introduzione di tutti gli altri nutrienti e non si hanno evidenze epidemiologiche di rischi associati ad apporto per difetto o per eccesso.
Per quanto riguarda i carboidrati è stato stabilito un intervallo di riferimento tra il 50 e il 60% dell'energia, mentre per i grassi tale valore va dal 20 al 35%. Non sono stati invece definiti gli intervalli di riferimento per le proteine ma certamente non possono essere desunti per semplice differenza, prendendo gli estremi inferiori e superiori della somma del contributo di carboidrati e grassi. Prendendo infatti ad esempio la somma dei valori superiori di carboidrati e grassi otterremmo un contributo proteico pari al 5%, che per un fabbisogno energetico di 2000 kcal corrisponderebbero a 25 grammi di proteine, valore estremamente basso per la maggior parte delle persone con tale fabbisogno.
La situazione attuale dei consumi degli italiani non è molto incoraggiante poiché non solo siamo lontanissimi dalla ripartizione energetica tipica della mediterranea classica o della DASH, ma siamo pericolosamente vicini al riferimento inferiore per i carboidrati e anzi gli adulti maschi sono sotto (44,3%) e oltre i livelli superiori per i grassi (più del 36%)
Si tratta di un modello alimentare che diventa nutritivo e salutista grazie all'organizzazione gastronomica dei vari alimenti che la compongono: pane, pasta, pane, riso, legumi, frutta, verdura, olio di oliva, carne bianca e rossa, pesce, latte e latticini e vino.
Cosa se ne evince? Che sono alimenti a basso tenore di grassi (non superiori al 30%), specie di tipo saturo, visto che i cibi di origine animale non sono prevalenti su quelli di tipo vegetale.
Tanta buona fibra vegetale dotata di attività preventive nell'insorgenza dei tumori e di malattie cardiovascolari e metaboliche, come il diabete.come avvengono queste azioni di prevenzione? Sfruttando la presenza nei vari alimenti di nutrienti, del tipo:

i) vitamine come: A; C; D ed E, che sono antiossidanti.
ii) Minerali antiossidanti, come SELENIO, MAGNESIO, MOLIBDENO, ecc.
iii) INDOLI
iv) GLUCOSINOLATI

v) FLAVONOIDI
vi) POLIFENOLI
vii) ISOTIOCIANATI
viii) INIBITORI DLELE PROTEASI
ix) FITOSTEROLI

Tutti questi elementi, che sono spesso di tipo *traccia* cioè minimamente presenti, attivano l'azione di molti enzimi che:

i) sconfiggono l'azione tossica di vari elementi presenti nei cibi
ii) esercitazione una forte azione antiossidante
iii) inibiscono la formazione di nitrosamine, notoriamente cancerogene
iv) sequestarno, legandoli, gli elementi carcinogeni in tutto l'apparato digerente
v) influenzano positivamente l'azione degli ormoni.

PERCHE' LA DIETA MEDITERRANEA PROTEGGE LA SALUTE

Nel passato si era accertato che in America, solo il 10% degli americani consumava le 5 porzioni quotidiane tra frutta e verdura, mentre il 43% non le mangiava affatto, né fresca né conservata, né sotto forma di succo. Ne è derivato che nel 1991 l'istituto dei Tumori americano (NCI) consiglò prima e poi impose il programma " *5 A DAY FOR BETTER HEALTH" cioè, 5 porzioni al giorno per una migliore salute,* che doveva portare gli americani a raggiungere l'obiettivo delle 5 porzioni di frutta e verdura al giorno, entro l'anno 2000.

Ciò purtroppo non è successo neanche oggi nel 2013 ma comunque una buona parte dei cittadini americani si è convinta a rispettare queste regole intelligenti e salutiste.

L'osservazione che oggi noi possiamo fare è che, negli ultimi 10 anni, in Italia si sta raggiungendo lo stesso grado di inosservanza americana, infatti il consumo di frutta e verdura giornaliera è calata, rispettivamente, del 18% e del 20%.

Oggi, in Italia, si consumano circa 2.500-3.000 Kcalorie, di cui solo il 36% è costituito dal consumo di pasta, cereali e derivati. Il 22% da grassi e olii, i 9% da patate, ortaggi e frutta, l'8% da carni e salumi, e resta sempre molto basso il consumo di pesce che all'epoca era del 3% ed oggi si è quadruplicato (circa il 13%) delle calorie totali.

Nelle regioni Centro-Meridionali, il consumo di pane, farina e derivati risulta più alto come anche quello dell'olio di oliva; nel Sud è aumentato anche il consumo di carni avicole, come il pollo e il tacchino, e anche equine, come il cavallo, mentre al Nord si mangiano più carni bovine e suine.

Una curiosità non salutista che ci ha allarmati è l'alta presenza di grassi che associata alla ridotta assunzione di pasta e cereali, spesso a causa delle terribili diete iperproteiche, ha sconvolto l'equilibrio che era proprio delle abitudini alimentari di noi italiani.

CO15 - REDOX REGULATION OF CELLULAR STRESS RESPONSE IN AGING AND NEURODEGENERATIVE DISORDERS: ROLE OF HORMESIS AND VITAGENES

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Protein quality control is a critical feature of intracellular homeostasis[1]. In addition, modulation of endogenous cellular defense mechanisms via the stress response signaling represents an innovative approach to therapeutic intervention in diseases causing chronic tissue damage, such as neurodegeneration and cancer[1]. Protein thiols play a key role in redox sensing, and regulation of cellular redox state is crucial mediator of multiple metabolic, signalling and transcriptional processes. Under optimal conditions long-term health protection is accomplished by protein homeostasis, a highly complex network of molecular interactions that balances protein biosynthesis, folding, translocation, assembly/disassembly, and clearance[2,3]. Efficient functioning of maintenance and repair processes is crucial for both survival and physical quality of life. This is accomplished by a complex network of the so-called longevity assurance processes, which are composed of several genes termed vitagenes[4-8]. The term vitagenes refers to a group of genes which are strictly involved in preserving cellular homeostasis during stressful conditions. The vitagene family is actually composed of the heat shock proteins (Hsp) Hsp32, Hsp70, the thioredoxin system and the sirtuin system[9]. Dietary antioxidants, such as polyphenols and L-carnitine/acetyl-L-carnitine, have recently been demonstrated in vitro to be neuroprotective through the activation of hormetic pathways, including vitagenes[10-12]. Over the past decade there has been a remarkable increase of interest in hormesis as a result of more significance being given to low dose effects and the use of more powerful study designs which have enabled to identify rational approaches to detect hormetic biphasic dose responses in the low dose zone. The hormetic dose–response, challenging long-standing beliefs about the nature of the dose–response in a lowdose zone, has the potential to affect significantly the design of pre-clinical studies and clinical trials as well as strategies for optimal patient dosing in the treatment of numerous diseases, including oxidant disorders. Given the broad cytoprotective properties of the heat shock response there is now strong interest in discovering and developing pharmacological agents capable of inducing stress responses. We have recently focused our research on the role of acetylcarnitine in the defense mechanisms against cellular stress and neurodegeneration. In addition, with a redox proteomics approach, we identified mitochondrial oxidatively modified proteins as a function of brain aging, specifically in those brain regions, such as cortex and hippocampus, that are commonly affected by the aging process. In all brain regions examined, many of the identified proteins were energy-related, such as pyruvate kinase, ATP synthase, aldolase, creatine kinase, and a-enolase. These alterations were associated with increased expression of Hsps, as well as carnosinase and thioredoxin reductase and with significant changes in both cytosolic and mitochondrial redox status in all brain regions analyzed. This findings are relevant to potential pharmacological interventions in healthy medicine strategy, pointing to maximize cellular stress resistance of the brain thus providing neuroprotection[9-12], and will be extended to other systemic oxidant disorders such as diabeties and cancer.

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CO16 - PREVENZIONE PRIMARIA, NUTRIZIONE , STRESS OSSIDATIVO E PERCORSI INDIVIDUALIZZATI

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In condizioni fisiologiche, le specie reattive dell'ossigeno (ROS) fungono da 'messaggeri redox' nella regolazione della segnalazione intracellulare, mentre l'eccesso di ROS può indurre, attraverso l' accumulo di mutazioni soprattutto a carico del DNA mitocondriale, l' alterazione della fosforilazione ossidativa, uno squilibrio nella espressione di enzimi antiossidanti con ulteriore sovrapproduzione di ROS, danni irreversibili a componenti cellulari, alla regolazione del metabolismo cellulare, della difesa antiossidante e alla modificazione post-traslazionale di proteine, portando anche alla morte cellulare.
L'eccesso di radicali liberi e l'ipossia cellulare vengono sempre più considerati oggi come fattori in qualche modo responsabili dell'insorgenza di invalidanti patologie cronico-degerative, del sistema nervoso, gastrointestinali, oculari, renali, cardiache, del sistema immune e multi organo. Queste possono essere in larga parte prevenute operando sulla nutrizione, sullo stile di vita e sulla gestione dello stress ossidativo. La prevenzione nel campo della nutrizione sembra rappresentare oggi il più importante intervento possibile per salvarci dai gravi problemi delle malattie cronico degenerative, da alcuni tipi di tumore in particolare e quindi dalle malattie non trasmissibili prima ritenute tipiche del mondo sviluppato ed oggi sempre più in aumento nel mondo in via di sviluppo.
In questo ambito sono sempre più ben accolti e considerati di grande utilità gli studi scientifici su: stress ossidativo, microbiota intestinale, lipidomica e, di conseguenza, le strategie nutrizionali/nutraceutiche adatte ad effettuare interventi che siano mirati e realmente efficaci.

CO17 - PHYTOCHEMICALS AS MODULATORS OF GENE EXPRESSION CONTROLLED BY DNA METHYLATION CHANGES: THE INDICAXANTHIN CASE

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Epigenetics refers to heritable changes that are not encoded in the DNA sequence itself, but play an important role in the control of gene expression. In mammals, epigenetic mechanisms include changes in DNA methylation, histone modifications and non-coding RNAs. Although epigenetic changes are heritable in somatic cells, these modifications are also potentially reversible, which makes them attractive and promising avenues for tailoring cancer preventive and therapeutic strategies. Nutrition plays a key role in many aspects of health directly influencing epigenetic mechanisms in humans and there is a growing realization that some of the dietary polyphenols may exert their chemopreventive effects in part by modulating various components of the epigenetic machinery. In this communication, we present data regarding epigenetic effects of the phytochemical Indicaxanthin from *Opuntia ficus indica* fruit on DNA of intestinal cancer cells. Incubation of proliferating Caco-2 cells with Indicaxanthin (10 □M to 50 □M) remarkably reduced the global DNA 5-methyl cytosine methylation, and caused de-methylation of the tumor suppressor $p16^{INK4a}$ gene promoter, with reactivation of the silenced mRNA expression and accumulation of $p16^{INK4a}$ protein, a major controller of cell cycle. A decrease of the hyperphosphorylated retinoblastoma in favour of the hypophospohrylated status was observed, with unaltered level of the cycline-dependent kinase CDK4. Analysis of cell distribution in the cell cycle phases after Indicaxanthin treatment showed arrest of Caco-2 cells in the S- G2/M-phase.

To rationalize the mechanism of DNA methylation changes induced by Indicaxanthin, we evaluated the effect of the phytochemical on the activity and the level of 5-cytosine DNA methyltransferase (DNMT). Ind induced a dose dependend inhibition of DNMT activity on Caco-2 cells, while did not affect DNMT1 and DNMT3b level. However a significant increment of DNMT3a was evident.

In conclusion, our findings show that Indicaxanthin from cactus pear fruit can arrest growth, revert epigenetic changes and reactivate the expression of the anticancer gene $p16^{INK4a}$ in Caco-2 cells, without toxicity for non-malignant cells. These data stimulate other research on the contribution of Indicaxanthin as a potential epigenetic modulator in the chemoprevention of colon cancer.

CO18 - IDENTIFICATION AND SUSTAINABLE EXPLOITATION OF WILD EDIBLE MUSHROOMS IN RURAL AREAS ("MYCOTICON", LDV-TOI PROJECT): DEVELOPMENT OF AN INNOVATIVE TRAINING PACKAGE TO MEET EDUCAT IONAL AND INCOME-GENERATING DEMANDS IN SOUTH EUROPE AND FOR IMPROVING THE USE OF MUSHROOMS AS HIGH-VALUE FOOD

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In Mediterranean and in southeast Europe the activities of a significant part of the population are traditionally linked with agriculture, forestry and animal husbandry. However, many rural communities are experiencing serious difficulties associated with low income per person and poor employment prospects combined with increased demographic decline. Alternative activities such as the collection and trading of wild edible mushrooms as well as the cultivation of choice species could contribute at providing valuable solutions both in financial and environmental terms.

The total number of fungal species which are considered having edible and/or medicinal value is over 2300 [1]. Most of them form large conspicuous sporophores (i.e. mushrooms) during their life-cycle, which are either harvested from the wild or cultivated on a wide range of plant and agro-industrial residues and by-products. Foraying and picking of wild edible mushrooms has a long tradition in most European countries; therefore it constitutes a significant socioeconomic activity, while at the same time reflects local knowledge and social practices that are worth preserving. Recent food market tendencies reveal a high demand potential for wild edible mushrooms among urban consumers. In those cases that wild fungi are not well-known because pertinent knowledge was not spread within families or local communities, people avoid their harvest; instead they are oriented at consuming cultivated mushrooms which become increasingly popular. This latter type of activity is tightly associated with environmental protection through recycling and valorization of low-value substrates together with the conservation of some highly sought-after mushroom species [2, 3].

The "Mycoticon" project (EU, LdV-ToI) involves Universities, Technological, and Research Institutions as well as local stakeholders and associated end-users from four European countries, i.e. Bulgaria, Cyprus, Greece and Italy. These partners combine their experience and expertise at developing an integrated educational and training package together with its respective tools to meet the demands of suitable target-groups willing to create collective entrepreneurship schemes for exploiting the economic potential of wild mushrooms in rural areas. Ultimately, the objective is to facilitate the generation of a new source of non-subsidized income and create new jobs in areas desperately in need of both. In parallel, local people are expected to be presented with incentives to adopt sustainable management and harvesting practices for wild edible mushrooms together with basic knowledge on mushroom cultivation.

Among other anticipated deliverables, national reports were compiled for each participating country as regards the current knowledge/situation on diversity, harvest and trade of wild edible

mushrooms as well as on commercial mushroom production. In addition, a voluminous textbook was prepared [4] which provided a detailed description of 22 choice edible and 11 selected poisonous mushrooms (together with many other related taxa) of significance in all four countries. Moreover, it included general information about biology and ecology of mushroom fungi, their common habitats/ecosystems, proper harvest practices and suitable food preservation methods, relevant legislation and conservation issues, and basic guidelines for the cultivation of the most popular species together with prospects for developing tourism activities associated with mushrooms. All of them formed the basis for the development of an innovative training material established both on paper and online by creating a moodle web-page (http://moodle.teilar.gr/). This electronic tool was assembled in four languages (English, Italian, Greek and Bulgarian) and it now provides a user-friendly and flexible modular training course through which e-self-assessment and e-accreditation could be also accomplished. The training package complies with EQF rules and it will be further structured according to EC-VET provisions. Its content is anticipated to enhance the development of pertinent skills and subsequently increase employment of qualified people in rural areas. Furthermore, it provides the prerequisites for combining local assets and resources into mushroom products that meet consumers' expectations. Such activities constitute a highly recommended approach in Europe since rural income could derive from integrated direct and indirect recourses (by also supporting conservation and environmental sustainability) and not only by the primary agricultural production.

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CO19 - LE POTENZIALITA QUALITATIVE DELL'OLIO DI OLIVA SICILIANO

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La coltivazione dell'olivo (*Olea europea*, subsp. *sativa*) ha origini remote, riconducibili al 3500 a.C., così come evidenziano i ritrovamenti archeologici di noccioli e legname da costruzione. Si ritiene, però, che già dal 5000 a.C. la pianta di olivo fosse conosciuta ed utilizzata dall'uomo. Altrettanto arduo risulta definire l'areale d'origine. Molti autori indicano l'Asia Minore, e più precisamente la regione compresa tra il Caucaso, l'Iran e le coste della Siria e della Palestina, come areale d'origine dell'olivo. La diffusione dell'olivo avvenne a partire dal XVI secolo a.C. ad opera dei Fenici, che lo introdussero nel bacino del Mediterraneo.
Il concetto di qualità riferito ad un alimento non può che essere dinamico, in quanto tende ad evolversi nel tempo in funzione delle mutate esigenze del consumatore sempre più alla ricerca di prodotti che rispondano a parametri di genuinità, qualità e tipicità. Risulta, quindi, alquanto riduttivo valutare la qualità di un prodotto solo attraverso i parametri di legge.
La Sicilia conta un gran numero di cultivar di olivo che suddivisi in; cultivar principali che costituiscono la base di alcune DOP riconosciute in Sicilia, cultivar minori che prevalgono in ristrette aree olivicole e possono entrare a far parte delle DOP siciliane e infine le cultivar neglette che rappresentano un limitato numero di esemplari a forte rischio di estinzione.
La qualità dell'olio di oliva, in definitiva, è determinata da aspetti ambientali, genetici, agronomici e tecnologici che esercitano la loro influenza sui diversi componenti chimici, sia su quelli saponificabili, sia su quelli insaponificabili. Nell'olio di oliva si ritrova un idrocarburo come lo squalene,che svolge un ruolo di antiossidante.
La qualità di un olio è determinata da fattori derivanti dalle cultivar (genetici), pedologici (caratteristiche chimico-fisiche del suolo), climatici e colturali. Dall'interazione di questi fattori dipendono le caratteristiche chimiche di un olio. Lo scopo del presente lavoro è quello di mettere in evidenza la qualità degli oli di oliva siciliani confrontandoli con oli monovarietali di cultivar spagnole.
Le analisi sono state condotte all'inizio dell'annata olivicola 2012 su 22 campioni di olio d'oliva seguendo il Reg. CEE 1989/03. Tutte le cultivar siciliane hanno riscontato un ΔK di 0,01 mentre alcune cultivar spagnole come la Picual ha ottenuto un risultato pari a 0, discorso molto simile per il K_{232} varialibi tra 1,18 dell'Aitana e un 2,31 per la Picual mentre il K_{270} ha visto come valore più basso quello della varietà Monaca mentre piu alto quello della Hoijblanca.
Per quanto riguarda i polifenoli totali dei campioni analizzati, i valori riscontrati hanno messo in evidenza una forte influenza della componente varietale sull'aspetto in questione. Le quantità di polifenoli variano dai 122 ppm, riscontrati della Manzanilla, e 607 ppm nella Vaddarica. Sono risultati carenti in polifenoli i campioni delle cultivar Aitana (242 ppm), Crastu (143 ppm) per le cultivar siciliane e Manzanilla (122 ppm) tra le cultivar spagnole.
Tali valori suggeririscono una ridotta conservabilità del prodotto. Per quanto riguarda la clorofilla ed i carotenoidi, i campioni analizzati hanno mostrato una forte variabilità riconducibile oltre che alla varietà anche al diverso grado di maturazione. La composizione acidica dei campioni è riportata nell'allegato 1. I dati riguardanti gli acidi grassi evidenziano percentuali in acido oleico comprese tra valori del 65,54% nella cultivar Abunara e 72,24% nella cultivar Verdello. In particolare, sono stati riscontrati contenuti elevati, superiori al 70 %, nelle cultivar Iacona, Biancolilla, Pizzutella, Monaca e Verdello.
Al contrario, la Barilara, l'Arbequina, la Manzanilla, l'Hojiblanca, hanno evidenziato contenuti di acido oleico inferiori al 65%, quindi con dotazione non ottimale. Sono stati riscontrati elevate percentuali di acido linoleico in Bottone di gallo, Monaca, Murtiddara, Pirunara, Verdello,

Biancolilla, Moresca dischi, Barilara, Nocellara belice, Erbano, Hojiblanca, Picual, Manzanilla, Arbequina,. Complessivamente il contenuto in acido linoleico è compreso tra il 12,64% della Royal de Cazorla ed il 7,44% della Pizzutella.
Per quanto riguarda lo squalene è stato rintracciato in quantità variabili dal 1,47% nella Montonica allo 0,14% nella Monaca. Valori minimi sono stati riscontrati anche in Verdello (0,16%), mentre i valori più elevati appartengono alla Montonica (1,47%), Vaddarica (1,45%), Crastu (1,36%) e Nebba (1,05%). Dall'indagine condotta su oli monovarietali di cultivar autoctono siciliano e spagnolo, è emerso che tutti gli oli rispettano i parametri europei di legge, ma al tempo stesso è stata riscontrata una diversa qualità complessiva che contrappone gli oli isolani a quelli iberici, a vantaggio dei primi.

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CO20 - INTEGRATORI NELLA SINDROME METABOLICA

L. Lozio

CO21 - PHYSIOLOGY OF NEMATOCYTES

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Nematocytes are high specialized cells from Coelenterates Cnidaria. They synthesize the nematocyst, a secretory product of Golgi apparatus, consisting of a capsule wall containing an inverted tubule and a capsule fluid in which various toxins are stored. The most relevant aspects of these cells are: *i*) the biophysics and control of discharge mechanism; *ii*) toxicology of venom and impact on humans; *iii*) cell volume regulation capability. These features underlie both aggression and defence strategies of Cnidaria, and, hence, their survival since Precambrian to date.

A general description of these features is provided by this presentation.

i) Under an adequate chemico-physical stimulus, the nematocyte ejects the tubule that in turn either adheres to or penetrates into the prey, injecting the venomous substances contained in the capsule fluid. This phenomenon is called discharge and it is the most rapid process of exocytosis known today. The complete mechanism of discharge involves signal recognition, transmission, transduction and response. It is observed both *in situ* and in isolated nematocytes and is under Ca^{2+}control.

ii) Toxicological aspects of Cnidaria have been extensively investigated owing to their notable impact on public health. The accidental contact of bathers with Cnidaria may induce both severe local and systemic pathologies, due to the release of venom. Research suggests that the large variety of envenomation should be ascribed mainly to either an aspecific change in membrane permeability or to a specific action of venoms on voltage-dependent channels. To prove the toxicity of capsule fluid, different types of biological assays have been performed, such as cytolytic assay on cultured cells and hemolytic test, which is the most used.

iii) The maintenance of cell volume in an anisosmotic medium is essential for cell survival and has been observed in a number of different cells. After exposure to hyposmotic medium (35% shock), cell volume of nematocyte increases rapidly, owing to water influx, and, thereafter decreases more or less slowly towards the control value, showing regulatory volume decrease (RVD). By replacing the hyposmotic medium with an isosmotic one, the cell volume decreases rapidly, reaching the control value. RVD mechanisms consist mainly in an increased conductance to K^+ and consequently Cl^-, as verified by specific inhibitors as quinine, NPPB, DIDS, gramicidin, and are triggered by Ca^{2+} from intracellular stores or from the external medium. Nematocytes also regulate their volume in hypertonic conditions (45% shock). After the exposure to hypertonic medium, cell volume decreases, owing to water efflux, and then increases towards the control value. Such a response is termed regulatory volume increase (RVI). RVI mechanism is mainly due to $Na^+/K^+/2Cl^-$ cotransport, as verified·by bumetanide and sodium free medium. Cell volume regulation, both in hypertonic and hypotonic conditions, show the morphological integrity and physiological viability of cell.

Based on these features, nematocytes isolated from jellyfish and sea anemones are a suitable model to perform physiological and toxicological investigations.

CO22 - THE ACUTE PHASE RESPONSE OF ATLANTIC COD (*GADUS MORHUA L.*): EXPRESSION OF IMMUNE RESPONSE GENES AFTER INFECTION *WITH AEROMONAS SALMONICIDA* SUBSP. *ACHROMOGENES*

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The Acute Phase Response (APR) is a core part of the innate immune response and is present in all animal species including mammals, fish and invertebrates. APR is a reaction of the body to injury, trauma or infection and the basic aim is to restore homeostasis. The APR can appear as a local reaction but mainly as systemic reactions including increased secretion of some hormones, activation of the complement system and increased synthesis and secretion of Acute Phase Proteins (APP).[1] Approximately 40 plasma proteins have been defined as APP.[2] Those proteins can have various functions and change in their plasma levels. APP's are classified as positive when their plasma concentration increases and negative when their plasma concentration decreases following the APR. Studies suggest that fish have an APR response that is delayed compared to mammals.[3,4] APR has been studied in several fish species and a large number of APPs have been identified in fish. In this study, APR was induced in Atlantic cod (*Gadus morhua L.*) by intramuscular injection of *Aeromonas salmonicida subsp. achromogenes* (Asa), a common bacterial pathogen in cod and other fish species, causing atypical furunculosis.[5] Asa is endemic in Iceland and caused serious losses of farmed salmonids before vaccination became a common practice.[6] Commercial Asa vaccines are as yet not available for cod and experimental vaccines show variable and often poor protection.[7] The aim of the current study was to examine the acute phase induced by Asa by mesuring the gene expression in cod's kidney and spleen as well as cortisol levels in serum. Cod, mean weight 97.3 g, were divided into three groups: two groups received intramuscular injections of two different concentrations of Asa and the third, a control group, was injected intramuscularly with PBS. Kidney and spleen were sampled from seven fish from each group at time 0 before treatment and at 1 hour, 24 hours, 72 hours and 168 hours after the injection. Blood was collected from the caudal vessel. An ELISA assay (Neogen Corp KY,USA) was used to measure cortisol. For the gene expression analysis of IL-1β, hepcidin and transferrin, total RNA was isolated from tissue samples from kidney and spleen with a NucleoSpin® RNA II kit following the manufacturer's instructions (Macherey-Nagel). Complementary DNA (cDNA) was prepared with the Revertaid™ First Strand cDNA Synthesis Kit, according to the manufacturer's instructions (Thermo). Quantitative real time PCR (qPCR) analyses were performed on a StepOne Plus™ real time PCR instrument (Applied Biosystems). The gene expression data were normalized to the expression of the reference genes ubiquitin or RPL4 with identical results. The expression of the reference genes in the tissue samples used was relatively stable. The results for gene expression in the controls and Asa injected fish were compared at each time point. Overall, the gene expression results showed a stronger response in the spleen than in the kidney. In Asa injected fish there was a significant increase in IL-1β and hepcidin gene expression at 24h, compared to controls, in both organs. Transferrin gene expression was also significantly elevated in both organs, reached a maximum peak at 72h in the kidney and at 168h in the spleen (Tab.1). The results of cortisol analysis showed a statistically significant increase of cortisol levels with a peak at 72h after injection (Fig.1). At the end of the experiment the cortisol levels were significantly elevated compared to the control fish. IL-1β is one of the earliest pro-inflammatory cytokines to respond to infection and induces a cascade of reactions leading to inflammation.[8] The observed early increase in IL-1β gene expression following an acute phase induction with Asa was in agreement with other studies in fish. The serum cortisol levels observed in this study reached a maximum concentration at 72h when the

IL-1β gene expression had started to decrease. This could mean that cortisol had a role in the suppression of the IL-1β gene expression as described by other.[9] Transferrin is the major iron binding protein and hepcidin is an antimicrobial peptide as well as an APP and both have central roles in the iron metabolism of the host. Iron acquisition is important during bacterial infections as it is essential for bacterial growth. The host responds by increasing gene expression of hepcidin and transferrin, especially in the spleen. In conclusion: Infection by Asa resulted in a significant increase in the stress hormone cortisol in the early stages of infection that stayed high until the end of the experiment. Hepcidin and IL-1β showed a strong response in spleen, with similar curves in both organs. Transferrin expressions increase significantly at 24 hours in both organs and remained significantly elevated throughout the experiment. The next steps will include gene expression in liver samples and iron levels measuring in serum at each time point.

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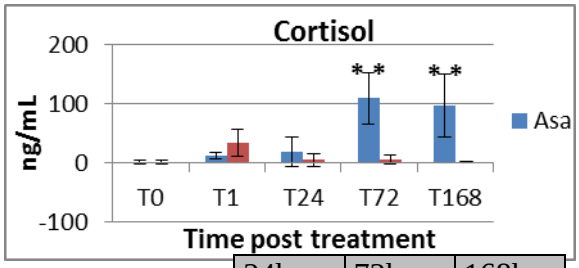

Fig.1 Bar graphs that shows the mean (+/-SD) serum cortisol concentration of untreated control fish or fish injected i.m with Asa and sampled at different times after injection. The asterisks indicate a significant difference (P<0.001) in the cortisol levels of the Asa treated fish compared to the control fish at the same time point.

		24h	72h	168h
IL-1β	spleen			
	kidney			
hepcidin	spleen			
	kidney			
t-ferrin	spleen			
	kidney			

Table 1. Expression patterns of the genes Il-1β, hepcidin and transferrin of both organs at main time points. The max (maximum) indicates the time point where each gene reached the maximum peak.

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CO23 - PHYSIOLOGICAL ROLE OF AMYLOID-BETA PEPTIDE AND HIS IMPLICATION IN ALZHEIMER DISEASE

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Amyloid-beta (Aβ) is a peptide produced in high amounts in Alzheimer's disease (AD) causing impairment of synaptic long-term potentiation (LTP), a widely studied cellular model of learning and memory, and cognition. However, Aβ is present in normal brains at low concentration but its physiological function is still poorly known. Recently, we have demonstrated that low picomolar amounts of exogenously applied Aβ42 enhance LTP in vitro and memory in vivo and that endogenous Aβ is necessary for synaptic plasticity and memory on mice. Moreover, we showed that Aβ action involves alpha-7 nicotinic receptors, which are known to be involved in different brain functions including synaptic plasticity and memory. Indeed, our findings strongly support a model for Aβ effects in which low concentrations play a novel positive, modulatory role on neurotransmission and memory, whereas high concentrations play the well-known detrimental effect, culminating in dementia. The knowledge of both the physiological role and the hormetic effect of Aβ (a biphasic dose-response phenomenon characterized by low-dose stimulation and high-dose inhibition), together with the clinical failure of anti-Aβ based therapy, raises several criticisms to the approaches aiming to decrease Aβ load, especially when suggested as prevention of the disease in healthy subjects.

CO24 - EFFECTS OF *PELAGIA NOCTILUCA* CRUDE VENOM ON CELL VIABILITY AND VOLUME REGULATION

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Among Cnidaria, *Pelagia noctiluca*, is one of the most dangerous jellyfish in the Mediterranean Sea, where its blooming has been very abundant for many years. Toxicology of crude venom from *P. noctiluca* stinging cells is reported in this presentation. Both *in vivo* and *in vitro* biological assays have been performed to verify and, possibly, measure the toxicity of *P. noctiluca* crude venom, whose composition is still not completely defined.
At first we tested the hemolytic activity of crude venom from single nematocysts discharged by a chemical non enzymatic method. The delivered venom induced a powerful and rapid hemolytic activity. As a second step, crude venom extracted from a population of isolated nematocysts, provoked a dose-dependent hemolysis in erythrocytes from different sources, including eel, rabbit, chicken and human. Moreover, *P. noctiluca* crude venom directly induced mitochondrial trans-membrane potential ($\Delta\Psi m$) collapse and Reactive Oxygen Species (ROS) generation in SH-SY5Y cells derived from human neuroblastoma.
In order to better characterize the biological effects of the crude venom, *in vivo* assays were also performed. Injection of crude venom into the rat paw evoked an inflammatory reaction in a dose-dependent manner. Immunohistochemical analysis showed a marked acute inflammatory response in the tissues, with accumulation of polymorphonuclear neutrophils. Treatment with melatonin as antioxidant significantly reduced the inflammatory response, thereby confirming that oxidative stress plays a major role in inducing the observed pathological changes.
In addition to hemolytic and cytolytic assays, a test on cell volume regulation capability was also chosen to describe the biological activity of *P. noctiluca* crude venom. As already demonstrated, isolated nematocytes of the sea anemone *A. mutabilis* exhibit Regulatory Volume Decrease (RVD) when stimulated with a 35% hypotonic solution. In nematocytes exposed to different concentrations of crude venom (corresponding to the amount contained in 10, 25 and 50 nematocysts/µl) RVD was partially inhibited 25 nematocysts/µl crude venom concentration and fully blocked at 50 nematocysts/µl. In the presence of 1□M gramicidin, RVD capability was completely recovered, therefore indicating that K^+ channels inhibition may account for the venom-induced RVD impairment. RVD tests were also performed on HEK293 Phoenix cells, a human embryonic kidney cell line. In control conditions, the cells stimulated by hypotonicity showed an initial swelling followed by RVD, whereas in 0.025 µg/µl crude venom-containing extracellular hypotonic solution, RVD was dramatically impaired. Furthermore, pre-incubation of cells in a crude venom-containing extracellular isotonic solution prevented RVD after hypotonic stress. Surprisingly, the presence of toxin in the extracellular isotonic solution led to cell swelling even in the absence of an osmotic gradient. This phenomenon was not observed in control conditions and the precise mechanism needs to be further elucidated.
We conclude that *P. noctiluca* crude venom extract has hemolytic activity, pro-inflammatory action, induces mitochondrial potential collapse and ROS production. In addition, crude venom inhibits RVD in both cnidarians and mammalian cells after hypotonic stress and leads to cell swelling in isotonic conditions. Our experiments add novel information to understand the mechanism of action of *P. noctiluca* venom.

CO25 - MEDITERRANEAN SEAWEEDS AS POTENTIAL DRUGS AGAINST LEISHMANIASIS

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Marine algae are a great sources of natural products that play an invaluable role in the drug discovery process. Many reports have been published about isolated compounds from algae with biological activity, demonstrating their ability to produce metabolites different from those found in terrestrial species, with high complexity and unlimited diversity of pharmacological and/or biological properties [1-4].
Among these, halogenated molecules and sulphated polysaccharides, produced by green, brown and red seaweeds, are numerous and with various structures. They are widely used in food and cosmetic industries, but also acknowledged as endowed with a rather low toxicity and numerous biological activities, including antiviral, anticoagulant, anti-tumoral, antimetastatic and anti-inflammatory effects, worthwhile for clinical uses [5-15]. Therefore, applications of macroalgal products are more and more frequent both in human and veterinary medicine
In this study, sulphated polysaccharides and biometabolites were extracted from different species of macroalgae: *Chaetomorpha linum* (O.F. Müller) Kützing, *Gracilaria bursa-pastoris* (S.G. Gmelin) P.C. Silva, *Gracilaria viridis* Sfriso, Wolf, Sciuto, M. Morabito, Andreoli et Moro, *Agardhiella subulata* (C. Agardh) Kraft et M.J. Wynne, *Hypnea cornuta* (Kützing) J. Agardh, *Asparagopsis taxiformis* (Delile) Trevisan, *Sargassum muticum* (Yendo) Fensholt and *Undaria pinnatifida* (Harvey) Suringar. The samples were collected in Venice Lagoon, in Lake Ganzirri and in Strait of Messina (Italy) and the algal extracts were tested against the protozoan *L. infantum* (MHOM/IT/80/IPT1), the prevalent agent of Leishmaniasis in the Mediterranean, endemic in Italy.
Leishmaniasis is a disease with a worldwide distribution affecting both humans and animals. There is increasing awareness that drug treatment can be complicated by variation in the sensitivity of *Leishmania* species to drugs, variation in pharmacokinetics, and variation in drug-host immune response interaction [16, 17].
Preliminary results showed that this algal extracts had remarkable antileishmanial activity revealing the studied species as a great source of natural antiprotozoal products.

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CO26 - REVERSE PHASE PROTEIN MICROARRAY TECHNOLOGY TO PROVIDE NEW DIAGNOSTIC MARKERS OF METABOLISM IN RARE DISEASES

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Energy metabolism offers a valuable gauge to monitor the genetic alterations that promote cellular dysfunction and hence, is a useful biomarker in human Rare Diseases.
Genetic alterations that result in cellular dysfunction and thus in an overt phenotypic presentation are usually accompanied by alterations in the proteome of energy metabolism. Cancer provides one example (1). The development of high-throughput OMIC techniques allows the simultaneous interrogation of a large number of genes, proteins and metabolites in the same assay. Reverse phase protein microarrays (RPPmA) is a high-throughput proteomic technique that allows the quantification (fentomolar range) of a given marker in minute amounts of protein from biological specimens. The application of this technique in oncology has been largely documented as it is most useful for the identification and quantification of biomarkers of survival and of the response to chemotherapy. Herein, we have studied the expression of twenty proteins of energy metabolism which include members of the TCA cycle, β-oxidation, electron transport, oxidative phosphorylation, glycolysis and oxidative stress using highly specific antibodies in a cohort of seventy three muscle biopsies of control donors and patients affected of neuromuscular diseases. The cohort included Duchenne (DMD), Becker (DMB), symptomatic forms of DMD and DMB in female carriers (Xp21 Carriers) and Limb Girdle Muscular Dystrophy Type 2C (LGMD2C) biopsies as well as of patients affected of glycogenesis type V (Mc Ardle disease), complex I mitochondrial myopathies, various intensive care unit myopathies (ICU) and neuronal ceroid lipofuscinosis (NCL) also known as Batten disease, a neurodegenerative disease. The samples were obtained with informed consent following the Declaration of Helsinki and coded for anonymity. The final aim of the study was to verify the potential applicability of RPPmA technique in the field of Rare Diseases for the identification of new molecular markers of diagnosis to contribute to the improvement of the clinical handling of these patients. The results indicate that the phenotype of energy metabolism offers relevant diagnostic markers in Rare Diseases.

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POSTER

P01 - NUTRITION IN IBD PATIENTS

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Inflammatory bowel disease (IBD) is a chronic disorder characterized by a relapsing-remitting course, which alternates between active and quiescent states, ultimately impairing a patients' quality of life.
The two main types of IBD are Crohn's disease (CD) and ulcerative colitis (UC). CD shows a transmural granulomatous inflammation that can involve any segment of the intestine affecting all layers of the intestinal wall while UC is limited to the mucosa and superficial submucosa of the colon.
In physiological conditions the gut is costantly exposed to various antigens, commensal microflora and pathogens and the inflammatory response is finely balanced. Anyhow in some individuals with genetic susceptibility an anomalous inflammatory response can arise due to the deregulation of the negative feedback mechanisms implicated in its self-regulation.
It is thought that a vast number of environmental risk factors may be implicated in the development of IBD, including smoking, dietary factors, psychological stress, use of non-steroidal anti-inflammatory drugs and oral contraceptives, appendectomy, breastfeeding, as well as infections.
Nutritional support as a primary therapy has a crucial role in the management of patients with IBD since it can control the inflammatory process, treat malnutrition and its consequences, and avoid the use of immune-modulating drugs and their side effects.
The gut microbiota is clearly manipulated by dietary components such as n-3 PUFA and coniugated linoleic acid (CLA) which favorably reduce endotoxin load via shifts in the composition and metabolic activity of the microbial community.
In particular, the beneficial effect of n-3 polyunsaturated fatty acids (PUFAs) and fermentable fiber, during the remission/quiescent phase of both CD and UC is highlighted. In fact, PUFAs are associated with a less grade of inflammation since they are metabolized to 3-series prostaglandins and thromboxanes and 5-series leukotrienes and, in addition, exert antiinflammatory effects when compared with their n-6 PUFA counterparts .
In similar action to dietary n-3 PUFA, coniugated linoleic acid (CLA) have been reported to ameliorate intestinal inflammation in animal models of IBD.
In contrast to corticosteroids, CLA suppresses gut inflammatory responses while enhancing antigen specific responsiveness of T cells against viral and bacterial pathogens.

Available data about nutritional interventions do not always match due to the incomplete knowledge of pathogenic mechanisms underlying IBD development. Further studies are therefore needed to improve nutritional therapeutic approach. In particular, is still unclear the role of the fiber in helping the remission of the disease. There are mainly two theories. On one hand, dietary fibers can act as effective prebiotics by altering the intestinal microbial composition and promoting the growth of beneficial bacterial communities within the large intestine.

Some authors reported a positive effect associated with the production by colonic microflora of short chain fatty acids (SCFA), able to down-regulate the production of pro-inflammatory cytokines, to promote the restoration of intracellular Reactive Oxygen Specie (ROS) balance, and the activation of NF-kB.

On the other hand, fibers can promote diarrhea, pain and gas aggravating the clinical state. We suggest that the consumption of fermentable fibers may have a good impact on patients' health.

Now is well known that various SNPs are linked to the risk of IBD development and therefore there is the possibility of predict if an individual is predisposed to the disease. The identification of some polymorphisms has an essential role because it allows the modification of diet in the hope of controlling symptoms or preventing relapse. As a consequence, foods that can potentially exacerbate symptoms are eliminated and substituted with those that promote a well-being state.

P02 - IDENTIFICAZIONE MEDIANTE SPME /GC/MS DI COMPOSTI VOLATILI NEI MIELI DI DIVERSE ORIGINI, PER LA DETERMINAZIONE DELL'ORIGINE FLORALE

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Introduzione

La composizione del miele è notevolmente complessa, le sostanze fino ad oggi identificate sono oltre 300 e diversi componenti minori non sono ancora noti. Alcuni gruppi di sostanze sono sempre presenti (zuccheri, acqua, sali minerali, acidi organici, enzimi, etc.), ma le proporzioni relative variano in relazione alla composizione del nettare e alla quota di melata da cui il miele deriva. Quindi la natura e l'origine stessa del miele non consentono una standardizzazione rigorosa della sua composizione e giustificano l'affermazione che due mieli identici non esistono. Un'analisi GC/MS dei composti volatili su una serie di mieli, utilizzando la tecnica "Purge and Trap", è stata effettuata da B.S. Radovic et all.[1] ed ha fornito dei risultati soddisfacenti. Scopo del presente lavoro è definire un metodo analitico semplice e relativamente rapido per la tipizzazione dei mieli, impiegando la spettrometria di massa abbinata alla Gas-Cromatografia e utilizzando l'analisi delle sostanze volatili del miele prelevate dallo spazio di testa mediante la microestrazione in fase solida "Solid Phase Microextracion" (SPME). Una review sui vantaggi e svantaggi di questa tecnica applicata al miele è stata già pubblicata[2]. In questo lavoro sono stati analizzati diciassette campioni di miele di quattro diverse origini botaniche: arancio (4 campioni), castagno (3 campioni), eucalipto (6 campioni), timo (4 campioni), allo scopo di distinguerli l'uno dall'altro.

Materiali e Metodi

Quattro grammi di ciascun campione di miele sono stati messi in un vial, questo è stato posto in un bagno ad olio termostatato alla temperatura di 65°C per un tempo di 19 h tenuto sotto agitazione magnetica. Attraverso il setto del vial è stata introdotta nello spazio di testa una fibra SPME ricoperta da un film di 100 μm di polidimetilsilossano (PDMS). Quindi la fibra è stata introdotta nell'iniettore del gascromatografo mantenuto alla temperatura di 250°C e tenuto in condizione di splitless per un tempo di 5 minuti. L'analisi è stata effettuata con il metodo della ionizzazione elettronica per mezzo di uno spettrometro di massa Autospec Micromass (Settore Magnetico e TOF Ortogonale) equipaggiato con un sistema GC HP 6890. Il riconoscimento dei composti è stato effettuato attraverso il confronto con gli spettri di massa riportati nella libreria NIST 2002, inoltre l'analisi degli spettri di massa con l'ausilio di misure in alta risoluzione ha consentito di determinare la struttura di alcuni composti di interesse.

Risultati e discussioni

Il metodo di analisi utilizzato non prevede laboriose manipolazioni del campione e complicati metodi estrattivi e/o di arricchimento. E' da notare che la quantità assoluta dei componenti volatili presenti nello spazio di testa di diversi campioni di miele e separati tramite SPME, è in verità abbastanza piccola, soprattutto nei campioni di miele non fresco. Questo apparente inconveniente si è invece rivelato un fatto positivo, nel senso che ha ridotto il numero di componenti da identificare, alcuni dei quali sono risultati caratteristici del tipo di fiori bottinati dalle api e che quindi, a buon titolo, possono considerarsi dei veri e propri marker, permettendo di distinguere il miele in base all'origine botanica. In tabella 1 sono riportati tutti i composti

trovati divisi in base, alla loro diversa origine botanica e diversa provenienza geografica prevalentemente siciliana salvo 3 di Trento e 2 di Foza (VI) .

Dati cromatografici			**TIPI DI MIELE**															
			ARANCIO				**CASTAGNO**			**EUCALIPTO**						**TIMO**		
RT		**Composti**	**1a**	**1b**	**1c**	**1d**	**2a**	**2b**	**2c**	**3a**	**3b**	**3c**	**3d**	**3e**	**3f**	**4a**	**4b**	**4c**	**4d**
11.28		Benzaldeide														X			
14,24		Benzacetaldeide	X	X	X											X	X	X	X
15.32		Tetraidrofurano	X	X															
15.49		Alfa,p-dimetil stirene					X	X	X										
16.43		Feniletilalcol														X	X		X
17.26		Benzeneacetonitrile														X			
19.34		$C_{10}H_{10}O_2$														X	X	X	X
19.52		3-Cicloesene-1-	X	X	X	X												X	
21.52		Acido Nonanoico								X	X	X	X	X	X				
21.52		Canfene							X					X					
22.13		Etanone1-(2amminofenil)					X												
22.41		Fenolo-2Etil-5-Metil								X	X	X							
23.28		$C_{10}H_{10}O$														X	X	X	X
24.02		4-metossi-fenil-alcool														X	X		
24.10		Bifenile								X		X							
26.59		Esaidronaftalene										X							
34.37		Benzaldeide Trimetossi														X	X	X	X
35.43		Idrocarburo					X	X	X										
35.53		Acido Dodecanoico	X																
35.60		Naftalene sostituito		X															
36.06		$C_{10}H_{12}NO_2$					X	X	X							X	X	X	
36.23		2 Cicloesene-1-One										X	X		X				
36.36		Benzofuranotetraidro								X	X	X	X	X	X	X	X	X	X
37.06		2-Cicloesene-1-one								X	X	X		X	X				
39.25		Trimetossibenzil,metil etere								X	X	X	X	X		X			
39.30		Acido tetradecanoico	X	X	X		X											X	
39.46		2 Cicloesene-1-One-			X					X	X	X	X	X	X	X	X		
41.44		Esadecanoato di metile																X	
41.49		$C_{16}H_{14}O$														X	X		
42.21		Acido Esanoico	X	X	X	X												X	
42.51		5 Fenilisochinolina														X			
43.05		Cinnolina,6Metil-4-Fenil														X			

Tabella 1 - Mieli analizzati di differente origine botanica: arancio (**1**), castagno (**2**), eucalipto (**3**), timo(**4**), e di differente provenienza geografica: Foza (**1a**), Termini (**1b**), Trento (**1c**), Vittoria. (**1d**), Etna (**2a**), Termini (**2b**), Trento (**2c**), Erice (**3a**), Foza (**3b**), Partitico (**3c**), San Cipirello (**3d**), Trento (**3e**), Vittoria (**3f**), Erice (**4a**), Termini 1 (**4b**), Termini 2 (**4c**), Vittoria (**4d**).

Conclusioni

Il presente lavoro si inserisce nella vasta tematica della ricerca di un metodo analitico adatto alla caratterizzazione dell'origine florale dei mieli. Il metodo proposto, basato sull'analisi delle sostanze volatili presenti nello spazio di testa di diversi campioni di miele, si è dimostrato promettente consentendo di diversificare le quattro diverse specie di miele. La metodica risulta affidabile, di semplice esecuzione e non necessita di alcuna laboriosa manipolazione dei campioni.

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P03 - TECNICHE DI DEFOGLIAZIONE APPLICATE A VITIGNI E VINI DI NERO D'AVOLA PER LA DETERMINAZIONE DELLE SOSTANZE NUTRACEUTICHE

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Introduzione

Studi condotti sui vini rossi, hanno dimostrato come un loro moderato consumo quotidiano possa ridurre l'incidenza delle malattie cardiovascolari[1] perché in essi sono contenute sostanze antiossidanti ed in particolare il transresveratrolo e il piceatannolo[2]. I meccanismi protettivi sarebbero indipendenti dall'effetto di quote equivalenti di alcool contenute nel vino ma riconducibili alle caratteristiche del vitigno e al contenuto di polifenoli, le cui concentrazioni dipendono molto dalla tecnica di vinificazione (flavonoidi, trans-resveratrolo e tannini polimerici)[3]. Con l'obiettivo di migliorare la quantità di polifenoli in una cultivar di Nero d'avola, e stata effettuata su alcuni filari di un Vigneto sito presso Castelbuono (PA), la tecnica di defogliazione eseguita alla fase fenologica di acino pisello su un campione di 500 piante con l'eliminazione manuale delle foglie fino al primo nodo sopra il grappolo. I confronti sono stati eseguiti con un testimone non defogliato (controllo).

Materiali e Metodi

Il contenuto in solidi solubili (°Brix) è stato determinato con un rifrattometro di Abbe. Il pH è stato misurato con piaccametro. Per la determinazione degli antociani totali, le bucce degli acini sono state poste in un tampone tartarico a pH 3,2 al fine di estrarre tutti i polifenoli, l'acidificazione del campione è stata eseguita con HCl conc., la diluizione con etanolo cloridrico, l'analisi spettrofotometrica (UV) misurando l'assorbanza a 540 nm utilizzando cuvette in quarzo con cammino ottico di 1 cm. Per la determinazione dei flavonoidi totali si misura la densità ottica a 280 nm e l'assorbanza dipendendo dalla concentrazione. La determinazione delle proantocianidine viene fatta per differenza fra l'assorbanza a 540 nm, misurata prima e dopo l'idrolisi e confronto del Δ di assorbanza con una curva di calibrazione determinata con soluzioni standard di proantocianidine. Lo studio della componente polifenolica è stato eseguito su campioni di bucce e polpa, acino e raspo, rachide e vino. Le analisi sono state condotte con tecnica HPLC con cromatografo liquido Agilent 1100, rilevatore a serie di diodi (DAD) posizionato a 305 e 325 nm, autoiniettore con volume di iniezione 50 µl, colonna Phenomenex Luna C18, eluenti: acetonitrile e tampone fosfato (KH_2PO_4 + H_3PO_4) 0,02 M a pH=3,0, flusso 1,0 ml/min., per calcolare i tempi di ritenzione sono stati preventivamente iniettati i rispettivi standard. Buccia e polpa, acino intero e raspo, rachide hanno subito lo stesso trattamento: macerazione con CH_3OH al 95%, omogeneizzazione e agitazione tramite agitatore orbitale, filtrazione con filtro in microfibra di vetro, evaporazione in rotovapor, prima estrazione con $NaHCO_3$ al 5% seguita da tre estrazioni con acetato di etile, evaporazione dell'estratto e risospensione con CH_3OH 80%. Sul vino sono state effettuate le analisi in HLPC senza trattamenti preliminari.

Risultati e discussioni

Gli obiettivi dello studio sono stati, analizzare l'andamento dei componenti dell'uva nelle differenti tesi e valutare l'influenza dei fattori studiati sul biochimismo dei polifenoli.
Dalla determinazione nel vino del contenuto di antociani totali (controllo = 201 mg/l, defogliato = 267), flavonoidi totali (controllo = 1832 mg/l, defogliato = 2122) e proantocianidine (controllo = 1761 mg/l, defogliato = 1767), si deduce che l'aumento di esposizione degli acini nelle prime

fasi di sviluppo ha prodotto un incremento degli antociani e degli altri flavonoidi. La defogliazione ha però causato uno squilibrio nelle dinamiche di maturazione, maturità fenolica raggiunta prima di quella tecnologica. La tesi controllo si è comportata in maniera più equilibrata presentando dinamiche di maturazione più regolari.

Dai parametri di altre analisi riportati, in Tabella 1, si evince che la tecnica della defogliazione non ha comportato un aumento di antociani, flavonoidi e prontocianidine nelle bucce.

	16 Agosto		24 agosto		19 settembre	
Tesi	Controllo	Defogliato	Controllo	Defogliato	Controllo	Defogliato
Solidi solubili (°Brix)	21,00	21,60	24,35	24,51	24,10	24,51
pH	2,89	2,95	2,98	2,94	3,43	3,37
Acidità titolabile (g/l ac. Tartarico)	13,30	10,70	11,70	10,00	8,10	8,40
Alcool % V	12,20	12,65	14,55	14,70	14,40	14,70
Antociani totali nelle bucce	0,93	1,44	1,47	1,82	1,62	1,51
Flavonoidi totali nelle bucce	2,75	3,54	3,48	4,10	4,10	3,98
Prontocianidine totali nelle bucce	2,23	2,64	2,89	2,39	2,06	2,66

Tabella 1 - La scala in gradi Brix mostra la concentrazione percentuale di tutte le sostanze disciolte nell'acqua. L'indice degli antociani totali (mg/acino) esprime il contenuto di antociani monomeri e polimeri. L'indice dei flavonoidi totali (mg/acino) esprime il contenuto di antociani e tannini. Indice di prontocianidine totali (mg/acino).

Dall'analisi della componente di alcuni polifenoli, Tabella 2, si può notare che il trattamento di defogliazione effettuato ha comportato un aumento delle concentrazioni dei composti piceatannolo glicosilato, trans-resveratrolo glicosilato e piceatannolo negli acini di grappoli provenienti da viti defogliate mentre nel vino i valori relativi agli stessi composti nelle le due tesi sono comparabili. Per quanto riguarda il trans-resverratrolo, la sua concentrazione è maggiore nella tesi controllo.

Composto	Vino Controllo	Vino Defogliato	Polpa e Buccia Controllo	Polpa e Buccia Defogliato	Acini e Raspo Controllo	Acini e Raspo Defogliato	Rachide Controllo	Rachide Defogliato
Piceatannolo Glicosilato	0,44	0,45	78,39	93,58	0,21	0,34	13,72	10,74
Trans-Resveratrolo glicosilato	0,16	0,15	39,37	55,92	46,60	51,52	57,13	40,51
Piceatannolo	0,17	0,15	3,58	5,48	3,045	4,19	91,32	12,53
Trans-Resveratrolo	0,20	0,13	0,130	0,03	0,04	nd	0,13	0,15

Tabella 2 - Analisi della componente polifenolica. Concentrazione espressa in ng / µl

Conclusioni

Le analisi condotte per la determinazione dei flavonoidi hanno evidenziato che il controllo ha presentato dinamiche di maturazione più regolari. Ciononostante, il vino della tesi defogliata ha presentato un maggior contenuto di antociani. Le analisi condotte sugli stilbeni hanno evidenziato che i valori del piceatannolo glic., trans-resveratrolo glic. e piceatannolo sono confrontabili nel vino (defogliato e controllo), mentre si trovano in maggiore concentrazione nel grappolo defogliato. Il trans-resveratrolo è invece presente in maggiore quantità nel controllo. La defogliazione influisce più sull'uva rispetto al vino.

In base ai dati ottenuti, considerando le condizioni pedo-climatiche del luogo dove è stata eseguita la sperimentazione, la defogliazione risulta essere una operazione non necessaria, non influenzando la componente polifenolica del prodotto finale.

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P04 - A 3D TOTALLY ASSORBABLE SYNTHETIC MESH IN ANTIREFLUX SURGERY: GORE BIO-A TISSUE REINFORCEMENT FOR HIATAL HERNIA REPAIRING

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Introduction
Hiatal hernia, defined as "transitory or stable dislocation of a part of the stomach in mediastinum through the diaphragmatic crura delimiting esophageal hiatus". Its appearance presupposes anatomic anomalies or weakening of structures and mechanisms able to maintain esophago-gastric junction and stomach in the abdominal cavity [1]. Classically hiatal hernia was classified in four types using Hill's classification: Type 1 hiatal hernia is associated with GERD in 50-90% of cases, in facts its presence gradually compromises esophago-gastric junction's continence favouriting the backwater of acid secretion and its reflux in contact with esophageal mucosa during transient relaxations of the LES and also reducing clearing systems overall for large hiatal hernias [2, 3]. Several randomized controlled trials with long-term follow-up comparing surgical with medical therapy for the treatment of GERD, strongly support surgery as an effective alternative to medical therapy [4]. Fundoplication has also been demonstrated to lead to improved or at least comparable quality of life to that of medically treated patients and it is associated with high patients satisfactions rate [5]. A laparoscopic total fundoplication is considered today the procedure of choice increasing the resting pressure and length of the lower esophageal sphincter, decreasing the number of transient LES relaxations and improving quality of esophageal peristalsis and follow-up demonstrates complete symptoms control in 80-90% of patients 10 years later [6]. However primary laparoscopic hiatal hernia repair is associated with up 42% recurrence rate [7]. Several level data suggest that mesh reinforcement of the crural closure for hiatal hernia repair decreases the recurrence of hernia, but can lead to esophageal erosion and stenosis or disphagya, above all non-absorbable mesh [8, 9]. For this clinical case, we experiment a new totally absorbable Gore Bio-A® mesh [10].

Materials and methods: Clinical Case
Female patient; 65-year old; 6-year classic history of GERD (regurgitation, belching, bloating, "acid in the throat" treated for several years by multiple proton pump inhibitors); BMI 22. An EGDS revealed a > 3 cm hiatal hernia, grade B Los Angeles esophagitis. 24-hour pH study was positive for acid reflux and esophageal manometry revealed LES intrathoracic dislocation. With laparoscopic 5-trocars approach, the hiatal hernia defect was identified and primarily repaired, by crural closure, with size 0 permanent suture (ETHIBOND). GORE BIO-A® Tissue Reinforcement was trimmed to fit the defect with a "U" shape cutout to accommodate the esophagus. It was secured using two absorbable sutures (VICYRL). At least A Nissen fundoplication was performed without incident. Result: Gore BIO-A® mesh was easily placed through a 10-12 mm trocar. It had good handling characteristics laparoscopically, and no pre-operative preparation was required of the prosthetic. It can be cut and tailored intraoperatively to an optimal adaptation. There were no short-term complications from the mesh. The patient had not significant post-operative sequelae.

Conclusion

Crural closure reinforcement during hiatal hernia repair can be done readily with this new totally absorbable Gore Bio A Tissue Reinforcement: it is a 3D web of completely absorbable synthetic polymers replaced by soft tissue over six months; it is a mix of glycolic acid and trimethylene carbonate and its function consistes in stimulating collagens deposition and ingrowth of new connective soft tissue [11]. It was demonstrated that Gore Bio-A increases cellular in-growth in 7-30 days more and more previously than biologics mesh; it also increases new blood vessels formation in 7-14 days reaching the greatest vascular in-growth. Instead the biologic meshes gore BIO-A seems to induce the least inflammatory infiltrate. Gore BIO-A tissue reinforcement seems to have all the best characteristics to hernia hiatal laparoscopic repair reducing both recurrence rates and post-operative mesh-related complications, even if several other cases and studies are necessary. However further data and studies are needed to evaluate long-term efficacy and complications associated with its use.

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P05 - SYNTHESIS AND SAR STUDIES OF NEW 3-METHYL-5-(5-PROPYL-1*H*-1-R'-3-PYRAZOLYL)-1*H*-1-R-4-NITROSOPYRAZOLES AS ANTIMICOTIC AGENTS.

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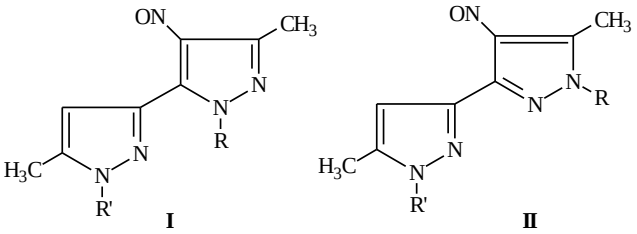
Many ubiquitous yeast are primarily pathogens for immunocompromised patients, individuals with AIDS and organ transplanted are at high risk of cryptococcosis and candidiasis.
In this setting, fungal infections are particularly difficult to treat because antifungal therapy usually does not eradicate the infection and require lifelong treatment with antifungal drugs.
Consequently, the need for novel antifungal agents for opportunistic infections is apparent in light of significant problems associated with current drugs and makes the development of new drug entities all more urgent.
We have reported that some 3-(3-alkyl-4-nitroso-1*H*-5-pyrazolyl)-5-R-isoxazoles [1] and the isomeric 5-(1-alkyl-4-nitroso-1*H*-3-pyrazolyl)-3-R-isoxazoles [2] showed in vitro potent antifungal activity at non cytotoxic concentrations.
This antifungal activity was correlated to: 1) the interaction of the isoxazolic nitrogen with the alkyl group bound to the pyrazolyl nitrogen; 2) the *cis* or *trans* configuration adopted by the nitroso group with respect to the alkyl chain bound to the pyrazolic nitrogen and perpendicularly folded to the molecular plane.
To verify this hypothesis, we synthesized compounds in which the isoxazole was substituted by a pyrazole moiety, leading to the new isomeric series **I** and **II**.



R':$CH_2CH_2CH_3$; R: a=H, b=CH_3; c=CH_2CH_3

The title compounds tested *in vitro* for antifungal activity against C. Neoformans and C. Krusei, displayed an interesting antifungal activity, in particular compound **Ib** was 2 and 32 fold more potent than Amphotericin B and Fluconazole, respectively, against *C. krusei,* fungus with intrinsic resistance to many of antifungal azoles
These results suggest that, depending on the heterocyclic molecule bound to the 5 position of 1*H*-1-R-4-nitrosopyrazoles, it is possible to modulate the antifungal activity of 4-nitrosopyrazoles.
In vitro metabolism studies and in vivo assay are in progress for all described compounds.

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P06 - DISTINCT BIOLOGICAL EFFECTS ARE OBSERVED IN HT-29 COLORECTAL CARCINOMA CELLS INDUCED TO EXPRESS K-RASG12V OR K-RASG13D

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p21 RAS are small membrane-bound GTPase proteins involved in signalling pathways that regulate proliferation, differentiation and apoptosis in all cell types, and whose activating mutations have oncogenic effects. The three major isoforms of p21 RAS (H-, K- and N –RAS) have a high degree of homology, especially in the regions involved in interactions with GDP/GTP and with regulatory proteins and effectors, but differ significantly in the C-terminal 25 amino acids region. This hypervariable domain is the site of post-translational modifications specific for each isoform, which result in distinct intracellular trafficking routes and final subcellular localizations, where the type and concentration of regulators and effectors may differ (1). This may explain the observed non-overlapping functions of these proteins, the different biological effects of their physiological activation, and their differential involvment, when mutated, in specific tumor types. In almost all cases, the genetic alterations detected in tumoral cells are missense point mutations in codons 12 or 13, more rarely in codon 61, and they always result in a constitutively active protein by inactivating its GTPase activity. Mutations in the K-RAS isoform are a frequent, early event in colorectal tumorigenesis and their occurrence is considered to be a resistance factor to therapies based on anti-EGFR monoclonal antibodies (2). However, molecular epidemiological studies in different primary and metastatic tumors suggest that mutations in different codons or different mutations in the same codon of Ras may have diverse biological consequences (3) and may lead to a different response to drug treatments. In particular, one of the new drugs developed for the treatment of colorectal carcinoma is Cetuximab, a monoclonal chimeric human/mouse antibody IgG1, which acts against the extracellular domain of EGFR. The binding of this antibody to the receptor causes a direct inhibition of its tyrosine kinase activity resulting in the inhibition of several pathways of signal transduction mediated by RAS, such as those of PI3K/AKT, and RAF/MAPKs. This stimulates pro-apoptotic mechanisms and the inhibition of cell proliferation. Several clinical trials conducted in recent years have shown that patients with CRC who have mutations in K-ras codon 12 or 13 respond heterogeneously to Cetuximab treatment and for this reason are currently excluded from treatment with this drug. However, it has recently been reported that tumors bearing the K-RASG13D mutation may show some response to the therapy (4). It is also currently unclear whether mutations in BRAF (an effector of RAS) affect the response to Cetuximab (2).
To shed more light on the molecular mechanisms responsible for the different effects of Ras mutations, we established an experimental system by isolating stable clones of HT-29 cells (a human colorectal adenocarcinoma cell line characterized by mutations in the BRAF and PIK3CA genes and in which the endogenous Ras genes are wild type) transfected with cDNAs codifying K-RASG12V (clone K12) and K-RASG13D (clone K13) under the control of a Mifepristone-inducible promoter. Cell proliferation assays and cytofluorimetric analysis reveal that activation of the expression of K-RASG12V and of K-RASG13D have distinct biological effects on the cells. We have also analysed the response of the induced and not induced cells to treatment with inhibitors of the two main RAS effectors (MEK and PI3K) and with the anti-EGFR monoclonal antibody Cetuximab.

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P07 - VALUTAZIONE DEGLI EFFETTI ANTIOSSIDANTI DEL RESOLVIS OMEGA™ IN UN MODELLO DI DISFUNZIONE CORNEALE *IN VITRO*.

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EPA (acido eicosapentaenoico) e DHA (acido docosaesaenoico) sono derivati metabolici degli AGE (acidi grassi essenziali) e in particolare dell'acido linolenico (omega-3). Gli acidi grassi omega-3 (DHA ed EPA) sono considerati AGE perché il nostro organismo non è in grado di sintetizzarli e il loro apporto è possibile solo attraverso l'assunzione di alimenti che li contengono.
Gli acidi grassi omega-3 sono molecole antinfiammatorie e come tali sono state associate ad una riduzione del rischio cardiovascolare e delle patologie correlate ad una inappropriata attivazione del sistema immunitario. Grazie alle loro caratteristiche chimico-fisiche contribuiscono a mantenere la "flessibilità" delle pareti dei vasi arteriosi, delle membrane cellulari e a ridurre i livelli di lipidi nel sangue. Infine, un aumento della loro concentrazione nei tessuti e'stato associato ad una inibizione dei processi biochimici associati alla senescenza e alle patologie oculari su base infiammatoria e degenerativa (1, 2).
Alla luce delle ormai note interconnessioni tra le reazioni infiammatorie e lo stress ossidativo, il presente studio ha valutato le proprietà antiossidanti del *Resolvis Omega*™, un nuovo farmaco a base di EPA/DHA in un modello sperimentale di disfunzione di cellule corneali umane (HCE) da perossido d'idrogeno, *in vitro*.
Da un punto di vista sperimentale, gli effetti antiossidanti sono stati analizzati, tramite analisi citofluorimetrica delle specie reattive di ossigeno e azoto intracellulari (3).
I nostri risultati dimostrano che HCE stimolate con H_2O_2 200 µM producono ROS intracellulari con una cinetica che raggiunge il picco massimo a 5 ore per poi raggiungere i livelli controllo entro 8 ore. Durante questo intervallo temporale, il pretrattamento delle HCE per 1h con *Resolvis Omega*™ determina una significativa riduzione dei ROS endocellulari a 3, 4, 5, e 6 ore ($P<0.05$) (Figura 1).
Il trattamento con il solo veicolo, in assenza di H_2O_2 e il pretrattamento con il veicolo in assenza di *Resolvis Omega*™, non determina alcun aumento o diminuzione dei livelli di ROS endocellulari, durante l'intervallo di tempo di osservazione (dati non mostrati).
Questi risultati dimostrano che *Resolvis Omega*™ alle concentrazioni usate comunemente nella pratica oculistica, esercita dei significativi effetti antiossidanti e costituisce un solido *rationale* per una ulteriore investigazione delle proprietà antiinfiammatorie di questo farmaco nelle patologie oculari.

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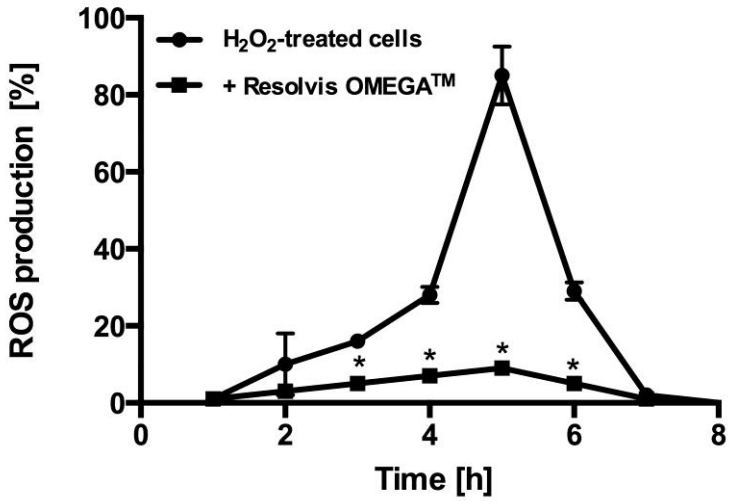

Figura 1.
Valutazione degli effetti del *Resolvis Omega*^{TM} sulla cinetica di produzione di ROS in HCE attivate da H_2O_2. Le cellule sono state stimolate con H_2O_2 (200 µM) in un intervallo temporale compreso tra 0 e 8 h, in assenza e in presenza di Resolvis Omega. I valori sono la media ± SE di 3 esperimenti separati condotti in triplicato.

P08 - CARBOHYDRATE DIGESTIBILITY ON WHEAT DURUM BREAD: PRELIMINARY HYPOTHESES ON RAW MATERIALS ROLE

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The 1997 FAO/WHO Expert Consultation suggested that the Glycaemic Index (GI) might provide an useful help to select the most appropriate carbohydrate-containing foods in order to reduce several diseases. The glycaemic response to food, which in turn affects the insulin response, depends on the rate of gastric emptying, digestion and absorption of carbohydrate from the small intestine, as well as on the effects of the other food factors to potentiate non-glucose mediated insulin secretion. A range of food factors has been identified as important determinant of the glycaemic response to carbohydrate foods. Therefore, different food products or composition of meals with the same amount and type of carbohydrates show differences in glycaemic and insulinemic responses.
In this view, the aim of this research was to identify the technological factors which influence the glycaemic index (GI) in bread making of reground semolina and wholemeal flour. Five ancient accessions (Russello, Timilia, Bidì, Biancolilla and Senatore Cappelli) were compared to five modern commercial blends and an ancient mix between Timilia and Russello accessions under technological and nutritional profiles. The parameters under study were: moisture, protein, total starch, ash, farinographic profile, wet and dry gluten, gluten index, α-amylase activity, particle size and damaged starch.
Our results showed the best attitude of wholemeal flour by old accessions in a health point of view, due to the higher gluten content, poor quality of gluten and greater absorption of water. On the contrary, modern varieties of reground semolina are more suitable for breadmaking, owing to the higher technological attitude and the greater alpha-amylase activity.
This work is considered the first step of a PhD project conducted within the *Università degli Studi di Foggia*.

P09 - ANTICANCER DRUG DELIVERY SYSTEM BASED ON VATERITE PARTICLES

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Photodynamic therapy (PDT) is a promising therapeutic option in the management of cancer and other diseases. The mechanism of photodynamic therapy is based on the selective combination of non-toxic dyes or photosensitizers with harmless visible light which, in the presence of oxygen, leads to the generation of cytotoxic species and consequently to cell apoptotic or necrotic death [1]. Since in clinical PDT it is common practice to irradiate wider fields that include healthy tissues which might contain microscopic malignant foci, the photosensitizer selectivity (target versus healthy tissue) become crucial. Otherwise a high concentration of photosensitizer is required to ensure enough concentration at the tumor site, with consequent incidental toxicity in healthy tissue and increased treatment cost. Both side effects and treatment costs could be significantly reduced by targeted delivery of the photosensitizer to the region of interest at a well-defined time.
In this work we use "Photosens" (a mixture of sulfonated aluminum phthalocyanines $AlPcS_n$, with n=2, 3 or 4) as photosensitizing drug [2] and calcium carbonate ($CaCO_3$) as delivery system. $CaCO_3$ exists in three different anhydrous crystalline polymorphs: calcite, aragonite, and vaterite. Vaterite particles have a large porosity, large surface area, and can decompose fast under relatively mild conditions. Vaterite is the least stable phase of $CaCO_3$ since it slowly dissolves and recrystallizes to form calcite in contact with water. Previous studies described the possibility of synthesizing spherical mono-dispersed vaterite particles in the size range from 2 to 10 μm [3] and could very recently be downscaled to 400 nm [4] which will strongly improve the cellular uptake efficiency. A controllable release mechanism based on a crystal phase transition has recently been demonstrated [5,6]. Preliminary tests have been performed to assess the particles cell toxicity, cell uptake and drug release [5]. Also, as a proof of principle, the pH sensitivity of the delivery system has been demonstrated [7], as well as the possibility to be used as a sensor platform [8].
The release mechanism depending on the surrounding pH has been studied, showing a fast degradation of the carriers in buffers below pH 7. These results hold out the prospect of a novel photodynamic therapy drug delivery system. Variations of particle size or additional coatings allow to custom-design workload release curves. An intrinsic cancer-sensitivity can be expected from the pH-dependent release in the acidic microenvironment of cancer tissue.
During our work, we performed a detailed study of the cumulative release and recrystallization process in different media and in the pH range from 3 to 7. Modification of particle size and pH will allow customizing the workload release curves. A drug delivery system with pH-controlled release promises intracellular delivery with a high selectivity to cancer cells. In combination with an otherwise poorly selective photosensitizer, this could become a strong cancer-therapeutic tool, where the carrier degradability could be tuned to control the rate of drug release.

We investigated the encapsulation efficiency for the photosensitizer in micrometer- and sub-micrometer-sized carriers. Release mechanism dependent on the surrounding pH was studied, showing a fast degradation of the carriers in buffers below pH 7.
These results hold out the prospect of a novel drug delivery system. Cancer-sensitivity can be achieved due to the enhanced uptake and fast release in the low pH endocytic vesicles of viable cancer cells.

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P10 - ASSESSMENT OF THREE DIFFERENT ANTICOAGULANTS AND STORAGE TIME INFLUENCE ON HAEMATOLOGICAL PARAMETERS IN *MUGIL CEPHALUS (LINNEAUS, 1758)*

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Interpretation of fish haematological data is quite difficult due to internal and external variation. It is routinely recommended that haematologic determinations on blood samples are carried out immediately after blood collection, and if not possible, the samples should be refrigerated until determination to minimize artifactual changes.[1] Moreover, as fish blood tends to clot rapidly and clotting becomes faster when it is warm or is under stress condition related to experimental procedures,[2] the use of anticoagulants is necessary to obtain reliable results of blood analyses. The effects of various types of anticoagulants in haematology were studied in various fish species[3,4,] but no information's for *Mugil cephalus* (*M. cephalus*) haematology were found. The aim of the present study was to evaluate the effect of three different anticoagulants and storage time on haematological parameters and differential leukocyte count of *M. cephalus*. Twenty-six adult male mullets (*M. cephalus*), caught from Faro Lake (Sicily, Italy), were immediately subjected to blood sampling from caudal vein. Blood samples were collected in different microtubes containing three different anticoagulants: ethylenediamine tetraacetic acid (EDTA), heparin and sodium citrate respectively. All samples were analyzed immediately (T0) and 24h (+4 °C), (T24), after blood collection to assess the follow parameters: red blood cell count (RBC), haematocrit (Hct), haemoglobin (Hb), white blood cell count (WBC), thrombocyte count (TC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) mean corpuscular hemoglobin concentration (MCHC) and manual differential leukocyte count (Lymphocytes, Monocytes, Neutrophils, Eosinophils and Basophils). Mean values ± Standard Deviation (SD) of studied haematological parameters and the percentage of leukocyte identification and counting were reported in Table 1. Two-way analysis of variance (ANOVA) for repeated measures showed statistical significant effects of different anticoagulants and storage time for some parameters studied. In particular, statistical analysis showed significant effect of anticoagulants on Hct, Hb, TC ($P<0.001$), WBC ($P<0.05$), Lymphocytes and Monocytes ($P<0.005$), and significant effects of storage time (T0 and T24) on MCH, MCHC, TC, WBC, Lymphocytes, Monocytes and Neutrophils ($P=0.005$). By the results of this study, it was found that haematological parameters and differential leukocyte count of *M. cephalus* were influenced by anticoagulants used and that, according to our previous study,[5] haematologic determinations should be assessed within 24 hours after collection, because long-term storage modifies the results of the analysis. All haematological parameters values obtained in EDTA, heparin and sodium citrate treated samples at T0 fall within values obtained in our previous study on *M. cephalus*[5] and leukocytes differential count of the samples treated with the three anticoagulants were within fish reference range.[6] Thus, haematologic determination and leukocyte differential count showed no cellular alteration depending on used anticoagulants. However studied parameters showed a higher reliability using EDTA as anticoagulant. Comparison of literature data indicates that modifications of blood parameters induced by internal or/and external factors, such as anticoagulants and storage time, could represent a species-specific responses. Further studies designed specifically to investigate the impact of different anticoagulants and storage times on these parameters could be still needed in various

fish species to validate an appropriate method for haematological analysis useful for the evaluation of the health status of animal living in captivity and in aquaculture.

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Table 1. Mean values ± Standard Deviation (SD) of haematological parameters and percentage of leukocyte identification and counting obtained in *26 Mugil cephalus.*

	T0			**T24**		
Parameters	***EDTA (M±SD)***	***Sodium Citrate (M±SD)***	***Heparin (M±SD)***	***EDTA (M±SD)***	***Sodium Citrate (M±SD)***	***Heparin (M±SD)***
RBC (x10^6/µL)	2.55±0.4	2.26±0.8	2.34±0.9	2.30±0.6	2.13±0.6	2.28±0.7
Hct (%)	30.14±5.7^{a}	26.89±4.9	25.43±4.8^{a1}	30.22±5.7	24.17±3.0	26.25±5.8
Hb (g/dL)	7.34±1.1	6.13±1.8^{b}	6.54±1.9	7.57±1.0	6.60±1.7^{b1}	7.27±1.6
WBC (x10^3/µL)	8.02±2.1	7.95±3.1	8.03±3.3	7.25±2.0	5.08±2.3^{c1}	7.20±2.9
Lymphocytes (%)	90.8±2.6	90.1±2.6	91.4±3.8	89.8±5.0*****	84.4±7.4^{c1}*****	88.3±6.6*****
Monocytes (%)	2.5±1.0	2.9±2.1	2.9±2.8	3.3±1.9*****	6.0±3.9 ^{c1}*****	4.0±2.6*****
Neutrophils (%)	6.7±2.3	7.0±2.4	5.7±2.3	6.9±3.3	9.2±3.9*****	7.3±4.5*****
Eosinophils (%)	0.0±0.0	0.0±0.0	0.0±0.2	0.0±0.2	0.1±0.3	0.2±0.5
Basophils (%)	0.0±0.0	0.0±0.0	0.0±0.2	0.0±0.2	0.3±0.3	0.2±0.5
TC (x10^3/µL)	22.36±3.9^{a}	24.59±3.6	24.99±3.1	26.25±3.5^{a1}*****	29.86±2.9*****	28.65±3.4*****
MCV (fL)	119.84±22.6	130.67±44.2	117.93±32.3	147.95±69.9	122.10±34.9	121.38±37.2
MCH (pg)	28.89±1.8	27.88±5.2	28.99±3.7	38.39±16.5*****	33.23±10.7*****	34.22±12.5*****
MCHC (g/dL)	24.65±2.8	23.19±7.2	25.65±4.9	25.59±4.0*****	27.56±7.4*****	27.89±3.6*****

Significances (Anticoagulants)*: ^{a}vs Heparin and Sodium Citrate at T0; ^{a1}vs Heparin and Sodium Citrate at T24; ^{b}vs EDA at T0; ^{b1}vs EDTA at T24; ^{c1}vs Heparin and EDTA at T24.*
Significances (Storage Time): *vs T0.

P11 - ALGAL PIGMENTS AS DYE SOURCES IN THE SOLAR PHOTOVOLTAIC TECHNOLOGY

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Macroalgae show a broad range of applications and their importance in several sectors is steadily increasing worldwide. Aquaculture produced 15.8 million tonnes of aquatic plants, with a total estimated value of US$ 7.4 billion [1]. About 47% of the total production is used for human consumption, 43% in the extraction of colloids, 7% in the production of maërl and the remaining 3% in the fields of pharmacology, cosmetics, agriculture and waste water treatments from aquaculture, sewage, agricultural and industrial run-off. Algal biomass are also employed in the field of renewable energies for the production of biogas. Among alternative energies, the solar photovoltaic technology is one such source that can looked up to as vast research is being carried out and a significant improvement in performance has been achieved. However, macroalgae are an underexploited resource in the photovoltaic technologies [2, 3].
Dye-sensitized nanocrystalline solar cells (DSSCs) has become an important topic in solar cell research. DSSCs was inspired by the energy and electron transfer mechanisms in natural photosynthesis, and their are based on the photosensitization of nanocrystalline TiO_2 semiconductor electrodes by dyes [4]. In nature, some fruits, flowers and leaves contain several pigments that can be easily extracted and employed in DSSCs. Many reports have showed that chlorophyll, which acts as an effective photosensitizer in photosynthesis, has the potential to be an environment friendly dye sources [5-7]. Presently, macroalgae are an underexploited resource in comparison to crop plants.
In the present study, we utilized chlorophylls from samples of the brown alga *Undaria pinnatifida* as sensitizer in DSSCs to investigate the light to electron conversion efficiency. Samples of *U. pinnatifida* were collected in Venice Lagoon (45°26' N; 12°20' E); chlorophylls were extracted by treatment with acetone, according to the protocol of Wang *et al.* [3]. The dye, extracted by frozen seaweeds and used without any chemical purification, showed a very good fill factor (0.69). Even the photelectrochemical parameters if compared with the existent literature are very interesting.
U. pinnatifida is a highly invasive species and has caused concern all over the world because it has invaded coastal environments, has the potential to displace native species, significantly alters habitat for associated fauna, and disturbs navigation. An exploitation of *U. pinnatifida* would result in the conversion of a waste into a valuable biomass.

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P12 - AN ASSOCIATION STUDY OF TWO SNPS IN THE SPARC AND EGF GENES WITH HEPATOCELLULAR CARCINOMA

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Background: Hepatocellular carcinoma (HCC) is one of the most frequent malignancies and causes of cancer-related deaths worldwide. It has a variable incidence depending primarily on the local environmental risk factors. However today it is clear that also genetics has role in HCC onset and development.
Secreted Protein Acidic and Rich in Cysteine (SPARC) is a multifunctional matricellular glycoprotein involved in extracellular matrix remodelling. Recent experimental studies suggested that SPARC expression is upregulated in HCC compared with non-tumourous liver and that its expression could be related to HCC angiogenesis and tumour progression.
Epidermal growth factor (EGF) has many biological functions involving stimulation of proliferation, differentiation and tumorigenesis of epidermal and epithelial tissues. In recent years, numerous studies have associated a single-nucleotide polymorphism (rs4444903) of the EGF gene with the risk of tumorigenesis in multiple human cancers among which HCC.
The aim of this study was to evaluate the association between the rs2304052 and the rs4444903 single nucleotide polymorphisms (SNP), located respectively on the SPARC and EGF genes and HCC susceptibility.
Methods: 75 HCC cases and 170 healthy controls were collected from a Southern Italian population. For each sample the DNA concentration was evaluated. Every sample was genotyped for the rs2304052 and the rs4444903 SNP through an amplification followed by a restriction reaction (PCR-RFLP).
A statistical analysis was performed, *p values* and ORs were determined.
Results: For both the SNPs we did not obtained any statistically significant *p values.* For the rs2304052 SNP we obtained ORs that are pointer of no association. For the rs4444903 SNP we obtained an OR of 1,12 (95% CI = 0,72-1,74) and an OR of 0,99 (95% CI = 0,43-2,33) for the dominant model. A result of weak association was obtained with the recessive model (OR: 1,41, 95% CI = 0,65-3,05) and with the recessive model for HCV positive and male subjects (OR: 1,72, 95% CI = 09,62-4,66). A moderate association was obtained with the male and HCV positive G/G vs A/A carriers (OR: 2,57 95% CI = 0,53-13,89).
Conclusions: The rs2304052 SNP seems not to be associated with the risk of HCC in a Southern Italian population. Male and HCV positive rs4444903 SNP G/G carriers have an increased risk of HCC compared with that of the A/A carriers in a Southern Italian population.

P13 - DAILY RHYTHM OF TOTAL ACTIVITY/REST PATTERN IN SMALL AND LARGE DOMESTIC ANIMALS

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Circadian rhythms represent an inherent property of living organisms that seem to guarantee an optimal functioning of the biological system, with maximum efficiency, performance and welfare [1]. In mammals, a master clock located in the suprachiasmatic nuclei (SCN) of the hypothalamus adjusts the timing of other self-sustained oscillators in the brain and peripheral organs [2]. In most species, the daily light-dark (L/D) cycle is the primary environmental stimulus (Zeitgeber) for the entrainment of the SCN pacemaker. The SCN receives light information from the retina and regulates several physiological processes by synchronizing molecular clockwork mechanisms consisted by a core group of clock genes in each cell [3]. Among all physiological processes, the total locomotor activity (TLA) is one of the most susceptible to the L/D cycle. Light acutely suppresses locomotor activity in nocturnal (night active) animals such as rats and owls but promotes activity in diurnal (day active) animals like dogs and eagles [4]. Since animals have a species-typical organization of activity patterns [5], the aim of this study was to compare the TLA in small and large domestic animals like rabbits, cats, dogs, goats, sheep, cows, donkeys and horses. Five clinically healthy female subjects from eight different species: rabbits (body weight 2.5±0.2 kg), cats (body weight 4.5±0.3 kg), dogs (body weight 13.5±1 kg), goats (body weight 40±2 kg), sheep (body weight 45±2 kg), cows (body weight 390±10 kg), donkeys (body weight 395±20) and horses (body weight 565±42) were enrolled in the study with owners consent. Animals were housed under natural photoperiod (March) 12:12 hours L/D cycle (5.30 am sunrise, 5.30 pm sunset) according to specific farm management, except for cats and dogs that lived outdoors. Water was available *ad libitum* and feeding was suitable for each species. Total activity pattern was recorded for 10 days using actigraphy-based data loggers Actiwatch-Mini (Cambridge Neurotechnology Ltd, UK) placed on each animal through collars or halters according to the species. Activity was monitored with a sampling interval of 5 minutes. Total daily amount of activity, mount of activity during the photophase and the scotophase were calculated using Actiwatch Activity Analysis 5.06 (Cambridge Neurotechnology Ltd, UK). The Cosine peak of a rhythm (the time of the daily peak) was computed by cosinor rhythmometry [6] as implement in the Actiwatch Activity Analysis 5.06 program. The temporal resolution of the locomotor activity data was reduced to t h bins by the averaging of all 15 data points within each 1 h bin to apply the statistical analysis. To analyze the locomotor activity a trigonometric statistical model was applied to each time series to statistically describe the periodic phenomenon, by characterizing the main rhythmic parameters according to the single cosinor procedure [6]. Four rhythmic parameters were determined: mean level, amplitude, acrophase (the time at which the peak of a rhythm occurs), and robustness (strength of rhythmicity). For each animal, the mean level of the rhythm was computed as the arithmetic mean of all values in the data set (24 data points). The amplitude of the rhythm was calculated as half the maximum-minimum range of the oscillation, which was computed as the difference between peak and trough. Robustness was computed as the percentage of the maximal score attained by the chi-square periodogram statistic for ideal data sets of comparable size and 24-h periodicity [7]. Two-way analysis of variance (ANOVA) was used for the assessment of effects due to species and days on the daily amount of activity per 24 h. Statistical analysis showed significant differences among domestic species. The highest daily

amount of activity was observed during the photophase ($p<0.0001$) in dogs, sheep, goats, cows, donkeys and horses, and during the scotophase ($p<0.0001$) in rabbits and cats. Our results show different pattern of locomotor activity in every domestic species (Figure 1), underlining a diurnal pattern of locomotor activity in dogs, goats, sheep, cows, donkeys and horses while rabbits and cats have a main nocturnal pattern. As previously observed by several authors [8,9,10], our study confirms that locomotor activity exhibits a robust daily rhythmicity during the photophase in dogs, cows and horses, therefore in these species the rhythm can be poorly affected by external stimuli. On the contrary, other domestic species can spontaneously shift from diurnal to nocturnal activity pattern. Sheep with restricted night time feeding can shift the main bout of activity during the night [8] or cats, that are considered mainly nocturnal, use to loose their rhythm when they live in symbiosis with humans [11]. Therefore, the daily pattern of TLA does not depend only on L/D cycle but it can be affected by several environmental variables including different activities such as feeding, drinking, walking, grooming, playing as well as all conscious and unconscious movements.

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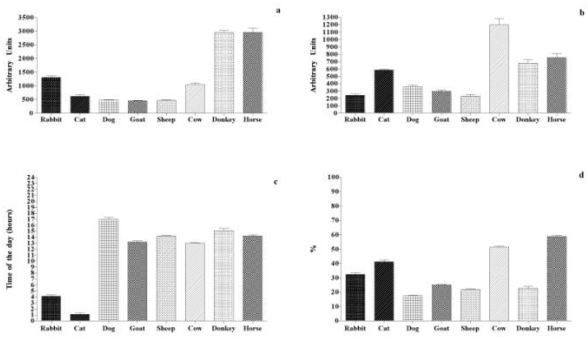

Figure 1. Analysis of rhythm parameters in domestic species: **a.** Total Locomotor Activity, **b.** Amplitude, **c.** Acrophase, **d.** Robustness.

P14 - ANALISI VOCALE DI EMISSIONI FONATORIE SOTTO SFORZO SUB-MASSIMALE: UN CASO STUDIO

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La laringe rappresenta il principale organo vocale, ma riveste anche un ruolo determinante nell'attività muscolare. E' noto infatti che la funzione di fissazione della laringe partecipi alla stabilizzazione dell'intera gabbia toracica, durante il compimento di uno sforzo attraverso l'accollamento cordale e la manovra di Valsalva, la laringe, dunque, ricopre un ruolo primario anche in questa funzione, come dimostrato dagli studi sui soggetti laringectomizzati, i quali riferiscono difficoltà nel sollevare oggetti pesanti dopo l'intervento (1). In ambito sportivo atleti ed istruttori possono evidenziare, durante la pratica, emissioni di sforzo sfocianti nel malmenage vocale esponendosi al rischio di disfonia muscolo-tensiva. Reich et al. (1986) ha condotto uno studio sul comportamento vocale dei cheerleaders, rilevando modalità ipercinetiche dei meccanismi fonatori tendenti ad innescare disfunzioni nodulari delle corde vocali (2). Heidel et al. (1993) ha ricercato l'insorgenza della sintomatologia fonopatica negli istruttori di aerobica, rilevando come questi esibissero raucedine, disfonia o afonia, tensioni muscolo-scheletriche, colpi di glottide e livelli di intensità e di F0 che superavano i range limite (3), dati confermati dalle successive ricerche condotte da Long et al. (1998) (4), e paragonando queste modalità vocali a quelle dei cheerleaders, evidenziando un elevato decremento della sintomatologia disfonica nei partecipanti alle lezioni (3).
Lo scopo di questo studio è stato quello di indagare le modificazioni glottiche, incorrenti nella produzione di emissioni fonatorie sotto sforzo sub-massimale, al fine di comprendere i meccanismi di avvio del malmenage vocale di atleti ed istruttori ed elaborare un protocollo di riscaldamento vocale in un' ottica preventiva-rieducativa.
Abbiamo condotto uno studio di osservazione video-laringo-strobo-scopica, eseguita con rino-faringo-laringo-scopio a fibre ottiche flessibili di emissioni fonatorie sotto sforzo sub-massimale, durante l'esecuzione del curl con bilanciere in posizione ortostatica. Ha partecipato allo studio un soggetto di sesso maschile con un età di 21 anni, un'altezza di 1,75 m, un peso di 71 kg che non presentava patologie a carico dell'organo vocale. Lo stile di vita del soggetto era attivo, in quanto praticava un'attività aerobica (corsa di lunga durata) due volte a settimana da almeno 1 anno. Il soggetto prima di essere sottoposto alle valutazioni è stato familiarizzato all'esecuzione del gesto tecnico e sottoposto al calcolo del massimale, effettuato con il metodo indiretto delle ripetizioni, che prevede l'esecuzione del maggior numero di ripetizioni con un carico predeterminato. Il soggetto prima di effettuare l'analisi ha svolto un riscaldamento dei principali distretti corporei, successivamente ha svolto l'esercizio per 2 serie da 4 ripetizioni a carico naturale sollevando un bastone sul quale imprimeva uno sforzo minimo imitando il gesto tecnico del curl, e al 50% e al 60% del massimale, con carichi equivalenti di 24 kg e 30 kg. Ogni singola serie prevedeva l'emissione vocale durante il sollevamento del carico, rispettivamente di 3 /e/, durante le prime tre ripetizioni, e di una /i/ durante l'ultima ripetizione. L'analisi si componeva di una valutazione video-laringo-strobo-scopica, una valutazione percettiva della voce e una valutazione spettro-acustica digitale eseguita con il software Praat.
A scopo di confronto è stata eseguita una valutazione laringoscopica del soggetto, in condizioni baseline, che evidenziava un'attività cordale eufonica con attacco vocale dolce e assenza di ogni elemento organico o funzionale segno di patologia. Dalla comparazione delle valutazioni con il

baseline si evidenzia come progressivamente all'aumentare del carico, aumenti il grado di chiusura glottica e aumenti la velocità di avvicinamento lungo la linea mediana delle corde vocali, diminuendo a sua volta il tempo di contatto cordale. L'analisi endoscopica mostra inoltre la comparsa di tre posture laringee scorrette, già osservabili nel sollevamento del bastone e di grado progressivamente maggiore al 60% del massimale: ipercontrazione e/o iperadduzione laterale con affrontamento glottico ipertonico e ipercontrazione e protrusione delle bande ventricolari che possono giungere a contatto; contrazione antero-posteriore della sopraglottide con ipertonia dei muscoli vocali e basculamento in avanti delle aritenoidi; ipertonia e iperadduzione della glottide posteriore con contatto fra le aritenoidi. Abbiamo, inoltre, rilevato endoscopicamente la presenza di un aumento di essudato mucoso a livello dei seni piriformi e della regione aritenoidea, refluito esofageo massimamente richiamato al 60% del massimale. L'osservazione semi-obiettiva in stroboscopia rileva un aumento dell'ampiezza e della progressione dell'onda mucosa all'aumentare del carico; non si evidenziano caratteri patologici nella morfologia e nella motilità cordale. Dall'analisi percettiva della voce la qualità risulta progressivamente pressata, con tenuta instabile, attacchi vocali duri e aumenti di intensità con l'aumentare del carico. L'analisi spettro-acustica effettuata sulle 3/i/ finali, infatti, rileva un aumento della F0 nelle tre prove, a carico naturale, al 50% e al 60% del massimale, passando per i valori di 160 Hz, 178 Hz, 192 Hz rispettivamente; abbiamo rilevato anche un aumento progressivo dei range di intensità nelle tre prove, a carico naturale, al 50% e al 60% del massimale, passando per i range 54/55 dB, 64/69 dB, 65/70 dB rispettivamente. Si evidenzia, inoltre, un aumento dei Pulse e Periods, mentre una tendenza a stabilizzarsi degli indici di perturbazione Jitter l.% e Shimmer l.% e del rapporto HNR.

Lo studio condotto ha evidenziato i quadri glottici del malmenage vocale a cui sono soggetti istruttori ed atleti, non parlare durante lo sforzo fisico è una norma di igiene vocale, poichè l'attività fonatoria incorrente durante lo sforzo muscolare scatena il manifestarsi di iperpressioni glottidee. I dati mostrano come anche uno sforzo minimo, come il sollevamento del bastone, porti all'assunzione di posture laringee scorrette e modalità ipercinetiche di condotta vocale, da cui, a lungo andare può derivarne una disfonia muscolo-tensiva con possibile esito fono-traumatico. Si potrebbe speculare la pratica di un protocollo di riscaldamento vocale, come attività fono-respiratoria preparatoria all'attività fisica e vocale, come punto da cui non prescindere per atleti ed istruttori, specialmente delle categorie che facciano uso della voce durante le attività. I dati ottenuti non ci permettono di definire con precisione la provenienza dei refluiti esofagei e la loro composizione, informazioni in tal senso potrebbero trovare riscontro nella prevenzione e nella presa di misure di adeguatezza di condotta vocale connessa all'attività fisica, per i soggetti affetti da Gerd, infatti, in accordo con lo studio condotto da Jozkow et al. (2006) (5), in questi soggetti aumenti pressori a livello addominale potrebbero ripercuotersi a livello pilorico, causando un incremento del reflusso, e richiamare materiale gastro-esofageo in laringe. La scelta di utilizzare un approccio multidisciplinare per studiare le emissioni fonatorie sotto sforzo sub-massimale, potrebbe permettere di elaborare ed adottare progetti per la prevenzione della disfonia in ambito sportivo.

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Keywords: Sport, Analisi vocale, Disfonia muscolo-tensiva, Prevenzione.

P15 - SELECTION OF THE BEST OOCYTES FOR INTRACYTOPLASMICSPERM INJECTION (ICSI) USING APOPTOTIC ANALYSIS OF CUMULUS CELLS

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Introduction: We studied the apoptosis rate of the cumulus cells of individual cumulus-oocyte complex (COC), to verify a relationship with clinical outcomes, in terms of pregnancy and implantation rates. Usually oocytes are selected using morphological criteria. We tried to verify if cumulus cell apoptotic rate could be used as molecular criteria in selecting oocytes with higher implantation potentiality (1;2).

Materials and Methods: The study design consisted in two different trials: in the first, we investigated apoptosis rate in cumulus cells of the three selected oocytes, to be fertilized by intracytoplasmic sperm injection (ICSI); in a second trial, average apoptosis rate of the cumulus cells coming from the three selected oocytes to be fertilized by ICSI and the pooled remaining oocytes were compared, when more than 5 COCs were aspirated. In a first trial we included 22 consecutive couples undergoing ICSI cycles, 20 in a second one, for a total of 42 patients. We selected the three oocytes for (ICSI) on the basis of the morphological appearance of the cumulus, according to Veek's criteria. The cumulus cells of each COC were submitted to apoptotic assays (3). The patients were classified, on the basis of pregnancy success, in A Group (pregnant patients) and B Group (patients with negative βhCG).
Results: Both trials showed that apoptosis in the cumulus cells was remarkably lower in the A Group if compared with B Group. The apoptosis rate in the selected COCs was similar to pooled COCs for each patient, confirming that apoptosis rate in cumulus cells is characteristic for patient. Out of 22 patients involved in the first trial, 8 were pregnant (36.3% A Group) and 14 were not pregnant (B Group). In the second trial 4 of a total of 20 patients were pregnant (20%). In the first trial a total of 58 metaphase II oocytes and 56 in the second trial were studied. In the second trial 38 oocytes where pooled to compare apoptosis rate with the three selected oocytes pools. In the first trial the incidence of DNA fragmentation, evaluated by TUNEL assay (fig. 1), of the cumulus cells from individual treated oocytes, was lower in A Group than in B Group (6.7% ranging between 2.2–13.3 vs 13.19% ranging between 6.2–34.9 respectively, $p<0.05$). To confirm if DNA fragmentation was related to apoptosis process, we performed caspase-3 immunoassay in the same cells (fig. 2). Data showed a lower capase-3 activity in cumulus cells of pregnant than in those of non-pregnant patients (5.2% ranging between 1.2–8.6 vs 11.8% ranging between 5.6–14.8, $p<0.05$). It is noteworthy to underline that pregnant patients usually exhibited, at least, one COC with a DNA fragmentation rate (TUNEL) less than 10% and caspase-3 activity rate less than 7%. Four (A Group) of 20 patients involved in the second trial were pregnant but two aborted at 8–9 weeks. The low number of pregnant patients did not allow us to have a powerful statistical analysis of apoptotic rate in cumulus cells, but it seems evident that a higher apoptotic rate in cumulus cells is associated to the pregnancy failure (B Group) and in aborted patients of A Group, ranging from 10 to 60.3%.
Conclusion: The data seem to demonstrate that apoptosis may be a marker for the selection of the best oocytes to be submitted to ICSI treatment. All pregnant patients showed a lower apoptosis rate in cumulus cells if compared with patients with pregnancy failure.

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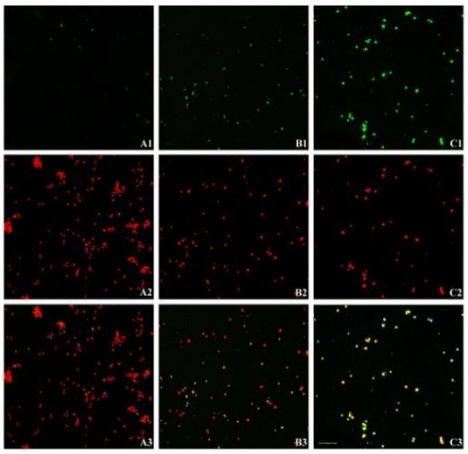

FIGURE 1. Apoptosis evaluation using TUNEL assay in human cumulus cells. (A1, A2, A3) A group; (B1, B2, B3) B group; (C1, C2, C3) positive control for TUNEL assay. (A1, B1, C1) fragmented DNA; (A2, B2, C3) propidium iodide staining; (A3, B3, C3) merge. Scale bar = 15 μm.

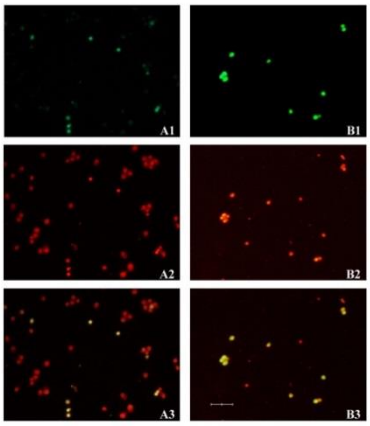

FIGURE 2. Apoptosis evaluation using Cleaved caspase 3 immunofluorescence *in situ* assay in human cumulus cells. (A1, A2, A3) A group; (B1, B2, B3) B group; (A1, B1) Cleaved caspase 3; (A2, B2) propidium iodide staining; (A3, B3) merge. Scale bar = 15 μm.

P16 - PROGETTO ASSO: UNO STRUMENTO WEB-BASED PER VALUTARE LE SCELTE ALIMENTARI DEGLI ADOLESCENTI

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Introduzione: Le abitudini alimentari della popolazione si sviluppano preferibilmente nell'adolescenza, periodo della vita caratterizzato da profonde modificazioni dell'aspetto fisico, da un'intensa evoluzione psicologica, ormonale e comportamentale. E' già da tempo noto che le cause dell'obesità sono molteplici, prima fra tutte un'alimentazione non adeguata, con una stretta interazione di fattori socio-economici, biochimici, genetici e psicologici; inoltre, un ruolo importante nello sviluppo, nella progressione e nel perpetuarsi dell'obesità negli adolescenti viene attribuito all'inattività fisica. Allo scopo di pianificare e implementare adeguati interventi per la prevenzione dell'obesità, risulta essenziale indagare tutti questi fattori.

Obiettivi: Il Progetto ASSO (Adolescenti e Sistema di Sorveglianza per la prevenzione dell'Obesità) individua l'adolescenza come periodo da studiare attraverso un innovativo sistema di sorveglianza nutrizionale basato su tecnologia web, che permetta una continua e sostenibile raccolta di dati su obesità e stili di vita degli adolescenti. Nel presente studio viene preso in considerazione uno degli aspetti che influenza l'insorgenza di obesità, relativo alla adeguatezza delle scelte alimentari.
Metodi: Il target è rappresentato da un campione di 919 studenti (571 maschi e 348 femmine) che frequentano le scuole superiori della città di Palermo e compresi in una fascia di età tra14 e 17 anni. Dopo aver ottenuto il consenso informato dei genitori, sono stati somministrati via web i questionari contenuti nel software "ASSO-NutFit" creato dal team del Progetto. Per il presente studio si sono presi in considerazione due dei questionari: l'ASSO-PIQ, per la raccolta di dati relativi alle informazioni personali, e l'ASSO-FHQ per le abitudini alimentari. Il database ottenuto è stato elaborato con STATA 12.0. Sulla base delle indicazioni nutrizionali contenute nelle Linee guida dell'INRAN e rappresentate graficamente nella Nuova Piramide Alimentare del 2005, è stata valutata l'adeguatezza delle scelte alimentari effettuate dai partecipanti durante i 5 pasti giornalieri.

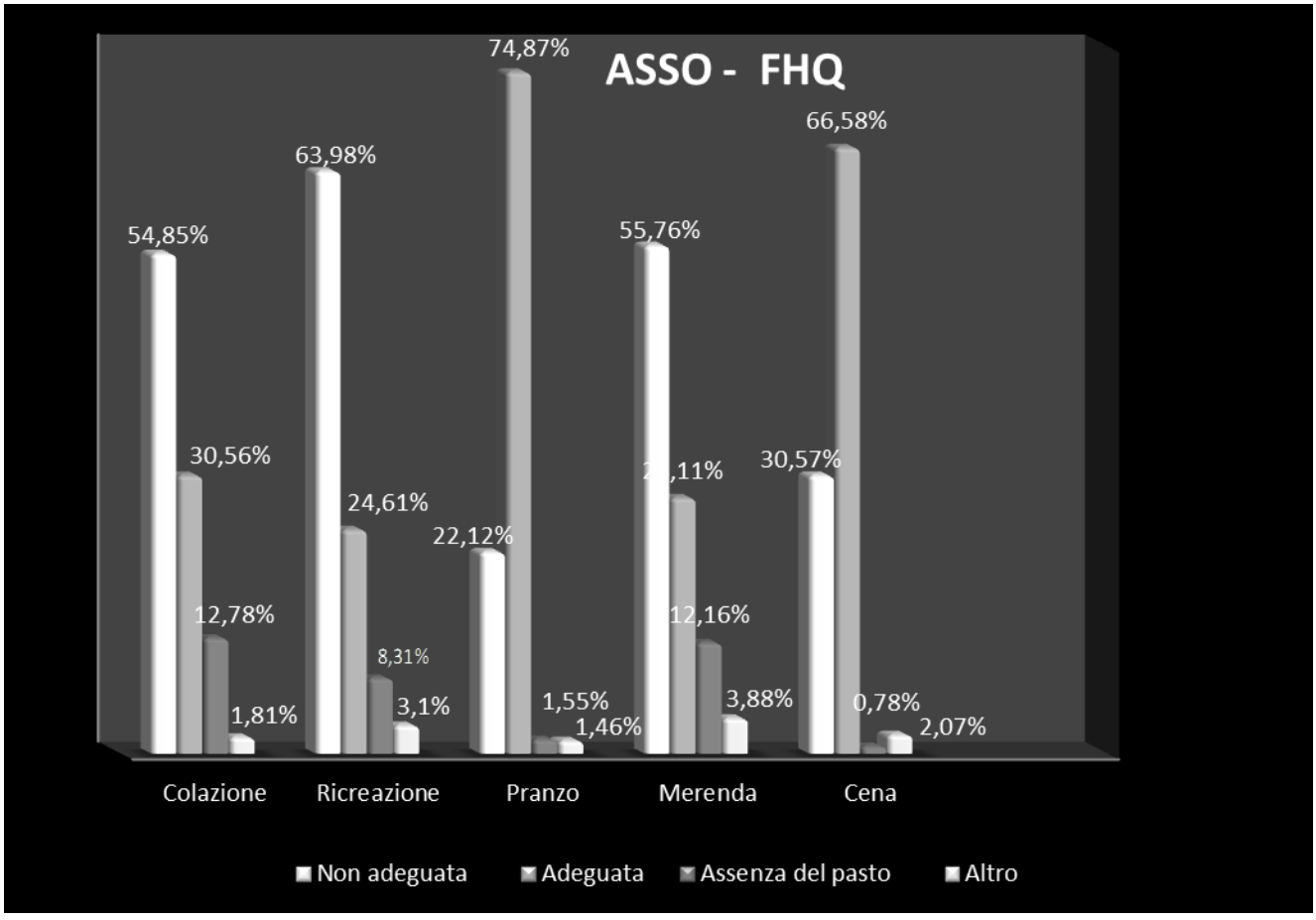
Risultati: Complessivamente sono stati raccolti 747 ASSO-PIQ e 772 ASSO-FHQ.
Il 61,53% dei ragazzi consuma la colazione in modo regolare; il 25,65% in modo sporadico; il 12,82% non fa mai la colazione, spesso motivandone come causa la fretta. In più della metà (54,85%) di coloro che la consumano, la colazione non è adeguata (Figura 1), poiché include alimenti quali merendine, snack confezionati o prodotti da forno del bar. Anche durante gli spuntini mattutini e pomeridiani gli alimenti scelti risultano non adeguati rispettivamente per il 63,91% ed il 55,76% dei ragazzi (Figura 1). Tuttavia, migliori risultati in termini di adeguatezza di scelte alimentari sono stati ottenuti relativamente al pranzo ed alla cena; infatti, il 74,87% ed il 66,58% degli adolescenti investigati consumano alimenti consigliati rispettivamente durante il pranzo e la cena, quali un primo piatto, contorno e frutta per il pranzo ed un secondo piatto, contorno e frutta per la cena (Figura 1).

Discussione: I risultati ottenuti confermano che la stragrande maggioranza della popolazione scolastica investigata mostra una positiva attitudine a consumare la prima colazione, ma più della metà di questo campione sceglie cibi non idonei per l'elevato contenuto di grassi saturi e zuccheri semplici, come merendine, snack o prodotti da forno del bar. Analogamente alla

colazione, anche gli spuntini mattutini e pomeridiani sono i pasti che registrano le più alte percentuali di alimenti non adeguati, in quanto i cibi più consumati risultano essere snack dolci o salati, prodotti da forno, rosticceria, bevande gassate e/o zuccherate. Pertanto, l'introito calorico ed in nutrienti di questi pasti secondari diventa eccessivo rispetto a quello del pranzo e della cena; non rispettando la giusta ripartizione consigliata dalle Linee Guida dell'INRAN si verificano "reazioni a catena" che incidono non solo sul bilancio nutrizionale quotidiano ma anche sul rendimento psicofisico dei ragazzi.

Conclusioni: Sulla base dei risultati ottenuti in questo studio, si ritiene di potere sperimentare questo sistema di sorveglianza nutrizionale per indagare le scelte alimentari della popolazione scolastica sull'intero territorio nazionale.

FIGURA 1. Distribuzione dell'adeguatezza dei cinque pasti giornalieri negli adolescenti, rilevata con l'ASSO-FHQ



P17 - UNO STRUMENTO WEB-BASED PER RILEVARE LE ABITUDINI alimentari degli ADOLESCENTI: IL PROGETTO ASSO

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Introduzione: Il progetto ASSO è nato dalla necessità di sperimentare un sistema di sorveglianza nutrizionale sugli adolescenti in grado di fornire una fonte permanente di dati affidabili e coerenti. Un sistema di questo tipo rappresenterebbe il primo passo per comprendere i problemi di salute pubblica correlati all'alimentazione della popolazione, al fine di implementare azioni adeguate. Considerato che l'Italia è uno dei paesi europei con la più alta prevalenza di obesità infantile, il progetto ASSO ha scelto di studiare l'adolescenza, periodo della vita fino ad oggi poco studiato e caratterizzato da profondi cambiamenti che riguardano, non solo l'aspetto fisico e l'evoluzione psicologica ed ormonale, ma anche lo stile alimentare.
Obiettivi: Il Progetto ASSO (Adolescenti e Sistema di Sorveglianza per la prevenzione dell'Obesità) propone un innovativo sistema di sorveglianza nutrizionale basato su tecnologia web, che permetta una continua e sostenibile raccolta di dati su obesità e stili di vita degli adolescenti. Tenuto conto che molteplici sono le cause dell'obesità, nel presente studio viene preso in considerazione uno degli aspetti che influenza l'insorgenza di questa patologia: le abitudini alimentari degli adolescenti.
Metodi: Il campione esaminato in questo studio è di 919 studenti (571 maschi e 348 femmine) che frequentano le scuole secondarie superiori della città di Palermo e che hanno un'età tra 14 e17 anni. Dopo aver ottenuto il consenso informato dei genitori, sono stati somministrati via web i questionari contenuti nel software "ASSO-NutFit" creato dal team del Progetto. Il presente studio prende in considerazione i risultati ottenuti dalla compilazione dei questionari ASSO-FHQ, contenenti dati relativi alle abitudini alimentari della popolazione scolastica intervistata, come il consumo di cibi pronti industriali o di prodotti biologici, l'acquisto di prodotti dai distributori automatici o l'abitudine a mangiare fuori dai pasti. Il database ottenuto è stato elaborato con STATA 12.0.
Risultati: Complessivamente sono stati raccolti 772 ASSO-FHQ, dai quali risultano informazioni incoraggianti: quasi la metà dei ragazzi mangia fuori casa solo una volta a settimana e non consuma fuori pasto; poco più della metà non consuma prodotti alimentari dei distributori automatici presenti a scuola e sceglie prodotti biologici; la stragrande maggioranza (76,71%) preferisce mangiare prodotti freschi dell'orto o di allevamento (Figura 1). Per quanto riguarda i fattori che influenzano le scelte alimentari degli adolescenti, le percentuali più significative riguardano il sapore con il 37,25%, l'abitudine con il 18,8% ed, infine, i genitori e la famiglia con il 14,76% (Figura 2); scarsa influenza, invece, hanno l'informazione (solo il 2,39%) e la pubblicità (appena l'1,31%). Un altro aspetto molto importante delle abitudini alimentari degli adolescenti riguarda l'attenzione che prestano al consumo dei pasti e, quindi, la valutazione di ciò che fanno mentre mangiano. Contrariamente a quanto si pensa, soltanto il 4,14% dei ragazzi intervistati utilizza il cellulare mentre mangia; invece, le abitudini più diffuse sono quelle di parlare con i commensali e guardare la televisione rispettivamente per il 49,68% e il 37,52% dei soggetti (Figura 3).
Discussione: I risultati ottenuti confermano che circa la metà della popolazione scolastica investigata mostra delle abitudini alimentari positive, in quanto mangia di solito a casa, consuma prodotti biologici, non si concede dei "fuori pasto" e non sceglie i prodotti dei distributori automatici, perché probabilmente non sono presenti all'interno dell'istituzione scolastica. Altra abitudine positiva frequentemente riscontrata è il consumo di cibi freschi dell'orto e di

allevamento. I fattori che più influenzano le scelte dei soggetti intervistati sono il sapore e l'ambiente familiare, aspetti di fondamentale importanza per la crescita, lo sviluppo degli adolescenti e l'acquisizione di corrette abitudini alimentari in grado di prevenire il sovrappeso, l'obesità e le patologie correlate in età adulta.
Conclusioni: Sulla base dei risultati ottenuti in questo studio, si ritiene di potere sperimentare questo sistema di sorveglianza nutrizionale per indagare le abitudini alimentari della popolazione scolastica sull'intero territorio nazionale.

FIGURA 1. Abitudini alimentari degli adolescenti, rilevate con l'ASSO-FHQ

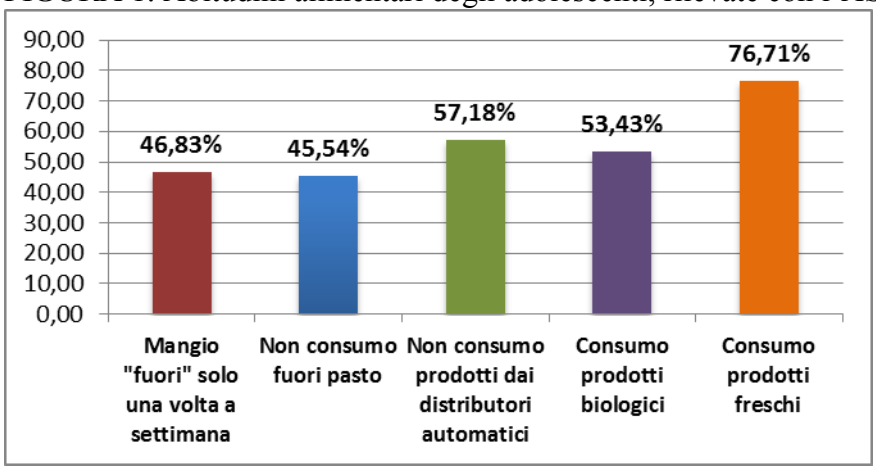


FIGURA 2. Influenza sulle scelte alimentari degli adolescenti, rilevata con l'ASSO-FHQ

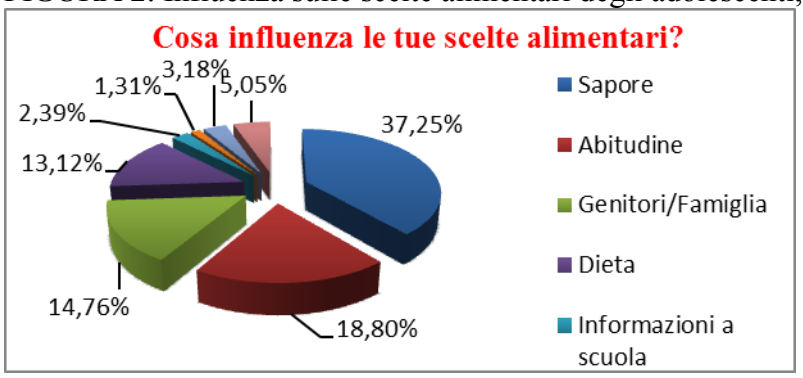
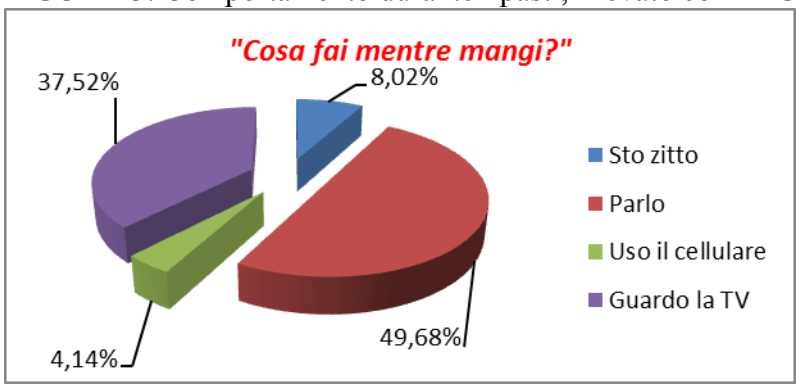


FIGURA 3. Comportamento durante i pasti, rilevato con l'ASSO-FHQ



P18 - APPLICATION OF MULTIVARIATE T-PATTERN ANALYSIS IN THE STUDY OF SOCIAL INTERACTION IN RATS

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Rationale: the social interaction test, introduced by File and Hyde in 1978 [1], is a well known behavioral model used to investigate anxiety-related behaviors in rodents. Basic premise of the test is that the behavior of a rodent influences the one of the other. An increased interaction between the two subjects is indicative of a reduced anxiety condition, in contrast, a reduced interaction indicates a higher anxiety. Albeit the test has been largely used to study anxiolytic and neuroleptic drugs, no data are available on the temporal structure of the behavioral interactions. In addition, interesting questions still remain unanswered. For instance, is it possible to identify recurring temporal sequences from the two interacting rodents ? If so, do such sequences encompass specific behavioral events ? Could such sequences have an ethological meaning ?
Method: in this preliminary study, four male Wistar rats (220 - 250 gr) were used. Each subject was housed in a thermo-regulated room. The day of the experiment, pairs of rats, randomly taken from housing room, were placed in an open field apparatus (50 x 50 cm) and observed for 15 min. The behavior of the animals was recorded with a digital video camera and video files stored in a personal computer. Using an ethogram, obtained on the basis of previous observations [2] [3] [4] [5], video files were coded by means of a software coder and the obtained event log files used for following analyses. Both quantitative and multivariate t-pattern analyses were carried out. The latter is a multivariate approach based on the utilization of a specific software that, by means of an advanced search algorithm, processes event log files evaluating possible significant relationships among the events in the course of time [6]. Theories, concepts and procedures concerning such a multivariate analysis of behavior can be found in our previous articles [6] [7] [8].
Results: the ethogram is presented in tab. 1. The behavioral activities are classified taking into consideration their characteristics: "non social" (produced in absence of interactions), and "social" (produced during interactions).

Non Social	Abbr.	Description
Walking	Wa	The rat walks around sniffing the environment
Climbing	Cl	The rat maintains an erect posture leaning against the Plexiglas wall. Usually associated with sniffing.
Rearing	Re	The rat maintains an erect posture without leaning against the wall. Usually associated with sniffing.
Front Paw Licking	FPL	The rat licks or grooms its forepaws
Hind Paw Licking	HPL	The rat licks or grooms its hind paws
Face Grooming	FGr	The rat ribs its face with the forepaws
Body Grooming	BGr	The rat rubs the body combing the fur by fast movement of the incisors
Shaking	Sh	The rat shakes its head and body with rapid semicircular movements
Immobility/Resting	Imm	The rat maintains a fixed posture
Immobile Sniffing	ISn	The rat sniffs the environment, standing on the ground
Social	**Abbr.**	**Description**
Withdrawing	Wit	One rat walks or runs away from the other rat.
Following	Fol	One rat follows the partner while the other is walking away
Approaching	App	One rat walks in the direction of the partner, while the other rat is immobile or is alredy approaching him
Crawling over	CrO	One rat walks over the partner
Crawling under	CrU	One rat walks under the partner
Boxing/Wrestling	Box	Offensive/aggressive behaviors such as pawing, pouncing, nosing, biting, boxing, kicking, wrestling
Leaning on	LeO	One of the rats leans with its forelimbs on the other rat that, in turn, maintains all the four paws on the ground.
On-top	Top	One of the animals stands over the partner that lies with its back on the floor
On-back	Bck	One of the animals lies with its back on the floor with the other animal standing over it
Mounting	Mnt	One of the rats holds the other rat's trunk with the forelimbs
Social grooming	SoG	One of the rats grooms its partner's body, neck or face
Social sniffing	SoS	One of the rats sniffs the partner's face and/or body
Ano-genital sniffing	GeS	One of the rats sniffs the partner's anogenital area

Tab. 1. Ethogram. First column: behavioral element. Second column: abbreviation. Third column: description.

Preliminary results, obtained from the analysis of two pairs of rats, are presented. Per cent distribution, evaluated both for social and non social activities are illustrated in fig. 1. ISn, Wa, Cl, Re, FPL, Imm and Sh represent 95.50% of the non social behavioral repertoire; on the other hand, SoS, App, Wit, LeO, SoG and GeS represent 84.50 % of the social one. T-pattern analysis demonstrated, in both pairs of rats, the presence of significant constraints among numerous events in the course of time. Fig. 2 illustrates a t-pattern detected in one pair of rats.

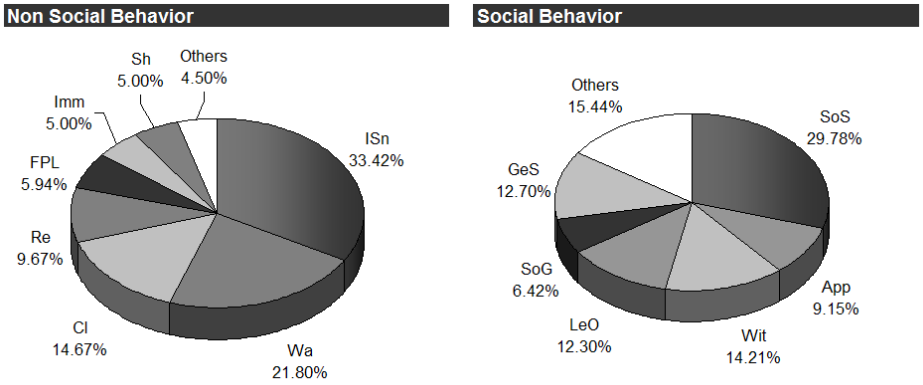

Fig. 1. Per cent distribution of non social (left pie) and social (right pie) behavioral elements carried out by the rats. Others = behavioral elements < 5%. For abbreviations see tab. 1.

Discussion: The current research represents the first effort to study the temporal structure of social interaction in rats by means of multivariate t-pattern analysis. Per cent distribution (fig. 1) shows that sniffing related (ISn, Cl, Re, SoS, GeS) and walking related (Wa, App, Wit) activities (tab. 1) are the most represented both in non social and social behavior.

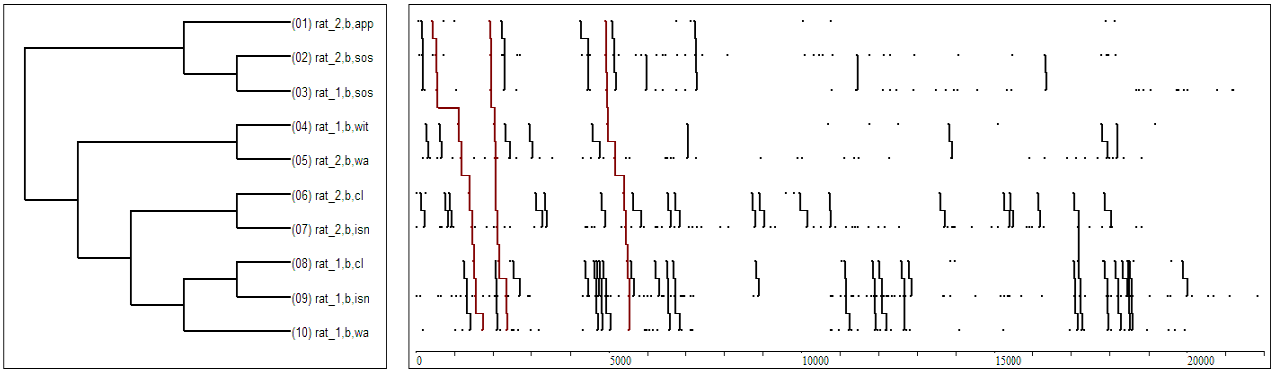

Fig. 2. T-pattern detected in one of the two pairs of rats. Left box: tree structure. Number in brackets indicates the order of appearance of each event. Right box: connection diagram. Dots show the occurrences of the corresponding events reported in the left box. For abbreviations see tab. 1.

Coherently, the t-pattern presented in fig. 2, encompassing only sniffing- and walking-related activities, well depicts the role of these behavioral events in moulding the temporal structure of the behavior. Interestingly, some kinds of behavioral symmetries were observed, where a behavior, carried out by one of the rats, was followed by the same behavior carried out by the other animal (fig. 2). To sum up, preliminary results obtained from t-pattern analysis demonstrate that the behavior of rats in the social interaction test is extremely complex and structured on the basis of numerous, recurring and statistically significant sequences of events.

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P19 - IL-1β INDUCES DNA DEMETHYLATION, AT GENOME LEVEL AND IN SPECIFIC CpG SITES OF IL-6 AND IL-8 GENES IN HUMAN INTESTINAL EPITHELIAL CELLS

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Inflammation is a complex physiological response that requires the activity of a sophisticated regulatory network involving the activation of specific genes for defense, tissue repair and remodeling.
Although transcriptional activation has been shown to be critical in the regulation of inflammatory genes (1) the role of epigenetic phenomena in the modulation of the inflammatory response is now emerging (2). Specifically, it has been recently reported that proinflammatory stimuli induce DNA demethylation in the interleukin IL-1 promoter of human articular chondrocytes (3). IL-1 cytokine, among several proinflammatory agents, represents an essential player in the inflammatory conditions of the gut (4): functioning as the strongest signal transduction to NF-kB, IL-1 increases in intestinal paracellular permeability and over-expression of proinflammatory genes (5). In this tissue, moreover, inflammatory response is crucial to maintain its structural integrity and function, thus, alteration and deregulation of inflammatory pathways contribute to tissue damage and ulceration, and are pivotal factors in the pathogenesis of several inflammatory gut diseases.
In the present study we evaluate both wide-ranging and gene-specific epigenetic changes in the inflammatory response of Caco-2 cells differentiated into intestinal epithelial cells and exposed to the inflammatory actions of IL-1β. Our results clearly show that IL-1 induces changes in the DNA methylation either at genome and gene level and that the local methylation changes are induced in two pro-inflammatory genes that are IL-1-regulated. In particular, we show that a cell exposure to IL-1β for 24 h induces, in a dose-dependent manner, hypomethylation of genomic DNA in respect to untreated cells. We also observe a reduced DNA methyltransferases activity of cell lysates obtained from IL-1 treated cells. Finally our data show that IL-1 is able to induce hypomethylation of specific CpG sites in IL-6 and IL-8 genes.
These preliminary results suggest that IL-1 in intestinal epithelial cells is able to act as an epigenetic modulator towards the entire genome and specific genes.
Modulation of epigenetic changes in inflammation may provide a new "reading frame" of the inflammatory diseases molecular basis. Since epigenetic modifications are potentially reversible, a thorough understanding of these changes during inflammatory response opens opportunities to develop efficient agents for specific targets.

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P20 - DESIGN AND SYNTHESIS OF FLUORESCENT GPER LIGANDS AS USEFUL TOOLS IN MOLECULAR BIOLOGY AND DRUG DISCOVERY PROCESS

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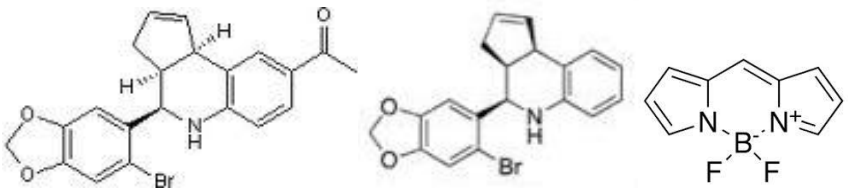
Breast and ovarian tumors are among the major cause of death in Western Countries' women. Estrogens play a pivotal role in the development of these hormone-sensitive tumors. Recently, a seven-transmembrane G-protein-coupled receptor (GPCR), named GPER, has been identified as membrane estrogen receptor able to mediate rapid estrogen signalling in a variety of normal and cancer cell types [1]. Different studies showed that GPER promotes the up-regulation of the oncogene *c-fos* and stimulates proliferative effects induced by estrogens and antiestrogen in cancer cells such as breast, endometrial and thyroid [2]. GPER is a 7 helices transmembrane protein (7TM) and its cellular localization is still a matter of debate: although it has been found within the Endoplasmic Reticulum membrane, further studies demonstrated the presence of this receptor on the cellular membrane. GPER has been associated with the proliferative effects induced by 17β-estradiol (E2) and a selective ligand of GPER, G-1, (A in Figure 1) through a functional cross-talk with ERα in ovarian cancer cells, playing an important role in tamoxifen-resistant breast cancer cells [3].



A B C

Figure 1. A: GPER agonist G-; B: GPER antagonist G-15; C: the skeleton of fluorescent BODIPY.

Taken together, these data indicate that GPER plays a role in a complex transduction network which mediates the biological responses to estrogens. However, the mechanism of activation of signals, the structure-function relationships, the pharmacological spectrum regarding GPER still suggest questions to be solved. The possibility to develop small molecular probes, to facilitate elucidation of mechanistic pathways and enable specific manipulation of the activity of GPER, provides an extraordinary tool in the complex field of drug discovery. Biological information can offer a more detailed scenery if the classical and efficient method of investigation, based on the exposition of cells to a fluorescent dye, such as one of the commonly used Alexa family,[3] can be flanked to the use of luminescent ligands of the protein under study.
In this communication we describe the synthesis of a family of small GPER ligands with intrinsic fluorescent properties. This approach can be regarded as a useful tool for exploiting structural changes of a protein upon interaction with specific target molecules and developing new targeted imaging agents for the intracellular receptor. We started in designing small molecules (Figure 2) showing the structural characteristics of some already known GPER ligands, such as the agonist G1 and the antagonist G15 (B in Figure 1), but with fluorescent elements incorporated in their skeleton, trying to avoid alteration in their properties as GPER agonists or, better, antagonists. For the fluorescence, we have been inspired by the dipyrromethene boron difluoride (BODIPY) dyes (C in Figure 1) as strongly UV-visible absorbing small fluorophores that exhibit relatively sharp fluorescence with high quantum yields

[4], are reasonably stable under physiological conditions and have been widely investigated as fluorescent probes for biological studies.

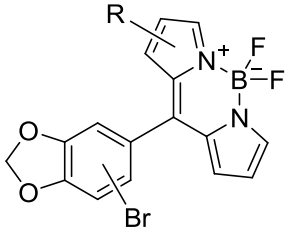
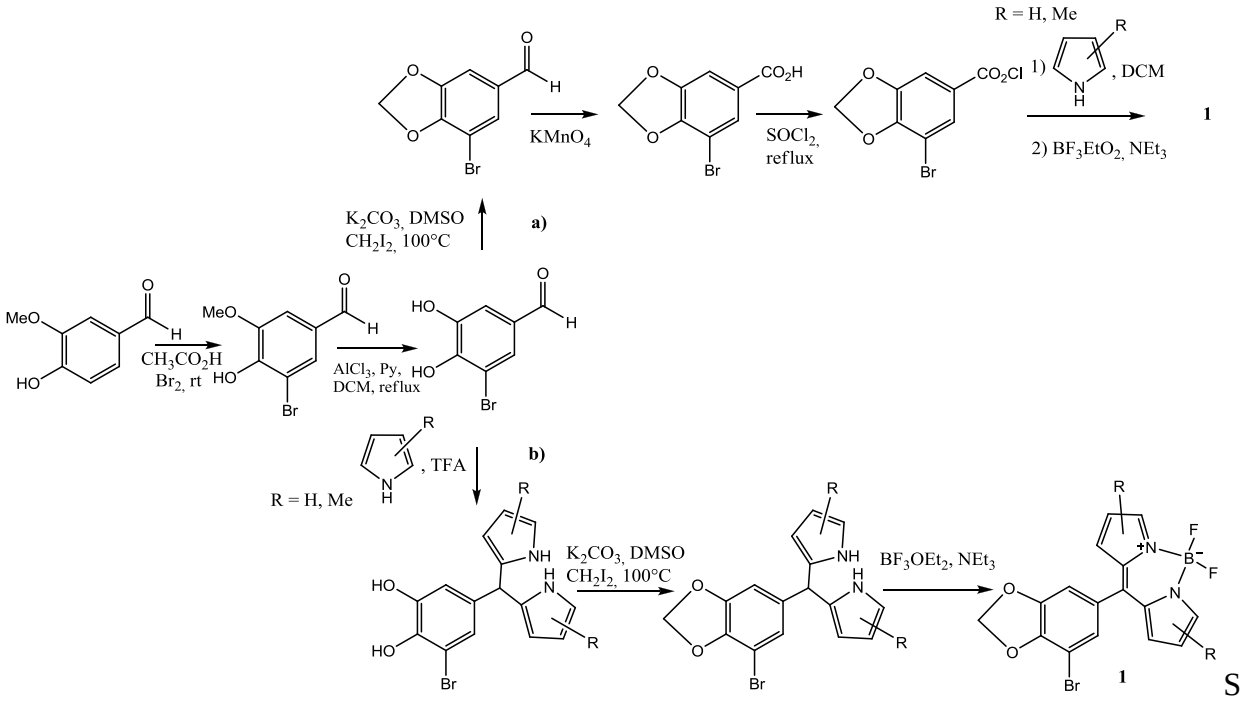


Figure 2. The general skeleton of the projected GPER fluorescent ligands.

In scheme 1 the two synthetic pathways a) and b), for the preparation of compounds 1, are shown. This communication will describe the details of these syntheses, the results gained and the work that has to be ended.



Scheme 1. Synthetic pathways to fluorescent GPER ligands.

The design of the fluorescent GPER ligands has been supported by virtual screening of their potentially effective molecular skeletons.

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P21 - FORENSIC TECHNIQUE TO ALLEGED HUMAN EMBRYO: THE FIRST CASE REPORT IN ITALY

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As commissioned by Judicial Authority, it was done an inventory of human cryo-frozen biological material, DNA typing of alleged human embryo and verification of compliance of the findings of PHRs acquired to Prosecution to reconstruct the path followed by gametes and the care of patients who have received medically-assisted reproduction treatments in four Centres highlighting any significant irregularities in contravention of the current legal framework on Assisted Reproduction Technology (ART). This article describes the first forensic investigation in Italy who involves forensic science in Reproductive Medicine [1, 2], taking into account the implementation of the Law 40 of 2004 on ART that has not changed with the pronouncement of The Constitutional Court in May 2009 [3, 4, 5]. In fact, embryos can be created only for the purpose of procreation and the prohibitions on their cryopreservation, suppression and selection for eugenetic purposes remain.
The inventory of liquid nitrogen containers of human material (semen, oocyte and embryo) has been done in cooperation with police who was video-recording the entire activity. This was done for two reasons: first, in order to be able to demonstrate that the manipulations of canisters, globets, visiotubes and straws, had not affected the cryo-frozen process. On the other hand, this would have allowed reviewing the manipulation, to check what has been writing for the identifications of patients who have received medically assisted reproduction treatments. However, the huge amount of documentation required in seizure had to define correctly the number of cryo-frozen biological material (oocyte and embryo) for each couple of patients to verification of compliance of the findings of PHRs acquired to Prosecution. Eight databases in Access format were found with no information of which centres of Assisted Reproductive could belong to. In addition, the customer registrations of clinical data records were identified by local, idiosyncratic, and sometimes redundant and/or ambiguous names (or codes) rather than unique, well-organized codes from standard code systems. This would have required a process of cleaning up of the data and mapping of the variable names and codes in collaboration with specialists in software programming . We were able to identify the databases corresponding to the 4 centers and we performed a second inventory of the cryo-frozen material to verify the previous data. A few discrepancies were highlighted.
At the same time, a genetic analysis was conducted on an alleged human frozen thawed embryo no more suitable to development. At Prosecution was declared that the human embryo at four cells was irreversibly compromised in its development: the aim of the investigation was to verify the genetic compatibility of a married couple who was waiting for frozen thawed embryo transfer (FET) and had done a complaint to the Prosecutor in order the possibility of replacement with heterologous embryos by medical staff. During the planned Embryo-Transfer (ET) the police put in seizure, one of the three frozen thawed embryo declared in advanced lysis state, into a straw. The laboratory activity was conducted in the presence of three specialists in the interest of the eight Suspects : when we analyzed the straw under microscopy we couldn't recognize any morphological characteristic of embryo but only small particles in a medium (fig. 1). For some suspects such evidence could fit well but for other suspects didn't fit well. Before performing the forensic genetic analysis the cell degeneration was investigated using an animal model (the sea urchin embryo) to understand, after stress conditions (freezing, thawing and physiological involution after death), when the morphological identification of embryo was no more possible. The results demonstrate that for the eggs of sea urchin after 8 days it was impossible to recognize them through microscopy analysis, differently from the embryo at 20

days where small cells could be seen, except that the lysis process was being induced by proteolitic enzymes.

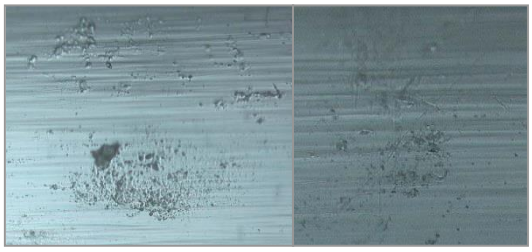

Figure 1 – A view of alleged human frozen thawed embryo into seized straw; magnification 80X - Stereo Microscope LEICA M165FC .

The case was taking aspects of particular relevance since it could not be excluded that the material into the straw in seizure was an oocyte not visible at microscopy. In addition, after the inventory it was proved that three straws with frozen-oocyte belonging to the couple of married patients who had done the complaint were missing. Therefore, only in theory it is simple to demonstrate this, since single cell degradation associated to small amount of forensic DNA is the best situation to obtain complex DNA profiles. A method of collecting the alleged embryo residues from the straw has been developed conforming to forensic procedure to ensure the repetition of analysis. The sample was collected using Whatman 3MM filter strip paper and was divided in 7 parts under absolute sterile conditions; each strip was extracted using a performed low copy number (LCN) extraction procedure. Low copy number (LCN) typing, particularly for current short tandem repeat (STR) typing, refers to the analysis of any sample that contains less than 200 pg of template DNA. Generally, LCN typing simply can be defined as the analysis of any DNA sample where the results are below the stochastic threshold for reliable interpretation [6, 7]. The extracted DNA was amplified using reduced amplification volume and higher PCR cycle numbers. Autosomal DNA profiles were obtained from most of the 3MM strips. These profiles were concordant with the profiles obtained from the couple of married patients proving the presence of human lysis embryo into the seized straw.
Random analysis on IVF-ET sheets, Clinical Records, IVF Registers, etc… combined with the computerization for each type of PHRs of such records: date of pick up, women, man, oocyte, IVF technique, MII oocyte , semen, embryo, discarded oocyte, discarded embryo, frozen-oocyte, frozen-embryo, embryo-transfer ET, MD, Biologist, clinical analysis, note, has permitted to reconstruct the path followed by gametes of patients who had received medically-assisted reproduction treatments highlighting significant irregularities in contravention of the artt.13 and 14 of the Law 40 of 2004.

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P22 - EFFECT OF Γ-AMINOBUTYRRIC ACID (GABA) EXPOSURE ON EMBRYOGENESIS OF *PARACENTROTUS LIVIDUS* AND IDENTIFICATION OF GABA-RECEPTOR GENES IN SEA URCHINS

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Developmental processes are controlled by regulatory genes encoding for transcription factors and signaling molecules. Functional relationships between these genes are described by gene regulatory networks (GRN), models which allow integration of various levels of information [1]. The sea urchin embryo is an experimental model system which offers many advantages for the analysis of GRN [2]. Recently, the GRN that governs the biomineralization of the sea urchin embryonic skeleton has begun to be deciphered [3-5]. Preliminary evidence suggest that the γ-aminobutyric acid (GABA) signaling pathway is involved in skeletal morphogenesis during development of the sea urchin. GABA is a molecule synthesized by nearly all organism, from bacteria to humans, and it acts through ionotropic and metabotropic receptors ($GABA_A$-Rs and $GABA_B$-Rs, respectively) [6].

We report that *Paracentrotus lividus* embryos exposed to GABA at concentrations ranging from 0.01 to 1.0 mM showed aberrations in axial patterning, with a dose dependent effect. In particular, at 24 hours post-fertilization (hpf) control embryos displayed two bilateral clusters of Primary Mesenchyme Cells (PMCs, Fig. 1Aa), which hold biomineralizing activity. By contrast, treated embryos contained a population of PMCs that was quite homogeneously distributed within the blastocoele (Fig. 1Ab). Moreover, at 48 hpf, when control embryos were normal angular-shaped plutei with the characteristic bilateral symmetry (Fig. 1Ac), GABA-treated embryos appeared spherical and contained supernumerary spicules (Fig. 1Ad).

Washout experiments allowed to determine that the period of sensitivity is restricted from the blastula to the gastrula stage.

In order to identify GABA-R genes we performed a comprehensive *in silico* analysis in selected sea urchin species (*P. lividus, Strongylocentrotus purpuratus,* and *Lytechinus variegatus),* and in phylogenetically related organisms, such as the hemichordate *Saccoglossus kowalevskii*, the chordate *Ciona intestinalis*, and the nematode *Caenorhabditis elegans*.

By combining iteration of *ab initio* predictions and pairwise comparative methods, we identified the orthologous genes encoding for $GABA_{B1}$ and $GABA_{B2}$, the two subunits which assemble $GABA_B$-R, and we confirmed that all of these organisms possess a unique α/β $GABA_A$-R gene pair clustered in the genome. Furthermore, we have observed that the reciprocal disposition of $GABA_A$-R genes is also evolutionarily conserved (Fig 1B).

Interestingly, in adjacent position to these genes, we have identified an additional gene, which shows significant sequence similarity to a invertebrate-specific $GABA_A$-R gene. Indeed, such a gene has been only identified in *C. elegans, Drosophila melanogaster*, and *Nematostella vectensis* [7-8].

We also retrieved several cDNA sequences from staged EST databases of the three sea urchin species inspected, indicating that these genes are actively transcribed during development. Some selected cDNA plasmids were also isolated from *P. lividus* total RNA samples and fully sequenced.

Hypothetical proteins were deduced and used for phylogenetic analysis, including a selection of vertebrate and invertebrate $GABA_A$-R subunit sequences. The resulting phylogenetic tree (Fig. 1C) strongly support the hypothesis that the sea urchins contain genes encoding for both canonical and invertebrate-specific $GABA_A$-R subunits. Such a collection of data should provide

a support to better understand the involvement of GABA-signalling pathway in the skeletal GRN.

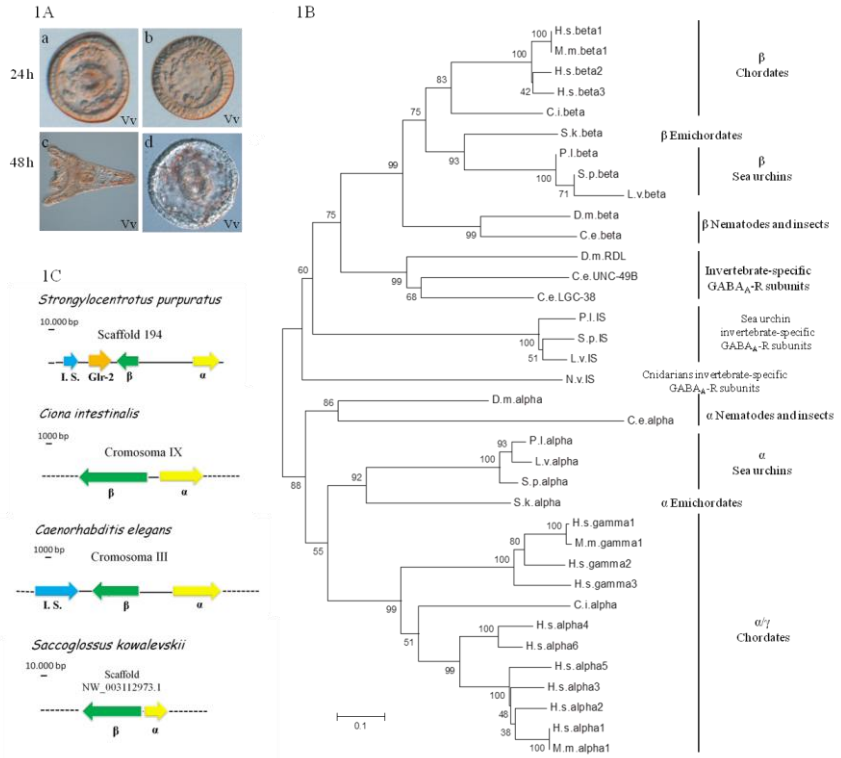

Figure 1. (A), Effect of GABA on embryogenesis of *P. lividus*. (a, c), control and (b, d) GABA-treated embryos observed at 24- (a, b) and 48- (c, d) hours post fertilization. Vv: vegetal view.
(B), Genomic configuration of $GABA_A$-R locus in different species of invertebrates. Glr-2: Glutammate receptor-2; I.S.: Invertebrate-specific $GABA_A$-R subunit gene.
(C), Neighbor-joining tree constructed with protein sequences of representative $GABA_A$-R subunits. Number above nodes indicate bootstrap values (1000 replicates). M.m., *Mus musculus*; H. s., *Homo sapiens*; P.l., *Paracentrotus lividus*; S. p., *Strongylocentrotus purpuratus*; L.v., *Lytechinus variegatus*; C.i., *Ciona intestinalis*; S. k, *Saccoglossus kowalevskii*; D.m, *Drosophila melanogaster*; C.e., *Caenorhabditis elegans;* N. v., *Nematostella vectensis*.

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P23 - EPITOPE MAPPING OF A CROSS-REACTIVE MONOCLONAL ANTIBODY AGAINST THE FACTOR H-BINDING PROTEIN OF *NEISSERIA MENINGITIDIS*

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Abstract
Neisseria meningitidis is an encapsulated gram-negative bacterium, major cause of bacterial meningitis and sepsi worldwide. Although polysaccharide-protein conjugated vaccines are available for the prevention of diseases caused by strains with group A, C, W-135 or Y capsules, no broadly protective vaccine is available against group B strains. The factor H binding protein (fHbp), a 27-kDa membrane-anchored lipoprotein of *Neisseria meningitidis,* is a promising vaccine candidate that elicits serum bactericidal antibodies in humans. The presence of factor H (fH) on the bacterial surface is critical to circumvent host defense while, in the absence of bound fH, the organism becomes susceptible to bacteriolysis. Based on sequence variability of the entire protein, fHbp has been divided into three variant groups or two sub-families. A panel of anti- fHbp mAbs has been produced from mice immunized with the 3 variants of fHbp and their epitopes were previously mapped, except for the mAb designated JAR36, a murine IgG mAb isolated from a mouse immunized with variant 3. In this study, we report epitope mapping of JAR36, this mAb cross-reacts with all fHbp sequences in V.2 and V.3 groups, binds to the bacterial surface and elicits complement-mediated bactericidal activity in combination with other anti-fHbp mAbs. We screened bacteriophage-displayed random peptide libraries to identify amino acid residues contributing to the JAR36 epitope. Mapping predictions were validated by constructing, through site-specific mutagenesis, corresponding rfHbps single-point variants, and analyzing their reactivity with the mAb.

Methods
The epitope recognized by JAR36 is located in the variable E (VE) segment of modular groups II, III, V, VI, VII in the C-terminal region of a chimeric *fHbp*. A multiple sequence alignment of the VE segment, comprising residues 186 to 255, from these *fHbp* variants [1; 2].
Peptides binding to JAR36 mAb were selected by panning five phage libraries (pVIII-9aa, pVIII-9aa.cys, pVIII-12aa, pVIII-Cys.Cys, pVIII-15aa) constructed in the two-gene/phagemid vector pC89 [3].
Mutants were constructed using the Phusion Site-Directed Mutagenesis Kit. Factor H binding protein was expressed from pET21b based plasmids in *E.coli* as described previously [4; 5].

Results
Forty-five positive clones were identified by screening phage libraries with JAR36. Among these clones there were 15 independent sequences. From the comparison of amino acid composition, obtained by characterization of the nucleotide sequence of the positive clones insert, it was not possible to identify a common consensus sequence among the peptides that reacted with JAR36, suggesting that the epitope is discontinuous. We hypothesized that the most abundant amino acids in the bound peptides might be important for the interaction between the immunogen and the mAb. Since JAR36 reacts with *fHbps* from variant groups 2 and 3, and more specifically with those sequences containing a variable E (VE) segment from lineage 2 [6; 7], we focused our attention on the amino acids more frequently occurring in the peptide sequences, and located between positions 186 and 255 in the sequence of *fHbp* ID 28 (Fig. 1). Consequently, we predicted a major contribution of the electrostatically charged residues aspartate and/or lysine to

the JAR36 epitope. We examined the positions of relevant aspartate and lysine residues in a homology model of the *fHbp* ID 28 protein. We then substituted the residues K199, D201 and K203 with alanine and expressed and purified the *fHbp* mutants. Binding of JAR36 or human *fH* to site-specific mutants of *fHbp* ID 28 was measured by ELISA.

Conclusions

- Three single amino acids positions composing the JAR36 epitope were predicted and the corresponding *fHbp* mutants were prepared.
- The K199A and K203A point mutants show decreased binding to human fH, but not to JAR36.
- The amino acid residue substitution, D201A in fHbp affects the binding of JAR36, but not that of human fH, thus Aspartate 201 is a necessary component of the JAR36 epitope.

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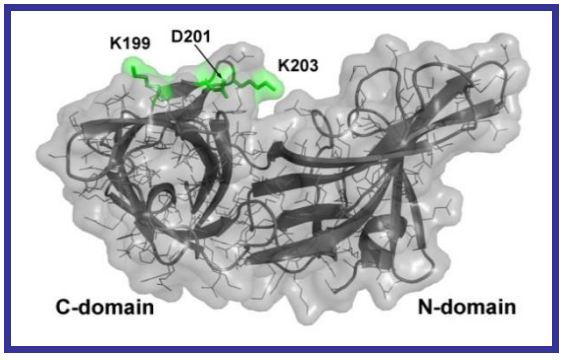

Figure 1. Location of residues predicted to affect binding of JAR36 mAb. A homology model of *fHbp* ID 28 was constructed using Swiss-Model [6] based on the atomic coordinates of the crystal structure of *fHbp* ID 1 (PDB accession number 3KVD) [7]. The protein is represented with the C-terminal domain on the left and the *fH*-binding surface at the top. The locations of the three residues tested by site-specific mutagenesis are shown in green. The figure was generated by using PyMol (http://www.pymol.org).

P24 - OXIDATIVE STRESS RESPONSES IN EARLY LIFE STAGES OF COMMON CARP (*CYPRINUS CARPIO*) AFTER SUBCHRONIC EXPOSURE TO NEEMAZAL T/S (AZADIRACHTIN 10 G/L)

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Increased use of chemical pesticides results in the excess inflow of toxic chemicals into the aquatic ecosystem and may affect aquatic flora and fauna. Recent emphasis is on the use of natural pesticides, which are usually of plant origin. Use of plant based pesticides is generally less harmful and more ecofriendly. Plant based pesticides contain easily biodegradable molecules which are more target specific than the highly persistent broad-spectrum synthetic chemical moieties[1]. Azadirachtin is an active compound extracted from the neem tree (*Azadirachta indica* A. Juss) grown widely in Africa and Asia. Its anti-insecticidal**,** antiviral, antibacterial, and antifungal properties have been known for long time[2]. Azadirachtin is the active substance of insecticide formulation NeemAzal T/S (concentration of active ingredients, 10 g/l), which is used to control whitefly, leaf miners, and other pests including pear psylla. In foreign countries, it has been generally distributed and widely used for a long period of time. Nevertheless, NeemAzal T/S has been registered in the Czech Republic since 2010. NeemAzal T/S is classified as highly toxic to aquatic animals.

The objective of our study was to evaluate changes in antioxidant defense enzymes and detoxifying system in early life stages of common carp after exposure to NeemAzal T/S. Subchronic toxic effects on embryos and larvae were investigated during a 31-day embryolarval toxicity test, which was carried out using a modified protocol according to the OECD guideline 210 (Fish, Early-Life Stage Toxicity test). Testing solutions were prepared from NeemAzal T/S with the nominal concentration of azadirachtin: 30, 100, 300 and 600 μg/l. Twenty-four hours after fertilization, one hundred fertilized eggs were separated and randomly distributed into crystallization dishes containing one of the four concentrations of azadirachtin solution, or a control dishes (azadirachtin-free tap water). The experiment was conducted in triplicate (a total of 300 fertilized eggs for each concentration and control). A semistatic method was used and the testing solution was replaced twice daily. During the test, larvae were fed freshly hatched *Artemia salina ad libitium* twice a day. The temperature, pH, and oxygen saturation were recorded daily. At the end of the experiment, the fish were killed, immediately frozen, and stored at –85 °C until analyses of biomarkers. Whole body samples were weighed and homogenised using phosphate buffer. Supernatant fraction of homogenate was used for determination of activity of glutathione S-transferase (GST) [3], glutathione reductase (GR) [4], glutathione peroxidase (GPx)[5] and catalase (CAT)[6]. Protein concentration was determined by Bicinchoninic Acid Protein Essay Kit (Sigma-Aldrich). Statistical analysis was performed using Statistica 8.0 for Windows software. Biomarkers were tested for normal distribution and after testing of homogeneity of variance across groups, an analysis of variance (one-way ANOVA) was used. The differences among groups were assessed with the Tukey-HSD test.

Effects of subchronic exposure to NeemAzal T/S on selected oxidative stress indices and activity of detoxifying enzyme are presented in Table 1. In all experimental groups, increases of the GR, GPx and GST activities were observed compared to the control group but only in the groups exposed to azadirachtin at 300 and 600 μg/l the level reached significance ($p < 0.05$). In case of CAT activity we did not obtain differences among groups.

Concentrations of azadirachtin	***GPx (nmol/min/mg protein)***	***GR (nmol/min/mg protein)***	***GST (nmol/min/mg protein)***	***CAT (μmol/min/mg protein)***
0 μg/l (control)	77.8±6.0^{a}	7.7±0.3^{a}	97.8±2.1^{a}	25.0±1.0^{a}
30 μg/l	84.3±7.1^{ac}	8.2±0.4^{ab}	104.0±7.9^{ab}	24.5±1.4^{a}
100 μg/l	84.7±4.2^{ac}	8.3±0.5^{ab}	100.4±1.6^{ab}	23.6±1.3^{a}
300 μg/l	108.1±5.1^{b}	10.1±0.2^{b}	109.1±1.9^{b}	27.4±1.3^{a}
600 μg/l	99.46±3.0^{bc}	10.1±0.4^{b}	108.5±2.6^{b}	23.2±0.9^{a}

Table 1. Results of oxidative stress indices (mean ± SEM). Significant differences ($p < 0.05$) among groups are indicated by different alphabetic superscipts.

In our experiment, we found higher activities in almost all enzymes (GR, GPx and GST) in all experimental groups compared to the control group. An increase in antioxidant enzymes contributes to the elimination of reactive oxygen species, which are induced by pesticide exposure. The increase in GST activity is connected with activation of detoxifying system after NeemAzal T/S exposure. Although botanical pesticides are being considered as less toxic and safe, our results indicate that azadirachtin may affect antioxidant defence system and detoxifying ability of fish organism. Negative effects of azadirachtin were also reported by other toxicology studies. Kumar et al. (2010)[7] observed morphological changes in vital organs such as gill of *Heteropneustes fossilis* after short- and long-term exposure to purified neem extract. Winkaler et al. (2007)[8] reported effects of acute lethal and sublethal exposure to neem leaf extract on the neotropical freshwater fish *Prochilodus lineaus*. Plasma glucose levels were higher in fish exposed to neem extract relative to controls, indicating a typical stress response. Neem extract did not interfere with osmoregulation capacities of the fish, as plasma sodium, chloride, total protein, and osmolarity did not change. It was shown to affect the antioxidant defence system of *P. lineatus*, as there was a decrease in liver CAT activity at all neem concentrations, and the detoxifying enzyme GST was activated in fish exposed to the highest concentration. At all concentrations, exposed fish exhibited damaged gill and kidney tissue.

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P25 - SPECTROSCOPIC CHARACTERIZATION AND IN VITRO ASSAY ON HUMAN BLOOD OF NOVEL PORPHYRIN DERIVATIVES

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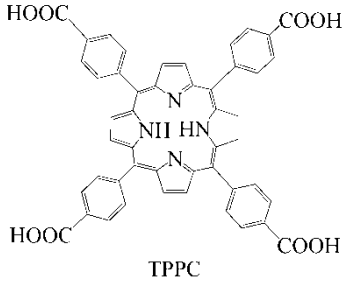
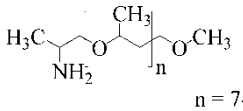
Porphyrins are an important class of natural and artificial pigments which play an important role in largely different area of both fundamental and technological interest. In particular, porphyrin metal derivatives have been exploited as models for enzymes and artificial blood. Charged porphyrins are able to interact with several relevant biomolecules, i.e., nucleic acids, polypeptides, and proteins. This property together with their ability to localize into tumor cells and to photosensitize the production of singlet oxygen led to the development of several compounds actually in use or under investigation for photodynamic therapy applications (PDT) [1]. In this approach visible light is used to active a photosensitizer, generating the highly reactive singlet molecular oxygen. To date, a great variety of porphyrins have been extensively studied. Interestingly, quite recently, meso-tetrakis(4-carboxyphenyl) porphyrin (TPPC) and its derivatives have been exploited as a marker for the rapid detection of tumor cells by fluorescence imaging. It is important to note that many of the physicochemical properties of this class of pigments, and in particular the electronic absorption and the luminescence, are strictly dependent on their aggregation state. A common feature of these molecules is their propensity to interact to form dimers, oligomers, or more extended aggregates. To develop efficient systems for biomedical applications or for PDT (in which aggregation should be prevented) or to stabilize monomeric porphyrins in a very water-soluble form, novel systems based on biocompatible delivery systems are highly desirable. In this framework, recently some of us reported on the employment of biocompatible amino-terminated polypropylene or poly(ethylene oxide)s generally termed as Jeffamines, to prevent porphyrin aggregation, allowing to reach millimolar concentration of TPPC in a monomeric form in solution [2]. In biological media, cell membranes seem to be important targets for many antineoplastic photosensitizer agents. Red blood cells have been often used for in vitro PDT studies. Here we report on two different porphyrin derivatives, TPPC-Jeff and ZnTPPC-Jeff. In Scheme 1 is reported the structure of TPPC-Jeff. Photodynamic action was then evaluated in vitro using human red blood (HRB) cells under different conditions to obtain information about the effect produced by these porphyrin derivatives upon irradiation.



TPPC



n = 7-9

Jeffamine M-600

Scheme1

These novel compounds were tested on human red blood cells (RBCs), with the purpose to see an haemolytic effect on the erythrocytes after exposure to Vis irradiation and overnight incubation. Human blood samples were collected from four healthy donators, were drawn into syringes filled with sodium citrate, as an anticoagulant and were used within 24 h after bleeding. Each, of the two compounds, was dissolved in a buffer, a saline solution at pH 7.4 with the following composition (mM): 125 NaCl, 5 KCl, 1 $MgSO_4$, 32 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 5 D-(+)-glucose, 1 $CaCl_2$; pH 7.4. The samples (HBR cells, saline solution and molecule) were placed in glass flasks (10 ml) and were exposed to 30

minutes to Vis irradiation (halogen lamp with a light dose of ~5 joule/cm^2). The molecules were tested at four different concentrations spanning from $5 \cdot 10^{-6}$ M up to 10^{-4} M. In all studies, using HRB cells, control experiments were carried out without porphyrin compounds in presence and absence of Vis irradiation.

We used an hemolysis test to see the hemolytic effects of the porphyrin compounds, that evaluates hemoglobin release in the plasma following molecule and irradiation exposure [3]. After overnight incubation at 37 °C, the tubes were centrifuged (10 minutes at 1500 rpm at 4 °C) and the supernatants were determined photometrically with a colorimeter and after with a spectrophotometer at the absorption of hemoglobin (540 nm). The spectroscopic measurements of haemoglobin released are much easier and faster to carry out than cell staining and counting [4]. The absorption of the supernatant of erythrocytes lysed in distilled water was defined as 100% haemolysis. The molecules under study induce hemolysis in human erythrocytes at the concentrations tested and then overnight incubation, as show in Table 1. The concentrations $5 \cdot 10^{-6}$ M and 10^{-4} M are respectively one too low and the other too high to see a reliable hemolytic effect. While we observed that TPPC-Jeff induce an hemolytic effect at the concentration of $5 \cdot 10^{-5}$ M, and the Zn TPPC-Jeff induce the release of hemoglobin at the concentration of 10^{-5} M. We have seen that Vis irradiation does not increase the efficacy of the compounds in inducing hemolysis.

Then we assayed the morphology of erythrocytes by an optical microscope after incubation with porphyrin compounds, 30 minutes of irradiation and overnight incubation and the result was that they lost their normal biconcave profile and presented a spiny configuration with blebs in their surfaces. A normal profile was observed in the control. Representative results are show in Fig. 1. HRB cells obtained from four subjects were used to assess an hemolytic activity of porphyrins. Results suggest that all the molecules under study show an hemolytic effect. Further studies are required to optimize potential therapeutic dosing strategies to inform and encourage clinical trial design.

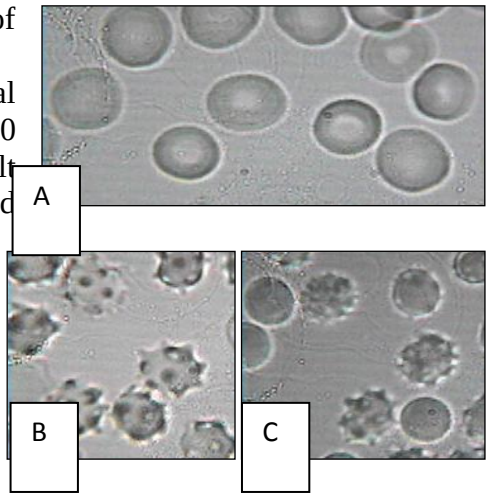


Fig. 1. Photograph showing morphology of HRB cells (A) control without tretracycline and irradiated for 30 min; (B) TPPC-Jeff and irradiated for 30 min; (C) Zn TPPC-Jeff and irradiated for 30 min.

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Table 1. Percentage of hemolysis by compounds TPPC-Jeff ($5 \cdot 10^{-5}$ M), Zn TPPC-Jeff (10^{-5} M)

Osmolarity (mOsm/kg)					
Compounds	**300**	**200**	**150**	**100**	**0**
Control	*0%*	*1%*	*2%*	*18%*	*100%*
TPPC-Jeff	12%	11%	12%	30%	100%
ZnTPPC-Jeff	17%	16%	23%	100%	100%

P26 - MITOCHONDRIAL MASS, DISTRIBUTION AND ACTIVITY DURING SEA URCHIN OOGENESIS

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The sea urchin egg is a favourite model for studies of the molecular biology and physiology of fertilization and early development, yet we know sparingly little of its oocytes and of mitochondria behaviour during oogenesis.
The process of oogenesis in most echinoderms is asynchronous so each ovary lobe has hundreds of oocytes at all stages of development. At the beginning of oogenesis, the oocyte is about 10 µm in diameter. During the vitellogenic phase of oogenesis, the oocyte accumulates yolk proteins and grows to ten times their original size to 80 to 100 µm in sea urchins. The oocyte, arrested at the prophase of the first meiotic division, is apparent with its large nucleus, the germinal vesicle (GV), containing a prominent nucleolus. Echinoid (such as sea urchin) and Holothurian oocytes complete meiotic maturation prior to fertilization, distinct from other echinoderms and almost all others animals. As maturation progresses, it occurs the GV breaks down (GVBD). These eggs may then be stored for weeks to months within the female before they are spawned, and the proportion of eggs in the ovary increases from early to late season, as the numbers of oocytes decline [1].
Mitochondria, generally known as the powerhouses of eukaryotic cells, play a primary role in cellular energetic metabolism, homeostasis and death. These organelles, with their multicopy genome maternally inherited, are directly involved at several levels in the reproductive process since their functional status influences the quality of oocytes and contributes to the process of fertilization and embryonic development.
It has been demonstrated that the number of maternal mitochondria is sufficient to support development until late stages without new synthesis of mitochondrial DNA or production of new organelles [2]. During embryogenesis mitochondrial mass does not change, whereas mitochondrial respiration increases [3]. The behaviour of these organelles during oogenesis remains at moment unclear.
In the present paper we studied, by Confocal Laser Scanning Microscopy tecnologies (CLSM), the mass and distribution, the activity and the DNA content of sea urchin *Paracentrotus lividus* mitochondria during oogenesis, by *in vivo* incubating oocytes of different size with cell-permeant probes specific for mitochondria and for DNA and by immunodetection of hsp60 chaperonine, a well known mitochondrial marker.
In particular the oocytes were grouped in six classes: < 10, 20/30, 40/50, 60/70, 80/90 µm, and 90 µm ovulated egg, on the base of diameters. Microscopic observations were performed capturing 2 µm thick layers of oocytes. Of the several thousands oocytes we observed, 20 for each different oogenesis stage were analyzed and processed. In order to interpret results and to draw unequivocal conclusions, we measured by IMAGE J software analysis the intensity values of fluorescent signals, as suggested in Agnello et al 2008 [4].
The mitochondria of oocytes with a diameter between 20 and 70 µm, appeared to give rise to clusters that disappear in that of 80 µm. In the oocytes between 60 and 90 µm the red fluorescence seems to be more evident around the germinal vesicle (the merge tends to red), suggesting an increasing oxidative phosphorylation activity.
In the ovulated eggs, red and green fluorescence are uniformly distributed suggesting that mitochondria are dispersed in the cytoplasm. In addition the merge of green and red colours

shows that the whole mitochondrial population is consuming oxygen at the same level (the resulting colours tends to yellow), figure 1.
In order to calculate the total mitochondrial mass and activity we integrated the values of pixel intensities for all captured sections and used the arithmetic means to draw a statistical analysis. Results suggest a parallel rise of mitochondrial mass and activity, suggesting that the amount and activity of organelles change remarkably during oogenesis.

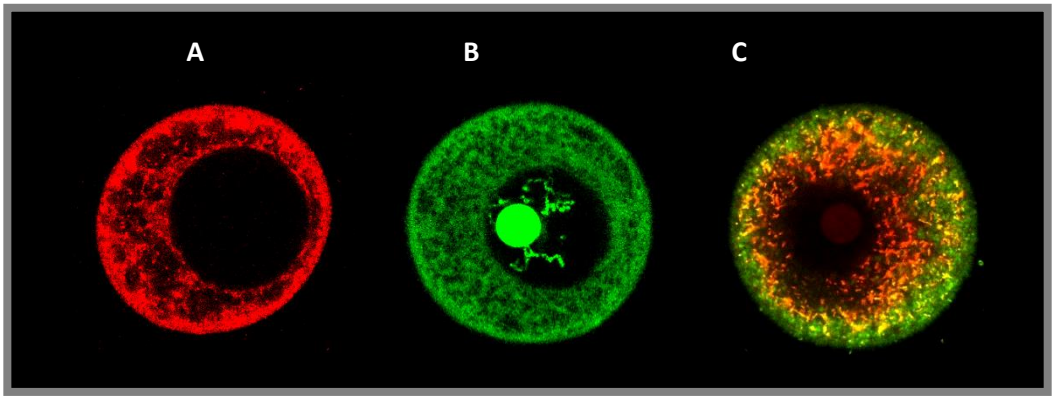

Figure 1 shows the distribution of hsp60 protein, detected by immunofluorescence analysis (A), the mitochondrial and genomic DNA, after in vivo incubation with PicoGreen probe (B) and the merge of green and red fluorescence signal, respectively due to mitochondrial mass and activity, after in vivo incubation with Mitotraker Green and Orange (C). The size of the oocytes reported is 80 µm.

Results suggest that mitochondria are actively duplicating and that mitochondrial DNA is replicating during the different oogenesis phases. It is noteworthy that around the germinal vesicle, especially in the larger oocytes, next to the germinal vesicle breakdown, the organelles are more active in oxygen consumption, probably due to the major energetic needing in this key moment of gametogenesis.

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P27 - OBTAINING MESENCHYMAL STEM CELLS FROM ADIPOSE TISSUE OF MURIN ORIGIN: EXPERIMENTAL STUDY

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Conflict of interest: none declared for all the Authors

Stem cells have a key role in regenerative medicine and tissue engineering. Although not immortal, they are able to expand manyfold in culture retaining at the same time their growth and multilineage potential. They also show a migratory capacity when transplanted systemically in animal model with injuries.
Thanks to their properties and their plasticity stem cells are of great importance since they can be used as a tool for repair damaged tissues and organs.
Mesenchymal stem cells, in particular, have the ability to differentiate into lineages of mesodermal tissues, such as skeletal muscle, bone, tendons, cartilage, and fat under appropriate culturing conditions.
Recent evidence suggest that the adipose tissue is a promising source of mesenchymal stem cells attracting the interest of researchers and clinicians. It is rich of pluripotent stromal cells , available in large amounts and more readily accessible than bone marrow. Furthermore, comparative analysis of mesenchymal stem cells of bone marrow and adipose tissue show that cells are not different regarding morphology, immune phenotype, success rate of isolating and differentiation ability.
Our experience at the Experimental Zooprophylactic Institute of Sicily "A. Mirri allows us to define a protocol for stem cells isolation of murine origin .
We used 6 Winstar breed male rats whose average weight was 350g.
All animals were sedated with an intramuscular injection of midazolam and anesthesia was maintained with isoflurane and oxygen gas mixture administered with a mask.
The adipose tissue has been taken from the root of the animal's thigh with a small incision.
Different steps are needed for processing and digesting the tissue. First it is washed several times in a solution enriched of antibiotics and then is mechanically fragmented. The homogenate is therefore digested enzymatically under permanent shaking.
We obtain an heterogeneous population of cells that were subsequently selected through the plastic adhesion. These cells are able to grow and proliferate and show all the characteristics typical of stem cells. In conclusion, we report a multistep and reproducible technique for providing a substantial number of mesenchymal stem cells and for maintaining them in culture.

P28 - ROLE OF ERK1/2 PROTEIN IN THE REGULATION OF HERPES SIMPLEX VIRUS TYPE I REPLICATIVE CYCLE

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Introduction and objectives: Herpes simplex virus type 1 (HSV-1) is a double stranded DNA (dsDNA) virus that causes a variety of infections in humans (1). HSV-1, as many DNA viruses, has developed different strategies during the evolution to modify the cellular environment in favor of its replication. Different aspects of HSV-1 biology render this virus a good model to study the complexity of virus-host cell interactions. The eukaryotic cells, indeed, respond to external stimuli through the activation of different signal pathways, as the Ras/Raf/MEK/ERK signal pathway. Among these protein kinases, the extracellular-signal-regulated-kinases (ERK) have proven critical in the control of the progression G1/S that involved specific regulator proteins, such as cyclins and cyclin-dependent kinases (CDKs) (2, 3). That HSV-1 infection requires involvement of ERK1/2 and mitogen activated proteins kinases (MAPKs) signal pathway and controls cell cycle proteins is already known (4, 5 6, 7, 8, 9). In fact, the activity of CDK involved in the transition from late G1 to early S phase seems to be required for viral DNA transcription and replication. However, the evidence for the overall understanding of networks and gene products involved in these interaction requires further investigations. Based on these knowledge the current work was focused to study the activity of ERK1/2 protein during viral replication and the correlation between ERK protein recruitment and G1/S phases regulation by HSV-1 infection.
Materials and Methods: Western Blot analysis was used to evaluate nuclear and cytoplasmic protein accumulations. The MAPK/MEK-ERK pathway was modulated using the inhibitor U0126 to study replication in HEp-2 (human larynx epidermoid carcinoma cell line). A stably transfected cell line was derived from wild type (wt) HEp-2, by transfection of plasmid coding for dominant negative form of ERK protein (HEp-dnERK). Standard Plaque Assay was done on VERO cells. Immunofluorescence assay and quantitative Real Time PCR were used to evaluate the levels of viral and cellular gene transcription and viral DNA synthesis .
Results and Discussion: We have analyzed the activation of ERK1/2 protein during wt HSV-1 infection in HEp-2 wt cell line. HSV-1 leads to the activation of ERK1/2 protein during the first phases of infection, and subsequent decrease during late phases compared to uninfected cells, suggesting involvement of ERK1/2 activity during infection. HSV-1 replication was studied in wt HEp-2 cells where ERK1/2 activity was chemically inhibited. The data showed a defect in viral progeny production in treated and infected cells as compared with non-treated and infected cells. These data were confirmed by the differences in the accumulation of ICP0 (immediate early) and Us11 (late) viral proteins. Moreover, we evaluated the phosphorylated forms of key regulators of G1/S progression, such as cyclin E and CDK2 proteins in presence or in absence of U0126. The results demonstrated that the treatment inhibits the accumulation of cyclin E and CDK2 proteins. These results were further confirmed by using HEp-dnERK cell lines. In this cell system HSV-1 replication was compromized compared with parental cell lines. Indeed, using q-PCR, viral DNA the cellular genes CDK2 and cyclin E, and the viral immediately early gene (ICP0) and the late gene, (gB) were evaluated in HEp-dnERK infected by wt HSV-1 compared to control. A decrease was observed in viral DNA synthesis, as well as in cellular gene transcripts, in cells where ERK1/2 activity was compromised, demonstrating that MAPK-ERK

proteins plays a fundamental role during HSV-1 replication. However, further investigations are necessary.
Conclusion: The new information obtained could be contribute to development of new pro-host tools that would be useful to set up effective prevention strategies, such as therapeudical approaches for severe HSV-associated infections. Because the Raf/Ras/MEK/ERK pathway is modified in 60% of solid tumors, understanding the interactions between HSV-1 and this pathway could contribute to the design of HSV-1-based oncolytic vectors.

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P29 - EFFECT OF CONDITIONED MEDIA FROM OSTEO- AND ADIPO-DIFFERENTIATING MESENCHYMAL STEM CELLS ON TRIPLE NEGATIVE MDA-MB231 BREAST CANCER CELLS

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It is know that mesenchymal stem cells (MSCs) actively secrete multiple biologically-active factors during their process of differentiation which gives rise to a variey of cytotypes including bone and fat cells. It is also acknowledged that the chemokines secreted throughout MSC differentiation may play an important role in the development and growth of tumor cells, although literature data appear somewhat indeterminate due to the contradictory evidence often found [1].
The purpose of this study was to evaluate the effect of conditioned media (CMs) from MSCs, cultured for 7, 14, 21 and 28 days in osteo-, adipo-differentiating and undifferentiated conditions, on MDA-MB231 breast cancer cells, an "in vitro" model system derived from a triple-negative breast cancer (TNBC). MTT assay showed that the CMs collected after 28 days of both osteo- and adipo-differentiation induced growth inhibition on MDA-MB231 cells after 24 h of incubation. In light of such evidence, these CMs were used to treat cells and perform cytofluorimetric assays to better evaluate their biological effects on viability/proliferation, cell cycle progression, apoptosis/autophagy induction and mitochondrial activity/reactive oxygen species (ROS) accumulation of MDA-MB231 cells.
The most interesting results regard the ability of CMs from osteo-differentiating MSC to induce an alteration of cell proliferation with an arrest in the G_2/M transition phase of the cycle coupled to both apoptotic and autophagic promotion. No accumulation of ROS and impairment of mitochondrial respiration was observed at the end of treatment. On the other hand, preliminary indications suggest that the CMs isolated from adipo-differentiating MSCs have different effect from those obtained by osteo-differentiating cultures, being the lethality unlinked to apoptosis and autophagy, and thereby prompting to get more insight into the anti-TNBC activity shown by the different CMs at the molecular level.

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P30 - 3D-TISSUE ENGINEERED CARTILAGE PRODUCTION ON ANIMAL MODEL. PRELIMINAR RESULTS.

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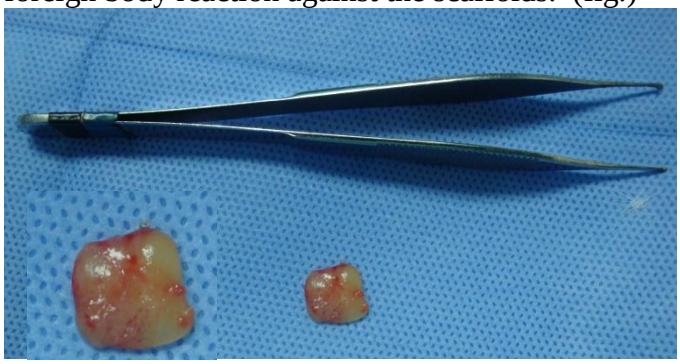
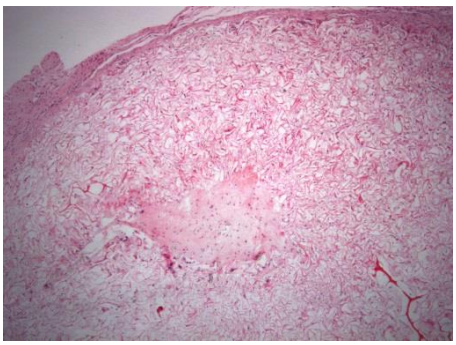
Keywords: cartilage tissue engineering, chondrocyte, 3D-scaffold

Introduction. Tissue or organ transplantation is severely limited by the problems of donor shortage and immune rejection from the patients. In plastic surgery, implants and autografts are frequently used to repair congenital or traumatic defects. For example, in nasal and auricular reconstruction surgeons harvest cartilage from a donor site in order to reconstruct the defect, but current therapies and techniques require invasive surgery, seem inadequate and present many problems (scaring, pain, infection, resorption and structural failure, poor aesthetic results et al). The development of tissue engineering allows to transplant cells from a patient's own tissue to regenerate damaged tissue or organ without causing immune response. The essential requirements for a bio-engineered cartilage are: cell source and 3D-biocompatible scaffold that allows cell replication and chondrogenesis. Several scaffold materials have been investigated for tissue engineering cartilage. It is necessary to find a 3D-scaffold that could define the shape of the engineering tissue, support the cell proliferation, maintaining their differentiation. The authors used a 3-dimensional porous matrix of cross-linked collagen and glycosaminoglycans for bio-engineered cartilage production on animal model.
Materials and methods. The experimentation was made on 12 New Zeeland white rabbits and was divided in 4 phases.
I) Culture of Human Chondrocytes onto 3D scaffold. Pieces of 3D scaffold (1 cm^2 size) were placed in a 12 well plates. After this, human chondrocytes cell suspension (human Chondrocytes kit, easy to be bought) was placed in each piece.
II) Surgical phase for template implantation. Under general anesthesia each rabbit received one template that was placed in subcutaneous pockets of the trunk.
III) Explantation of the new tissue. After 8 weeks the neocartilage was explanted.
IV) Histological analysis with light microscopy of the neocartilage was made.
Results. All animals tolerated the cartilage implanting without evidence of wound breakdown or infection. The implants maintained the appearance of the rectangular template and had a white, glistening appearance. Macroscopic and histological examination with hematoxylin-eosin staining of tissue-engineered specimens showed evidence of well organized cartilage. There were no signs of immune response in tissue engineered constructs against autologous cells or the foreign body reaction against the scaffolds. (fig.)





Conclusion. Cartilage is avascular aneural and alymphatic tissue, composed of chondrocytes embedded within a dense extracellular matrix, composed by collagen and GAG. Due to its limited ability to self-repair, cartilage is an ideal candidate for tissue engineering. In their

experience the authors used the 3-dimensional porous matrix of cross-linked collagen and glycosaminoglycans as scaffold for the chondrocytes seeding and the cartilage production. This scaffold has a good biocompatibility. It retained the pre-designed shape during the chondrogenesis and no grow-factors were added. In all the samples neocartilage was produced and was similar to native articular cartilage. This experience suggests that this scaffold could be applied in cartilage tissue engineering with success. Perspectives of this procedure are: 1) producing the cartilage that retains the original 3D shape of the scaffold 2) the tissue engineered cartilage could be created into the desired shape preoperatively using an easy-to-model scaffold. 3) In addition, the engineered cartilage is an autogenous tissue that would avoid the risk of graft rejection or extrusion while encouraging long-term durability and even proportional growth. This preliminary data suggest to repeat the same experimentation using chondrocytes isolated from elastic cartilage specimens taken from the human ear. Further long term in vitro and in vivo studies are necessary in order to find the best method for a specific clinical application for example before engineered cartilage can be applied in reconstructive surgery of the ear.
Fig.1a) e b) The new tissue is a white, glistening appearance, with rectangular shape. It was hard to cut by scalpel as native cartilage. 1c) Microscopic aspects of the engineering cartilage: well organized cartilage, without signs of foreign body reaction.

P31 - STUDY OF ALGAL BLOOMS OF *ALEXANDRIUM* SPP IN AN IONIAN BAY OF SICILY HOSTING BIVALVE MOLLUSCS AQUACULTURE

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Bloom impact on aquaculture may be dramatic, with fish kills, economic losses due to temporary harvest closures of contaminated shellfish, and risks for human health (1). It is known that live bivalve molluscs may concentrate dangerous toxins produced by different microalgal species, and so the man received as a result of their consumption, even after cooking: the presence of toxins in bivalve molluscs beyond the limits set by law, it prohibits the harvesting and marketing in order to protect public health. One of the main problems in such regions worldwide, also in the Mediterranean Sea, is the increased frequency of HABs (Harmful Algal Blooms), including blooms of toxic dinoflagellates, such as several *Alexandrium* species as *A. minutum*, *A. catenella*, *A. tamarense* and *A. taylori*, which produce potent neurotoxins (saxitoxins and/or gonyautoxins) (2,3). The presence of toxic algae for PSP (Paralytic Shellfish Poisoning), with recurrent blooms of *A. minutum*, has been known for some years also in the waters of an Ionian bay of Sicily, the Syracuse harbour (4) (Fig. 1), in which are located shellfish aquaculture practices, in waters classified as "Area B". Our previous work reported in this area positivity for PSP toxin in mussels (*Mytilus galloprovincialis*) with saxitoxin concentrations above the limit of the law (5), and the simultaneous presence of toxic species (*A. minutum*) in the waters (5,6). In this work are reported recent episodes of algal blooms by *Alexandrium* spp. - *A. minutum* and first records of *A. catenella* - in the same area.

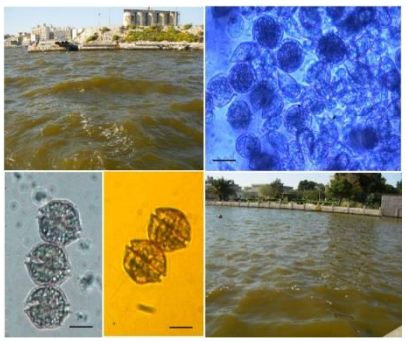

FIG.1.Water discolourations in Syracuse bay, Sicily, and phytoplankton assemblages (upper photo) containing *Alexandrium catenella* (lower photos). Spring 2012. Scale bars=15µm

The study (2010-2013) has being focused on a multi-approach, including: 1) identification of toxic species, such as *Alexandrium* spp. based on morphological traits (microscopy) and isolation of cells in culture for further studies (molecular and toxicity); 2) identification and confirmation of target species by molecular approaches using quantitative Real Time PCR technique; 3) research of PSP toxins in mussels sampled in the same area by biological method (MBA)-AOAC Official Method 959.08, 2005. The first molecular identification, by PCR using species-specific primers targeting the ITS-5.8S rDNA (7), allowed the rapid confirmation of the identity of several suspected toxic *Alexandrium* species, such as *A. minutum* and also *A. catenella*. The PCR amplification conditions were used as described in Penna et al (3). Evidence of the occurrence in surface waters of *Alexandrium catenella* (Fig.1) was gained, that is a new record for this Mediterranean locality. In this period the research for algal toxins PSP, carried out on samples of mussels with the mouse test, was negative.

Marine coastal areas are ecosystems increasingly subjected to intense human pressure, nutrient inputs, eutrophication, pollution, as well as to introduction of NIS non-indigenous and IAS invasive alien species, with adverse effects on biological diversity and ecosystem functioning.

The area of the Syracuse harbour is more suitable to the study, in terms of area at risk due to the presence of various phytoplankton species that producing Harmful Algal Blooms (HAB). This website confined used for mussels aquaculture, among other things, is subject to the onset recurrent harmful or toxic blooms of *A. minutum* and other toxic species of Dinoflagellates, blooms that occur each spring, with a maximum density of 10^6 cells/l (2).
A recent introduction of the species in the region is thus hypothesized, since previous data from toxic phytoplankton monitorings (microscopical and molecular surveys) have never shown the presence of *A. catenella* in this harbour, or in other Sicilian locations.
The problem of toxic algae blooms, and recurring in various zones of the Italian coast, locations of shellfish, has long been known: the resulting health risk is subject to continuous attention due to the current national and European regulations that impose systematic monitoring of water and MBV to detect respectively the possible presence of toxin-producing plankton and biotoxins in production areas, as well as measures of early closure of fishing areas when accumulated to levels above legal limits: for this reason for several years also this area is monitored periodically. Efforts are being addressed to trace toxin profiles in cultures and intensify the analyses of PSP-toxins in farmed shellfish, in order to control and limit the heavy impact of these blooms on shellfish farming and public health.
Data obtained arise from an Italian national project on "Algal toxins contaminating water and fish products: monitoring, characterization and study of innovative methods" supported by the Ministry of Health, Project RF-IZI-2008-1139874, and co-ordinated by Istituto Zooprofilattico Sperimentale of Sicily.

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P32 - STUDIO SULLA CONTAMINAZIONE MICROBIOLOGICA DEI MANGIMI E SVILUPPO DI METODI DIAGNOSTICI AUTOMATIZZATI FINALIZZATI ALL'IDENTIFICAZIONE DI MUFFE MICOTOSSINOGENE

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Tra le diverse problematiche riguardanti gli alimenti ad uso zootecnico, la contaminazione da micotossine rappresenta una delle principali fonti di preoccupazione per l'Autorità Sanitaria essendo queste ultime fra le sostanze naturali ad elevata tossicità, sia per l'uomo che per gli animali. I danni provocati da tali sostanze sono solitamente localizzati a livello di specifici organi e tessuti: alcune esplicano azione nefrotossica, epatotossica, immunotossica, mutagena, teratogena e cancerogena. La presenza di micotossine nei mangimi è strettamente correlata alla presenza e crescita fungina anche se la presenza di muffe su una derrata alimentare non implica necessariamente la presenza di questi metaboliti. La contaminazione da muffe tossinogene e da micotossine può avvenire lungo tutta la filiera: in campo, durante la coltivazione, durante lo stoccaggio, la lavorazione e la distribuzione delle derrate alimentari(1,2). La contaminazione e la produzione di micotossine da Fusarium avviene prevalentemente durante la coltivazione, mentre quelle da Aspergillus e Penicillium si verificano soprattutto durante lo stoccaggio (3,2) . Le muffe che producono micotossine sono innumerevoli, ma i ceppi che per la loro diffusione e pericolosità assumono maggiore importanza sono i generi Aspergillus, Fusarium e Penicillium produttori di Aflatossine, Ocratossine, Zearalenone, Fumonisine, Deossilivalenolo (DON) e Tossina T-2 (4,5). Risulta necessario attuare piani di monitoraggio per la valutazione delle condizioni igienico sanitarie dei mangimi al fine di adottare misure necessarie ad impedire la crescita di muffe e quindi la eventuale formazione di micotossine. Nel nostro studio le analisi effettuate hanno riguardato complessivamente n° 54 campioni di alimenti ad uso zootecnico destinati a diverse specie di animali da reddito. E' stato valutato il livello di contaminazione da muffe, con particolare riferimento alle specie tossigene. Sugli stessi campioni è stato altresì effettuato un esame batteriologico completo al fine di valutarne lo stato igienico. La ricerca è stata eseguita per: Salmonella spp., Carica Microbica Totale, Enterobatteri , E. coli, Clostridi solfito riduttori, Stafilococchi coagualsi positivi e Streptococchi fecali. Per ottimizzare la numerazione delle muffe si è proceduto ad una valutazione preliminare dei terreni di coltura disponibili, sia previsti dalle normative, ISO21527-1: Dichloran-rose bengal chloramphenicol agar (DRBC) e ISO21527-2: Dichloran 18% glycerol agar (DG18), sia disponibili in commercio e specifici per funghi filamentosi quali Sabouraud agar, Malt agar ed ODYE agar (estratto di lievito destrosio cloranfenicolo). Si è ritenuto opportuno utilizzare il solo terreno DG18 (ISO21527-2) in quanto ha dimostrato essere quello più rispondente ai requisiti di selettività e specificità oltre ad essere il terreno d'elezione per mangimi con attività dell'acqua (aw) inferiore a 0.95. I terreni ODYE e Sabouraud, per la loro scarsa selettività determinano il sopravvento delle sole specie fungine a crescita "esplosiva" quali gli Zigomiceti impedendo ad altre specie a lenta crescita di manifestarsi. Il Malt agar, invece, benché non selettivo, risulta essere quello più adeguato allo sviluppo delle colonie di muffe destinate all'identificazione di specie, in quanto consente l'espressione di tutte le caratteristiche sia morfologiche che e biochimiche. Dopo aver effettuato un conteggio delle colonie fungine, le differenti tipologie morfologiche sono state sottoposte ad identificazione di specie mediante l'ausilio di un sistema automatizzato quale il MicroLog System BIOLOG (AES Chemunex). Il MicroLog System è un sistema identificativo utilizzato per l'identificazione e caratterizzazione di più di 2100 microrganismi tra i quali i funghi filamentosi. Il metodo si basa sull'utilizzo, da parte del microrganismo, di una serie fonti di carbonio, adsorbiti nei pozzetti di una piastra microtiter (MicroPlate). Utilizzando un

opportuno brodo colturale viene preparata una sospensione microbica che verrà inoculata nei 96 pozzetti della Microplates ognuno contenente una fonte di carbonio differente. L'eventuale utilizzo del substrato è evidenziato da una reazione redox a carico di un indicatore (sale di tetrazolio) che riducendosi cambia colore. I sali di tetrazolio possono, infatti, essere utilizzati come indicatori colorimetrici dell'attività respiratoria a livello cellulare. L'ossidazione biologica di un substrato organico da parte di un microrganismo origina NADH ridotto, se gli elettroni sono donati ad una catena di trasporto, il sale di tetrazolio può funzionare come accettore finale artificiale, riducendosi e formando un prodotto colorato, il formazolo. Dopo idoneo periodo di incubazione si procede alla lettura della piastra utilizzando uno spettrofotometro (Biolog MicroStation) in grado di leggere alla lunghezza d'onda di 590 nm. Ad ogni ceppo microbico saggiato corrisponde un profilo metabolico o profilo di utilizzo dei substrati di carbonio sulla base dei test biochimici risultati positivi o negativi: la somiglianza o l'equivalenza del profilo ottenuto con i profili registrati nel database Biolog permette di identificare il microrganismo risalendo al genere e alla specie. In particolare, nel nostro caso, il metodo prevede cinque fasi successive: la semina di una singola colonia da identificare su terreno Malt agar; l'allestimento di un vetrino per poter procedere ad una prima caratterizzazione morfologica; la preparazione dell'inoculo, in opportuno medium (FF broth) e ad una specifica densità ottenuta con l'ausilio di un turbidimetro; l'incubazione delle microplate FF a 25°C per un max di 5 giorni; la lettura allo spettrofotometro ed l'interpretazione dei risultati. Le analisi effettuate suggeriscono come le condizioni igieniche dei mangimi non siano eccellenti, si osserva infatti una presenza fungina rilevante (10^4 ufc/g) ed eterogenee. E' stato possibile identificare numerose specie appartenenti a generi produttori di micotossine quali: muffe del genere Cladosporium, Aspergillus, Pernicillum, Fusarium e DON (deossinivalenolo). Sono state altresì identificate specie appartenenti agli Zygomiceti, non produttori di micotossine. Anche i risultati della numerazione batteriologico mostrano come la Carica Microbica Totale risulti sempre con valori elevati (10^5 ufc/g) indipendentemente dalla tipologia del mangime. Tuttavia, le altre tipologie batteriche risultano assenti o scarsamente rappresentate, suggerendo dunque una contaminazione da flora batterica ambientale. Inoltre l'utilizzo del sistema automatizzato Biolog ha dimostrato essere un valido supporto nell'identificazione delle specie fungine presenti nei mangimi in popolazioni molto eterogenee, consentendo di porre maggiore attenzione alle specie micotossinogene.

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P33 - SYNTHESIS OF ENANTIOPURE SUGAR-DECORATED SIX-ARMED TRIPTYCENE DERIVATIVES AND A BIOLOGICAL EVALUATION ON HUMAN RED BLOOD CELLS

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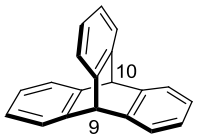
Molecular recognition phenomena occurring between carbohydrates and proteins are responsible for the initiation of critical events in many biological processes such as cell-cell communication, immune response or cancer metastasis. However, individual sugar units have generally weak binding affinity to their complementary proteins, while systems incorporating several carbohydrate units, attached to an appropriate scaffold or self-assembled in nanoparticles, lead to greater affinity properties due to the sum of the constitutive interactions [1]. The efficient construction of molecular systems bearing multiple carbohydrate appendages has become a necessary tool in glycobiology and glycomic fields [2]. The general prototype of a glycoconjugate comprises a core molecule serving as an oligovalent scaffold, a number of sugar moieties and suitable spacers to link the sugar moieties to the central core. Several significant examples of such molecular architectures have been obtained and demonstrated to be perfectly suited, for example, to the binding of lectins, through the glycoside cluster effect [3]. These glycomimics have attracted attention because of their great potential not only to gain insight into the molecular recognition events but also for their capabilities in biotechnology, pharmaceutical and medical applications. The geometric features of triptycene (**1**) with a D_{3h} symmetry has attracted much attention and its derivatives have been used for significant applications in supramolecular chemistry, material chemistry, and as molecular machines [4].



1

Triptycene derivatives have been used also as building blocks for the synthesis of new host molecules in host-guest chemistry and investigated for their potential applications in molecular recognition and assemblies, showing powerful complexation abilities toward different kinds of organic guests [4]. In accordance with our research interests devoted to the synthesis of new glycoconjugates and study of their biological properties [5], we have developed a methodology for obtaining molecules showing a rigid lipophilic core represented by the triptycene skeleton that is linked, by the arene rings, to six sugar moieties, through six triazole units as spacers ((Figure 1). The relationship between their molecular structure and biological activity was investigated with respect to their ability to induce apoptotic cell death, taking into account the pivotal role of apoptosis regulation in both the controlled expansion and removal of immune cells and cancer progression and therapy. [6]. The synthetic pathway was based on the efficient azide/alkyne 1,3-dipolar cycloaddition. The six-armed triptycene azide (2,3,6,7,14,15-hexakis(azidomethyl)-9,10-dihydro-9,10[1',2']-benzenoantracene) was prepared from the corresponding hexakis(bromomethyl)-substituted triptycene, that was obtained by an original procedure. Different 2-propyn-1-yl □-D-glycopyranosides represented the unsaturated acceptors. All products were isolated in good yields and fully characterized.

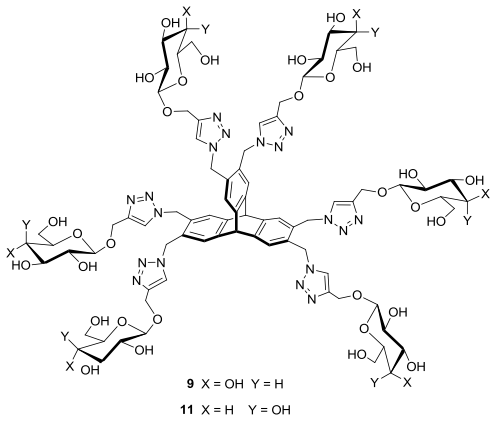


Figure 1: The molecular skeleton of sugar-decorated six-armed triptycene derivatives.

In order to assess the potential biological activity of the obtained compounds, tests were performed to know both the cytotoxicity (trypan blue assay) and the hemolytic activity on human red blood cells by overnight exposition at 37°C to the galacto- and glucoconjugates. The experiment showed the absence of both cytotoxicity and erythrocyte hemolysis as shown in Figure 2. This supports the rational design of hemocompatible molecules for biomedical applications[7].

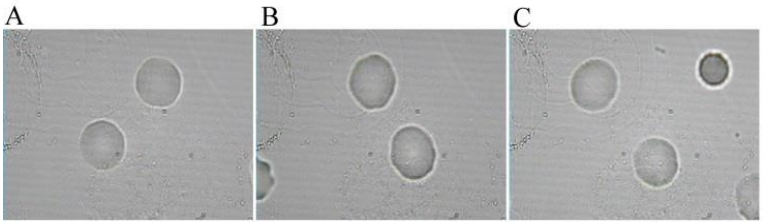


Figure 2: A) Control: Erythrocytes in saline solution; B) Erythrocytes exposed to deprotected glucoconjugated triptycene (10^{-4} M); C) Erythrocytes exposed to deprotected galactococonjugated triptycene (10^{-4} M).

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P34 - SIMPLE AND FAST OROTRACHEAL INTUBATION PROCEDURE IN RATS

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Keywords: endotracheal intubation in the rat, simple and fast orotracheal intubation, rat model, inhalation anesthesia, experimental surgery.

Conflict of interests: none declared for all the authors

Abstract: Introduction: Endotracheal intubation in the rat is difficult because of the extremely small size of anatomical structures (oral cavity, epiglottis and vocal cords), small inlet for an endotracheal tube and the lack of proper technical instruments. Material and Methods: In this study we used seventy rats weighting 400–500 g. The equipment needed for the intubation was an operating table, a longish of cotton, a cotton tip, orotracheal tube, neonatal laryngoscope blades, KTR4 small animal ventilator and isoflurane for inhalation anaesthesia. Premedication was carried out by medetomidine hydrochloride 1 mg/mL; then, thanks to a closed glass chamber, a mixture of oxygen and isoflurane was administered. By means of a neonatal laryngoscope the orotracheal tube was advanced into the oral cavity until the wire guide was visualized trough the vocal cords; then it was passed through them. The tube was introduced directly into the larynx over the wire guide; successively, the guide was removed and the tube placed into the trachea. Breathing was confirmed using a glove, cut at the end of a finger, simulating a small balloon. Results: We achieved a fast and simple orotracheal intubation in all animals employed. Conclusions: We believe that our procedure is easier and faster than those previously reported in scientific literature.

P35 - EFFECT OF COPPER CHELATION ON HEPCIDIN EXPRESSION IN THE HUMAN HEPATOMA CELL LINE, HEPG2

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Hepcidin, a peptide hormone produced mainly by the liver (1), has an essential role in iron homeostasis. It is produced as prepropeptide of 84 amino acids and, after two consecutive cleavage, a form of 25 amino acids is secreted in the bloodstreem (2, 3, 4). Hepcidin is able to control the body iron concentration by the binding to the only known protein involed in iron export from the cells, ferroportin, that is present on the basolateral membrane of enterocytes, on macrophages, hepatocytes and other cells types (5, 6). After the binding, ferroportin is degraded and the iron uptake by the diet and iron recycling from senescent red blood cells, is inhibited (7). View the important role of hepcidin in iron metabolism, its expression is strictly regulated; in fact, dysfunctions or mutations that modify its expression, lead to different pathologies, such as hemochromatosis and anemia (8, 9, 10). Some crystallographic studies have demonstrated that an other important metal for the life of all the organisms, copper, could be important for hepcidin funtion; in fact, the presence of copper in solution can influence the aggregation state of hepcidin and studies in which a cell line was used as model system, have highlighted the importance of this element in the capacity of hepcidin to induce ferroprtin degradation (11, 12). The link between copper and iron is demonstarated also by an other important plasma protein, ceruloplasmin, a ferroxidase enzyme that contain copper in the catalytic site and is responsable of the transport of 90% of plasma copper (13) Different studies have demonstrated that animals fed with a copper deficient diet, have low levels of plasma ceruloplasmin and, its characteristic oxidase activity, is strongly reduced (14, 15); this kind of situation is normally present in Wilson disease patients, in which a defect in copper transport in the Golgi, is cause of the lack of plasma ceruloplasmin (16). To better understand if the copper has a role on hepcidin expression, we have used a human hepatoma cell line, HepG2, to investigate the influence of copper chelation and inflammation, by the use of the citokine interleukin-6 (17), on the level of hepcidin expression. To corroborate our results, we have also studied ceruloplasmin expression. Our results have demonstarted that the use of the chelation substrate, Bathocuproinedisulfonic acid (BCS) has a slight effect on ceruloplasmin mRNA levels, both in normal and stimulated IL-6 cells, while the intracellular level of protein is markedly reduced when BCS is added in both conditions. The inhibitory effects is more evident if the secreted form of the protein is considered; in fact, western blot and in gel oxidase activity analysis, have shown a disappearence of the protein and of its activity in the growth cellular media, results coherent with bibliographic data. The effect on hepcidin is also evident. Even if no statistical significative differences were found at mRNA level, the effect on the concentration of the intracellular prepropeptide is more evident, especially when the citokine and BCS are used togheter. The presence of both substrates leads to a decrease of the protein level when it is compared to the use of interleukin-6 alone and this effect was more evident when the concentration of the peptide in the cellular growth media was assayed. Our results demonstrate that a copper deficiency could negatively influence hepcidin expression even if other studies are necessary to better understand this mechanism.

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P36 - TOTAL INTERNAL REFLECTION FLUORESCENCE MICROSCOPY (TIRFM) AS A POWERFUL TOOL TO FOLLOW DYNAMIC EVENTS AT THE CELL MEMBRANE

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Cell membranes are involved in a variety of cellular processes such as cell adhesion, ion conductivity and cell signaling. Understanding how these processes are dynamically regulated is fundamental. Total internal reflection fluorescence microscopy (TIRFM) is ideally suited to study these events. It is based on the use of fluorescent labeled proteins associated to a microscope set-up that allows selective excitation of molecules at the plasma membrane. Indeed, fluorescent molecules alone provide information about the expression and localization of proteins and other molecules, but the temporal and spatial resolution is confounded by signal from outside the area of interest. TIRFM overcomes this limitation by using an evanescent wave generated by the reflection of a laser beam; this wave is relatively low powered and it is able to illuminate just a narrow (<100 nm) strip at the surface of a cell, thereby excluding the signals arising from structures in the cell interior[1] (FIG1).
As a result, spatial and temporal resolutions are increased, thus making it possible to measure dynamic events occurring at or immediately below the plasma membrane such as exocytosis and endocytosis, single molecule interactions, and ionic changes. This technique allows not only qualitative analysis, but also a quantitative measure of these events, by evaluating variation in fluorescence intensity during time-lapse recording. Analysis of these processes may open novel perspectives in the study of cell signaling, membrane trafficking and cytoskeleton remodeling.

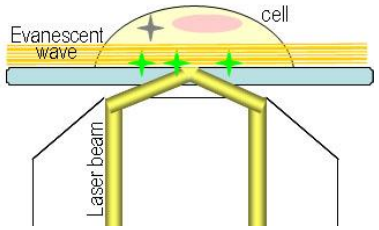

Fig. 1. Schematic representation of TIRF microscopy. When the laser beam is reflected by the glass slide, it generates an evanescent wave that diffuses in the specimen with the same wavelength but decades in a short distance, thereby illuminating only fluorescent molecules at or immediately below the plasma membrane.

In our laboratory, we have taken advantage of TIRFM for evaluating the glutamatergic signaling in the nervous system and in peripheral organs, in particular we have investigated:
1- Dynamic modulation of glutamate transporter density at the plasma membrane. The Excitatory amino acid carrier 1 (EAAC1) is a plasma membrane high affinity glutamate transporter expressed in the nervous system and in absorptive epithelia. EAAC1 activity can be rapidly regulated by its redistribution between intracellular compartments and the plasma membrane, a process controlled by protein-protein interactions and extracellular signals. We analyzed the molecular mechanisms of this modulation in two different physiological contexts: in epithelial cells, where EAAC1 is important for absorption of dicarboxylic amino acids, and in Schwann cells, where it may participate in cell myelination and proliferation by regulating the level of extracellular glutamate or by providing Schwann cells with glutamate.
In Epithelial cells, we investigated the molecular mechanisms that control the surface density of EAAC1. We detected in its cytoplasmic C-tail a consensus sequence for interaction with class I PDZ proteins and a tyrosine-based internalization signal (-^{503}YVNG^{506}-). To understand their role in transporter trafficking, we generated green fluorescent protein (GFP)-tagged transporters-lacking the PDZ target motif (□TSQF) or carrying the Y503A substitution. We expressed them in the Madin Darby Canine Kidney epithelial cell line, and we monitored their residence on the

plasma membrane by time-lapse TIRF imaging. In these experiments, if the GFP transporter is internalized, the fluorescence signal recorded by TIRFM should progressively decrease. Quantification of the fluorescence changes in the different mutants indicated that the PDZ target sequence controls the transporter resident time at the plasma membrane and that Y503 is involved in the constitutive endocytosis of EAAC1 (Fig.2) [2].

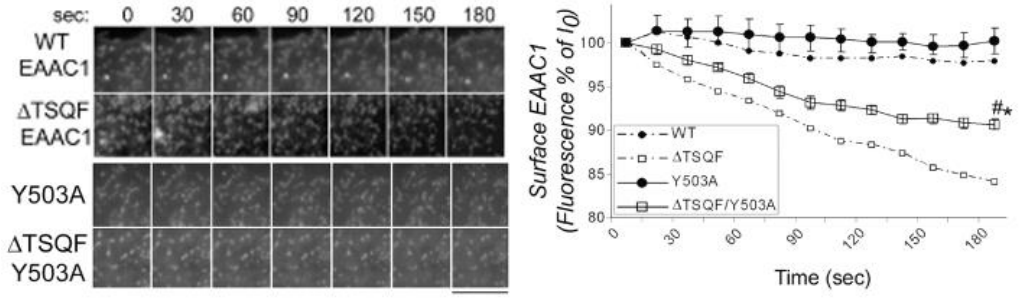

Fig.2. Representative TIRFM image sequences showing the membrane resident time of the indicated transporters (left), together with the averaged fluorescence intensity curves (right). (©Wiley & Sons, Inc.)

In Schwann cells, we investigated the mechanisms of action of allopregnanolone (ALLO), a steroid with neuroprotective effects, synthesized by Schwann cells. We found that incubation with ALLO rapidly increases the activity of the glutamate transporter EAAC1, with a mechanism that involves protein trafficking to the plasma membrane. We investigated this phenomenon by time lapse TIRFM imaging in primary cultures of Schwann cells transfected with EGFP-EAAC1. By alternatively blocking the exo- or endocytic pathways, we found that ALLO promotes the surface delivery of EAAC1 and increases its plasma membrane residence time by tethering it to the submembrane cytoskeleton. This recruitment is important to control Schwann cell proliferation[3].

2- Vesicle dynamics. Vesicle exocytosis is a common mechanism to control neurotransmitter and hormone release in different biological systems. To investigate the molecular mechanisms of vesicle exocytosis modulation, we labeled mice cortical synaptosomal membranes with the fluorescent organic dye FM1-43. Under TIRFM, we monitored the effect of corticosterone treatment on dynamics of vesicles docking and fusion and we found that the glucocorticoid promotes the docking of vesicles to the synaptic plasma membrane[4].

In addition to conventional fluorescent dyes, genetically engineered fluorescent proteins such as vesicles-resident proteins, cargo molecules (neurotransmitter and hormones) are increasingly being used to measure membrane trafficking and to monitor cell signaling[5]. We are currently setting up the experiments to measure the dynamics of hormone release in endocrine cells of the pancreas.

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P37 - PHYSYOLOGICAL ROLE OF t-PMET IN ERYTHROCYTES REDOX HOMEOSTASIS:INFLUENCE OF FLAVONOIDS

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Background
In the last decade the trans-Plasma Membrane Electron Transport (t-PMET) has been subject to more research. Ever growing evidence has demonstrated that t-PMET occurs in all types of organisms, including bacteria, yeast plants and animals (1). This system regulates distinct cellular functions and its malfunction relates to some diseases such as cancer, cardiovascular diseases, aging, obesity, neurodegenerative diseases, pulmonary fibrosis, asthma (1). The activity of t-PMET is critical to redox homeostasis in blood. In particular**,** a close link between t-PMET and metabolic status of erythrocytes has been reported (2). In hypoxic condition the activation of t-PMET may serve to compensate the impaired pentose phosphate pathway, thus ensuring a functional reducing capacity; in this conditions t-PMET may use ascorbic acid or polyphenols as electron donator, since NADH derived from enhanced glycolysis is preferentially utilized by meta-hemoglon reductase (3).Some authors have found a relationship between the dietary flavonoids and trans- plasma membrane oxidoreductase activity, suggesting an additional mechanism whereby dietary flavonoids may exert beneficial effects in human (4).

Aim of this work was evaluate whether some of flavonoids, enclosed in sub-class of flavonols (Quercetin and Kaempherol) and phenolic acids, are able to modify the erythrocytes redox homeostasis. Quercetin has been used as control because other authors had previously described its activity to enter erythrocytes and donate electrons to the PMOR system (4).

Methods
Human venous blood from different healthy volunteers of both sexes between the ages of 25-50 years was obtained by venipuncture in heparin after an over-night fast and centrifuged. The plasma and buffy coat and the upper 15% of the packed red blood cells (RBC) have been removed. The antioxidant capacity of plasma was analyzed by crocin bleaching assay (5) and FRAP. A stock solution (20mM) of each flavonoid was prepared in dimethyl sulfoxide and then diluted 1:2 with PBS. Packed RBC (10%v/v) were incubated in PBS containing 5mM glucose at 37°C for 10 minutes with a 50 µM concentration of each flavonoids. After this time the suspensions was centrifuged, the RBC were washed and then analyzed. The extracellular concentration of the flavonoids was measured in the medium at the end of the incubation period with the compounds (4). The assay was performed spectrophometrically by measuring the absorbance at the wavelength corresponding to the maximal absorption spectrum. The flavonoids intracellular content was measured on the erythrocytes lysate after extraction three times with ethyl acetate and they have been quantified spectrophometrically. The activity of the erythrocyte plasma membrane redox system (PMRS) was estimated by reduction of ferricyanide according to Fiorani method (4). The catalase activity was measured by catalase assay kit purchased from the SIGMA-ALDRICH.

Results
All compounds were taken up by the erythrocytes and displaying significant FIC-reducing activity. Their ability to act as intracellular substrates of PMOR is structure-dependence. In

physiologic condition the catalase activity varies from 28.6 mU/g protein to 40,6 mU/g protein. Catalase activity decreased with increasing concentration of flavonoid and related compounds. The activity of catalase after incubation in the presence of luteolin is lower by 30% than control (erythrocytes without luteolin). This results is not dose-dependence. In fact, no-significance difference has been observed at both luteolin concentrations (10 and 100µM). Not any correlation has been found between catalase and plasma antioxidant capacity.

Conclusions

This study shows that the flavonoids are able to form stable complexes with the erythrocytes and to influence the intracellular redox homeostasis. Therefore, it could affirm that the polyphenols are able to increase the defence of erythrocytes against ROS. This work underlines that the RBC plays a pivotal role in the distribution and bioavailability of circulating polyphenols which contribute to the defence against injury induced by ROS in various clinical disorders.

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P38 - ERYTHROCYTE CATALASE ACTIVITY IN EVER-SMOKERS AND NO-SMOKERS HEALTHY SUBJECTS: INFLUENCE OF FLAVONOIDS (QUERCETIN AND LUTEOLIN)

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Background. Oxygen free radicals are highly reactive species that can cause cell damage including lipid peroxidation, enzyme inactivation and DNA damage. Inactivation and removal of highly reactive species depend by the antioxidant defence systems. The catalase (CAT) belongs to the primary antioxidant defence system of the cell which catalyses the decomposition of hydrogen peroxide to water and molecular oxygen. Flavonoids are a group of polyphenolic compounds with different chemical structure and properties. They are widely distributed in fruits, vegetables, nuts, seeds and beverages such as wine and tea. The major flavonoide classes include flavonols, flavone, flavonones, flavanols, anthocyanidins and isoflavones. These compounds may scavenge free radical species and other oxidants. In previous studies, we have observed that some polyphenols are able to cross the erythrocyte's membrane and this process is influenced by the plasmatic albumin; in fact quercetin intracellular concentrations is albumin dose depending. The influence of flavonoids on catalase activity has been reported in some papers but the results are contradictory. Some authors have found an increase of catalase activity in cell in the presence of flavonoids (1,2). Others have observed any effect or even a decrease of catalase activity (3). The present work is based on a study of Krych's, (4), in which the influence of flavonoids on catalase in model system has been evaluated.
Aim of this work was to study the role of red body cells in the antioxidant balance. The primary goal was to evaluate the antioxidant status of no-smokers and ever-smokers healthy subjects by the determination of the plasma antioxidant capacity and of the catalase activity of erythrocyte and then to evaluate if flavonoids (quercetin and luteolin) are able to modify the enzyme activity.
Methods. This is a pilot study. Nine healthy subjects, aged 24-55 years, of which six females (3 no-smokers and 3 ever smokers) and 3 males (2 smokers and 1 no-smokers) were recruited. None of the subjects had any pathologies at the time of sampling. We assayed the CAT activity in erythrocytes isolated from whole blood of the subjects by the colorimetric assay and the plasma antioxidant capacity using the spectrophotometric method known "crocin bleaching assay".
The catalase activity was performed in human erythrocytes (control) and after the incubation of them with the flavonoids (quercetin and luteolin). Human venous blood (in heparin) from healthy volunteers was obtained by venepuncture. The blood was centrifuged and then plasma, buffy coat and upper 15% of the packed erythrocytes were removed. The isolated erythrocytes isolated were washed twice with cold PBS and then re-suspended and incubated with flavonoids according Fiorani (5) method. The catalase activity was analysed by a catalase assay kit purchased from Sigma-Aldrich. All results are presented as mean ± SD or mean ±SEM. Correlation statistics between variables were assessed by calculating the Pearson coefficient. Differences in means between groups were analyzed by the unpaired t-test. Differences were considered statistically significant at $P<0.05$. Multiple regression analysis was used to investigate the influence of different variables on the enzyme activities.
Results. The flavonoids were efficiently taken up by human erythrocytes in dose-depending manner. There was no significant difference in the percent accumulation of both molecules

(quercetin and luteolin) inside the erythrocytes when incubated at the same concentration of 50µM. In physiologic condition the catalase activity varies from 28.6 mU/g protein to 40,6 mU/g protein. Data have shown that CAT activity of erythrocytes was significantly lower in ever smokers than in no-smokers (Figure 1). It was also found that Quercetin at the concentration of 100µM is able to increase the catalase levels in ever-smokers up to the normal values observed in no smokers (Figure 2). The study on the luteolin has not produced the same effects. In fact, Luteolin is able to reduce the CAT levels in no-smokers subject according with the data from Krych, 2013. The different actions of compounds on catalase can been explained as consequence of flavonoid interaction with enzymatic protein. The inhibiting action of the luteolin can be a consequence of a conformational change which occurs upon the flavonoid binding to catalase. This interaction changes the geometry of the substrate channel and thus inhibits the reaction of H_2O_2 with the heme center. Plasma antioxidant capacity was lower in no smokers than in smokers. An inverse correlation has been found between age and plasma antioxidant capacity.

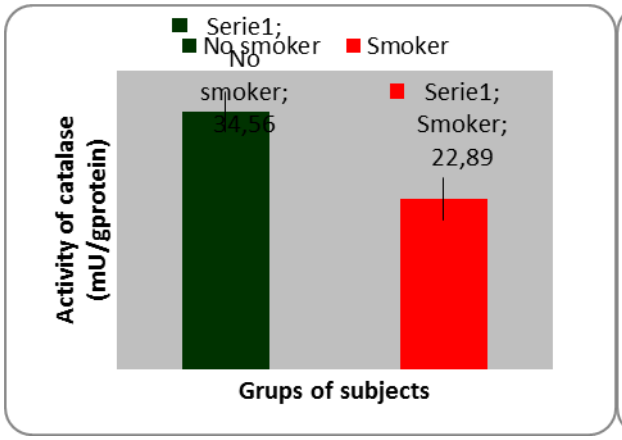

Figure 1: Catalase activity of erythocytes in non-smokers and ever-smokers. Mean ±SEM, (unpaired t-test p=0,007)

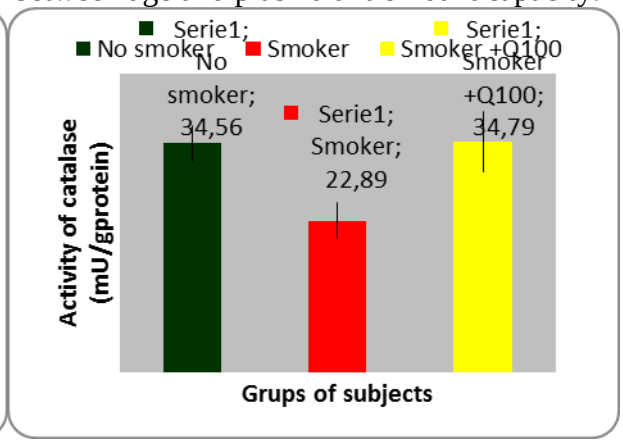

Figure 2: Effect of quercetin (100µM) on the catalase activity in ever-smokers. Data are expressed as mean ± SEM.*Non-smokers vs smokers +Q100 (p=0,09)

Conclusion from the results of this study affirms that an oxidative stress condition is present in ever-smokers respect to non-smokers, but the quercetin is able to restore the erythrocyte oxidative stress condition of ever-smokers back to the normality. Further studies are necessary in the future to better investigate the role of luteolin on the catalase activity of human erythrocytes.
Wu et al. have warned that microenvironment can shift erythrocytes from friendly to a harmful behaviour. The release of iron-hemoglobin following RBC hemolysis can cause excess accumulation of free iron to catalyze the generation of the highly toxic hydroxyl, peroxyl and alkoxyl radicals. Therefore by virtue of their antioxidant and chelating properties for divalent metals both free and polyphenols bound to RBC might also act to neutralize the toxic effects of ROS.

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P39 - APPLICAZIONE E SVILUPPO DI UN METODO DI IDENTIFICAZIONE DEI MANGIMI IRRADIATI E VALUTAZIONE DELLA CAPACITà SANITIZZANTE DEL TRATTAMENTO RADIANTE

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La qualità igienico-sanitaria degli alimenti ad uso zootecnico rappresenta una base imprescindibile per il benessere degli animali e quindi per la sicurezza alimentare . Tra le diverse strategie di controllo per il contenimento delle contaminazioni microbiologiche, l'uso delle radiazioni ionizzanti può rappresentare un metodo valido ed efficace. Sebbene esistono norme comunitarie e nazionali che regolamentano l'uso delle radiazioni ionizzanti negli alimenti ad uso umano e sono stati validati metodi per l'identificazione degli alimenti irradiati, nessuna regolamentazione è stata invece emanata per l'uso del trattamento radiante nella conservazione e sanitizzazione degli alimenti ad uso Zootecnico né tanto meno esiste alcun metodo per l'identificazione dei mangimi irradiati. Scopo del presente lavoro è stato quello di studiare l'efficacia del trattamento radiante sulla contaminazione microbica dei mangimi e sviluppare e mettere a punto un metodo per l'identificazione di mangimi irradiati con particolare riferimento alla Spettroscopia di Risonanza Spin Elettronico (ESR).
In questo studio sperimentale campioni di Avena, Grano duro e mangime completo, che presentavano alte cariche batteriche e fungine, sono stati sottoposti a trattamento con radiazioni ionizzanti alla dose di 1 e 5 kGy. Tale intervallo di dose è stato scelto in quanto è quello tipicamente utilizzato per ridurre la contaminazione microbica nelle matrici alimentari di natura vegetale. Le analisi batteriologiche effettuate dopo irraggiamento hanno mostrato come le dosi utilizzate siano state sufficienti per determinare l'abbattimento della carica microbica ed inoltre è stato evidenziato una progressiva riduzione delle cariche all'aumentare delle dosi di radiazioni impartite .
Per la messa a punto del metodo di Spettroscopia di Risonanza Spin Elettronico (ESR) sono state scelte matrici con differente contenuto di cellulosa: Avena, Grano duro e mangime completo. Sono state condotte analisi sia su campioni non irradiati , usati come bianco campione, che su campioni irradiati alla dose di 1 e 5 kGy. Lo studio ha evidenziato che l'intensità e la stabilità del segnale ESR è fortemente influenzato dalla quantità di cellulosa , oltre che di umidità, presente nel campione in esame.
Da questa prima esperienza si evidenzia come la spettroscopia ESR può essere utilizzata come tecnica per l'identificazione di mangimi irradiati anche se la sua applicabilità dipende dalla composizione chimica del mangime .

P40 - THE APPROACH OF MICRORNA EXPRESSION ANALYSIS IN THE DETECTION OF AUTOLOGOUS BLOOD TRANSFUSION IN DOPING CONTROL

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Abstract
Blood transfusion (BT) as blood doping practice is banned by the World Anti-Doping Agency (WADA) and can be abused by cheating athletes to increase the rate of oxygen transport to tissues with the aim to improve sport performance. At present, a method for the detection of Homologous Blood Transfusion (HBT) has been implemented by the WADA accredited antidoping laboratories worldwide, while no internationally recognized method has been finalized so far for the direct detection of autologous blood transfusions (ABT), which can at present be only detected indirectly by targeting longitudinal profiling of key hematological parameters. In this perspective, several researches approaching different fields are underway to find reliable biomarkers to be suitable in the development of a method to directly detect autologous blood transfusion. In this work we have experimented the possible role of microRNA (miRNAs) as biomarkers related to the execution of an autologous blood transfusion practice. MiRNAs are a class of 18-24 nucleotides long non coding RNAs acting as post-trancriptional modulators of mammalian gene expression. Normally they are involved in the regulation of many physiological processes (such as erythropoiesis), and recent evidences shown that they have a role as useful biomarkers in certain diseases such as cancer and heart malignancies. This study aims to experiment their possible role as biomarkers of blood doping.

Materials and methods: A total of eight miRNAs (mi923, mi150, mi144, mi96, mi196a, mi30b, mi197, mi451) have been extracted and quantified from six whole blood samples from healthy athletes at three different times (T=0 within 24h from sample taking, T=1 after 15 days, T=2 after 30 days). Another blood sample was withdrawn fresh from a donor, then stored as erythrocyte concentrated and, after 30 days, used to get an *ex-vivo* autologous blood transfusion with new fresh blood from the same donor. All miRNAs were extracted with a specific kit (miRneasy, Qiagen), then quantified with a specific Chip Electrophoresis System (Bioanalyzer 2100, Agilent), then retro-transcribed to cDNA and analyzed to quantitative PCR (qPCR) using PCR7500 Fast system (Applied biosystems). Analysis of expression was made with the technique of "relative quantification" that was choosen as the more suitable for the aims of this experiments. In data analysis, a key step was the identification of the most appropriate housekeeping gene who resulted mi150 as its variability was found to be the most stable among all miRNAs studied. The study of the variation of the expression was evaluated estimating the relative quantities (RQ) of the miRNAs expressed by the samples compared to a calibration sample used as reference.
Results: we observed a gradual tendency of the miRNAs analyzed to increase their expression in samples at T=2 compared to T=0 where mi144 and mi923 shown the most consistent differences however with great variability among the samples. A marked differences has been observed for the erythrocytes concentrate sample (analyzed after 30 days of storage) where expression levels of mi923, mi30b, mi197, mi96 and mi451 resulted higher compared to the fresh samples at the time of the withdrawal. Moreover the most important result relies on the

observation that the expression of some miRNAs (such as mi197, mi30b, mi451, mi96 and mi923) is very high in the erythrocytes concentrated sample and it is detectable also in the ex-vivo transfused sample with levels higher compared to the fresh non-transfused sample (even though lower compared to the concentrated erythrocytes sample because of the post-transfusion dilution effect). Moreover, two miRNAs (mi144 and mi923) show the most significant increasing in the transfused sample compared to the fresh one. As result, it is very important to note that, from the data obtained, the possibility to use miRNAs expression both as biomarkers of storage and biomarkers of effect in blood doping detetction emerges.

Results we achieved in this work seem to be significant for several reasons. Firstly, at methodological level, the development of a specific protocol for the extraction and the quantitation of miRNA and a proper strategy in data analysis (including a correct strategy in the choice of the most suitable "housekeeping gene") have allowed for the first time our laboratory to experiment and apply molecular biology techniques in the field of doping control. More in detail, an accurate and sensitive quantification of the extracted miRNAs represented a first key step to subsequently achieve accurate and reproducibile genetic expression data by quantitative real time PCR. Finally, the methodological approach here followed and results obtained in this work can be considered as preparatory to the opening of new research perspectives in the field of doping control. The next step we aim for the future is first of all to extend both the panel of miRNAs to quantitate and the number of samples to be analyzed also considering the important issue of ethnical diversity between individuals. Moreover, the strategies and studies we intend to apply in the near future also include the extention of this approach to different hematological matrixes considering for example circulating miRNAs in serum and plasma and, in a further step, considering miRNAs extracted from urine samples also to the final scope to experiment and apply miRNAs expression data as biomarkers for the detection of other banned doping practices and drugs as for example the abuse of synthetic erythropoietins, insulin and analogs, growth hormone and related growth factors.

P41 - IDENTIFICAZIONE TASSONOMICA DI *AOTUS* (PLATYRRHINAE) MEDIANTE LA CITOGENETICA

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Le scimmie del genere *Aotus* (Platyrrhine, Cebidae) presentano caratteristiche peculiari essendo notturne e monogame. Hanno un'ampia distribuzione geografica che si estende dallo stretto di Panama al nord dell'Argentina; questo determina una notevole complessità nella distribuzione e di conseguenza nell'interpretazione sistematica e filogenetica. Lo studio del genere *Aotus* mediante l'analisi di caratteri morfologici e genetici ha prodotto una tassonomia controversa. Inizialmente era riconosciuta solo la specie *Aotus trivirgatus*, successivamente in base alla colorazione del pelo del collo, alla diversa suscettibilità alla malaria e ai dati citogenetici sono state identificate fino a nove specie, oltre a due *"sibling species"*, per un totale di 11 (1-2). Il numero diploide di cromosomi in *Aotus* varia da 46 a 56; nel cariotipo sono presenti molti polimorfismi dovuti all'ibridazione che si verifica in specie simpatiche; in alcune specie è presente una traslocazione Y-autosoma. Le undici specie sono state suddivise in due gruppi monofiletici: il gruppo *grey-black neck* distribuito a nord e il gruppo *red neck* distribuito a sud del Rio delle Amazzoni. Le specie del gruppo *red neck* sono omogenee da un punto di vista del cariotipo con un numero diplolide 2n=49 (maschio)/50 (femmina) e una traslocazione Y-autosoma. Le specie del gruppo *grey-black neck* presentano numero diploide di cromosomi variabile, con il più basso 2n=46 in *A. vociferans* e il più alto 2n=56 in *A. lemurinus*. Solo due specie (*Aotus nancymae* e *Aotus lemurinus*) sono state analizzate mediante la citogenetica molecolare (*painting* cromosomico). Quest'analisi ha permesso di dimostrare che le specie del genere *Aotus* posseggono un cariotipo piuttosto derivato se confrontato con quello ipotetico ancestrale delle Platyrrhinae, da cui si è originato attraverso fusioni, fissioni, traslocazioni ed inversioni (3,4,5).

L'identificazione tassonomica di taxa di *Aotus* mediante l'analisi cromosomica rappresenta, in cattività, il prerequisito per programmi di *"breeding"* in quanto la ricostruzione del cariotipo bandeggiato è l'unico approccio per identificare la maggior parte delle specie del genere *Aotus*. Mediante le tecniche citogenetiche vengono identificati individui compatibili da un punto di vista cromosomico che possono essere incrociati tra loro al fine di evitare ibridazioni interspecie (6) e favorire la conservazione delle diverse specie.

L'obiettivo del presente lavoro riguarda l'identificazione mediante bandeggio cromosomico di individui di una colonia di *Aotus*, al fine di avviare un programma di conservazione delle specie mediante*"breeding"*. Il gruppo di scimmie, originario della Bolivia, è presente in Giappone dal 1977 presso "The Primate Research Institute" di Tokyo; tra gli individui della colonia si è inavvertitamente verificata la produzione di ibridi prima che fossero riconosciute le diverse specie del genere. Si riportano dati preliminari sull'identificazione di individui idonei da incrociare, in particolare il cariotipo bandeggiato di un maschio di *A.l griseimbra* (2n=54) (Fig.1). Inoltre si sono revisionati dati citogenetici presenti in letteratura su *Aotus* al fine di sottolineare l'importanza della citogenetica classica e molecolare negli studi filogenetici e in quelli riguardanti la conservazione delle specie (Fig. 2).

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Fig. 1 Cariotipo ricostruito mediante bandeggio g di un individuo di *Aotus l. griseimembra* (2n = 54) proveniente dal centro Giapponese; un maschio con un grande cromosoma submetacentrico risultato di una fissione (prima riga, primo cromosoma) e due cromosomi non omologhi (seconda riga ultimi due cromosomi); ricostruzione in accordo con Ma et al., (1976).

Fig. 2 Elenco delle specie analizzate ad oggi mediante la citogenetica classica e molecolare con il relativo riferimento bibliografico.

NAME	2N=	METHODS	REFERENCES	DATE
Aotus (from Colombia)		R,Q,G-NOR-banding	Torres et al.,	1998
Aotus trivirgatus	56	G- banding	Chiarelli and Stanyon	1985
Aotus trivirgatus-from Peru	49, 50 female	G , C banding	Pieczarka and Nagamachi	1988
Aotus		Review.	Galbreath	1983
Aotus trivirgatus form Peru	46,47,48	G- banding	ShuiFong Ma et al.,	1985
Aotus		chromosome evolution	shui Fong and Ma	1981
Aotus (from Northern Argentina)	50 female, 49 male	C, G- banding	Mudry and Colillas	1984
Aotus		Chromosome Nomenclature	Reumer, De Boer	1980
Aotus (from Bolivia)	50 female, 49 male	C, Q, G- banding,	Ma et al.,	1976
Aotus (from Rondonia, brazil)	48	G, C, NOR- banding	Pieczarka et al.,	1993
Aotus nancymae, ***A. vociferans***	54 46	G, C, NOR- banding	Pieczarka et al.,	1992
Aotus			de Boer	1974
Aotus nancymae	54	FISH	Stanyon et al.,	2004
Aotus nancymai ***Aotus sp***	54 50	FISH	Ruiz Herrera et al.,	2005
Aotua jorgehernandezi	50	G- banding G- banding	Defler TR, Bueno Torres et al.,	2007 1998
Aotus lemurinus	54	FISH	Stanyon et al.,	2011

P42 - FEEDING AND CORTISOL ALTER BRANCHIAL Na^+/K^+ ATPase ACTIVITY AND GROWTH PERFORMANCE DIFFERENTLY IN COMMON CARP *Cyprinus carpio*

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Feeding is the key of growth and survival, granting sufficient nutrients in all living life. Feeding is under endocrine monitoring, and the role of cortisol on feeding modulation is poorly understood: whether the growth-suppressing is due to a cortisol-mediated decrease in food intake is not clear [(1)]. On the other hand, cortisol is the main circulating corticosteroid in teleost, plays a significant role in monitoring homeostasis balancing in freshwater fish. However, most of the researches were focused on freshwater salmonid and not much is known about cyprinid.
Therefore, the aim of this study was to investigate the effect of cortisol on the growth performance, branchial Na^+/K^+-ATPase (NKA) activity and plasma Na^+ levels in common carp *Cyprinus carpio.*

Carp was fed at low and high ratio (0.5% and 3.0% body weight (BW)) for 6 weeks. Carp was injected intraperitoneally with coconut oil implant (sham), or cortisol in coconut oil implant (cortisol – 250 mg cortisol per kg fish) while control fish received no injection, and was monitored at 12, 24, 72 and 168 hours after injection (h-PI).

- Cortisol decreased growth rate compared to sham and control groups, in the high-fed group (Table 1).
- High feeding significantly increased gill NKA activity in sham and cortisol implanted fish at 24h-PI and in all groups at 72h-PI compared to low-fed fish (Fig. 1A & 1B).
- Cortisol injection did not affect plasma Na^+ level, although a decreasing trend was observed in the high-fed group at 24h-PI. Furthermore, plasma Na^+ levels were increased at 168h-PI in the high-fed group after cortisol injection compared to the low-fed group. Plasma Na^+ levels were enhanced in the high-fed group due to dietary intake (Table 1).

Cortisol increased branchial NKA activity: the upregulation of this activity further confirms the role of cortisol in modulating ionoregulation capacity in freshwater fish.
The lower growth rate observed in cortisol treated-fish fed to high ratio reflected a high living cost [(2,3,4)], despite the high feeding ratio ensures an appropriate nutrient supply.

As a conclusion, cortisol impaired weight gain in common carp but reallocated energy to compensate increasing NKA activity for basal homeostasis needs.

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Table 1: Growth rate (%) and Plasma Na^+ (mmol/L) of common carp fed at different ratio without (control) and with implantation (sham and cortisol) over 168h-PI. All values were presented as mean ± SEM, *n*=8. Significant level was set at *P*<0.05. A plus (+) indicates significant differences between feeding regimes.

		Low feeding			High feeding		
		Control	Sham	Cortisol	Control	Sham	Cortisol
Growth rate (%)	12h-PI	3.2±0.9	0.7±0.4	1.0±0.4	9.6±0.4 +	2.2±0.8	1.5±0.9
	24h-PI	4.2±0.6	1.6±1.0	1.8±0.5	6.2±0.7	2.2±0.7	2.4±0.8
	72h-PI	2.3±1.0	1.1±0.3	0.8±0.5	5.3±0.4 +	2.0±0.4	2.2±0.6
	168h-PI	2.2±0.7	0.8±0.1	0.8±0.1	7.0±1.0 +	4.1±0.9 +	2.5±0.7
Plasma Na^+ (mmol/L)	12h-PI	119±4.6	113±4.9	107±2.9	131±5.8	126±5.3	118±4.6
	24h-PI	122±1.5	114±5.5	110±2.7	136±1.8 +	123±5.1	115±7.2
	72h-PI	121±2.2	116±4.3	111±3.7	133±4.1 +	127±6.9	119±6.3
	168h-PI	118±7.0	113±6.1	109±2.9	130±1.8	125±4.6	124±4.9 +

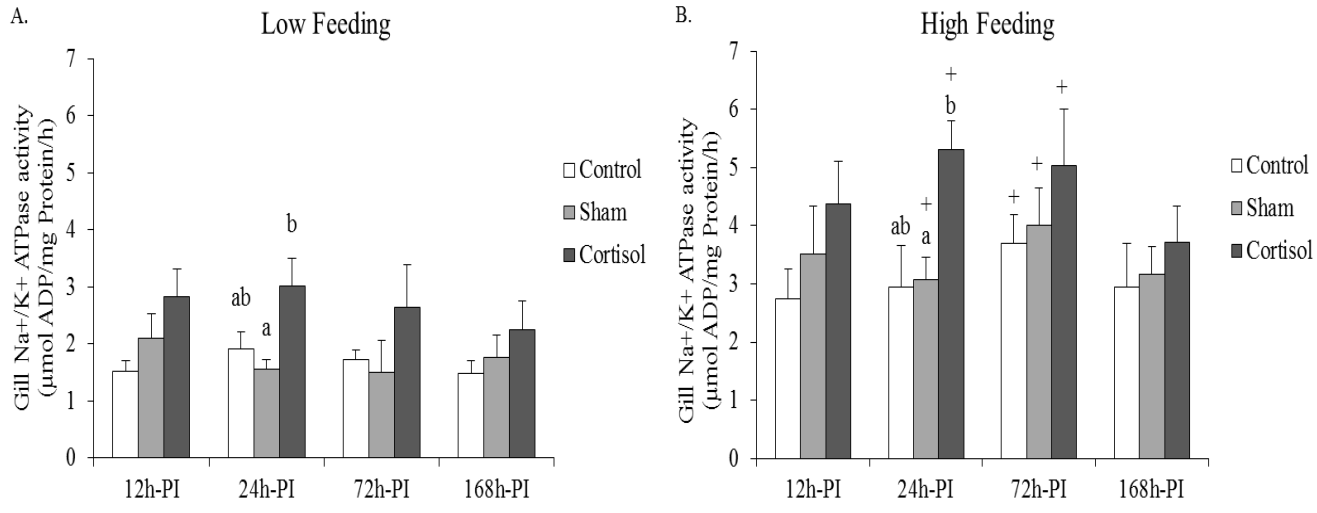

Fig. 1A &1B: Gill Na+/K+ ATPase activity of common carp fed at low (1A) and high (1B) ratio. All values were presented as mean ± SEM, *n*=8. Significant level was set at *P*<0.05. A plus (+) indicates significant differences between feeding regimes.

P43 - IL SENTIMENTO DI VITA IN S. K. LANGER
UNA RIFLESSIONE FILOSOFICA

D. Svorova

«What am I, Life? a thing of watery salt, held in cohesion by unresting cells…?», il domandarsi masefieldiano racchiude in poche parole la problematica centrale di tutte le scienze biologiche: Che cosa è vita?

Una visione della natura puramente quantitativa, che neghi la fondamentale categoria ecologica della qualità e l'importanza dell'elemento estetico, mostra oggi i suoi limiti di fronte alla complessità dell'ecosistema globale, dinamiche basate su molteplici relazioni in coevoluzione fondate sulle forme, sui colori, sui suoni, sugli odori. Anche Susanne K. Langer, studiosa americana, nella sua ambiziosa ricerca filosofica legata alla comprensione della natura umana, intravvede in ogni atteggiamento di tipo riduzionista nei confronti dei sistemi viventi un serio impoverimento dell'intero fenomeno chiamato vita. Ella, quindi, conduce verso un atteggiamento *interdisciplinare,* ancora all'epoca poco diffuso, per cogliere quel profondo sentire umano strettamente legato allo straordinario fenomeno definito *vita.*

Nell'opera intitolata *Mind: An Essay on Human Feeling* (1969-1982) Langer incentra, dunque, l'attenzione alla ricerca e alla teorizzazione dell'essenza più intima dell'essere umano: il *sentimento di vita.* Esso, a suo avviso, rappresenta il culmine del lungo processo evolutivo in cui l'essere umano, senza dubbio, occupa il vertice dell'intero albero evoluzionistico. Il *sentimento di vita,* secondo Langer, quindi costituisce la parte essenziale di quel complesso flusso coscienziale in cui confluiscono tutti i processi vitali dell'organismo rendendolo unico e irripetibile. La ricerca, volta alla comprensione dell'essenza della natura umana, si rivelò lunga e tortuosa: l'Autrice spazia tra logica, antropologia filosofica, pragmatismo, psicologia della Gestalt, biologia, fisiologia, neurologia, evoluzionismo, etologia, zoologia, paleontologia, etnologia, ecc.; in altre parole, Langer abbraccia tutti i campi del sapere che in un certo qual modo trattano l'argomento relativo al fenomeno umano. Ella, quindi, per raggiungere il suo obiettivo recluta un'intera "squadra" di specialisti disposti a collaborare all'ambiziosa "avventura" intellettuale che le consentì di correlare gli studi umanistici con le più svariate posizioni scientifiche costruendo in tal modo un'opera di straordinaria valenza. Langer, con atteggiamento critico, infatti mette "in gioco" le più attuali teorie scientifiche all'epoca disponibili individuandovi contemporaneamente le loro inevitabili lacune e integrandole con le sue più acute osservazioni.

Langer fu ben consapevole che il suo approccio interdisciplinare con grande probabilità avrebbe suscitato numerose obiezioni all'interno del dibattito culturale ma come sottolinea l'Autrice stessa «io non cerco in queste pagine di dimostrare la correttezza del mio procedimento» bensì «di concepire la mente come un fenomeno naturale, o meglio come la più grande meraviglia della natura».

In *Mind* quindi si assiste a un approccio *olistico* applicato alla comprensione della natura umana che svela appunto la complessità di innumerevoli interazioni tra le singole parti corporee, tra l'organismo e l'ambiente, tra l'organismo e le varie forme culturali, ecc., ovvero tutto ciò che influisce sui processi fisiologici che infine confluiscono nel vortice vitale lasciandone l'impronta sul profondo sentimento di vita. Tale processo neurofisiologico non soltanto prefigura l'individualità di ogni singolo soggetto ma ne determina anche la straordinaria capacità simbolizzatrice, di cui sono "testimoni" appunto le più svariate forme culturali. L'arte in tale contesto, in quanto portatrice emblematica del sentimento di vita, svolge un ruolo di grande importanza poiché proprio essa, come sostiene Langer, consente di attingere in modo prescientifico al «grande segreto della vita stessa». Così in *Mind* la mente non rappresenta più un'entità misteriosa e imperscrutabile bensì una complessa attività fisiologica intesa come lo straordinario esito della peculiare *individuazione* filogenetica: per dirla con Langer un vero e proprio «natural wonder», che si riflette, a sua volta, nelle più svariate forme culturali. Queste

ultime vengono, a pieno titolo, chiamate in "causa" a chiarire alcuni lati oscuri del cervello umano non omettendo l'integrità funzionale dell'intero organismo.

Langer quindi delineò una nuova linea d'orizzonte conoscitivo lasciando ai posteri un dichiarato invito a una sfida intellettuale di vasta portata.

P44 - EFFETTO DEL PROCESSO DI MACINAZIONE DEL FRUMENTO DURO SUL CONTENUTO IN POLIFENOLI E SULLA CAPACITA' ANTIOSSIDANTI

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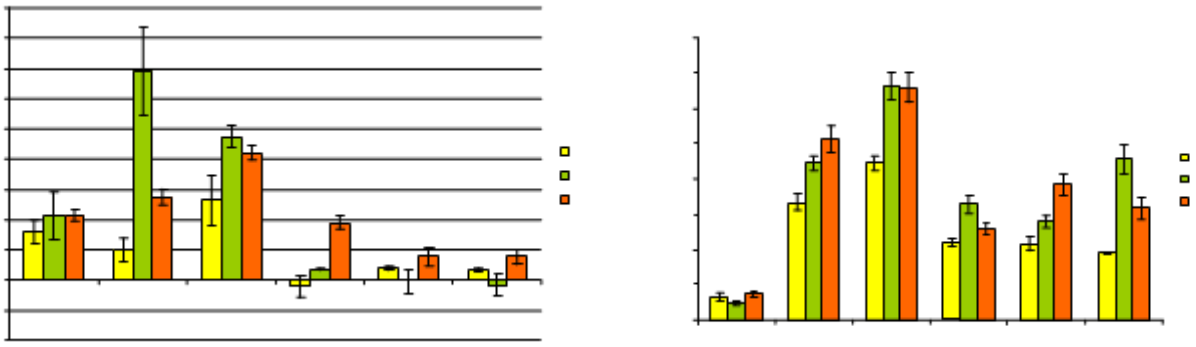
Introduzione
I polifenoli sono presenti negli alimenti di origine vegetale e sono gli antiossidanti più abbondanti nella dieta. Le principali fonti alimentari di polifenoli sono frutta e bevande, difatti, nella frutta come mele, uva, pere, ciliegie sono contenuti fino a 200-300 mg di polifenoli per 100 grammi di peso fresco.
Questi componenti sono considerati antiossidanti in quanto sono in grado di proteggerci dai danni ossidativi e di conseguenza riescono a limitare l'insorgenza di varie malattie degenerative causate dallo stress ossidativo. Da studi condotti su animali, infatti, è stato dimostrato che quando vengono aggiunti alla dieta i polifenoli si ha una riduzione dello sviluppo di cancro, malattie cardiovascolari, malattie neurodegenerative, diabete ed osteoporosi (1).
L'azione antiossidante dei polifenoli è determinata dai gruppi fenolici che accettando un elettrone formano radicali fenolici relativamente stabili che interrompono così le reazioni di ossidazione a catena (2).
Nel frumento duro i polifenoli sono presenti negli strati periferici della cariosside (3).
Dal punto di vista chimico queste molecole presenti nel grano sono derivati degli acidi cinnamici (acido p-cumarico, acido ferulico, acido caffeico e acido sinapico).
Come noto il frumento subisce un processo di trasformazione (molitura) che consente di ottenere dalla granella prodotti come: crusca, cruschello, semolato, fino ad arrivare alla semola fine, che viene prevalentemente destinata alla produzione di alimenti quali: pane, pasta ecc.
Lo scopo di questo studio e' stato quello di verificare l'impatto del processo di trasformazione nel frumento duro sui fenoli liberi totali e sulla capacita' antiossidante idrofila.
Materiali e metodi
La concentrazione dei polifenoli totali liberi e' stata eseguita mediante il Folin-Ciocolteau (4).
La capacita' antiossidante e' stata misurata mediante lo sbiancamento della crocina (5).
Risultati e Discussione
In figura 1 sono riportati i risultati della determinazione della capacità antiossidante idrofila. Come si può osservare, i valori più alti della capacità antiossidante idrofila si riscontrano nella crusca e nel cruschello, in quanto proprio in queste due frazioni sono maggiormente presenti i polifenoli.. I valori maggiori si riscontrano nella varietà S.Agata, intermedi nel Simeto e più bassi nella varietà Duilio. Invece nel semolato, nella semola e nella farina, i valori sono decisamente piu' bassi, questo perché in queste fasi di lavorazione (molitura) tutta la crusca è stata tolta e quindi la capacità antiossidante idrofila deriva solo dai composti presenti nell'endosperma e nel germe. I valori più alti sono nella cultivar Simeto, le altre due cultivar presentano valori molto bassi.



Nella Figura 2, sono riportati i valori dei polifenoli liberi totali nelle tre varietà. L'andamento della concentrazione dei polifenoli totali liberi è, in buona approssimazione in accordo con gli andamenti della capacità antiossidante idrofila. Come si può vedere, nella crusca e nel cruschello si hanno i valori più alti di queste sostanze, e più precisamente, nella crusca del Sant'Agata si riscontrano valori maggiori, di viene segue il Simeto e con valori più bassi il Duilio; per quanto riguarda il cruschello, i valori del Sant'Agata e del Simeto si equivalgono, mentre valori nel Duilio sono più bassi. Nel semolato, semola e farina i valori sono più bassi con un'inversione di tendenza tra Simeto e Sant'Agata. La cultivar Duilio rimane costante.
Nei polifenoli liberi totali l'effetto del processo di molitura è più evidente, infatti dal seme intero (granella) abbiamo il valore più basso di polifenoli, in quanto l'estrazione di questi composti avviene esclusivamente a carico di quelli superficiali, in quanto il seme è ancora intero. Con il procedere della molitura, si ha la separazione della crusca e del cruschello. In queste due frazioni abbiamo i valori massimi di polifenoli liberi in quanto è proprio in queste frazioni che si ha la massima concentrazione di queste sostanze. L'abbattimento di questo valore si ha nelle fasi successive (semolato, semola e farina) dovute alla raffinazione operata dalla molitura per avere questi prodotti, con conseguente perdita di queste sostanze.

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P45 - STUDIO PRELIMINARE SULLA CARATTERISTICHE NUTRIZIONALI DELL'OLIO DI *MORINGA OLEIFERA*

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Introduzione
Tutte le specie di Moringa sono originarie delle regioni Sub-Himalaiane e la più diffusa risulta essere la *Moringa oleifera* v. Lamarck attualmente coltivata in diversi paesi dell'Africa, dell'Asia e Sud-America. Dalla fine degli anni ottanta si è registrato un crescendo di lavori centrati sulla possibile applicazione in campo alimentare, farmaceutico, cosmetico (1) nonché agricolo delle radici, delle foglie, dei semi oppure dell'olio di moringa. Sono apparse una miriade di pubblicazioni che, spesso attingendo dalla tradizione e/o medicina popolare, evocano per i vari derivati di moringa proprietà iperboliche, miracolistiche se non magiche (2). Di fatto pochi lavori esaminano rigorosamente la chimica dei costituenti e rari sono gli studi clinici (2) che ne documentano l'efficacia nutrizionale in modo puntuale (3 e 4). D'altra parte, nel sahel senegalese, zona da cui provengono le foglie e i semi oggetto di questo studio, l'albero di moringa è considerato una risorsa polivalente. Il legno è impiegato in falegnameria o come legna per ardere, le foglie fresche vengono utilizzate per integrare l'alimentazione se seccate e ridotte in polvere vengono impiegate per "combattere" il diabete. Dell'olio ne fanno un uso culinario nonché cosmetico.
Scopo di questo studio preliminare e quello di monitorare e le capacità antiossidanti e il profilo degli acidi grassi introducendo un semplice, ma efficace modo per calcolare la relazione tra saturi ed insaturi, che aiuta a chiarire la notevole resistenza dell'olio di moringa all'ossidazione.
Materiali e metodi: Il profilo degli acidi grassi e' stato effettuato mediante trans metilazione (6) e analizzati in gas-cromatografia con rivelatore di massa. La concentrazione dei polifenoli totali liberi e' stata eseguita mediante il Folin-Ciocolteau (7). La capacitaà antiossidante e' stata misurata mediante lo sbiancamento della crocina (8).
Risultati e Discussione: come si può osservare dalla tabella 1 l'olio di moringa presenta un profilo in acidi grassi molto simile a quello dell'olio di oliva, specialmente nel C18:1, in più nell'olio di moringa è presente in modo cospicuo il C22:0. In tabella 2 sono riportati i valori dei polifenoli e della capacità antiossidante dell'estratto acqua:metanolo delle foglie di moringa, comparati con quelli di altri prodotti alimentari. I valori per vini e per il te bianco sono riportati in (9). Come e' possibile osservare dalla tabella l'estratto alcolico presenta eccellenti valori sia di capacità antiossidante che di concentrazione di polifenoli totali liberi. In questo studio abbiamo voluto introdurre un diverso approccio matematico rispetto ai calcoli che comunemente si fanno per quanto riguarda il rapporto Sat/Ins. A nostro avviso questo tipo di calcolo e' fuorviante, in quanto non tiene conto del grado d'insaturazione dei diversi acidi grassi insaturi. Però anche il solo grado d'insaturazione non e' esaustivo, perche' non considera assolutamente gli acidi grassi saturi, perciò abbiamo introdotto un altro tipo di rapporto e precisamente il rapporto Saturi/Grado d'insaturazione.
In conclusione, mediante questo nuovo rapporto si evidenzia come l'olio di moringa si discosta nettamente dall'olio extravergine di oliva, cosa non molto evidente con gli altri due indici (Sat/Ins, e Grado d'insaturazione), collocandolo tra l'olio extravergine di oliva e lo strutto. Si è, inoltre verificato il buon corredo antiossidante custodito dalle foglie di questa pianta

Tabella 1: Valori degli acidi grassi, espressi in percentuale, dell'olio di moringa a confronto con altri oli alimentari.

Acidi grassi	Moringa (%)	Oliva (%)	Arachidi (%)	Palma (%)	Girasole (%)	Mais (%)	Burro (%)	Strutto (%)
C16:0	12.12	20.97	9.91	41.21	5.92	11.69	20.86	24.02
C16:1	1.87	1.85	0	0.37	0.27	0.4	1.9	2.55
C18:0	10.27	2.28	2.53	4.34	4.74	2.3	9.4	16.67
C18:1	62.50	69.59	51.3	38.45	32.91	29.88	20.68	39.06
C18:2	1.35	5.31	27.87	9.28	49.89	49.83	1.57	8.95
C18:3	0.00	0.00	0	3.3	0.33	0.6	1.18	0.92
C20:0	0.00	0.00	2.28	0.4	0.54	0.4	0	0
C20:4	0.00	0.00	0	0	0	0	0	1.83
C22:0	5.83	0	3.25	0	0	0	0	0
Sat/Ins	0.43	0.30	0.23	0.89	0.13	0.18	1.19	0.76
Insat. Grade	0.67	0.82	1.07	0.67	1.34	1.32	0.29	0.70
Sat/Inst.grade	**42.09**	**28.32**	16.79	68.30	8.36	10.92	25.33	58.47

Tabella 2: Contenuto in polifenoli totali e capacità antiossidante dell'olio di moringa a confronto con altri alimenti

Prodotti	Ka/Kc	Polifenoli Tot. (mg/g)
Moringa	3,03	52,2
Olio extra vergine d'oliva	0,3	51
Radicchio	4,34	13,9
Mela	0,09	4,08
Pere	0,34	2,7
Cioccolato latte	1,47	8,54
Cioccolato fondente	1,98	18,59
Grano duro	0,6	0,31
Te Bianco	6,53	0,62
Vino Cabernet-Souvignon	7,94	2,72
Vino Nero D'Avola	12,23	3,11
Vino Syrha	6,32	3,01
Vino Merlot	16,46	3,03

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P46 - IMPACT OF A SINGLE, INTENSE PRENATAL STRESS ON ETHANOL DRINKING BEHAVIOUR AND COGNITION IN ADULT MALE RATS

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Early exposure to stressful stimuli is crucial for developing varied behavioural patterns in adulthood such as anxiety, cognitive dysfunction and abuse disorders [1]. The alteration of the hypothalamic–pituitary–adrenal (HPA) axis represents the neurobiological substrate responsible of the behavioural consequences of prenatal stress (PS). Indeed, prenatal manipulation of the HPA axis impacts on cognitive performance of the adult offspring [2], but also on vulnerability to alcohol consumption [3]. Prenatal acute, moderate restraint stress has proved to facilitate HPA axis development of the offspring, since maternal corticosterone secretion leads to the reduction of anxiogenic behaviour and an improvement in the ability to cope with stress, increasing both the rate of learning [2] and long-term potentiation [4], but also gestational stress blunts initial alcohol-induced HPA axis activation [3]. In our recent study, moderate alcohol intake proved to reduce emotionality and facilitate the adaptive responses to stress, enhancing behavioural flexibility [5]. Based upon these findings, we aimed at assessing the impact of a single, intense prenatal stress on exposure to alcohol preference and on the effects exerted by ethanol on behavioural reactivity, anxiety-like behaviour and spatial learning in adult male Wistar rats.

Methods

36 adult male Wistar rats were separated into three groups: prenatally stressed, alcohol free-access (PS-FA); free access (FA) and control group (CTR).

Prenatal stress procedures involved immobilization of pregnant dams for 120 minutes on Gestational Day 16 [6]. Male adult PS-FA and FA offspring were subjected to a three-bottle choice paradigm with free access to ethanol 10% (v/v), white wine (Tavernello 11 v%, Italy, diluted with water until 10 % v/v) and water, along a four-week period. The volumes consumed were recorded daily.

To assess the influence of ethanol self-administration on behavioural patterns we used respectively: the open field test (OFT) for behavioural reactivity, the elevated plus- maze test (EPM) for anxiety-like behaviour and the Morris Water Maze (MWM) test for spatial learning.

Results

Our results showed that there is no statistical difference in ethanol consumption between PS-FA and FA rats, both consuming moderate doses (3.00 ±0.8 g/kg/day), apart from the first week when the intake was higher.

Results from the OFT and EPM displayed a reduction in anxiety-like behaviour in PS-FA and FA rats, when compared to CTR. Also, PS-FA group further displayed a reduced anxiety-like behaviour, compared to FA.

Indeed, the OFT showed a significant increase in the number of transitions in the center of the arena ($p<0.0005$) and amount of time spent on the center of arena ($p<0.0073$) in FA rats, compared with controls. The time spent on the center of arena was significantly increased ($p<0.0318$) in PS-FA, compared with FA rats.

Data from the EPM showed a significant increase in the percentage of time spent ($p<0.045$) and of the number of entries ($p<0.0001$) in the open arms of the FA group, compared with CTR. No significant differences was found between PS-FA group, compared with FA.

Finally, PS-FA and FA rats displayed a significant reduction in latencies and distance travelled to find the platform in the place learning of the MWM ($p<0.001$), with respect to controls. In

particular, PS-FA group showed significant improvements in the MWM ($p<0.01$), when compared to FA.

Conclusion

Our study proved that both PS-FA and FA groups had an irregular trend in alcohol consumption, representing an initial binge-like drinking behaviour, then ensued by a voluntary reduction in alcohol intake to moderate values [5,7], evidencing that prenatal stress does not influence ethanol consumption in adulthood.

Moderate ethanol intake exerts anxiolytic properties, as showed by the OFT and EPM, improving the response to stress in the adversative situation in the MWM. Furthermore, ethanol facilitating effect on cognitive performance was enhanced by prenatal attenuation of HPA axis. Indeed, corticosterone levels are inversely correlated with mechanisms of hippocampal neuroplasticity such as BDNF release [8].

In conclusion, our findings further highlight the role of prenatal experiences on ethanol-induced mechanisms of neuroadaptation, indicating that a single, intense stress during early gestational period interacts with alcohol effects in adulthood.

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P47 - ANTI-INFLAMMATORY EFFECTS OF SICILIAN PISTACHIO (*PISTACIA VERA* L.) NUT IN AN *IN VITRO* MODEL OF HUMAN INTESTINAL EPITHELIUM

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Intestinal epithelial cells play an important role in the mucosal inflammatory response. These cells synthesize and secrete inflammatory mediators, and selectively modulate the permeability of the epithelial monolayer thus exposing immune cells to antigens. Although intestinal inflammatory response is crucial to maintain gut structural integrity and function, alteration and dysregulation of inflammatory pathways contribute to tissue damage and ulceration, and are thought to be pivotal factors in the pathogenesis of different inflammatory gut diseases [1]. The limited efficacy of conventional pharmacological therapy in the intestinal inflammatory conditions has fostered research on alternatives and, at the same time, stresses the importance of prevention. In this context, the influence of dietary components, becoming in a physiological close proximity to intestinal cells and then to inflammatory processes within the intestinal mucosa, appears of nutritional and clinical interest [2]. Among natural preventive and complementary approaches to improve inflammatory symptoms, dietary polyphenols represent potential candidates and proanthocyanidins are particularly interesting [3]. For their relatively high concentration in a number of edible plants and their high digestive stability and limited intestinal adsorption [4, 5], proanthocyanidins reach the colon at relatively high concentrations and may have direct effects on the intestinal mucosa through their interaction with the intestinal epithelial cell membranes [6, 7].
The edible pistachio nut has been ranked among the first 50 food products highest in antioxidant potential [8]. A number of data show that the pistachio nut consumption has positive effects in human serum lipid profile and cardiovascular disease (CVD) risk factors [9,10] and significantly improves oxidative status and reduces circulating inflammatory biomarkers [11]. Our previous research provided evidence that a hydrophilic extract from Sicilian pistachio nuts (HPE) contains substantial amounts of polyphenols, including proanthocyanidins, and possesses radical scavenging and antioxidative properties in *in vitro* models of lipid oxidation [12]. Moreover we also demonstrated that HPE has anti-inflammatory activities in lipopolysaccharide (LPS)-activated macrophages interfering with the NF-*k*B activation, and that the high molecular weight proanthocyanidin fraction (PF) can play a major role as the bioactive component of HPE [13].
In the present study we investigated the activity of HPE, and of its polymeric proanthocyanidin fraction as well, in an *in vitro* model of intestinal inflammation, consisting of Caco-2 cells differentiated into epithelial intestinal cells and exposed to the inflammatory actions of interleukin (IL)-1□. Our results clearly show that HPE effectively inhibits the inflammatory response in intestinal epithelial cells, and that highly polymeric proanthocyanidin components exhibit qualitative and quantitative effects substantially comparable to those of whole extracts when tested at the same concentration found in the extracts. The protective effects are expressed through a marked decrease in release and expression of inflammatory mediators and occur in parallel with a reduced activation of the nuclear factor-□B. Moreover, our results clearly show that HPE can partially prevent the IL-1□-induced gap formation with perturbation of the monolayer integrity, as shown by a limited IL-1□-induced increase of paracellular permeability. Finally we provide evidence that HPE treatment increases transepithelial electrical resistence of Caco-2 cells monolayer, demonstrating that protective effects of HPE under our conditions occur in parallel with molecular interaction of nut components with the epithelial cells membranes.

To assess the physiological relevance of the tested concentration it is worth noting that a single serving of pistachio nut (28.34 g) [USDA National Nutrient Database for Standard Reference] contains around 62,34 mg polymeric proanthocyanidins (cyanidin equivalents). Once diluted in a gastrointestinal volume of 600 mL, this results in a 3,5 □M concentration (104 □g/ml) (as cyanidin equivalents) of polymeric proanthocyanidins which represent a plausible concentration in the human gut [14]. This concentration is one order higher than the concentrations selected in our cell model and this suggests that our results might be physiologically relevant in the gastrointestinal tract.

Data here presented may further remark the potentially beneficial health effects that may arise by daily intake of small quantity of pistachio nut. Widely available, inexpensive and frequently consumed, this nut for its favorable fatty acid profile and high content in bioactive antioxidant compounds, positively influences the plasma lipid parameters and oxidative status, and elicits antinflammatory properties. In this respect high content in large proanthocyanidins consumption of pistachio nut can exerts locally significant beneficial effects to physiology of gastrointestinal tract.

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P48 - DEVELOPMENT OF SMART PROBIOTICS AGAINST *CLOSTRIDIUM PERFRINGENS*

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Probiotics are living microorganisms which confer health benefits to the host. Our work was initially focused on investigating the possibility of producing "SMART" probiotics, which are probiotics with modified extra functions, such as the heterologous expression of an antimicrobial. Our first aim was to identify antimicrobial activities or agents which could act against *Clostridium perfringens. C. perfringens*, one of the most pathogenic species in the *Clostridium* genus, is causing increasing concern because it is responsible for severe infections both in human and animals, especially poultry. It is considered the third leading cause of food poisoning death in the UK and USA and causes necrotic enteritis (NE) in poultry [1]. Bacteriophages and their endolysins have been used to treat human infections and to control antibiotic-resistant pathogenic bacteria in animal models [2-4]. Bacteriophages infecting *C. perfringens* are both lysogenic and virulent and show either long tails if members of the Siphoviridae family or short tails if members of the Podoviridae, both in the order of Caudovirales. Several putative bacteriophage endolysins have been identified, both from *C. perfringens* bacteriophages and by genome mining, producing a rich resource of enzymes [5]. The use of endolysins as antimicrobials has been explored. In fact recent studies showed the efficiency of these proteins in killing or controlling pathogenic bacteria when used alone, by a synergistic action with antibiotics or also in combination with other proteins such as the holin. The first test conducted to observe the presence of bacteriophages which contain an endolysin, was a plaque assay test. The appearance of a plaque is the oldest, but at the same time the most useful and direct confirmation way of a phage presence [6]. The nutrient agar layer method was first described by Gratia to enumerate phage particles [7]. A thin layer of soft agar, containing host bacteria and bacteriophages, is poured on a thick layer of higher concentrated agar, used as nutrient medium by bacteria. The phages infect the bacteria and after the production of new phage particles, which are released after bacterial lysis, start a new infectious cycle. To investigate the presence of prophages in *C. perfringens* strains, bacteriophage release was induced by mitomycin C. The supernatants were then concentrated by PEG precipitation then both observed by TEM and used for plaque assays. In the anaerobic cabinet 25 µl aliquots of filtered mitomycin C-induced supernatant were spotted on plates of BHI agar which had been overlaid with 4 ml BHI top agar (0.7% agar) seeded with 100 µl of *C. perfringens* overnight culture. Plates were incubated for up to 48 h and checked regularly for plaque formation. TEM observations on PEG-precipitated supernatants obtained after bacteriophage induction showed the presence of bacteriophages both in *C. perfringens* strains 54116-97 and 6081-97 (**Fig 1**). The bacteriophages in these supernatants did not produce plaques on any of the 25 strains tested. However, the mitomycin C-induced supernatant from strain 6081-97 did show antimicrobial activity on several *C. perfringens* strains, evident because of the zones of clearance around the supernatant dropped on plates (**Fig. 2**). This activity appeared to be variable so to elucidate this behavior, strain 6081-9736 was streaked close to other *C. perfringens* strains such as 562118-98, 4519-98, 2151-88, and DP3. Bacteria which were potential producers of antimicrobials were streaked across BHI agar plates and potential sensitive strains were cross-streaked at a 90° angle with regard to the first streak of the indicator organism or parallel to this one and incubated overnight. The aim was to check if this strain was able to inhibit the growth of other *C. perfringens* strains. Among the strains tested, strain 2151-88 was shown to be able to inhibit the

growth of strain 6081-97 (Fig. 2B,C), but its antimicrobial activity was not constant in repeat tests. In the same way strain 5416-97 showed an antimicrobial activity against other *C. perfringens* strains, but again with non-constant responses. It has been previously reported that mitomycin C can induce the production of bacteriocins from *C. perfringens* [8]. The genomes of both *C. perfringens* strains have been sequenced using Illumina technology and are currently being mined for genes associated with bacteriocin production. Further studies are in progress to investigate bacteriocin production and to assess the meaning of the observed variability in the antimicrobial activity of *C. perfringens* strains tested. The genome sequencing also allowed the identification of an active endolysin against *C. perfringens* from strain 5146-97 [9] and we will investigate the possibility of a further lysin from strain 6081-97.

Fig.1. Tailed bacteriophages found in mitomycin-C induced supernatants of strain 6081-97 (A) and 5416-97 (B,[10]). Scale bar represents 100 nm

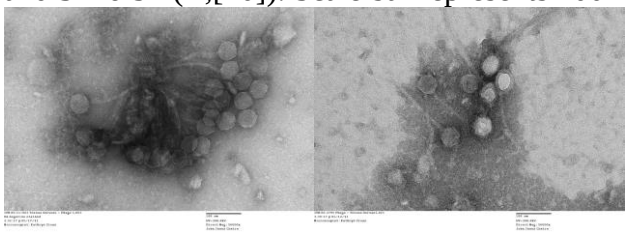

Fig. 2**.** A)Antimicrobial effect of *C. perfringens* 6081-97 mitomycin-C induced supernatant on *C. perfringens* NCTC3110 and (B-C) on *C. perfringens* 2151-88 and 6081-97

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P49 - HOW CBT (COGNITIVE BEHAVIORAL THERAPY) CAN MODULATE NEGATIVE EMOTIONAL FACTORS AS ANXIETY OR DEPRESSION IN OBESE PATIENTS

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Emotional factors have great influence on eating habits and increase the risk of obesity (1). Few studies demonstrate that patients affected by binge eating disorder felt negative emotions before overeatings; the main emotions reported were anxiety, then sadness and tiredness. Emotional eating is related to the stress (2,3), so when subjects feel anxiety and depression, is more probable that they use food as modulator of their emotions, in this way the weight develops fast with all risks of obesity.
The obesity is often attended by a specific syndrome called metabolic syndrome; the main symptoms are insulin resistence, hyperinsulinemia, not-insulin-dependent diabetes mellitus, dyslipidemia, central obesity, hyperuricemia, hypertension; this clinic condition predisposes to cardiovascular diseases (4). So the aim of our study is to observe how CBT can help to manage negative emotions as anxiety e depression (5) and in this way modulate the emotional eating. We began to select a sample of obese subjects, and gave them few questionnaires to evaluate the degree of anxiety and depression (STAI/BDI), the level of self-esteem (BASIC SE), and if there was a diagnosis of Binge Eating Disorder (BED) by a Binge Eating Scale (BES). The first results show a significant relation between anxiety and depression, low level of self-esteem, and their relation with BED. We analyzed the results after an year of treatment with CBT and dietotherapy and we found that patients without BED had a more significant loss of weight,
In the subjects with BED the binges disappeared and the self-esteem increased.

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P50 - PLASMA ANTIOXIDANT CAPACITY STATUS AFTER RED WINE CONSUMPTION

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Several studies report that the plasma antioxidant capacity (PAC) increases after a single ingestion of red wine. However, data on the different behaviour of PAC after food intake in subjects of the same sex are still lacking. On the basis of these observations some investigations were undertaken in order to evaluate the effects of red wine intake on PAC in a homogeneous groups of healthy female volunteers (n=18). Additionally, the possible correlation between increase of PAC values and increase in uric acid levels was also assessed. PAC and uric acid concentrations were determined before wine intake and 50, 120 and 240 minutes thereafter. The results obtained following these studied highlighted two different patterns of variation of plasma AC values after red wine intake in women. Group “A” exhibits a significant increase in PAC at 120 minutes after wine consumption while group “B” showed a peak level of AC 50 minutes after wine intake. However, no significant correlation was highlighted between increased levels of uric acid and PAC. These results provide a strong argument for the hypothesis that sampling procedures may be one of the confounding factor in studies on the plasma antioxidant status after food or beverage consumption. These preliminary observations indicate that sex-based selection of volunteers should be considered in further investigations.

P51 - DOES MATING GROUP SIZE NEGATIVELY AFFECT FEMALE INVESTMENT IN THE SIMULTANEOUS HERMAPHRODITE *APLYSIA PUNCTATA*?

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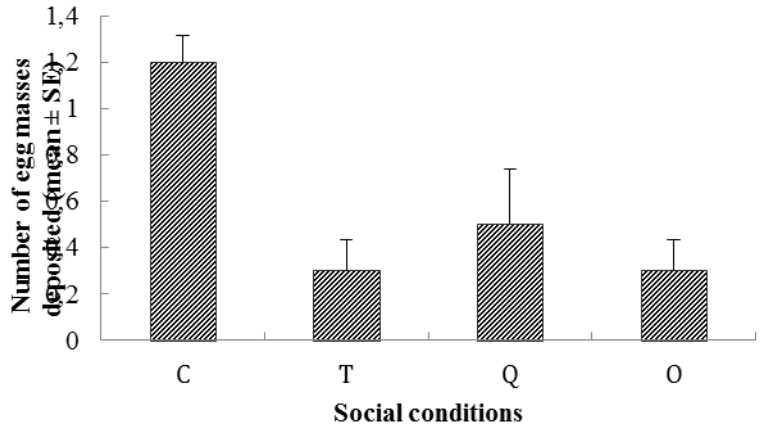
Theoretical models and empirical studies have supported the idea that hermaphroditic sex allocation is sensitive to social conditions [1; 2]. In general, when the mating group size equals two, both partners are expected to allocate as few resources as possible to sperm production, just enough to fertilize each other's ova, and devote all excess energy to egg production [1]. When the average number of possible partners increases, sperm competition for fertilization leads to greater investment in sperm, and resource allocation to female function is expected to decrease. However to our knowledge the relationship between mating group size and sexual behavior has been investigated in only a few species of outcrossing marine hermaphrodites.
The research presented here explores whether the sea hare *Aplysia punctata* (Cuvier, 1803), a simultaneous hermaphrodite, adjusts its investment in female function in response to social conditions. We experimentally manipulated the group size of mature *A. punctata* to determine whether individuals diminish female investment under different social conditions. We predicted that individual *A. punctata* would diminish egg mass production in larger social groups.
A total of 200 adult *A. punctata* (from 40 to 50 mm long) were collected within the rocky shore of the Ustica Island (Cala Sidoti 038'42.50N; 013°9.00E) from January through March 2006. After collection, sea hares were isolated over a period of 30 days in 35 L aquaria with circulating natural seawater, at ambient light (12 hours light:12 hours dark) and temperature regimes (22 °C). *Ulva rigida* (C.Agardh 1823) was supplied daily *ad libitum*, to ensure continuous access to food.
A laboratory experiment was carried out in order to determine if egg mass production of *A. punctata* is related to the size of the social group. Following isolation, animals were randomly assigned to a group of two (P), three (T), four (Q) and eight (O) animals, with 10 replicates of each group size for a total of 170 animals, and constantly monitored for one month. These groups were maintained in tanks (50 cm in diameter, 40 cm in deep) filled with running seawater at a stable temperature and controlled light regime as described previously. All treatments were performed simultaneously and no *A. punctata* was used more than once to ensure the independence of data (Underwood 1981).
Following the treatments, each individual that had been observed mating as a sperm recipient was isolated and monitored for an additional 30 days in separate cages with continuous access to food. Every day we checked for the presence of a spawned egg mass. When an egg mass was detected, it was gently removed from the cage and its wet weight was recorded to the nearest 0.01 g. The proportions of egg masses showing no development (non viable eggs) and normal development (viable eggs) were determined for each egg mass. Differences in number and width of egg masses (a measure of female investment) were analyzed by two separate one-way Analyses of Variance (ANOVA), with "mating group" (MG), including its four levels (P, T, Q, O) as a fixed factor. Data were tested for normality with a Bartlett test (Bartlett, 1937). Homogeneity of variances was also checked with Cochran's C-test (Winer, 1971). Following the ANOVA, means were compared (at $\alpha = 0.05$) with Student-Newman-Keuls (SNK) tests (Underwood, 1997). The GMAV 5·0 software (University of Sydney, Australia) was used to perform the statistical tests.
All egg masses produced subsequent to the experiment contained fertilized eggs indicating that exogenous sperm transfer had been successful. Egg masses were always laid by individuals that were observed to be the first member of a mating chain (the terminal sperm recipient) and never

by an animal that acted as both sperm recipient and sperm donor at the same time. We observed that *A. punctata* laid fertilized egg masses during copulation, after few hours or, at most, 36 hours after copulation.

A total of 23 egg masses were produced: 10 in the P treatment (1 from each pair), 3 in the T treatment (2 from individuals that mated in a pair and 1 from a chain of three), 4 in the Q treatment (all from individuals that mated in a pair) and 4 in the O treatment (2 from individuals that mated in a pair,1 from a chain of three and 1 from a chain of four). All egg masses showed normal cleavage, development of embryos and the hatching of planktonic larvae within 7-10 days. Group size strongly influenced egg production ($F_{3,39}$= 6.15; P = 0.0017) and SNK results revealed that the number of spawned egg masses was greater for the P treatment than any other treatment (P>T=Q=O) and ranged from a maximum of 1.2 ± 0.1 egg masses per individual (SE) (laid by individuals from P treatments; Fig. 1) to a minimum of 0.3 ± 0.1 (SE) (laid by individuals from T and O treatments; Fig. 1). Moreover, egg mass size varied significantly as a function of the social condition ($F_{3,39}$= 8.26; P = 0.0003). In particular, SNK results showed that egg mass width was greater for the P treatment than any other treatment (P>T=Q=O) and ranged from a maximum of 26 ± 3.8 (SE) mm (laid by individuals in the P treatment) to a minimum of 8.0 ± 3.8 (SE) mm (laid by individuals in the T treatment).



Our findings showed that group size strongly influenced female function and that number and width of egg masses were greater for individuals maintained in pairs than for those maintained in larger social groups. These results indicate that *A. punctata* respond as expected according to sex allocation theory for simultaneous hermaphrodites [3]. Furthermore, our experiments reveal that female allocation in this species is phenotypically flexible, as fully mature individuals were able to adjust their investment in the number and size of egg masses with the size of the social group.

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P52 - ALLEVAMENTO A CICLO BIOLOGICO NATURALE DI CORNU ASPERSUM (MÜLLER, 1774): PROPRIETÀ ALIMENTARI ED USI DEL SECRETO O BAVA DI LUMACA.

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Cornu aspersum (Muller, 1774), specie che in passato era attribuita al genere *Helix* (*Helix aspersa*),è un mollusco gasteropode terrestre appartenente alla famiglia degli Helicidae E' diffuso nel bacino del Mediterraneo e nell'Europa nord-occidentale. In Italia è presente in tutta la penisola, isole comprese. Il diametro della sua conchiglia può arrivare a 35 mm., con un'altezza di 27–31mm; il suo peso è di circa 13-15 gr. La colorazione di fondo va dal verdastro al giallastro; può essere uniforme, ma più spesso sono presenti da 1 a 5 bande spiralate marroni, con screziatura gialla o bianca. Come molte chiocciole, *C. aspersum* è ermafrodita, cioè ogni individuo possiede sia organi riproduttivi maschili che femminili; non è tuttavia in grado di autofecondarsi (ermafroditismo insufficiente).
L'azienda elicicola " La Lumaca del Belice" sita nel territorio di Sambuca di Sicilia (AG) su una superficie di circa 10.000 mq, usa un sistema di allevamento a ciclo naturale biologico in campo aperto che dà un aumento notevole della produzione per metro quadrato ma soprattutto dà la possibilità di portare le chiocciole alle caratteristiche ottimali nel più breve tempo possibile. Con questo sistema di allevamento le chiocciole nascono e crescono dentro lo stesso recinto, evitandosi i lavori di spostamento dei piccoli causa di mortalità elevata dovuta allo stress provocato dal cambio di habitat ed allo schiacciamento durante il trasferimento a causa della fragilità del tenero guscio che le protegge. Il ciclo di allevamento inizia con la coltivazione di bietola e cavolfiore per l'alimentazione e di trifoglio per ricreare l'habitat naturale nei mesi di marzo/aprile, fino ad arrivare al mese di novembre quando la raccolta delle chiocciole sarà completata ed il terreno verrà preparato per il successivo ciclo. Durante questo periodo gli ortaggi non verranno trattati né con concimi chimici né con pesticidi. Le prime chiocciole riproduttrici verranno immesse nei recinti nel primo periodo primaverile, quando la vegetazione avrà raggiunto le caratteristiche ottimali di vegetazione. Qui le chiocciole si accoppieranno e concepiranno le nuove chiocciole che, già nei primi mesi di settembre-ottobre, avranno raggiunto le caratteristiche ottimali di pezzatura e peso e saranno pronte per essere commercializzate.
Diversi studi hanno contributo ad approfondire le conoscenze utili ai fini della valorizzazione del prodotto alimentare "lumaca", non solo in termini alimentari ma anche economico-sociali in virtù della crescente richiesta del prodotto da parte del mercato interno e della prospettiva di poter integrare, attraverso l'attività di allevamento del mollusco, l'attività agricola. L'indagine analitica finalizzata all'approfondimento conoscitivo delle proprietà dietetico-nutrizionali di cinque diverse specie di molluschi polmonati del genere *Helix* di interesse alimentare, condotta da Novelli (1) permette di trarre alcune considerazioni. Il contenuto in sostanza secca della parte edibile è prossimo al 20%, i due-terzi del quale sono rappresentati da proteine. Il contenuto lipidico è decisamente ridotto (inferiore all'1%) e ciò non è sorprendente in ragione della predisposizione di tali molluschi a prediligere riserve energetiche tissutali in forma di polisaccaridi piuttosto che di lipidi. La quota parte costituita da acidi grassi è per quasi il 75% rappresentata da acidi grassi insaturi, tre-quarti dei quali sono polinsaturi. Da un punto di vista strettamente nutrizionale gli acidi grassi della serie *n6* e della serie *n3* sono fra loro in rapporto sbilanciato a favore dei primi, in cui sono significativamente rappresentati l'acido linoleico e l'acido arachidonico. Ugualmente elevata è risultata la componente in sali minerali (più del doppio del normale contenuto in ceneri delle carni dei Vertebrati terrestri). Differenze interspecifiche sono state rilevate soprattutto a carico del contenuto in proteine e ceneri; il

confronto intra-specifico fra prodotto d'allevamento e prodotto raccolto in natura non ha evidenziato sostanziali differenze. Le caratteristiche alimentari della "lumaca" si distinguono per un ridotto contenuto calorico conseguenza della limitata quantità di grasso di deposito nelle masse muscolari. L'apporto in acidi grassi di elevato valore nutrizionale da parte della massa muscolare del piede è ragguardevole.

Le lumache rispondono ai danni cutanei provocati dai predatori o dagli incidenti rigenerando le cellule danneggiate. Non sviluppano un'eccessiva reazione infiammatoria e inoltre le ferite sono riparate velocemente senza la formazione di cicatrici evidenti. I ricercatori hanno visto che la pelle delle lumache ha la stessa composizione di quella umana, con i medesimi elementi strutturali come il collagene e l'elastina. Quando la pelle del corpo umano è danneggiata o attaccata da microrganismi, la reazione infiammatoria che si scatena è molto differente e più forte di quella che si verifica nelle lumache. La lumaca reagisce ai danni cutanei producendo una grande quantità di muco che, attraverso la formazione di numerose bolle, bagna e aderisce completamente alla superficie della cute. Il fluido naturale idrata efficacemente la pelle e nello stesso tempo la protegge con i suoi peptidi antimicrobici, le sostanze antiossidanti e le molecole che incentivano l'ordinario processo rigenerativo che ristruttura e rinnova le cellule dei tessuti danneggiati (2). La bava di lumaca ha peculiari caratteristiche adesive.per cui grazie a una particolare componente proteica, anche in concentrazioni minime, aderisce in maniera efficace, in ambienti umidi anche a superfici irregolari (3). La bava di lumaca di *Cornu aspersum* (*Helix aspersa*) ha una composizione complessa di sostanze attive che la rendono un ingrediente cosmetico unico e non replicabile in laboratorio con un prodotto di sintesi o una miscela di essi. L'analisi chimica quali-quantitativa ha evidenziato la presenza in particolare di allantoina 0.3 - 0.5%, collagene 0.1 - 0.3%, acido glicolico 0.05 - 0.1%, acido lattico 0.05 - 0.1%, anti-proteasi 1.3 - 1.8%, vitamine e minerali in tracce. In un recente lavoro (4) è stato messo in evidenza che il secreto di *Crymptophalus aspersa*, utilizzato per applicazioni cutanee, possiede proprietà rigenerative per la pelle umana per le quali sono state fatte interessanti ipotesi di meccanismo d'azione Studi clinici hanno dimostrato che prodotti cosmetici a base di secreto di *Cornu aspersum* (*Helix aspersa*) favoriscono la cicatrizzazione delle ustioni dei bambini riducendo la formazione del cheloide, l'iperpigmentazione e migliorando complessivamente l'aspetto estetico della cicatrice(5). In altri studi (6) è stata confermata l'efficacia del secreto di *Cornu aspersum* (*Helix aspersa*) nella cicatrizzazione delle ustioni facciali di soggetti adulti. Sembra che la secrezione di *Cornu aspersum* (*Helix aspersa*) contribuisca con tutti i suoi componenti a promuovere la cicatrizzazione della ferita e la riduzione della formazione del cheloide. In particolare l'allantoina in essa contenuta ha spiccate proprietà cicatrizzanti già note anche per favorire la riparazione di ferite suppuranti, ulcere resistenti, emorroidi e varie infezioni dermatologiche. L'allantoina stimola la formazione tessutale e rende più rapida la cicatrizzazione delle ferite. Diverse preparazioni medicamentose topiche per la cicatrizzazione dei tessuti sono formulate con allantoina e si sono dimostrate particolarmente efficaci nel velocizzare la riparazione dei tessuti e nel ridurre la formazione delle cicatrici e dei cheloidi (7). L'acido glicolico e l'acido lattico, naturalmente contenuti nella bava di *Cornu aspersum* (*Helix aspersa*), contribuiscono a idratare (8) e levigare la pelle riducendo l'iperpigmentazione e prevenendo la formazione delle smagliature anche durante la gravidanza (9). Migliorano l'estetica delle cicatrici anche di vecchia data. L'acido glicolico promuove il turnover epidermico e favorisce la proliferazione dei cheratinociti (10).

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P53 - HIGH TURNOVER RATE OF CENTRAL HISTAMINERGIC SYSTEM IN PATIENTS WITH DOWN SYNDROME AND ALZHEIMER DISEASE

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It is well confirmed that a strong relationship exists between Down's syndrome (DS) and Alzheimer's disease (AD). Neurochemical investigations reported that many central neurotransmitter systems are similarly affected in aging Down and in Alzheimer patients, respectively. Airaksinem et al. (1) found numerous neurofibrillary tangles in the tuberomammillary area of the hypothalamus, where cell bodies of histaminergic neurons are located. While Mazurkiewicz-Kwilecki et al. (2) found deficits of the endogenous diamine, Cacabelos et al. (3) reported an increase of central histamine levels. In the present study, in order to test whether AD-like neuropathological changes involve the central histaminergic system, we measured the concentration of histamine, histidine as well as the activity of histidine decarboxylase (HDC) and histamine-N-methyltransferase (HMT) in temporal cortex (TC) of aging Down, Alzheimer and control patients.
Post-mortem samples (temporal cortex, TC; grey matter) of AD neuropathologically confirmed cases (72.1 ± 7.6 years old), of karyotyped patients with DS (56.1 ± 7.1 years old), and control adults (72,7 ± 9.7 years old) were obtained from the MRC London Brain Bank for Neurodegenerative Diseases, Department of Neuropathology, Institute of psychiatry, London, U.K.
Each block of brain tissue from AD, DS and controls were thawed on ice and homogenized in ice-cold HDC-solution of 0.1 M sodium phosphate buffer (pH 6.8) containing dithiothreitol and antipain protease inhibitor. Homogenates of brain specimens were centrifuged at 12,000 x *g* for 20 min at 4°C. The supernatants were poured into CENTRIPEP-3 concentrators (Amicon), and centrifuged at 2,000 x *g* for two 10 min periods at 4°C. The clear extracts were stored in small quantities in Eppendorf tubes at -80°C until analysis. HDC activity has been measured with the procedure described by Gueli et al. (4) and briefly summarized. Extract aliquots were pre-incubated for 10 min with HDC assay-solution (0.1 M PBS, 0.2 mM DTT, 0.01 mM PLP, 0.1 mM Aminoguanidine), then incubation was started by adding 0.5 mM L-histidine for 0-3 h at 37°C. At the established times, the reactions were stopped with 60 % ice-cold PCA, and stored overnight. Finally, the reaction mixture was centrifuged at 19,000 x *g* for 30 min at 4°C. The supernatants were withdrawn and filtered (0.45 μm Millipore filter). The HPLC system consisted of a 600E Waters pump with a Waters 474 scanning fluorescence detector (ex 350 nm, em 450 nm). Chromatograms and calculations were performed by Empower TM2 Data Software. Histamine was separated and quantified after pre-column derivatization with Shore's o-phthalaldehyde reaction (5), using a Spherisorb ODS2 analytical column, particle size 3 μm (20 x 0.46 cm,) (Waters, Milano), a 10 μl injection volume, and a mobile phase of methanol, 20 mmol/ L sodium acetate in water, acetic acid (55:43:2 v/v) and 0.33 mmol/L 1-octanesulfonic acid sodium salt. The flow rate was 1.0 ml/min. In order to measure HMT activity brain tissue was disperged with a glass Teflon homogenizer in 0.1 M PBS (pH 7.2). After centrifugation the supernatant was used for the radioenzymatic assay (6). Histidine contents were measured using the procedure described by Borum (7).
We observed a increase of histamine levels in temporal cortex of AD (+15%) patients. Down brains also showed a mild increase of the endogenous diamine concentration (+8%). HDC activity in both groups of diseased brains was significantly increased compared with controls (+59% for DS and +21% for AD, respectively). In accord to HDC actvity, HMT activity run in

parallel in both pathological groups. In contrast to histamine, histidine levels were markedly decreased in temporal cortex of both pathological groups. These results put together leads us to think of a similar high turnover rate in the metabolic happenings of the histaminergic system in the temporal cortex of the patients with Alzheimer's disease and Down's syndrome. The fast histaminergic changes may contribute to the clinical manifestation of dementia in both disorders.

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P54 - STUDY OF SAGITTAL SPINAL ROM IN ELDERLY WOMEN AFTER A SPECIFIC FLEXIBILITY-TRAINING PROGRAM

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The reduction of spinal mobility is one luckless consequence of the aging process. In particular, scarcity of exercise appears to be the principal reason of aging-related spinal dysfunction syndrome, which is characterized by adaptive shortening of soft tissue and a partial loss of range of spinal motion (1). Several recommendations promoting the spinal flexibility in elderly people are based on stretching programs that include static and ballistic exercises and PNF techniques (2). Several studies showed that specific training programs could improve body balance (3), bodyweight distribution on feet (4), muscle strength and flexibility (5) in elders. In particular, Imagama et al. (2011) showed that spinal flexibility and resistance training programs may be able to affect positively primary factors related to quality of life, such as lumbar lordosis angle, sagittal balance and spinal ROM in middle-aged and elderly subjects (6).
The aim of this study was to evaluate the effects of a specific flexibility training program on thoracic spinal range of motion (T-ROM) in female older subjects. Thirty older women were randomized into either a trained group [TG] (n: 17; age: 68.35±6.04 years; height: 1.54±0.06 m; weight: 64.78±10.16, kg, BMI: 27.28±3.08) and a control group [CG] (n: 13; age: 69.69±7.94 years; height: 1.57±0.06 m; weight: 68.42±8.18 kg, BMI: 27.88±2.81). Trained subjects were trained for 8 weeks by two sessions/week. In particular, every trained session included: a warm up period (~15 min), a training period (~50 min) including specific exercises to train spinal flexibility, cool down period (~15 min). Control subjects did not perform any programmed physical activity during the experimental period. Data were obtained before and after the experimental phase. Spinal ranges of motion were measured using SpinalMouse® (Idiag, Volkerswill, Switzerland), which is an electronic computer-aided device that measures sagittal spinal ROM and inter-segmental angles non-invasively (6). Each angle was measured three times in a neutral standing (nS) position, maximum extension (maxE) position and maximum bending (maxB) one, and average data were used.
At baseline and after experimental period TG and CG were equivalent in age, anthropometric characteristics and Berg balance score. Moreover at baseline two groups showed similar ROMs during sagittal maximum bending and extension of spinal. We found a significant increase in thoracic ROM from nS to maxB position ($p<0.05$) in TG compared to CG after the training period. Instead, we did not show any signifcant difference in T-ROM from nS to maxE position ($p>0.05$).
In conclusion, our results suggest that well-organized flexibility training programs executed for eight weeks can improve spinal ROM from nS to maxB position and enhance the quality of life in older women. In agreement with the 2009 American College of Sports Medicine (ACSM) position statement, we think there is a lack of consensus concerning the prescription of stretching

exercises for elderly people (ACSM, 2009). For these reasons it is necessary to increase studies on potential benefits of flexibility-specific training interventions on range of motion (ROM) of elders' spine.

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P55 - NITRITE AS DIRECT S-NITROSYLATING AGENT OF KIR2.1 CHANNELS: BIOTIN SWITCH ASSAY APPLICATION

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In both mammals and non-mammalian vertebrates, nitrite anion, the largest form of intravascular and tissue storage pool of nitric oxide (NO), represents a key player in many biological processes, including cardiac performance regulation [1, 2]. Nitrite affects numerous biological processes through NO-dependent and independent pathways [3], including the S-nitrosylation of thiol-containing proteins [4]. The mechanisms underlying these phenomena are of great interest because of their cardiovascular therapeutic potential. However, so far they are not fully understood. The purpose of this study was to analyse in the rat heart (as prototype of mammalian heart) whether nitrite affects S-nitrosylation of cardiac proteins and the potential targets for S-nitrosylation.
Among methods for studying protein S-nitrosylation, the biotin switch assay, successfully used in various mammalian tissues and cell types, has rapidly gained popularity because of the ease with which it can detect individual S-nitrosylated (SNO) proteins in biological samples. As illustrated in Fig. 1, the biotin switch assay consists of three principal steps: 1) "blocking" of free cysteine thiols by S-methylthiolation with MMTS (a reactive thiosulfonate); 2) conversion of SNOs to thiols via transnitrosation with ascorbate; and 3) in situ "labeling" by S-biotinylation of the nascent thiols with biotin-HPDP, a reactive mixed disulfide of biotin. The degree of biotinylation (and thus S-nitrosylation) is determined by either anti-biotin immunoblotting or streptavidin pull-down followed by immunoblotting for the protein(s) of interest.

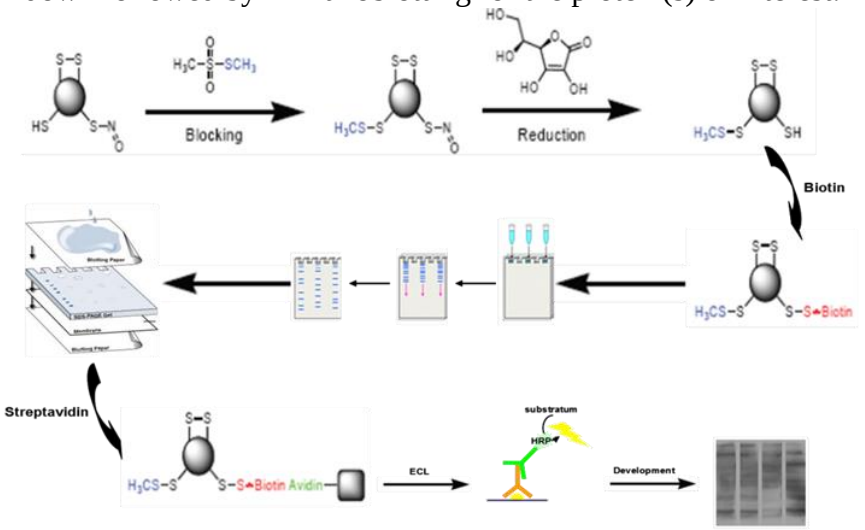


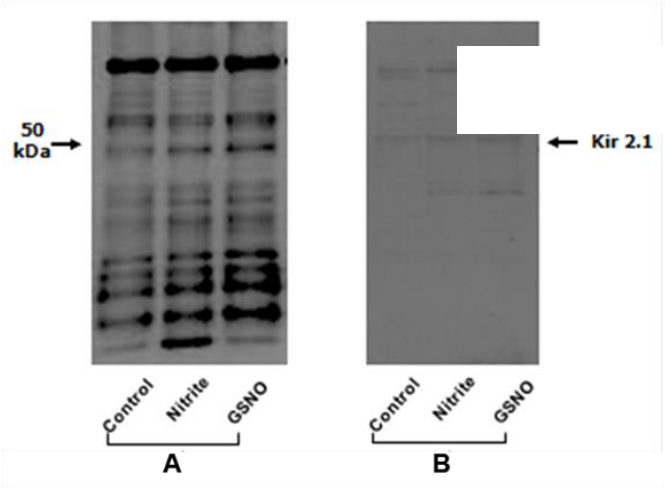
Fig. 1. *Over view of the Biotin Switch Assay: in this method, un-modified protein thiols are blocked while S-nitrosylated thiols are reduced to free thiols; the newly generated thiols are labeled with a biotin tag, followed by avidin capture of the labeled proteins. The final products then correspond to the originally S-nitrosylated proteins that can be identified by proteomic approaches.*

In our study, the biotin switch assay was performed essentially as previously described [5, 6] and utilized to assess whether exposure of the Langendorff perfused rat heart to nitrite induces proteins S-nitrosylation. We analysed, in both cytosolic and membrane cardiac extracts, the proteins containing S-nytrosilated cysteines showing that nitrite treatment increased the degree

of S-nitrosylation of a broad range of plasmalemmal proteins. Since a number of studies [1, 3, 7] showed that nitrite mediates its effects through its conversion to NO, the changes in S-nitrosylation of nitrite-treated rat heart were compared with those induced by the NO donor GSNO.

Further analysis, conducted on subfractioned proteins, allowed us to identify a high level of nitrosylation in a small range of plasmalemmal proteins (45-50 kDa) of nitrite and GSNO treated hearts. The increment in S-nitrosylation at this location was characterized by using an anti-Kir2.1 rabbit polyclonal antibody (Fig. 2). We also verified that this effect of nitrite is preserved in the presence of the NO scavenger P-TIO.

Our results suggest, for the first time, that nitrite represents a direct S-nitrosylating agent in cardiac tissues and that Kir2.1 channels are one of the targets. These observations are of relevance since they support the growing evidence that nitrite is not only a NO reserve but also a direct modulator of important functional cardiac proteins.



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P56 - NOVEL MECHANISMS OF PHOSPHOLAMBAN/SERCA2A MODULATION: PHOSPHORYLATION *VS* S-NITROSYLATION AND S-SULFYDRATION

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Phospholamban (PLN), a small protein closely associated with the cardiac sarcoplasmic reticulum (SR), has been identified and sequenced in many vertebrates, from fish to human. The very high homology among the sequence underlines the old evolutionary history of this protein, as well as its importance in myocytes dynamics. By modulating the intracellular calcium transient it represents the major determinant of cardiac contractility and relaxation. Alternate PLN phosphorylation/dephosphorylation determines SERCA2a on/off state, and thus the rate of SR refilling with Ca^{2+}, with a consequent impact on myocardial relaxation and contraction. In its dephosphorylated state, PLN inhibits Ca^{2+} sequestration by SERCA2a (1) and this induces more Ca^{2+} to be available for the contractile apparatus, thus decelerating relaxation; when PLN is phosphorylated this inhibition is relieved and Ca^{2+} is actively pumped into the SR causing an increased rate of myocardial relaxation (2). A phosphorylation-dependent activation of PLN was observed by us both in mammalian (rat: 3, 4, 5) and non-mammalian (fish: 6, amphibian: 7) vertebrate hearts.

Recently, we have identified alternative, phosphorylation-independent, mechanisms of PLN/SERCA2a regulated Ca^{2+} reuptake, such as S-nitrosylation (6, 4, 5) and S-sulfhydration (8).

S-nitrosylation, the covalent modification of a protein cysteine thiol by a nitric oxide (NO) group to generate an S-nitrosothiol (SNO), is recognized to be important in regulating protein function (9). This is particularly relevant in the heart, in which several proteins of critical significance were identified as potential targets for S-nitrosylation (10). Using the biotin switch method (11) (Fig. 1), we demonstrated that in the eel (6) and rat (4, 5) heart PLN represents an important target for S-nitrosylation. In particular, in the eel it has been observed that the Frank-Starling response is modulated by a nitric oxide-dependent S-nitrosylation of PLN, which in turn improves myocardial relaxation (6). In the rat heart, PLN S-nitrosylation appeared involved in the lusitropic action of several cardioactive substances, such as Catestatin and 17–β-estradiol (4, 5).

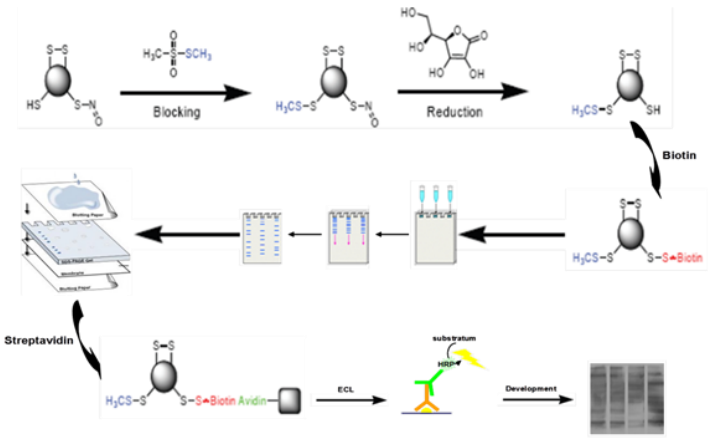

Fig. 1**.** Biotin Switch Assay

A modified biotin switch method, using S-methyl methanethiosulfonate (MMTS) as an alkylating reagent, was recently used to identify a large number of proteins that may undergo S-sulfhydration (12). Similarly to S-nitrosylation, S-sulfhydration represents an important signal

which modulates many biological processes. In frog and rat heart, NaHS (a donor of H_2S) increases protein S-sulfhydration (8). In the rat, Western Blotting of membrane fraction revealed two bands corresponding to the apparent molecular weights of PLN monomer (6kDa) and dimer (12 kDa) as putative targets for S-sulfhydration**.** This was confirmed by immunoprecipitation of the membrane protein fraction with anti-PLN antibody which revealed an increase of PLN S-sulfhydration in NaHS-treated hearts, particularly evident in the case of the 12kDa band (8) (Fig. 2).

Taken together, these results propose S-nytrosilation and S-sulfhydration of PLN as novel mechanisms for SERCA2a regulation which in turn modulates myocardial inotropic and lusitropic properties.

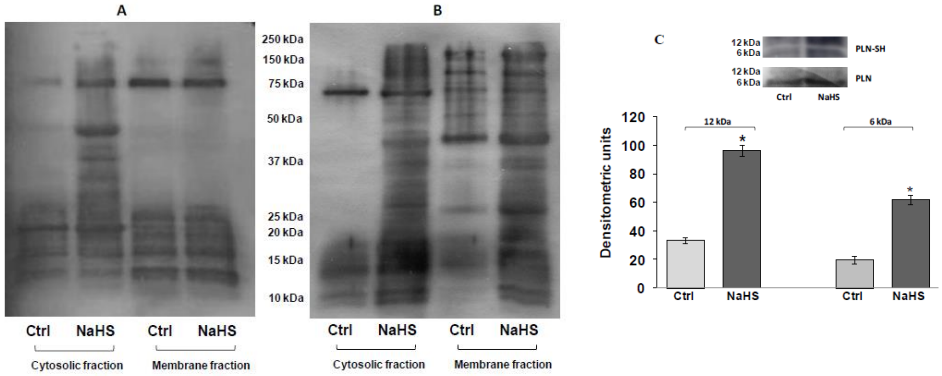

Fig. 2. Cardiac protein S-sulfhydration in frog (A) and rat (B). C: Western blot analysis of immunoprecipitated PLN (rat).

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P57 - THE OSTEOGENIC DIFFERENTIATION OF BONE CHIP-DERIVED MESENCHYMAL STEM CELLS IS CONTROLLED VIA SPECIFIC RECEPTOR SIGNALING

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Mesenchymal stem cells (MSCs) are an attractive cell source for Regenerative Dentistry in particular due to their ability to differentiate towards osteoblasts, among other lineages. Tooth and jaw bone loss are frequent sequelae of traumatic and pathological conditions in both the young and the elderly and must be met by appropriate prosthetic replacements. For successful osseointegration of the dental implant a sufficient bone level is necessary. Besides the utilization of bone autografts or synthetic biomaterials, medical research is more and more focused on the utilization of MSCs. Compared to cells obtained from liposuction material, ectomesenchymal stem cells derived from the head area e.g. out of dental follicles or particulate, non-vascularized bone chips show a higher differentiation potential towards osteoblasts. This implies that due to their different origin, ectomesenchymal stem cells are stronger committed towards hard tissues and are therefore interesting candidate cells for bone regeneration [1, 2].
Parathyroid hormone-related protein (PTHrP) is known to be involved in tooth eruption. It acts as a signaling molecule that stimulates local bone resorption [3]. Recently, PTHrP was found to affect the MSC differentiation process. The differential expression level of specific PTHrP isoforms might be considered as a molecular signature associated with the respective differentiation state during osteogenesis [4]. Moreover, we could show that in addition to the role of purinergic 2 (P2) receptors in cellular processes such as proliferation, migration and apoptosis, they are also involved in stem cell differentiation. Several P2 receptor subtypes play a role in key steps during osteogenic lineage commitment. Further development of MSCs into progenitor cells, pre-osteoblasts and osteoblasts seemed to be triggered via the alteration of their P2 receptor expression patterns [5].
Human mesenchymal stem cells were isolated from bone chip material harvested during oral surgery intervention. Their stem cell character was demonstrated by plastic-adherence and expression of the surface markers CD73, CD90, and CD105 following differentiation along the osteogenic lineage [6]. The mineralization process was monitored by Alizarin Red S staining of extracellular matrix components. Among the examined P2 receptor subtypes, down-regulation of P2Y14 appeared to be involved in the onset of this differentiation process. Today several artificial P2 receptor ligands are present in the market. The administration of a stimulating P2Y14 receptor ligand had a direct influence on the osteogenic differentiation potential. More precisely, the application of the potent and selective P2Y14 agonist MRS 2690 led to a dose-dependent reduction of the extracellular mineralization.
Taken together, bone chip-derived mesenchymal stem cells are promising candidate cells for bone replacement. Here we show that they are capable of differentiating into osteoblasts. The application of an artificial receptor agonist confirmed the functional role of P2Y14 during osteogenesis. Therefore, it is of major interest to develop selective and potent antagonists directed against this recently discovered member of the purinergic receptor family. Controlling the P2Y14 receptor signaling might improve future bone tissue engineering approaches in Regenerative Dentistry.

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P58 - IN VIVO ASSAY FOR THE IDENTIFICATION OF POTENTIAL INHIBITORS OF EPITHELIAL-MESENCHYMAL TRANSITION (EMT)

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We designed and developed a simple and reproducible screening assay to explore the ability of a number of small compounds, interfering with evolutionary conserved signalling pathways, to inhibit epithelial mesenchimal transition (EMT) *in vivo*. EMT is an important process during development by which fully polarized epithelial cells acquire mesenchymal, fibroblast-like properties and show reduced intercellular adhesion and increased motility. EMT mechanism is re-activated in tumor progression, tissue invasion, metastasis and the acquisition of resistance to therapy (1-2). Targeting EMT, therefore, represents an important strategy for cancer treatment.

Sea urchin embryos provide a useful system to study EMT progression, just few hours after fertilization. The process is well characterized. Prior to ingression, the future primary mesenchyme cells (PMC) are indistinguishable from the neighbouring epithelial blastomeres, but their regulatory apparatus is actively preparing new molecular programs by which cells are going to turn on the expression of mesenchyme-specific molecular markers. Thus, at the right time PMCs, previously adherent to the adjacent epithelial cells via cadherin and adherens junctions, loose cell adhesion, enter the basal lamina and move through the blastocoele. Later, after migration to the proper *loci*, the specified PMCs will give rise to the embryonic skeleton (3).

So far, potential EMT inhibitor compounds have been identified using a carcinoma cell line specifically induced to undergo EMT by the activation of growth factor signalling pathways (4). Nevertheless, this approach restricts the research to bio-molecules only affecting the selected growth factor induced signals and in some cases the reporter cell lines are not responsive to all exogenous growth factors known to be EMT inducers.

Here, we propose an *in vivo* screening assay, using sea urchin embryos (fig. 1); we picked out a selection of pharmacologically active compounds from a commercial library (LOPAC^{1280}TM, Sigma-Aldrich) and tested their ability to inhibit EMT in embryos. These molecules are known to interfere with some evolutionarily conserved signalling pathways: P38 mitogen-activated protein kinase (MAPK), platelet-derived growth factor receptors (PDGF-R) epidermal growth factor receptor (EGFR) tyrosine-protein kinase (Src), Glycogen synthase kinase 3 (GSK-3). We set up the experiments as follows: two different batches of *Paracentrotus lividus* embryos at zygote (just post-fertilization) or hatching blastula (12h post-fertilization and 4h prior PMC ingression) stages were incubated in multiwell plates in the presence of different concentrations of the selected drug. Treated or control embryos were then monitored under an inverted microscope and scored for timely precise PMC formation, number and migration capability. Embryos were then photographed, phenotipically classified and in some experiments assayed for the expression of specific antigens. We obtained evidence that some of these compounds inhibit EMT and prevent the expression of mesenchymal specific molecular markers. We propose this low-, medium-throughput Sea Urchin embryonic EMT Assay (SU-EMTA) as an affordable and useful method to screen a high number of compounds, with potential anti-metastatic activity.

Partially supported by a grant from the Italian Ministry of Economy and Finance to the CNR for the Project FaReBio di Qualità.

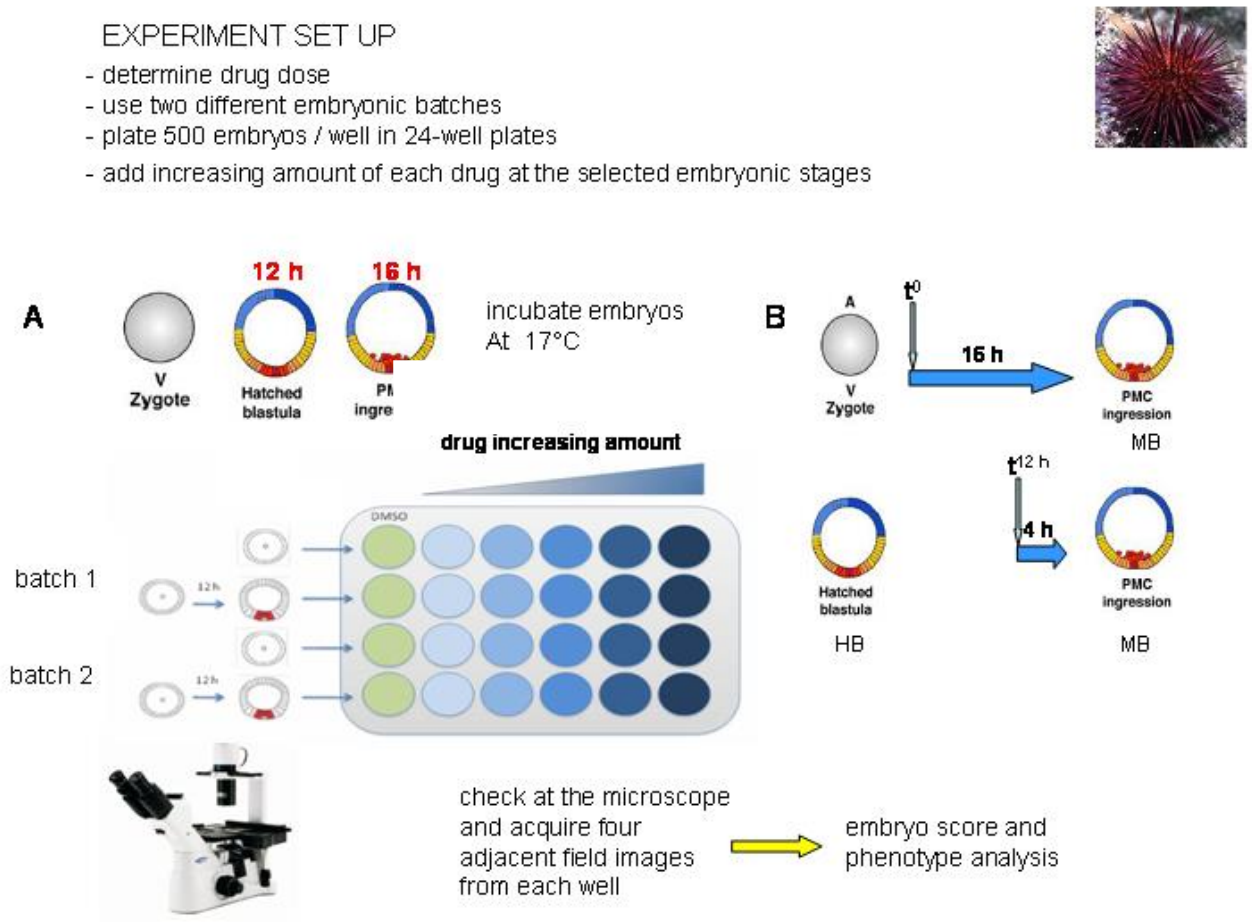

Fig. 1 A. Set up of a standard EMT assay on sea urchin embryos. B. Drug addition at fertilization (zygote) for 16 h or at hatching blastula stage (HB) for 4 h. Phenotype analysis at mesenchyme blastula stage (MB) and later (36 h after fertilization).

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P59 - PTC124 DERIVATIVES AS A NOVEL APPROACH TO IMPROVE THE READTHROUGH OF PREMATURE AMBER AND OCHRE STOP CODONS

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Nucleotide changes within an exon may alter the trinucleotide normally encoding a particular amino acid, such that a new "stop" signal is transcribed into the mRNA open reading frame. This causes the ribosome to prematurely terminate its reading of the mRNA, leading to the lack of production of a normal full-length protein. Such premature termination codon (PTC) mutations occur in an estimated 10% to 15% of many genetically based disorders (1).
Pathological nonsense mutations resulting in TAG (40.4%), TGA (38.5%), and TAA (21.1%) occur in different proportions to naturally occurring stop codons (2). Several genetic disorders are characterized by *opal* (TGA; Cystic fibrosis, Duchenne/Becker muscular dystrophy), *amber* (TAG; □-thalassemia, emphysema, cystic fibrosis) and *ochre* mutations (TAA; APC gastric cancer, Haemophilia B, Hypothyroidism) (3). Messenger RNA containing a nonsense mutation is often degraded rapidly through the process of nonsense-mutation-mediated decay (NMD) resulting in the lack of the protein (4).
A recent approach to directly overcome the deleterious effects caused by nonsense mutations is represented by readthrough strategies which take advantage of the known properties of aminoglycosides that can suppress stop codons (5). Several aminoglycosides (gentamicin, amikacin, hygromycin, etc.) can suppress the accurate identification of translation termination codons in cultured eukaryotic cells. Unfortunately, aminoglycoside action lacks specificity resulting in readthrough of many correctly positioned stop codons. Consequently, long- term use of aminoglycosides may originate toxic aggregates or dominant negative readthrough products (6).
By an high throughput screening it was identified the PTC124 (Ataluren), a small molecule that has been suggested to allow PTCs readthrough (7). However, despite the results obtained on opal mutation it was shown that it has a lower activity against *ochre* and *amber* nonsense mutations (7).
In the attempt to identify molecules with an activity against *ochre* and *amber* nonsense mutations, we designed and synthesized new PTC124 derivatives to be tested in human cultured cells to see if they have higher and wider activity towards PTCs than PTC124. To this aim we generated a reporter vector with non-sense mutation by introducing in the pBOS-H2BGFP plasmid a TAG codon (*amber*) and TAA codon (*ochre*) by site-directed mutagenesis. PCR and sequencing analyses confirmed the presence of the stop codons in the plasmids that were transfected in HeLa cells to explore the ability of the derivatives to promote the translational read-through. Immunofluorescence analyses showed that one of the analyzed derivatives was able to restore GFP fluorescence in HeLa H2BGFP-*amber* cells, as indicated by GFP-localization in the nuclei of treated cells (figure 1). This positive response also confirmed the correct functioning of the model system which will allow us to perform the screening of a greater number of molecules with read-through action.

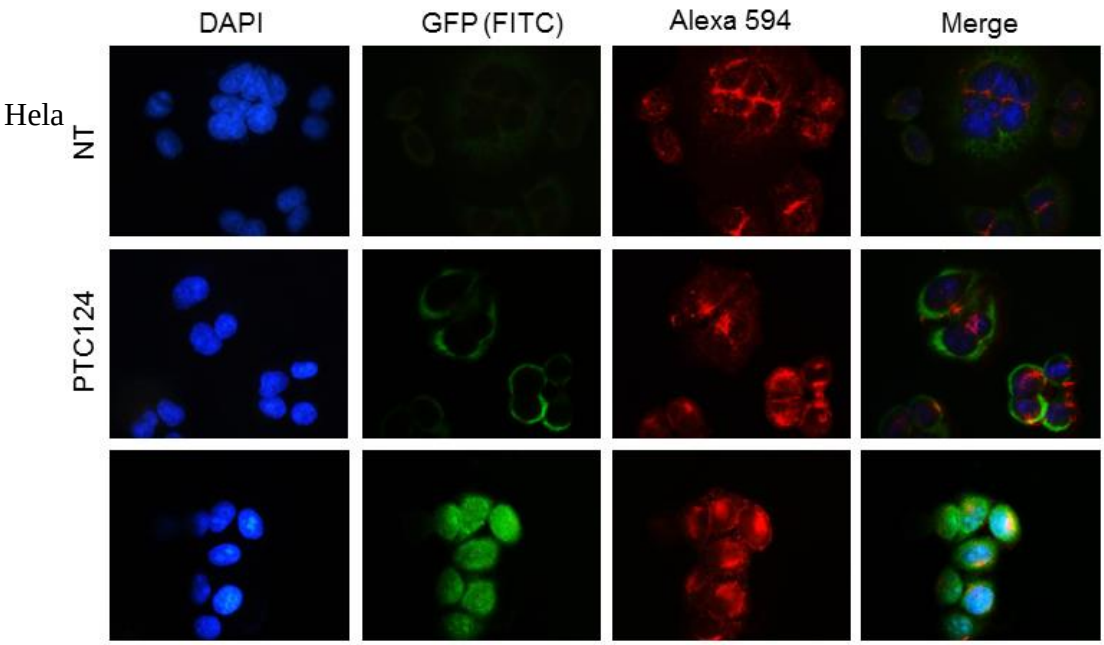

Figure 1. Immunofluorescence analysis of the H2B-GFP protein in H2B-GFP-*Amber* stably transfected HeLa cells after 24 hours exposition to PTC124 and N.1 derivative.

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P60 - JAHA, A NOVEL HISTONE DEACETYLASE INHIBITOR: CYTOTOXIC EFFECT ON TRIPLE-NEGATIVE BREAST CANCER CELLS

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Histone deacetylase inhibitors (HDACi) have emerged as effective anticancer agents in the clinical practice. Jay Amin hydroxamic acid (JAHA), is a metal-based analogue of the HDACi suberoylanilide hydroxamic acid SAHA [1] obtained by the formal replacement by a ferrocene bioisostere of the aryl *cap* in SAHA. In the present study, the effects of JAHA on MDA-MB231 cells, obtained from triple-negative human breast carcinoma, were evaluated. JAHA exhibits high cytotoxic activity on breast cancer cells, with an IC_{50} = 8.45 µM at 72 h. Following treatment with JAHA at this concentration, the viability of MDA-MB231 cells was analyzed using an MTT assay, and apoptosis onset, alteration of cell proliferative rate, intracellular reactive oxygen species (ROS) generation and mitochondrial membrane potential ($\Delta\Psi m$) alteration, if occurring, were evaluated by flow cytometry [2]. In addition, Western blot analysis was performed to examine the expression of autophagy-associated proteins. The results demonstrated that treatment with JAHA induced a non-apoptotic type of cell death, and an alteration of cell proliferation characterized by the accumulation of cells in the G_1 and subG_0 phases of the cycle. The most interesting results on JAHA mechanism of action regard its ability to induce early ROS production and subsequent dissipation of $\Delta\Psi m$ and autophagy inhibition, that were confirmed by the reversion of the cytotoxic effect obtained by co-treatment with either anti-oxidant (butylated hydroxytoluene) or autophagy-promoter compound (rapamycin). An in vitro "scratch assay" has also been performed to measure migration of cells treated for 24 h with 8.45 µM JAHA compared to control. Preliminary indications suggest that JAHA has no effect on the motile behaviour of MDA-MB231 breast cancer cells. In light of such results, it appears that JAHA may be a promising potential chemotherapeutic agent for triple-negative breast cancer.

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P61 - NEW MORE POLAR SYMMETRICAL CHOLINE KINASE INHIBITORS II: STUDY OF SETTING UP A NEW SCAFFOLD FOR THE CANCER THERAPY

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Research into the anti-tumour properties of biscationic compounds has received significant attention over the last few years. In the challenge to improve modern cancer chemotherapy, the search of new drugs with higher therapeutic index and lower capacity to induce resistance is an active field of investigation in medicinal chemistry. As part of our drug research program in searching modified biscationic compounds that show strong growth inhibitory activities against a two cancer cell lines (1-3), we were interested in more polar biscationic compounds derivatives, which should constitute an important class of new compounds for their potential pharmaceutical applications.
A novel family of 1,1'-[biphenyl-4,4'-diyldi(methylene)]dipyridinium salts containing a pair of pyridines as linker of the framework of the biscationic compounds, like hypothetical hydrogen bond acceptors with the enzyme choline kinase, were synthesized and they are being evaluated as inhibitors of choline kinase. Their antiproliferative activity will be evaluated in the future as well.

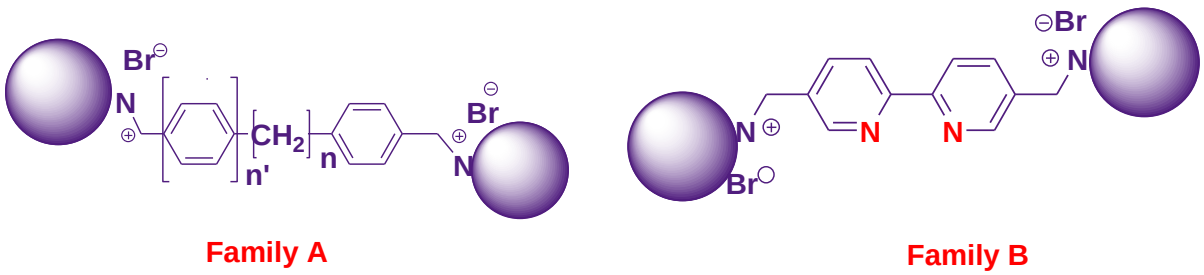

Fig. 1: General structures of symmetrical biscationic inhibitors of choline kinase Family A was previously published [1-3]. Family B are described in this work.

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P62 - TWO PROTON PUMPS ARE REQUIRED FOR REGULATION OF BICARBONATE SECRETION IN THE INTESTINE OF *SPARUS AURATA*

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Acid and base transport through cell membranes is essential for all cells in order to maintain acid-base balance. In some cells this transport is used for peculiar purposes. Two examples are the pancreatic duct cells and the enterocytes of marine teleosts. The former secrete a HCO_3^- -rich fluid necessary to increase the chime pH and to activate the intestinal enzymes. Marine teleost enterocytes actively secrete HCO_3^- in order to precipitate the large amounts of Ca^{2+}(and Mg^{2+}) introduced by swallowing seawater. Divalent cation precipitation has the role to reduce the osmotic pressure of the intestinal fluid, thus allowing water absorption, necessary to compensate water loss into the hyperosmotic external medium. In addition it inhibits Ca^{2+} over-absorption and may prevent renal stones formation (1; 2).
The mechanisms of base secretion and their regulation in seawater teleosts, including *Sparus aurata*, have been extensively studied (3;4;5;6). In this paper the involvement of proton pumps in bicarbonate secretion (BCS), already suggested in mammal pancreatic duct cells (7), was investigated in isolated tissues mounted in Ussing chambers, where the transepithelial electrical parameters (short circuit current, I_{sc}, and tissue resistance, R_t) were also measured.
We found that the apical addition of 10□M omeprazole, a specific H^+-K^+-ATPase inhibitor produced a significant decrease of BCS and a parallel increase in I_{sc} positivity in both the anterior intestine and the rectum, irrespective of the presence of serosal bicarbonate. Experiments performed with the aim of testing the reversibility of omeprazole effect on BCS led to an intriguing result: a large and transient increase of BCS following omeprazole removal from the apical solution. This response was strongly reduced in tissues pre-incubated with 100□M colchicine, a known inhibitor of microtubule polymerization.
Acetazolamide, a carbonic anhydrase inhibitor, which strongly inhibited BSC, was not able to modify I_{sc} in the anterior intestine but produced a small increase in I_{sc} positivity in the rectum.
Apical addition of the selective inhibitor of the V-type H^+-ATPase, Bafilomycin A1 (0.1□M), was not able to reduce BCS in the rectum while exhibited a significant inhibitory effect in the anterior intestine. The addition of omeprazole after Bafilomycin A1 produced a further BCS inhibition. In contrast Bafilomycin A1 was ineffective when tested in tissues in which omeprazole had produced its maximal inhibition of BCS.
The results of our experiments lead us to assume that two proton pumps are necessary for the regulation of bicarbonate secretion in the intestine of *Sparus aurata*.
Omeprazole experiments suggest that H^+-K^+-ATPase plays a role in BCS both in the anterior intestine and in the rectum, this role seems independent from the source of secreted bicarbonate (endogenously formed or extracellular). The possibility that omeprazole can inhibit carbonic anhydrase (8) and hence the intracellular bicarbonate generation, seems ruled out by the observation that acetazolamide, that inhibited BCS, produced effects on I_{sc} that are different from those produced by omeprazole both in the anterior intestine and in the rectum. However an effect of omeprazole on Cl^- channel (9) cannot be excluded.
The finding that the large and transient increase of BCS, observed when omeprazole was removed from the luminal solution, was reduced in tissues pre-incubated with colchicine could suggest that omeprazole removal stimulates the H^+-K^+-ATPase activity, due to an insertion of proton pumps into plasma membrane by a mechanism microtubule dependent, already demonstrated in the gastric parietal cells (10).

Bafilomycin experiments suggest that the V-type H^+-ATPase is also involved in the regulation of bicarbonate secretion in the anterior intestine but has a minor role in the rectum. However further studies are necessary to confirm our conclusions.
The sensitivity of intestinal BCS to omeprazole and bafilomycin points the functional involvement of two proton pumps in the simultaneous secretion of H^+ and HCO_3^- in the intestine that could be explained by two hypotheses:
1) the titration of luminal HCO_3^- with protons near the luminal surface of the enterocyte could reduce the net HCO_3^- gradient across the luminal membrane to sustain the action of a Cl^-/ HCO_3^- exchanger;
2) apical H^+ secretion could defend cytosolic pH in the apical region where carbonic anhydrase is abundant and catalyzes the hydration of CO_2 to form H^+ and HCO_3^-, necessary for Cl^-/ HCO_3^- exchange. Proton removal could prevent reversal of the hydration reaction (3).

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P63 - ECOLOGY AND ENERGY TRANSFER FROM PLANKTONIC ORGANISMS TO SMALL PELAGIC FISHES IN THE NW MEDITERRANEAN SEA

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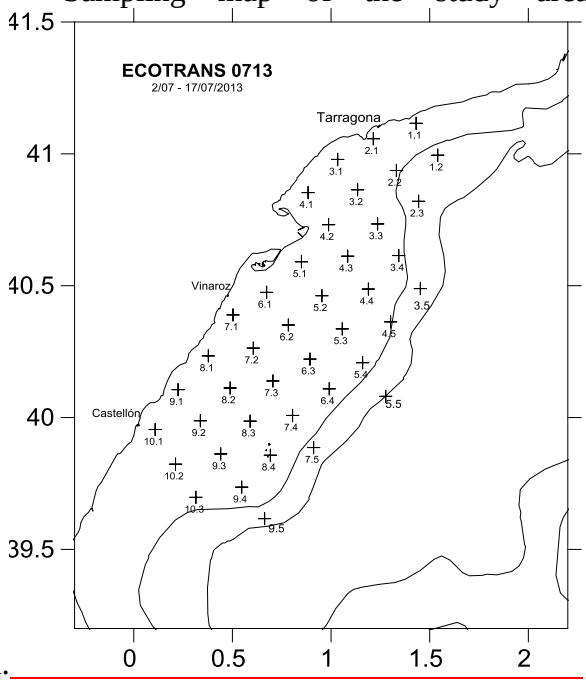
Ecosystem ecology is today one of the most important scientific tools that have direct applications for management and conservation of marine enviroment.
I had possibility to work as an Erasmus Placement student at Barcelona in the ICM – CSIC (Institute of Marine Sciences) in a project named ECOTRANS leadered by Dr. Isabel Palomera. The project will last for three years (2012-2014). It's a scientific project funded by the Spanish Ministry of Economy and Competitivness and the team of researchers which I was part of, are studying the trophic relations between different aspects of the pelagic ecosystem in the Western Mediterranean Sea, with a special focus on the dynamics and ecological role of small pelagic fish,in particular European anchovy (*Engraulis encrasicolus*), European sardine (*Sardina pilchardus*), and sardinella (*Sardinella aurita*) such as dominant and key species in terms of biomass and production, and their transfer of energy as trophic levels among the trophic chain .

The area of interest and study includes the continental shelf in front of the Ebro river delta from Tarragona northwards until Castellón de la Plana. The total study area is about 1800 squared miles (figure 1).

Fig.1 – Sampling map of the study area indicating the grid of planktonic station.



This project is articulated into two fundamental, parts, necessary to recollect all the informations and datas,the big one is the sampling by two oceanographic-fishing cruises, that will be conducted to cover the following objectives:1) Sampling micro and mesozooplankton; 2) Sampling larvae of selected pelagic and 3) Trawl sampling (pelagic and benthonic fish) to get

the species and functional groups biomass in the ecosystem that will allow to actualize the existing trophic models. I participate at the second cruise that was conducted during the spawning of anchovy as one of the objectives was to get their larvae, and also larva of gilt sardine, horse-mackerel and mackerel .

The plankton methods conducted at wich I mostly collaborate were the sampling with plankton nets aimed at obtaining the microplankton and mesozooplankton biomass, and the fish larvae .

We used various kind of nets everyone with a different technique of sampling , depending if we need to catch microplancton or mesozooplancton , like CALVET net and WP2 ;also Bongo net sampling for catching fish larvae in the same station and finally samples with RMT-1m network to capture larvae and crustaceans in depth. There will be also catches with PATIN neuston for capturing larger larvae, which are in the surface layers during the night. The plankton samples collected for the study of micro and mesozooplankton are being used to calculate the biomass (dry weight) of each group and another part for the corresponding biochemical analysis (calories, fat and stable isotopes) of the functional groups of plankton and the larvae. At same time the larvae samples recollected will be also conserved in cryovials at -80 ° C. The rest of each fish larvae will be conserved in 5% formalin for subsequent separation and determination of the size structure.

An other important part of this study was developed consequently at the laboratory to follow the protocols for plankton preparation and analysis divided into:

1) Calculate and obtain the data of biomass abundance and distribution of micro and mesoplankton.
2) Sorting functional group of zooplankton as Copepods, larvae of Decapods, Eufasiacea and Mysidiacea, Cladocers and Appendicularians that will be prepared for isotopic and calorimetric analysis.
3) Identification and sorting of eggs and larvae of sardine and anchovy for each station .

From all these data recollected we're attending a partial spatial-distribution on the area of study,like the use of SURFER software for plankton distribution analysis and know approximately the percentage of energy/biomass from intermediate trophic levels available to upper trophic levels.

P64 - INNOVAQUA "TECHNOLOGICAL INNOVATION FOR THE IMPROVEMENT OF PRODUCTIVITY AND COMPETITIVENESS OF SICILIAN AQUACULTURE": ASPECTS OF EXPERIMENTAL BIOLOGY

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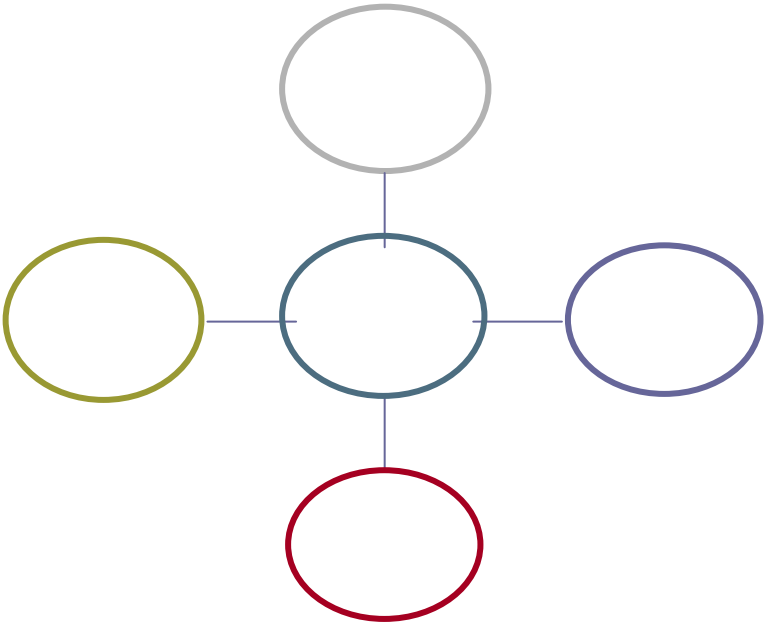
The INNOVAQUA project entitled "Technological Innovation for the improvement of productivity and competitiveness of Sicilian aquaculture" aims to stimulate the potential development of existing in Southern Italy aquaculture, through the definition of new technologies for the industry that require intensive researches and development in-house, and involving different stages of the production chain. The project holds in high regard the needs of health, safety and sustainability of consumers. The scientific partners of INNOVAQUA are: Acqua Azzurra SPA; University of Messina; University of Palermo; IAMC–CNR; IZS Sicily. The project has been founded by the Operative National Program Research and Competitiveness (PON R&C) to the Technological District "*Agro Bio Pesca Ecocompatibile*" of Sicily.

In order to maximize profitability and expansion in the increasingly competitive market, the project includes three specific work packages (WP 1-3):

- The activities planned in WP1 (Fish species diversification) aim to identify new fish species (umbra and amberjack) of commercial interest on which will be carried out tests of reproduction and larval rearing. A stock of breeding amberjack will be set up to develop suitable techniques for maintaining in captivity.
- The WP2 (Productivity increase of farmed fish) activities are designed to improve the productivity of seabass and seabream, representing almost the entire Mediterranean and European production. These activities represent an opportunity for industry development through important technical-scientific progress and commercial applications.
- The WP3 (Actions to support innovation and competitiveness) activities will focus on the improvement of fish farming conditions, through the use of active molecules extracted from marine algae. The following activities (A1, A2, A3 and A4) of Experimental Biology will be developed:

A1. The algal biomolecules are of considerable interest in different sectors characterized by a wide spectrum of antibiotic activity and immunostimulant, as pharmaceuticals. Such molecules can greatly differ among related species and also within the same species. For this reason, the taxonomic identification of the species is carried out by the DNA barcoding techniques. The results will yield a list of local species producing bioactive macromolecules and protocols for the extraction of phyto-derivates. Local species of macroalgae producing bioactive macromolecules

have been identified and obtained crude extracts will be characterized. Different methods of extraction on the basis of various active ingredients will also be tested.



A2. Diseases of bacterial origin are cause of considerable economic losses in aquaculture. The discovery of new bioactive molecules produced by marine organisms is still today a promising challenge in the field of biotechnology, mainly in pharmaceuticals. The increasing phenomenon of resistance of pathogenic bacteria of humans and animals to current antibiotics raises the need for discovering novel active biomolecules useful in human therapy, in veterinary medicine and in the aquaculture industry, and with not any side effects on human and environmental health. Since the compounds extracted from marine algae have already been reported to have antibacterial and immunostimulant activities, in this study we analyze the extracts of algae belonging to the genera most widely distributed along our coasts against bacteria of great significance for fish and human health. Preliminary results indicate that the studied algal extracts could be sources of novel antibacterial compounds with potential use in the prevention and treatment of diseases of farmed fish.

A3. In order to evaluate cytotoxicity of algal extracts the common cytotoxicity test will be performed: the trypan blue assay that measures cytotoxicity based on alterations in plasma membrane permeability and consequent dye uptake, normally excluded by viable cells and the hemolysis assay, a sensitive and accurate tool used as a guide to assess the safety and utility of a molecule or pharmaceutical preparations. If the algal extracts will be neither cytotoxic nor hemolytic they will be administered to fish and afterwards studies on fish hematological parameters and on the gastric and intestinal transepithelial parameters, by short circuit current, I_{sc}, a measure of transepithelial ion transport will be carried out.

A4. Algae of the genus *Gracilaria* have been studied to verify various types of cultivation (on nets, on ropes). Once tested the growth of seaweeds in natural environment, a protocol for culturing these algae in the wastewater of a system of intensive aquaculture is in course of development, thus verifying the ability of seaweed to reduce the pollution load of wastewater. Also, the extractability of phycocolloids and agar from *Gracilaria* will be evaluated. The goal is the selection of strains of *Gracilaria* able to provide products of industrial interest and with capacity of phytoremediation of breeding water.

The experimental activity described is preparatory to evaluate the efficacy *in vivo* in experimental aquaculture plant. In fact, the antimicrobials and immune-boosting obtained from selected algal cultures will be tested on teleosts to assess their potential use as therapeutic and prophylactic agents.

With the contribution of the project Innovaqua PON02_00451_3362185/1.

P65 - PRELIMINARY INVESTIGATIONS ON GRACILARIA GRACILIS CULTIVATION TECHNIQUES AND EXTRACTION OF BIOACTIVE COMPOUNDS WITH ANTIOXIDANT ACTIVITY

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It is know that macro algae can be a valuable source of food and industrial raw materials, such as natural polysaccharides. Furthermore seaweeds are the most promising sources of primary and secondary bioactive metabolites and represent about 9% of biomedical compounds obtained from the sea.
Antioxidants (carotenoids, polyphenols etc.) fatty acids, amino acids, polysaccharides etc., obtained from algae, are useful for many applications, such as nutrition, pharmacology and cosmetics.
These bioactive compounds belong to different classes of chemicals; thus, they can be obtained by several extraction techniques. The toxicity of the solvents used, the degradation of the compounds and the selectivity of the process are major constrains that must be considered in an industrial extraction process. As the bioactive industry is constantly seeking innovation, processes that can increase yield, the productivity, and selectivity are being considered. The Supercritical Fluid Extraction (SFE) is a technique, that allow to modify easily the characteristics of the extracted product, changing the process parameters such as temperature, pressure and co-solvent, ensuring very high yields (1).
Among cultivated algae either on small or large scale, species of the genus Gracilaria have been utilized in several countries, mainly India, Indonesia, Japan. In Italy there are only a few experimental evidences in brackish environments. The commercial interest for Gracilaria derives from the fact that the thallus of many species represent the raw material for the extraction of the agar (2). Agar is used as gelling agent in food and pharmaceutical industry and has an high commercial value.
In this study, we are approaching the experimental techniques of cultivation of Gracilaria gracilis, in the Capo Peloro lagoon (Eastern Sicily; 38°15’57” N; 15° 37’ 50” E), and the extraction of bioactive compounds from cultivated and wild specimens. Square frames (90 cm×90 cm) were used to provide the cultivation structure (3). Each raft held 8 parallel lines of polypropylene rope (3 mm) where vegetative fragments of thalli were inserted (Figure 1). The raft was positioned at about 50 cm from the surface.

Figure 1 – Square frame utilized for G. gracilis cultivation trial
Wild samples of Gracilaria were collected monthly from Margi Canal (the connecting channel between Ganzirri and Faro Lakes). During sampling main physical-chemical water parameters were monitored.
Samples of dry Gracilaria, wild and cultivated, were extracted by ethanol 96%, absolute methanol and supercritical CO2, SFE (4, 5).
Gracilaria extracts were utilized to determine: phenolic content by Folin-Ciocalteu reagent and expressed as gallic acid equivalents (GAE); DPPH radical scavenging assay to evaluate antioxidant activity of 1% extracts and reducing power, as potential antioxidant indicator (4).
Cultivation technique used allowed us to easy manipulate algae. During cultivation trial, although in the short term, it was possible to highlight the influence of environmental factors on the growth of algae. It was found a high mortality when the water temperature began to rise, while, regarding salinity, the algae has been shown to survive for up to salinity of 34‰ .

Table 1 - Antioxidant activities of G. gracilis extracts. EE (ethanol extract), ME (methanol extract), SE (supercritical fluid extract)

Extract	Total antioxidant power (% inib DPPH)		Reducing power	Total polyphenols (mg GAE)
EE	46	0.1	60	
ME	51	0.01	30	
SE	64	0.6	370	

The obtained results showed a significant effect of the method of preparation on the antioxidant power exhibited by the extracts (table 1).

The total antioxidant power has showed the higher value of radical inhibition (64%) for the SFE extract (SE), respect to the methanol (ME) (51%) and ethanol extract (EE) (46%) (table 1).

The total reducing power resulted also higher in the SE (0.5) respect to the ME (0.01) and EE (0.1) (table 1). The total polyphenols contents showed the significant higher value in the extract obtained by SFE (368 mg GAE), compared to ME (30 mg GAE) and EE (60 GAE).

The obtained results suggest that G. Gracilis can be easily cultivated and that it is a potential source of antioxidants; that phenolic compounds might be major contributors to the antioxidant activities and that SFE seems to be the most effective techniques in improve the yield of polyphenols.

Our preliminary results appear useful for further research aiming to isolate and identify the specific phenolic compounds responsible for the antioxidant activity of G. gracilis and underline the importance to select and standardize the extractions methods in order to gain the best yield and to adequately transfer the protocol from laboratory to the industry.

Keywords: Gracilaria; seaweed; biological activity; natural product; antioxidant; SFE.

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P66 - EFFECT OF STORAGE TIME ON HAEMATOLOGICAL PARAMETERS IN TWO DIFFERENT TELEOSTS, MULLET AD SEA BREAM

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Assessments of blood parameters in fish have thus far been performed manually, using a haemocytometer[1]. Unlike mammals, all fish cells are nucleated and this makes the calibration of and reading by automated systems difficult. Automated analysis should facilitate collection of the haematological parameters of other wild and farmed fish species although it may be necessary to validate the outcomes against those obtained from the established manual methods until the general applicability of the method is better understood. Previous studies on fish haematology revealed that the interpretation of blood parameters is quite difficult because variations in the blood are caused by both internal (species, sex, age) and external factors (temperature, water quality),[2] and that there are significant species differences in the stability of blood samples stored at room temperature or under refrigeration[3]. In view of this, the aim of this study was to evaluate the effect of storage time on two different fish blood and assess a good storage procedure of blood samples to obtain reliable results. Twenty *M. cephalus* and twenty *S. aurata* caught from Tyrrhenian Sea, from Messina Strait and from a sea cage of an aquaculture plant in Trappeto respectively, were used. All animals were considered healthy on the basis of an external examination for any signs of abnormalities or infestation. The fish were anaesthetized prior to blood sampling using 2-phenoxyethanol at the concentration of 400 mg/L and successively underwent venipuncture by the caudal vein using a 20 G x 1 ½ syringe. All blood samples, collected in Miniplast containing EDTA as the anticoagulant agent, were analyzed immediately (T0) by both manual and automatic methods and then divided into 4 different aliquots stored at 4°C for at 6, 24, 48 and 72 h from collection. To validate the reliability of the automatic method, a Paired t-test was applied between the values obtained by the manual and automated method at T0 for both species. The influence of sampling time and different species on the haematological parameters evaluated with the automatic method was assessed by two-way analysis of variance (ANOVA) for repeated measures. No statistical differences were observed between haematological parameters evaluated with manual and automatic method at T0 in both species (Table 1). These data show the reliability of the automatic method. ANOVA showed a statistically significant effect of specie on all haematological parameters and a significant effect of storage ($P<0.05$). Our results showed that baseline values of haematologic parameters differ in two species studied and that storage time affects in different way on blood cells. In sea bream a significant effect of storage time on all haematological parameters except haematocrit (Hct) was found, while in mullet haemoglobin (Hb), white blood cells (WBC), thrombocyte (TC), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) significantly change (Figure 1). In *S. aurata*, red blood cells (RBC) showed a significant decrease during the storage period whereas directly related parameters such as Hb and mean corpuscular volume (MCV) showed a significant increase. There is considerable evidence showing that the storage of blood affects RBC vitality, deformability, and geometry[4]. The significant rise in MCV during the storage period can be attributed to the increase in the volume of RBC due to swelling. Storage for long periods is related to degenerative changes which occur in RBC and leads to widening of the ‘‘pores’’ on the surface of the RBC. This is directly related to the significant increase found in Hb values and in MCH and MCHC. The significant decrease in the WBC count obtained both in mullet and sea bream could be attributed to cellular

degeneration as a consequence of the storage time, whereas the significant increase in the TC could be due to the presence of white cell nuclei that were erroneously added to the TC. In *M. cephalus* Hb increased after 24, 48 and 72h after collection, but RBC and MCV did not show statistically significant changes. Despite no significant change in RBC was showed, probably the long storage time involved a slight erythrocyte pain, which was reflected in the change of Hb. The results demonstrate that, even if in mullet erythrocytes resistance to storage time were higher than in sea bream, haematological parameters may be assessed within 6 h from collection when samples are stored at + 4°C because long-term storage modifies the results of the analyses. These results help veterinary practitioner in handling and storing blood samples of different fish species appropriately even if there is still a need for further studies involving other fish species, various storage time and temperature.

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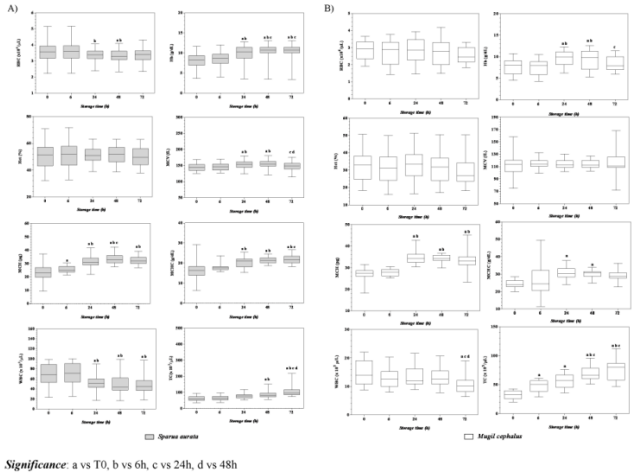
Table 1. Mean ± SEM values of haematological parameters obtained by two different methods of analysis (manual and automatic) immediately after blood collection (T0) in two species studied

Parameters	***Mugil cephalus***		***Sparus aurata***	
	Manual	**Automatic**	**Manual**	**Automatic**
RBC	3.00 ± 0.23	2.83 ± 0.14	3.40 ± 0.06	3.50 ± 0.08
Hb	7.82 ± 0.52	7.68 ± 0.48	8.10 ± 0.19	8.00 ± 0.23
Hct	32.51 ± 2.54	31.20 ± 1.64	50.80 ± 1.00	50.35 ± 1.18
MCV	109.6 ± 3.80	113.3 ± 5.00	146.10 ± 2.00	144.40 ± 1.63
MCH	26.65 ± 0.86	27.00 ±0.72	23.30 ± 0.75	23.42 ± 0.82
MCHC	24.45 ± 0.60	24.16 ± 0.63	16.00 ± 0.52	16.28 ± 0.58
WBC	15.06 ± 1.84	14.74 ±1.08	67.70 ± 2.10	68.08 ± 2.95
TC	32.94 ± 1.75	32.00 ± 1.81	62.00±2.60	60.91 ± 1.94

Figure1. Box and Whiskers plot graph of haematological parameters analyzed with the automatic method and recorded during the different time of storage in sea bream (A) and mullet (B).



Significance: a vs T0, b vs 6h, c vs 24h, d vs 48h

P67 - BRIEF MATERNAL SEPARATION PROCEDURES OCCURRING EARLY IN LIFE AFFECT LEARNING AND MEMORY IN ADULT WISTAR RATS: SEX-RELATED DIFFERENCES IN COGNITIVE BEHAVIOUR

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Adverse life events during the neonatal period result in long-term effects on physiology and behavior [1]. Early postnatal experiences, such as a modification of the mother–infant interaction, may influence the development of neural systems that underlie the expression of neuroendocrine and behavioural responses to environmental challenges, involving changes in the hypothalamic-pituitary-adrenal (HPA) axis [2] together with decreased levels of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) [3]. Disturbances in mother-infant interaction represent a natural stressor which may lead to maladaptive development [4]; indeed protracted Maternal Separation (MS) reduces maternal care thus induce in abnormal HPA axis responses, hippocampal BDNF down-regulation [5] impaired memory formation [6]. The opposite is observed when maternal care is increased by a daily brief, maternal separation. The polymorphism of neuroendocrine processes and behavioural responses following brief MS procedure 7includes gender-related differences; however very few studies exist on sex-specific behaviours [7]. Based upon these findings, the present study was carried out to investigate the consequences of a brief, maternal separation on declarative and spatial memory, focusing on sex related alteration due to the discrete effects that hormones may play on the brain circuits.

Materials and methods
24 adult male and female Wistar rats were divided in the following experimental groups: maternally separated males (MS-m) and females (MS-f), and non-separated males (NS-m) and females (NS-f). Maternal manipulation involved a 15- minutes daily separation of litters from the mothers from postnatal day 2 (PND 2) to 21 (PND 21). Non separated rats were left undisturbed in their home cages until weaning. To assess the influence of maternal separation on cognitive function we used respectively: the Object Recognition (OR) Test for declarative memory and the Morris Water Maze (MWM) for spatial learning and reference memory, performing respectively place learning and probe sessions.

Results
Our results indicate that a brief, daily maternal separation was able to induce in MS-f group an increase in the time spent exploring the novel object in the OR in both after 1h ($p<0,001$), and 24h ($p <0,05$) retention intervals compared to NS-f controls, while no significant differences were observed in MS-m group when compared to respective controls.
In the MWM, during the Place learning paradigm (day 1) MS-m rats showed a reduction in escape latency ($p<0.001$) and, during the probe phase, an increase in time spent in the target quadrant after platform removal ($p<0.05$), compared to NS-m group. On the contrary, MS-f group showed non-significant differences in escape latency in the place learning compared to NS-f controls. MS-f rats spent more time ($p <0,05$) in the target quadrant in probe phase, compared to respective controls.

Conclusions
The present study was designed to examine the effects of brief daily mother–offspring separation on learning and memory performance, focusing on sex-related differences in declarative and

spatial memory. In detail, our study showed that exposure to a brief, maternal separation results in sexually-dimorphic cognitive alterations that depend on the nature of the behavioral task: Iindeed we found that MS-f group outperformed MS-m group on Object Recognition Test, a working, non-spatial memory dependent task that utilizes both cortical and hippocampal input [8]. This result could be due to a potential improvement of the perirhinal cortex and dorsal hippocampus activity in females when compared to MS-m group and to controls [9]. In addition, in the Morris Water Maze, maternal separation affected learning in adult male and female rats in a task-specific manner. In particular, it was observed that this procedure enhanced the ability of processing spatial information only in male rats during the first day of place learning whereas, both groups showed improved performances during probe, confirming the discrete effects of maternal separation on mechanisms of memory storage. In conclusion, a brief, maternal separation affects learning and memory in adult rats not only in a sex-related, manner, but also in a task- specific way[10]. This study provides us with different outcomes useful to clarify how early life events can influence the behavioural adaptive mechanisms in adulthood in a sex specific manner probably due to differences in the modulation of hippocampal function and HPA axis response [11,12] highliting, overall, the main role played by a high mother-infant relationship in the correct development of physiology and behaviour in adulthood.

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P68 - EVALUATION OF ANTIFOULING ACTIVITY OF TIO2 AND AG DOPED TIO2 IN LABORATORY CONDITIONS TO BE APPLIED ON SUBMERGED ARCHEOLOGICAL MATERIAL

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The term of biofouling or biological fouling means the accumulation of microorganisms, plants, algae, or animals on wetted surfaces with consequent loss of the intrinsic characteristic of the material.
Biofouling occurs worldwide in various submerged substrates of industrial interest as well as on materials that have an archeological and historical importance such as wood, metals and stones [1]. The process begins with the formation of a biofilm produced by bacteria or other microorganisms [2] and once it starts archeological materials are subjected to an increase of deterioration due to the grazing action of epilithic and endolithic marine micro- and macroorganisms.
To avoid and/or to stop either micro or macro-biofouling processes, in marine environment conventional procedures include anti-fouling paints to prevent the attachment of sealife such as algae and molluscs [3]. However, these compounds slowly "leach" into the sea water, killing sealife, harming the environment and possibly entering in the food chain and in any case they cannot be directly applied on submerged archeological materials [4].
Hence, new products that are a) antifouling agents, b) harmless to the valuable materials and c) eco-friendly, are welcomed [3, 5].
In the last ten years, Titanium oxides are proven to have antibacterial activity, if exposed to certain light wave lengths (photocatalytic activation). In particular, TiO2 is activated at UV wavelength, while Ag-TiO2 is photoactivated by visible spectrum and thus being effective also to a certain depth [5].
The aim of this study was to compare the antimicrobial activity of Titanium oxide (TiO2) and a Titanium oxide doped with Ag (Ag-TiO2) as described by Ruffolo et al [5] against pure cultures of Gram positive and Gram negative bacteria as well as to evaluate the ability to interfere with the adhesion of bacteria on submerged treated marble slabs simulating a marine environment in laboratory conditions.
For this purpose we carried out different sets of experiment in agarized medium and in UP distilled water to evaluate the antibacterial activity of the titanium oxides suspensions and the preventive effect against microbial adhesion on submerged material after 24-72 hrs.
Laboratory experiments carried out on UP distilled water suspension showed that Ag-TiO2 activity was slightly higher than TiO2 (tested at concentration of 0,1% and 0,01%), perhaps due to the intrinsic antibacterial activity of the Ag alone. On marble slabs, both treatment with TiO2 and AgTiO2 were efficient to prevent the microbial colonization of the surfaces as shown by the SEM analysis.
Untreated marble showed a diffuse presence of Extracellular Polymeric Substances (EPS) while on marbles slabs treated with TiO2 or TiO2-Ag or Ag no EPS production and no microbial colonization was evidenced.

In conclusion, our preliminary results demonstrate that Titanium oxides could be successfully applied on the surface of submerged archeological items to prevent the initial microbial biofouling.

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P69 - 3D SPHEROID CULTURE FROM EQUINE AMNION MESENCHYMAL STEM CELLS (EAMSCs)

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Introduction - Currently, there is an increasing interest to investigate the presence of MSCs in extraembryonic tissues (1) due to easy collection after birth of the foal. Among fetal adnexa the human amnion expresses stem cells that show embryonic surface markers and are negative both for MHC I and MHC II (2). These cell populations display a fibroblast-like appearance, adhere to plastic culture dishes, form clonal colonies and, under appropriate culture conditions, they can differentiate into the chondrogenic, osteogenic and adipogenic lineages. We have already reported a protocol to expand equine fetal adnexa-derived MSCs collected by a non-invasive technique and cultured in EGF-supplemented media (3-4), in traditional two-dimensional (2D) culture. The use of three-dimensional (3D) culture recently gained some interest since the plastic surface is known to limit the potential of stem cells to recreate the in vivo conditions (5). The aim of this study was to perform a reproducible method for the isolation of 3D spheroid cultures of EAMSCs grown into the chondrogenic and osteogenic media, to obtain a source of cells with potential clinical use in the equine regenerative medicine field.

Materials and Methods - Amnion cells were obtained from 4 ten-to-thirteen years old standardbred mares. For the study of cellular stemness, immunocytochemistry on amnion sections with anti-C-Kit, -CD105 and -Oct-4 antibodies, involved self-renewal of embryonic stem cells, was performed as already reported (3). Flow cytometer analyses were also performed. For 2D culture, nucleated cells were isolated, centrifuged (500 rpm for 10'), suspended in α-MEM and supplemented as previously reported (3). Cells were cultured at 10^5 cells/cm^2 and the adherent ones were grown for about 14 days, until 90% of confluence (passage 0) was reached. For 3D culture $5X10^5$ cells were dissociated with 0.25% trypsin in 1mM EDTA, centrifuged at 1000 rpm for 5'and the pellet was left in 15ml tubes in an incubator with the chondrogenic and osteogenic differentiation media for 20 days. The pellets were thus collected, washed in PBS, fixed in formalin 10%, embedded in paraffin and sectioned at 4µm for the morphological study (Alizarin Red staining and Alcian blue and Alizarin Red double staining).

Results - Mesenchymal stem cells, isolated from equine amnion, were able to achieve chondrogenic and osteogenic differentiation in 2D cultures. Flow cytometer analyses of EAMSCs confirmed a mesenchymal stem cells phenotype (reported elsewhere).
EAMSCs cultured in 3D resulted in the formation of spheroidal structures (spheroids) that were 500-600 µm in diameter and gradually merged into a single one (1300-1800 µm in diameter) (Fig. 2a). They showed matrix glycosaminoglycans when grown into the chondrogenic differentiation medium and a network of apoptotic or necrotic cells in a not mineralized matrix (Fig. 2b). Mineralization of bone matrix and formation of mineralized nodules was observed from day 20 when cultured in the osteogenic medium. These latter were generally formed by confluent masses with an Alizarin reactivity at their border that showed a layer of fibroblastoid tightly packed cells, more elongated and flattened than cells located in the center (Fig. 2c).

Where the matrix was mineralized, it was possible to evidence a core of calcium precipitation (Fig. 2d).

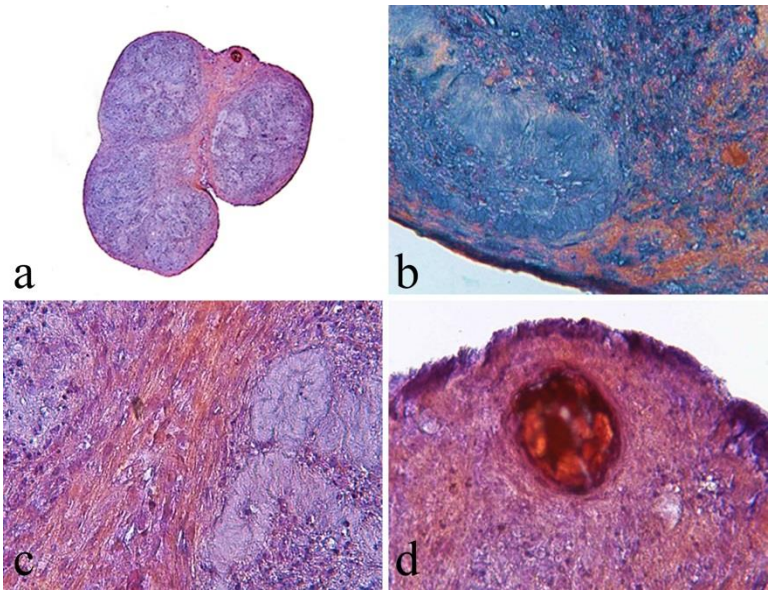

Fig. 2: EAMSCs differentiation in 3D culture: spheroid sections stained with Alizarin Red (a) (2X), Alcian blue and Alizarin Red (b) (25X); Alizarin Red (c) (40X), a core of calcium precipitation (d) (25X)

Discussion - When grown in specific culture media, EAMSCs differentiated into chondrogenic and osteogenic lineages. This study shows a technique for sampling, isolation and expansion of EAMSCs for 3D culture. Such scaffold-free spheroids are thought to better resemble the tissue microenvironment because of their cell to cell interaction and behavior (6). The observed masses forming spheroids, showed two cells populations that can be discriminated on the basis of their morphology; spindle shaped cells may be more differentiated while round cells located in the center may keep the features of a stem population. Further analyses are ongoing to better characterize EAMSCs spheroids and to obtain scaffold-free bone substitutes.

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P70 - AN AUTOLOGOUS PLATELET-RICH PLASMA MEMBRANE TO IMPROVE PERIPHERAL NERVE REGENERATION IN A RAT SCIATIC MODEL

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INTRODUCTION: Platelet-Rich Plasma (PRP) is defined as an "autologous concentration of platelets in a small volume of plasma" and has been shown to positively influence the healing of lesions affecting various tissues [1,2] because of the presence of several growth factors that play different roles in tissue regeneration. [3,4]. Activated platelets are involved in tissue healing/regeneration and promote cell proliferation, migration and differentiation [5,6]. The scarce regenerative capacity of the nervous tissue led the investigators to widely test the effectiveness of PRP on the regeneration of peripheral nerve fibers, particularly after re-anastomosis [7,8]. Our study rely on a commercially available medical device (Regen Extracell Membrane, RegenLab, CH) which is able to modify the normal consistency of PRP giving rise to a suturable membrane to be applied to the neurorrhaphy. This particular form of PRP might perform an action not only as a source of bioactive proteins but also as a nerve guide to hold the scar reaction and thus improve axonal regeneration.

METHODS: Sixteen female Wistar rats were utilized in the study; the suturable autologous PRP membrane was obtained from each animal using the Regen Extracell Membrane kit (RegenLab, CH). Fourteen animals underwent surgery and two did not sustain nerve injury (control). After the induction of anesthesia, the left and right sciatic nerves were cross-sectioned about 1cm from their distal bifurcation and reanastomized. PRP membrane was sutured around the left sciatic nerve neurorrhaphy whereas the right sciatic nerve was not treated. *Sciatic nerve sampling*: 6 weeks after surgery, animals were euthanized, the sciatic nerves were reproached and the previous neural sutures were located. A segment of 2cm, with the surgical site at the center, was excised and processed for histological examination. A segment of same length and localization was obtained from control animals. *Histology and morphometry:* serial cross sections (5 µm) of sciatic nerves distally to the neurorraphy were obtained and 5 random images for each nerve were acquired under a light microscope and analyzed. Myelinated nerve fiber diameter, myelin thickness and fiber density were obtained by analyzing not overlapping areas to cover 10-15% of the nerve section, normalized to the total nerve area, at a final magnification of 400X. The same procedure was followed for the control animals. Fiber density and myelinated fiber thickness are presented as mean and SD. Differences were considered significant if $p < 0.01$. Number of myelinated fibers were also described on the basis of their diameter in classes differing for 1 µm.

RESULTS: After surgery, all rats remained healthy throughout the study with complete recovery of motility two/three days after surgery. *Sciatic nerve sampling*: all nerves were in continuity, the sutures were easily identified and showed a slight increase of their diameter at the level of neurorraphy. *Histology and morphometry*: light microscopy observation of sciatic nerve sections from control animals showed fibers of circular shape uniformly distributed, organized in regular fascicules fiber. Their diameter ranged from 1 to 11µm and more than 40% of fibers showed a diameter greater than 6 µm. Six weeks post neurorraphy the histological sections of the nerve

sutured with or without PRP still showed an immature morphology, characterized by small diameter fibers when compared to control section. Such fibers were surrounded by abundant connective tissue (not arranged in regular fascicules) in which increased cellularity can be demonstrated and a large number of axons with Wallerian degeneration and abundant neovascularization with numerous small caliber blood vessels were present. The fiber density was slightly lower than the control; a statistically significant ($p<0.005$) difference was observed between treated and untreated animals with an higher observed density in treated nerves. No differences were observed in myelin thickness ($p=0.11$). The myelinated fibers diameter was always lower than 5µm and the most represented value ranged between 2-3 µm (~45%). The nerve fibers of diameter greater than 3µm were more numerous in the treated nerves sutured with PRP.

DISCUSSION: When complete severance of a peripheral nerve trunk does occur (neurotmesis) spontaneous recovery does not take place and a surgical approach is thus needed A surgical repair associated with the use of bioactive factors regulating the connective proliferation can perform axoplasmic migration into the distal stump [8]. In the literature many works demonstrated the effect of a gel of PRP in severe nerve injury for the bioactive proteins found in platelets, plasma and white blood cells [7,8]. The commercial medical device Regen Extracell Membrane was easy to use and consistently gave rise to a suturable membrane that was applied around the anastomosed nerve stumps; animals did not experience clinical and gross pathology changes associated to its use. While differences were not observed for the myelin thickness at different time-points, we found a statistically significant difference on fiber density at 6 weeks post-surgery. This latter finding may be due an "acute" effect of the sutured membrane, PRP use has indeed been reported to accelerate wound healing in several conditions particularly through the effects of growth factors on the inflammatory phase of tissue repair [9]. The fiber density was statistically different between treated and untreated animal with an higher density observed in treated nerves. No difference in the distribution of fiber diameters was observed. Our data demonstrate that the application of a PRP suturable membrane around the neurorraphy improves the nerve regeneration process in a rat sciatic nerve model not only as a source of bioactive proteins but also as a nerve guide to improve axonal regeneration.

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P71 - PCL SCAFFOLD IN A RABBIT CSD MODEL: HISTOLOGICAL OBSERVATIONS

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INTRODUCTION: Repair of "critical size defects"(CSD) (1) remains a serious problem as the associated loss of function considerably impairs the quality of life of the affected patient (2). Bone grafts remain an important part of orthopaedic surgeon's armamentarium to treat tissue loss related to disease or trauma (3). Although autologous bone graft still represent the gold standard for CSD treatment, particularly for its properties of osteoinduction, osteogenesis, osteoconduction, histocompatibility and absence of immunogenicity, it presents undeniable drawbacks such as an elevated postoperative morbidity of donor site, thus requiring additional surgical procedure, with well-documented complication and discomfort for the patient (4). Allogeneic bone graft, obtained from cadavers or living donors, is considered an alternative but for its limited regenerative capacity, risk of immunogenicity and rejection reactions, failure of vascularization, possibility of infection transmission or high cost of processing, its use is considered sub-optimal (5). Bone graft substitutes, consisting of scaffolds of either synthetic or natural biomaterials, were developed: their ideal features are considered to be osteoconductivity, osteoinductivity, biocompatibility, biodegradability, and a structure similar to bone (6).
AIM: Here we report the effect of three-arm star branched poly(ε-caprolactone) (*PCL) developed as an anatomically-shaped scaffold (by the computer-aided wet-spinning technique (7, 8) when implanted in a rabbit CSD model.
METHODS: Eighteen healthy 4-month-old male New Zealand White rabbits, weighing between 2.0 and 3.0 kg, were included in the study under a protocol approved by the local ethic committee of Pisa University. Under general anaesthesia, a segmental defect of 20 mm (CSD) of the radial diaphysis with periosteum of the right forelimb was removed and substituted with *PCL scaffold. After surgery digital X-ray of treated forelimb was performed to evaluate length of osteotomy, and radiopacity of each scaffold. Bone regeneration was evaluated using a modified system to score defect bridging and bone formation on X-rays (9, 10). At 4, 8 and 12 weeks after surgery, specimens of 40 mm were collected (scaffold, 10 mm proximal and distal radius bone and ulna) and processed for histological evaluation. Haematoxilyn-Eosin, Mallory thricrome, Toluidine blue and Congo red stained sections of samples selected on the basis of their radiological score were examined.
RESULTS: The X-rays performed immediately after surgery confirmed that the defects were indeed CSD (20mm ± 0,89). The epiphyseal plates at this time point were not completely closed. Mean assigned scores are reported in the following table:

Time after surgery	Mean assigned score	Standard deviation
4 weeks	7.75	3.8
8 weeks	9.33	2.71
12 weeks	7.66	4.72

Qualitative histological examination generally confirmed radiological observations. Sections obtained from samples with an high radiological score showed the presence of new-formed bone tissue invading the scaffold; some of them also showed the presence of a medullary canal occupied by fat cells and mononucleated elements resembling bone marrow cells (Figure 1a).

The scaffold was never completely invaded or replaced by bone tissue that was always projecting from the radial margin of ulna (Figure 1b). In sections obtained from samples with a medium radiological score the new-formed bone tissue was present as a bridge projecting from ulna to the junctions between radius and the scaffold (Figure 1c). Ulnar periostium was always the main osteogenic source both in high and medium score samples (Figure 1d). Sections obtained from samples with a low radiological score did not show new-formed bone tissue (Figure 1e); connective/granulation tissue was present among the scaffold fibers together with some areas of mononucleated inflammatory infiltrate (Figure 1f).

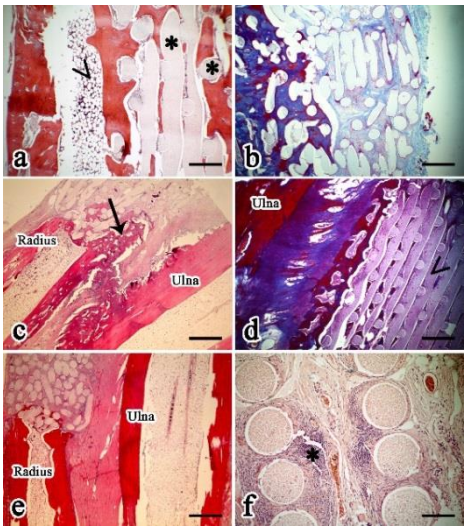

Figure 1: representative photomicrographs of high-score (a, b) medium score (c,d) and low score (e, f) samples. a) Asterisks indicate the implanted scaffols while the arrowhead indicates the fat cells populating the new-formed medullary canal; scale bar = 500 μm. b) The bone tissue (left) is growing into the implanted scaffold; scale bar 800 μm. c) The arrow indicates the new-formed bone as a bridge projecting from ulna to the junctions between radius and the scaffold; scale bar = 2 mm. d) The ulnar periosteum is the main source of new-formed bone invading the scaffold (arrowhead); scale bar = 800 μm. e) The scaffold (up-left) is not invaded by new-formed bone; scale bar = 2 mm. f) The asterisk indicates the presence of mononucleated inflammatory cells; scale bar = 200 μm.

DISCUSSION: *PCL guides tested in this study seem to be biocompatible: signs of rejection were never detected. The presence of a new-formed bone witness osteo-conductivity of the scaffold. Histological data showed that osteogenesis mainly arise from the periostium of ulna, initially reacting to form a bridge toward the scaffold. Further studies should investigate if modifications in the composition of the scaffold may increase its osteoconductivity and osteoinductivity.

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P72 - OPN IN ABERRANT WOUND HEALING OF HUMANS AND HORSES: AN IMMUNOHISTOCHEMICAL STUDY

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INTRODUCTION: Osteopontin is a matricellular protein involved in both physiological and pathological processes. It is a secreted integrin-binding glycophosphoprotein, involved in inflammation, wound healing, bone formation and remodelling, as well as atherosclerosis and cancer. Three different splice variants are reported in humans (1). With regard to wound healing, one of the main functions of OPN is reported to be recruitment, regulation, and differentiation of fibroblasts and myofibroblasts (2). In a mouse model of dermal bleomycin-induced fibrosis, OPN-deficient mice develop less dermal fibrosis, coupled with reduced TGF-β levels, compared to wild type mice (3). Also, decreasing OPN protein levels in mouse skin wounds accelerated healing and reduced granulation tissue formation and scarring (4). Horses suffer traumatic wounds, in which healing is often fraught with complications leading to a fibroproliferative disorder known as Exuberant Granulation Tissue (EGT). Both similarities and differences between equine EGT and human keloids are reported (5).

AIM: Here we investigated the presence of OPN in experimental wounds and naturally occurring EGT of horses, the latter compared with clinical samples of human keloids.

METHODS: cDNA from samples taken from experimental wounds of the body and limb at selected times during healing was used for PCR (housekeeping gene: GAPDH) Archival FFPE samples were used for histology. Haematoxylin&Eosin as well as Mallory's trichrome stainings were performed according to routine methods. Immunoreactivity against OPN was visualized by Diaminobenzidine according to the peroxidase method. Antigen retrieval was performed in Tris-EDTA pH 9 in a pressure cooker for 5 minutes. A commercial mouse monoclonal anti-OPN antibody was employed at 1:50 dilution (Santa Cruz Biotechnology - sc-21742). Equine kidney was used as positive control.

RESULTS: Expected splice variants were not detected in normal skin and in the inflammatory phase of equine wound healing. Clinical samples of equine EGT (Figure 1) stained for OPN showed a strong presence of the protein in the hyperplastic peripheral epidermis. Infiltrating neutrophils as well as endothelial cells and fibroblasts were immunoreactive to the OPN antibody. The slight brown staining of infiltrating mononucleated cells resembling macrophages in H&E section and the primary antibody omission leads to the hypothesis that the strong staining visible with the OPN antibody is not completely due to the protein presence.
OPN expression in clinical specimens of human keloids resembled that of clinical equine EGT (Figure 2).

DISCUSSION: Our preliminary data show an overexpresion of OPN protein in clinical samples of equine EGT and human keloids detected by immunohistochemistry. PCR data were produced to investigate the presence of splice variants: the presence of only one splice variant is reported in other species. Further analyses will be needed to understand equine OPN post-transcriptional regulation.

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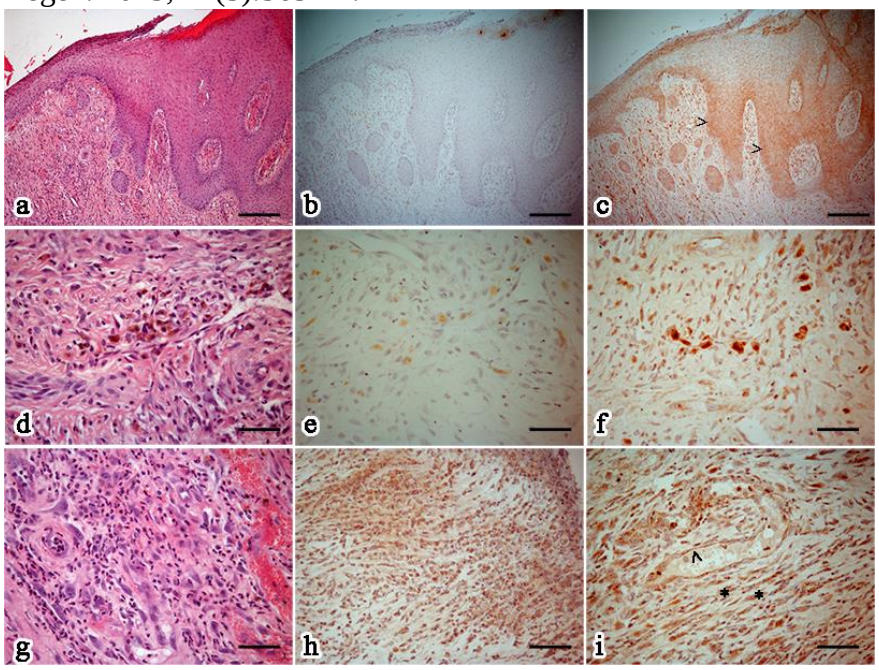

Figure 1: a) H&E stained equine EGT focusing on the epidermal compartment; b) primary antibody omission; c) OPN immunostaining showing strong epidermal reactivity at the periphery of the lesion (arrowheads); d) H&E stained equine EGT focusing on the dermal compartment; e) primary antibody omission: the stained mononuclear cells might represent macrophages with either melanin or ferritin phagosomes; f) OPN immunohistochemistry with strong staining of mononuclear cells analogous to (e); g) H&E stained equine EGT focusing on the acute inflammatory infiltrate (neutrophils); h) OPN immunoreactivity of neutrophils; i) OPN immunoreactivity of fibroblasts (asterisk) and endothelium (arrowhead). Scale bars: a-c = 200 mm; d-i = 50 mm.

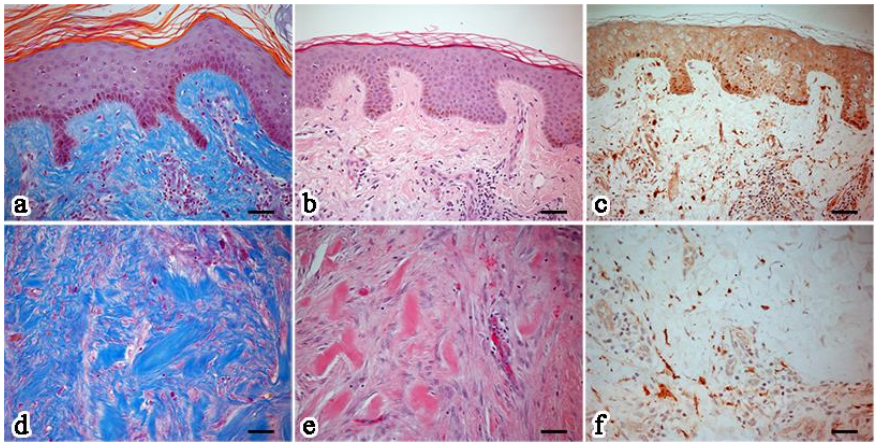

Figure 2: a) Mallory stained human keloid focusing on the epidermal compartment; b) same as (a), H&E stained; c) OPN immunostaining showing epidermal reactivity; basal staining mostly due to melanin presence; d) Mallory stained human keloid focusing on the dermal compartment; e) same as (d), H&E stained; f) Macrophages and scattered fibroblasts are immunoreactive to OPN. Scale bars: a-e= 50 mm; f = 80 mm.

P73 - EFFECT OF THIORIDAZINE ON ERYTHROCYTES

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Thioridazine, a phenothiazine drug with antipsychotic efficacy [1, 2] and anti-microbial activity [2], is particularly useful for the treatment of multidrug resistant tuberculosis [3, 4]. This drug is known to trigger anemia [5]. At least in theory, the anemia could result from stimulation of suicidal erythrocyte death or eryptosis [6], which is characterized by cell shrinkage, phospholipid scrambling of the cell membrane with phosphatidylserine exposure at the erythrocyte surface, increase of cytosolic Ca^{2+}-activity ($[Ca^{2+}]_i$) and activation of p38 kinase, a kinase expressed in human erythrocytes and activated by hyperosmotic shock, a known trigger of eryptosis [7].
The present study explored whether thioridazine triggers eryptosis. The concentrations required to trigger eryptosis are within the range of concentrations (6 μg/ml ≈ 15 μM) encountered *in vivo* [8]. Since different erythrocyte specimens used in distinct experiments are differently susceptible to triggers of eryptosis, the same erythrocyte specimens have been used for control and experimental conditions.
A 48 hours exposure to thioridazine was followed by a significant:
- increase of $[Ca^{2+}]_i$ (30 μM), estimated from Fluo3-fluorescence in flow cytometry (Fluo-3/AM);
- decrease cell volume, estimated from forward scatter (30 μM) in flow cytometry;
- increase of the percentage of phosphatidylserine exposure, estimated from annexin-V-binding (≥ 12 μM) in flow cytometry (Annexin-V-FITC, 1:200 dilution);
- increase of the percentage of hemolysed erytrocytes, estimated from the hemoglobin concentration in the supernatant This in turn was determined photometrically at 405 nm. The absorption of the supernatant of erythrocytes lysed in distilled water was defined as 100% hemolysis. As illustrated in Fig. A, thioridazine treatment significantly increased the hemoglobin concentration in the supernatant. The percentage of hemolytic erythrocytes was, however, clearly smaller than the percentage of annexin V binding erythrocytes. Both, cell membrane scrambling and cell shrinkage could have resulted from increase of cytosolic Ca^{2+} activity ($[Ca^{2+}]_i$). However, even in the absence of extracellular Ca^{2+}, thioridazine still significantly increased the percentage of annexin-V-binding erythrocytes pointing to additional mechanisms involved. In order to explore whether the additional mechanisms could include p38 kinase, erythrocytes were exposed in further experiments to 6-30 μM thioridazine for 48 hours in either the presence or absence of the p38 kinase inhibitor SB203580 (2 μM) (Fig. B).
In conclusion, thioridazine stimulates eryptosis and is partially effective by activation of p38 kinase and by increase of cytocolic Ca^{2+} activity. Nominal absence of extracellular Ca^{2+} and p38 kinase inhibitor SB203580 significantly blunted but did not abolish annexin-V-binding following thioridazine exposure.

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Fig.A

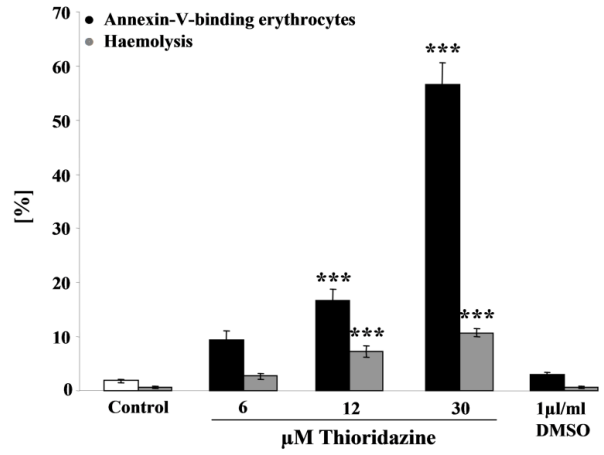

Fig.B

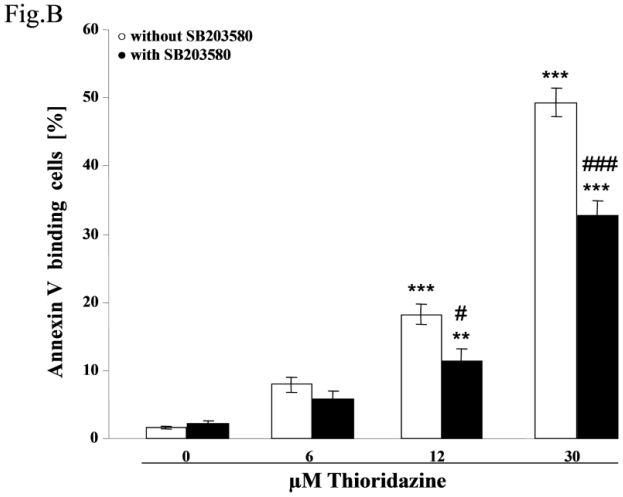

Fig. A: *Effect of thioridazine on phosphatidylserine exposure and hemolysis*
Arithmetic means ± SEM (n = 6) of erythrocytes annexin-V-binding following incubation for 48 hours to Ringer solution without (white bar) or with (black bars) presence of thioridazine (6-30 µM). For comparison, arithmetic means ± SEM (n = 5) of the percentage of hemolysis is shown as grey bars. *** ($p<0.001$) indicate significant differences from the absence of thioridazine (ANOVA).

Fig. B: *Effect of thioridazine on phosphatidylserine exposure in the presence or absence of p38 kinase inhibitor SB203580*
Arithmetic means ± SEM (n = 6) of erythrocytes annexin-V-binding following incubation for 48 hours to Ringer solution without or with presence of thioridazine (6-30 µM) in the absence (white bars) or presence (black bars) of 2 µM SB203580. ** ($p<0.01$),*** ($p<0.001$) indicate significant differences from the absence of thioridazine (ANOVA), # ($p<0.05$), ### ($p<0.001$) indicate significant differences from the absence of SB203580 (ANOVA).

P74 - UTILIZZO DELLA MICROSCOPIA ELETTRONICA A SCANSIONE PER L'ANALISI MORFOLOGICA E ULTRASTRUTTURALE DI CARIOSSIDI DI FRUMENTO DURO

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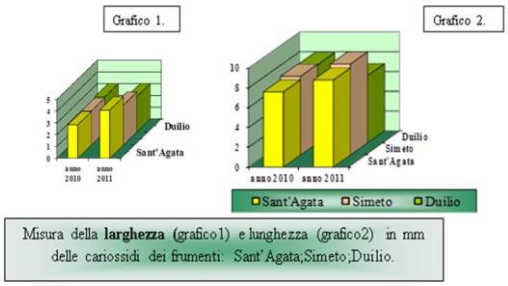
Abstract:
Questo studio è stato svolto nell'ambito del Progetto"ALISAL": Miglioramento delle proprietà igienico-sanitarie, salutistiche e funzionali di materie prime per l'alimentazione dell'uomo e/o degli animali, promosso dal Ministero delle Politiche Agricole, Alimentari e Forestali italiano (MIPAAF).
Lo studio in oggetto è parte del progetto sopra indicato il cui scopo era quello di selezionare nuovi genotipi di cereali, in particolare di frumento duro, al fine di migliorare le caratteristiche nutrizionali e tecnologiche dell' intera filiera produttiva. La fase preliminare dello sviluppo progettuale è stata quella di sviluppare un metodo per la caratterizzazione morfologica e ultrastrutturale delle granelle d'interesse allo scopo di fornire indicazioni qualitative e quantitative per la precisa caratterizzazione dei campioni. I risultati ottenuti sono di seguito riportati.
Materiali e metodi
Al fine di evidenziare eventuali differenze morfologiche e ultrastrutturali tra i campioni, le cariossidi di frumento duro sono state analizzate *tal quali* mediante microscopia elettronica a scansione in ambientale e a pressione variabile utilizzando il microscopio SEM EVO LS10 ZEISS. Questo microscopio è dotato di una sorgente di elettroni LaB6 in grado di fornire una luminosità del fascio elettronico uniforme, stabile e di conseguenza un alto contrasto; tali caratteristiche lo rendono particolarmente adattato all'osservazione di campioni sia disidratati che umidi. Sulle cariossidi di grano duro si è eseguito un adeguato taglio sagittale e le sezioni *tal quali*, senza alcuna preparativa sono state fissate su gli appositi *stub*, successivamente si è passati all'osservazione predisponendo il microscopio a pressione variabile ed utilizzando il detector CZBSD come rivelatore primario e il detector SE1 come rivelatore secondario. Il mix dei due ci ha fornito una precisa mappa ultrastrutturale di ogni cariosside testata.
Secondo le specifiche progettuali abbiamo preso in considerazione tre cultivar di frumento duro coltivate nel Sud Italia (Sicilia) negli anni 2010 e 2011. I campioni sono stati classificati come SA_1(*cultivar Sant'Agata*), DU_1(*cultivar Duilio*), SI_1(cultivar *Simeto*) per le produzioni dell' anno 2010 e SA_2 , DU_2 , SI_2 per le produzioni dell'anno 2011.

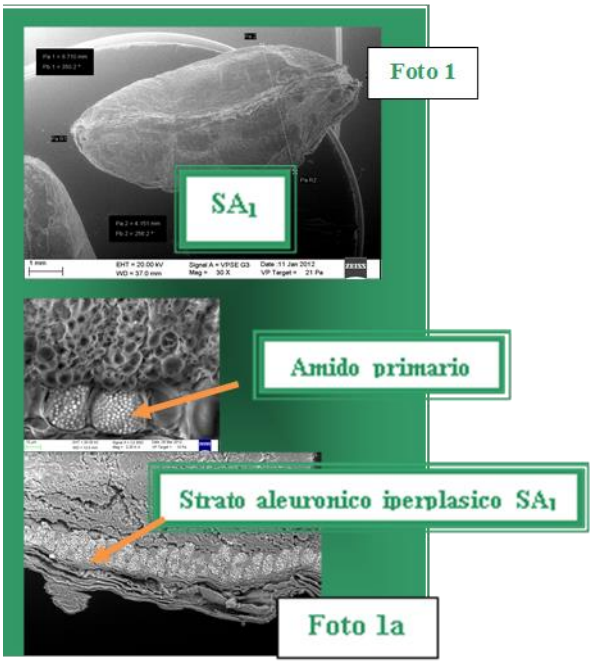


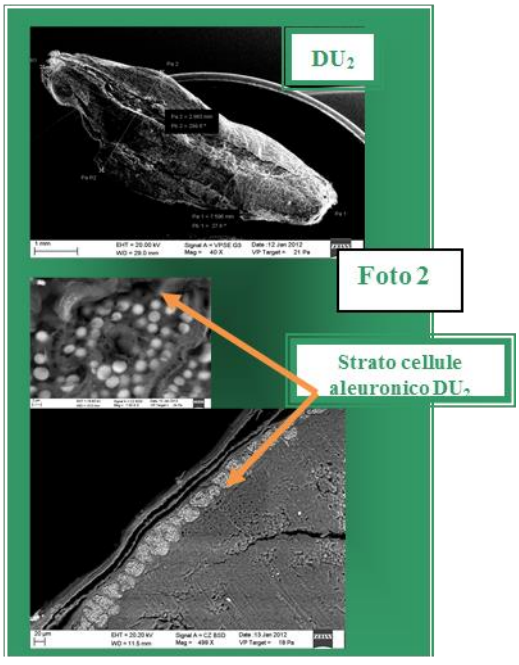
Misura della **larghezza** (grafico1) e lunghezza (grafico2) in mm delle cariossidi dei frumenti: Sant'Agata;Simeto;Duilio.

Risultati

I campioni di grano duro classificati, come detto in precedenza, sono stati sottoposti all'analisi in scansione elettronica che è stata eseguita in due fasi. Una prima fase è stata finalizzata allo studio della superficie delle cariossidi a basso ingrandimento per rilevare le caratteristiche morfologiche più grezze quali la lunghezza e la larghezza media (Grafico 1-2).

La seconda serie di osservazioni è stata realizzata ad alto ingrandimento in modo da evidenziare le componenti ultrastrutturali delle cariossidi, quali lo strato aleuronico, la camera embrionale, e la morfologia dell'endosperma, cercando per ogni campione di fare le osservazioni in posizioni di coordinate analoghe e quindi confrontabili. Non è stata rilevata alcuna variazione morfo-ultrastrutturale significativa per la maggior parte dei campioni: tuttavia nei campioni DU_2(Foto2) *cultivar Duilio* produzione del 2011 e nel campione SA_1(Foto1) *cultivar Sant'Agata* produzione del 2010, abbiamo osservato una serie di modificazioni di notevole entità. Nello specifico il campione DU_2 presenta una forte discrasia fra cariossidi dello stesso campione e, comunque, le dimensioni dei parametri macroscopici, come si evidenzia nelle grafico 1-2, sono inferiori a tutti gli altri campioni testati. Le dimensioni volumetriche ridotte e coartate dell'endosperma (foto2) fanno presupporre che il suddetto campione abbia subito uno stress da temperatura in fine stagione o una conservazione anomala. Il campione SA_1 (Foto1) invece presenta le cariossidi più omogenee fra loro, mentre presenta lo strato aleuronico iperplasico (Foto1a) in contrasto con quello della normale morfologia descritto in letteratura. Un'ipotesi è che tale campione abbia subito uno stress da temperatura in un momento precoce di crescita e che abbia poi innescato successivamente un parziale recupero realizzando una iperplasia delle cellule aleuroniche in maniera di aumentare la produzione di amido primaria e compensare il *gap* di crescita iniziale.





Conclusioni: Questo studio preliminare ci ha permesso di caratterizzare morfologicamente i campioni di grano duro fornitoci e ci ha consentito di individuare il campione più performante dal punto di vista morfologico e ultrastrutturale che è risultato essere il grano duro della *cultivar Sant'Agata*. La tecnica utilizzata in questo lavoro (microscopia elettronica a scansione) può rappresentare un valido supporto per caratterizzare campioni biologici con struttura definita e parametrizzabile, in questo caso grano duro, da destinare a ulteriori indagini di natura analitico-funzionale.

P75 - BIOLOGICAL EVALUATION OF THE ACTION OF *UNDARIA PINNATIFIDA* EXTRACT ON EQUINE RED BLOODS CELLS

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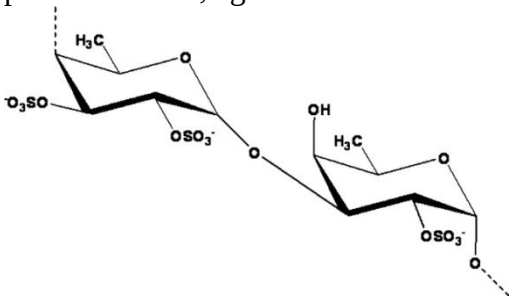
Recently, a great deal of interest has been developed to isolate novel bioactive compounds from marine resources because of their numerous health beneficial effects. Among marine resources, algae are valuable sources of structurally diverse bioactive compounds. The cell walls of algae are rich in polysaccharides, some of which sulphated (SPs) such as fucoidans in brown algae, carrageenans and agar in red algae and ulvans in green algae. These SPs exhibit many beneficial biological properties such as anticoagulant, antiviral, antioxidative, anticancer and immunomodulating activities. Therefore, they have great potential for further development as products in nutraceutical, pharmaceutical and cosmetics areas [1].
Biological activities of SPs depend on chemical structure, molecular weight and chain conformations. The fucoidans are polysaccharides containing relevant percentages of L-fucose and sulfate ester groups [2] Fig. 1.
Fucoidans display several physiochemical and biological features of potential interest for food, pharmaceutical, agricultural and chemical applications [3].



Among the properties of algal polysaccharides, the anticoagulant activity of collecting so much scientific interest in this direction are being conducted extensive research. Fucans have activities similar to those of heparin, a drug for excellence with anticoagulant action [4].

Fig. 1 Chemical structure of the repeating dimeric units of fucoidan
In the veterinary field, one of the diseases that can affect the species *Equus caballus* the most severe and debilitating, it is certainly laminitis, an inflammation of the laminae of the foot. The affection of the foot is only a local manifestation of a systemic metabolic disorder that affects the cardiovascular apparatus, endocrine system and urinary tract, blood clotting and the acid-base balance. It is believed that the basis of the decrease in capillary blood flow and ischemic necrosis jobs are dynamic vessels mangled and bleeding disorders [5]. To this between the various drugs is used heparin, for its anticoagulant action.
In recent years fucoidans have been investigated to develop novel drugs.
In this study was evaluated the toxicity of algal extracts from brown algae *Undaria pinnatifida*.
The polysaccharides extracts were tested on equine red blood cells to evaluate potential haemolytic effects on cell membranes. *Undaria pinnatifida* samples were collected in the lagoon of Venice in May of 2011, the component epiphytic clean, dried in the sun for several days and finally crushed. The sample was treated with 100% ethanol and placed in an oven at 70°C for one hour, it was centrifuged at 4000 rpm for 10 minutes and treated with 100% acetone to obtain a complete depigmentation. Then the material was centrifuged at 4000 rpm for 10 minutes at room temperature.
The pellet (dispersed in distilled water) was placed in an oven at 70°C for 24 hours to allow the passage of the polysaccharides in solution. The solution was centrifuged at 4000 rpm for one

hour at room temperature. The supernatant was mixed with ethanol 96% with a ratio V/V (volume of sample equal to the volume of ethanol). The precipitate, consisting of the crude polysaccharides, has been exposed to complete dehydration at room temperature and then pulverized by the use of a pestle.
Equine blood samples were collected from five healthy donators, were drawn into syringes filled with sodium citrate as an anticoagulant. The algal extracts was dissolved in a buffer, a saline solution at pH 7.4 with the following composition (mM): 125 NaCl, 5 KCl, 1 $MgSO_4$, 32 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 5 D- (+)-glucose, 1 $CaCl_2$; pH 7.4. Algal extracts was tested at two different concentrations 10µg/ml and 20µg/ml. In all studies, using horse red blood cells, control experiments were carried out without extract. We were evaluated the toxicity of algal extracts through two tests: Trypan blue test and hemolysis test. In order to evaluate the toxicity of each sample 1) by Trypan blue test, the cells were counted in Burker's chamber and the cells were expressed as percentages of viable cells; 2) the hemolysis test to see the hemolytic effects of the algal extracts, that evaluates hemoglobin release in the plasma following molecule. After incubation at room temperature, the tubes were centrifuged (5 minutes at 1500 rpm) and the supernatants were determined photometrically with a spectrophotometer at the absorption of hemoglobin (540 nm). As a measure of hemolysis, Hb concentration of the supernatants was determined photometrically. The absorption of the supernatant of erythrocytes lysed in distilled water was defined as 100% haemolysis. In the treatment of RBCs with polysaccharides extracted from *Undaria pinnatifida*, hemolysis occurence at identical concentrations of the control conditions suggest that the compounds does not shows toxicity effects in equine red blood cells at the concentration tested.Tab.1
Currently in the lagoon of Venice *Undaria pinnatifida* is removed and treates as waste stored in landfills and incinerated. This study shows a possible exploitation of *Undaria pinnatifida*, not toxic to equine red blood cells, as a source of anticoagulant drug with the aim of trasforming a waste into a valuable biomass.

Tab. 1: Percentage of hemolysis in control and after the additional of *Undaria pinnatifida* polisaccaride extract.

	300mOsm	200mOsm	150mOsm	100mOsm	0mOsm
Control	0%	0%	9%	85%	100%
10µg/1ml	0%	0%	10%	84%	100%
20µg/1ml	0%	0%	9%	81%	100%

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P76 - NUTRITIONAL QUALITY OF EXTRA VIRGIN OLIVE OILS FROM TWO DIFFERENT *OLEA EUROPEA* CULTIVARS: APPLICATION OF A FUNCTIONAL MATEMATIC INDEX

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Background The extra virgin olive oil is very important in the Mediterranean diet. From the nutritional point of view, the high energy content and an optimal ratio between saturated and unsaturated fatty acids, together with the presence of minor compounds such as phenols and tocopherols and the related antioxidant capacity, confer to this product a high healthy value [1]. The extra-virgin oil's nutritional properties depend on the pedoclimatic conditions, cultivar and the production process. In the last years many studies have been performed to remark and describe the safety and nutritional quality of the extra-virgin olive oils but it is difficult to compare different parameters representative of oil's nutritional quality and to establish which of them is more important to define the nutritional value of an oil. In 2007, Finotti et al.[2] developed a Functional Mathematic Index (FMI) in order to quantify the nutritional quality of extra virgin olive oil and then the same authors applied it at other foods, such as tea [3], potatoes [4]. This index is important because it is able to convert a concept as the "quality" into a numeric quantity. The FMI takes into account different nutritional, antioxidant and chemical parameters representative of oil properties. These parameters, suitably processed with the "FMI Workbench" free software, provide a unique value indicated as "global" quality index (I_{GQ}). We have calculated both the global FMI and the local FMIs.

Aim of this study is to compare the nutritional properties of extra virgin olive oils from different olive varieties, Biancolilla and Oglialora grown in Sicily, by the functional mathematic index [5]

Methods: samples of extra-virgin olive oils from two different cultivar (Biancolilla, Oglialora) and processed by different extraction methods (continuous cycle and hydraulic press) have been analyzed. The drupes are grown in the same climatic conditions. The parameters analyzed to calculate the FMI have been divided in three groups which are related to chemical, nutritional, technologic properties of oil.
The three groups are:

- ✓ Nutritional parameters: fatty acids (numbers from 1 to 4, in table 1).
- ✓ Chemical parameters: acidity and peroxide number (numbers 5 and 6 in table 1);
- ✓ Technological process parameters: lipidic antioxidant capacity, tocopherols and total phenols (numbers 7-9 in table 1).

The results of the analyses were processed by the software and the I_{GQ} and LQ of each oil sample have been compared in order to define which of them had the better nutritional properties.
The FMI varies from zero to one (good to poor). The global index has been expressed as "goodness percentage" (%FMI). The upper and the lower bounds for each group are established and they are report in the following table 1.

Results: the analyses allow to classify all oil samples as extra-virgin olive oils. All parameters are within the limits of the Italian (E.U.) law and those established in table 1. No difference

among two cultivars about acidity, peroxide number and fatty acids profile was found. The lipofilic antioxidant capacity was higher in Biancolilla variety than in Oglialora with both extractive methods (p=0,0001); sample oils extracted with continuous method have shown a lower of 16% content of tocopherols for both variety.

Table 1: parameters and methods used to analyse of the extra-virgin oil samples. Upper and lower bounds for each parameters established according Finotti et al. [2]

Index number	Parameters	Method used to analyze and related references	Lower Bound	Upper bound
1	Palmitic acid (C16:0)	Chromatographic analyses (EU Regulation 2568/91, All. Xa and All. Xb)	7,5%	20%
2	Stearic acid (C18:0)		0,5%	5%
3	Oleic acid (C18:1)		55%	83%
4	Linoleic acid (C18:2)		3,5%	21%
5	Acidity	EU Regulation 2568/91, all. II	0%	0,8%
6	Peroxide number	EU Regulation 2568/91, all. III	0 meq/O_2	20 mEq/O_2
7	Lipidic antioxidant capacity	(Finotti et al.1998,2000)	-1,6 K_a/K_c	+ 1,6 K_a/K_c
8	Tocopherol	HPLC, detector UV/Vis (method of SSOG)	1,2mg/100g	43mg/100g
9	Total phenols	HPLC, detector UV/Vis (method of SSOG)	20 mg/Kg	900 mg/Kg

The FMI of all oil samples varies from 0.27 to 0.34, therefore they satisfy the necessary conditions to be considered high quality extra-virgin oil; Nonetheless, the cultivar Biancolilla has shown a global index better than the Oglialora. From the study of local FMI we observed that the nutritional quality of oils extracted by continuous cycle is penalized in the total polyphenols and lipofilic antioxidant capacity parameters.

Conclusion: the FMI allows to detect that the Biancolilla variety is better than Oglialora and then to underline which parameter can be modified in order to increase the nutritional quality of the oil analyzed.

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P77 - ATTIVITÀ ANTIBATTERICA DI ESTRATTI ALGALI NEI CONFRONTI DI BATTERI ISOLATI DA TAMPONI AURICOLARI

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L'otite esterna consiste in un processo infiammatorio del condotto uditivo che può giungere ad interessare anche la membrana timpanica. Questa patologia, i cui sintomi più caratteristici sono otorrea ed otalgia, è causata generalmente da infezioni batteriche e, più raramente, da infezioni micotiche [1]; i principali microrganismi responsabili sono *Pseudomonas aeruginosa* e *Staphylococcus aureus* [2]. La diagnosi di otite esterna si basa, oltre che sull'esame clinico, sull'effettuazione di tamponi auricolari che, permettendo il riconoscimento dell'agente eziologico, costituiscono il supporto indispensabile per una corretta terapia; quest'ultima si basa essenzialmente sulla somministrazione di antibiotici, eventualmente associati ad antinfiammatori steroidei. Tuttavia, le infezioni da *P.aeruginosa* e da *S.aureus* pongono seri problemi terapeutici legati alla farmaco-resistenza, particolarmente sviluppata in ambiente ospedaliero, che in molti ceppi si estende alla maggior parte degli antibiotici in uso. Nell'ambito degli studi orientati alla scoperta e all'utilizzazione di sostanze ad attività antibiotica, interessanti molecole sono state isolate in microalghe [3, 4]; inoltre, si è osservato che la somministrazione di cellule algali favorisce l'attivazione dei macrofagi e l'aumento della produzione di cellule staminali nel midollo osseo accelerandone la differenziazione in cellule immunocompetenti [4, 5]. Questo studio intende valutare la popolazione microbica del condotto uditivo in pazienti affetti da otite esterna e verificare la sensibilità dei microrganismi responsabili ad estratti algali opportunamente approntati. Per l'indagine microbiologica sono stati utilizzati tamponi auricolari prelevati da 100 pazienti con presunta otite acuta esterna. Ogni tampone è stato seminato in opportuni terreni di coltura selettivi per Gram- (Columbia horse blood agar, Mc Conkey Agar), per stafilococchi (Mannitol Salt Agar) e per miceti (Sabouraud Agar); le piastre sono state tutte incubate a 37°C per 18/24 ore ad eccezione di quelle con Sabouraud per le quali l'incubazione è stata prolungata fino ad 1 settimana. I microrganismi sono stati identificati mediante esame microscopico con colorazione di Gram. In seguito, per i ceppi Gram+ sono stati eseguiti il test della catalasi, per discernere tra *Staphylococcaceae* (catalasi positive) e *Streptococcaceae* (catalasi negative) ed il test della coagulasi, per discernere tra *S. aureus* (coagulasi positivo) e gli altri stafilococchi (coagulasi negativi). L'attività antibiotica di estratti algali è stata verificata utilizzando colture della specie marina *Dunaliella tertiolecta* Butcher 1959 (Chlorophyceae) e della specie d'acqua dolce *Pseudokirchneriella subcapitata* (Chlorophyceae). L'estratto algale è stato preparato per centrifugazione; il pellet è stato lavato con tampone fosfato (per *P. subcapitata*) o con PBS (per *D. tertiolecta*), è stato successivamente sottoposto a 20 cicli di sonicazione di 30 secondi ciascuno e a successiva centrifugazione (3500 rpm per 30 minuti). Al pellet ottenuto è stato aggiunto metanolo al 60% (0,25 g/ml) e si è proceduto ad una nuova centrifugazione (3500 rpm per 20 minuti); il sopranatante ottenuto è stato filtrato (0,22 μm - Millipore GV) ottenendo l'estratto che è stato utilizzato nello studio. Colture batteriche pure in fase di crescita esponenziale (5×10^5 CFU/ml) ottenute da tamponi auricolari raccolti tra gennaio 2012 e gennaio 2013 presso il Pronto Soccorso dell'IRCCS Azienda Ospedaliera Universitaria San Martino IST da 100 pazienti (di cui 58,06% maschi e 41,94% femmine) di età compresa tra 15 e 92 anni (media = 48,15 anni) sono state trattate con diverse concentrazioni di estratti algali di *P. subcapitata e D. tertiolecta*. L'attività antimicrobica degli estratti è stata determinata dopo 18-24 ore di incubazione ed è stata calcolata la Concentrazione Minima Inibente (MIC) secondo

metodiche standardizzate [6]; 84 tamponi sono risultati positivi per la presenza di uno o più microrganismi (Tab. 1). *Pseudomonas aeruginosa* e *Staphylococcus aureus* sono risultati i patogeni più ricorrenti; le micosi hanno avuto un'incidenza molto inferiore (<9 %). I valori di MIC ottenuti trattando ceppi di *P.aeruginosa* e di *S. aureus* con estratto da cellule di *D. tertiolecta* sono risultati compresi rispettivamente tra $1,4 \times 10^9$ e $5,6 \times 10^9$ cell./ml e tra $2,8 \times 10^9$ e $1,1 \times 10^{10}$ cell./ml. I valori di MIC dei ceppi sottoposti all'estratto da cellule di *P. subcapitata* sono risultati compresi tra $6,2 \times 10^9$ e $1,2 \times 10^{10}$ cell./ml per *P. aeruginosa* e tra $1,6 \times 10^9$ e $1,2 \times 10^{10}$ cell./ml per *S. aureus*.

	Ceppo	n. ceppi isolati	%
Gram+	*Staphylococcus aureus*	14	11,20
	Stafilococchi coagulasi-negativi (*)	22	17,60
	Enterococcus spp.	1	0,80
	Kokuria spp.	2	1,60
	Streptococcus pneumoniae	2	1,60
	Micrococcus spp.	8	6,40
Gram-	*Pseudomonas aeruginosa*	31	24,80
	Escherichia coli	8	6,40
	Klebsiella spp. (^)	7	5,60
	Altri enterobatteri (§)	7	5,60
	Altri Gram- non fermentanti (#)	10	8,00
	Vibrionaceae	2	1,60
	Miceti (°)	11	8,80

Tabella 1. Microrganismi isolati dai tamponi positivi. (*) = *S. epidermidis* (14), *S. capitis* (4), *S. xylosus* (2), *S. haemolyticus* (1), *S. symulans* (1); **(^) =** *K. pneumoniae* (5), *K. oxytoca* (2); (§) = *P. mirabilis* (2), *E. cloaceae* (2), *E. aerogenes* (1), *R. terrigena* (1), *S. marcescens* (1); (#) = *W. paucula* (2), *A. xylosoxydans* (2), *A. baumannii* (1), *P. alcaligenes* (1), *B. cepacia* (1), *R. picketti* (1), *S. paucimobilis* (1), *A. hydrophylia* (1); (°) *Candida* spp. (6), *Aspergillus niger* (5).

Questo studio ha confermato che *P. aeruginosa* e *S. aureus* sono gli agenti principali responsabili delle otiti esterne. Nel complesso, *P. aeruginosa* è prevalente e presenta una maggiore incidenza nei mesi estivi. Gli estratti provenienti da *D. tertiolecta* e *P. subcapitata* hanno mostrato attività antibatterica, in particolare nei confronti di *P. aeruginosa*, ($MIC_{90} = 5,6 \times 10^9$ e $6,2 \times 10^9$ cell./ml rispettivamente) ed un minore effetto nei confronti di *S. aureus* ($MIC_{90} = 1,12 \times 10^{10}$ e $1,25 \times 10^{10}$ cell./ml rispettivamente). Considerate le difficoltà derivanti dalla presenza di ceppi resistenti, l'individuazione di nuove molecole bioattive di origine naturale può costituire una prospettiva terapeutica di notevole interesse. In questo ambito, i risultati ottenuti indicano che estratti da specie algali fitoplanctoniche possono risultare adatti per ottenere sostanze ad attività antibiotica utili per il trattamento di patologie otorinolaringoiatriche causate da agenti microbici.

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P78 - RELAZIONE FRA L'IPERTENSIONE E LE CADUTE ACCIDENTALI: I POTENZIALI EFFETTI POSITIVI DELL'ESERCIZIO FISICO SULLA PRESSIONE SANGUIGNA

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Introduzione

La letteratura scientifica supporta la relazione fra le variazioni di pressione sanguigna e il fenomeno cadute nell'anziano; in particolare l'ipotensione è associata a perdita di equilibrio. Una diminuzione di 20mmHg o più della pressione sistolica o una diminuzione di 10mmHg o più in diastolica sono associati a perdita di equilibrio e cadute. Altri studi suggeriscono una relazione fra l'ipertensione e l'ipotensione e in definitiva supportano l'idea che il rischio di cadute sarebbe più alto nelle persone con ipertensione[1]. Lo scopo del nostro studio è capire e valutare, tramite una scala validata, se uno stile di vita attivo influisce significativamente nell'equilibrio residuo in soggetti ipertesi.

Materiali e Metodi

La Berg Balance Scale (BBS) è stata somministrata a due gruppi di soggetti anziani che vivono nella zona città di Palermo, Italia. Il primo gruppo era costituito da centododici soggetti che riferivano di essere affetti da ipertensione o esserne in cura (HP-G); il secondo gruppo consisteva in novantasei soggetti che dichiaravano di non soffrire di tale patologia (NP-G). I criteri di esclusione sono stati: 1) persone con un'età inferiore a 65 anni; 2) persone con una diagnosi positiva di malattie fortemente debilitanti; 3) atleti ex professionisti. I punteggi sono stati utilizzati per correlazioni statistiche.

Risultati

Duecentotto persone hanno partecipato allo studio. 112 nel gruppo HP-G (Età: 74 ± 8,12 anni; Peso 70,05 ± 11,51; Altezza 162,58 ± 8,12), 96 nel gruppo NP-G(Età: 72,5 ± 7,62 anni; Peso 67,89 ± 12,06; Altezza 159,75 ± 9,60). I valori di BBS (Fig.1) erano nel NP-G 47,14 ± 11,53; mentre nel HP-G erano 39,96 ± 14,90 ($p < 0,0001$). Inoltre, il 52% di HP-G ha riferito di praticare una regolare attività fisica. I soggetti attivi di HP-G (Fig.2) hanno mostrato valori pari a 48,12 ± 9,08, mentre i soggetti sedentari di HP-G hanno registrato valori pari a 31,19 ± 14,99 ($p < 0,0001$).

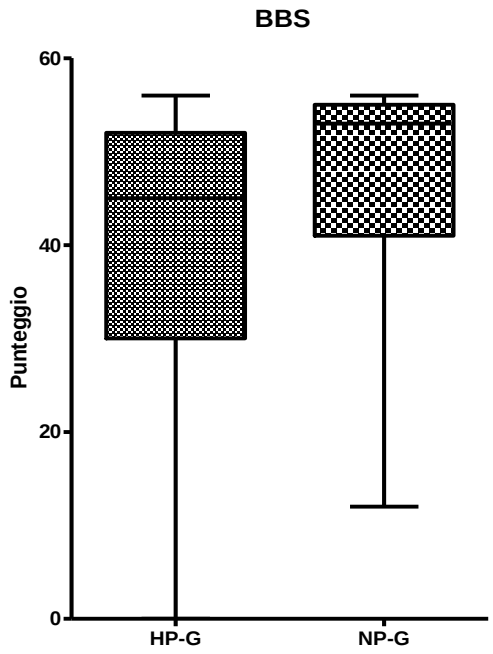

Figura 1

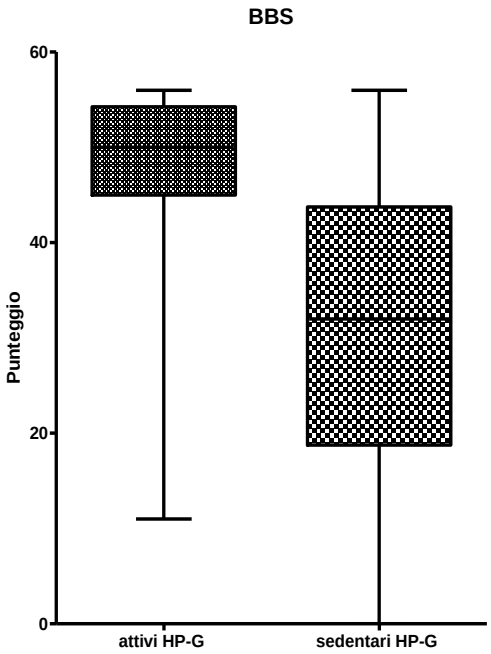

Figura 2

Risultati

L'attività motoria sembra influire positivamente sull'equilibrio residuo dei soggetti analizzati. I risultati suggeriscono che soggetti ipertesi dovrebbero partecipare a programmi di attività motoria volti a prevenire le cadute. Sarebbe interessante stimare con ulteriori studi se l'attività motoria agisca direttamente sui meccanismi fisiologici emodinamici[2] o migliora semplicemente la performance atletica agendo così sull'equilibrio residuo.

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P79 - ESPRESSIONE GENICA DELLE CITOCHINE NELLA TUNICA ALBUGINEA IN SOGGETTI AFFETTI DA MALATTIA DI LA PEYRONIE. STUDIO PILOTA CON GRUPPO DI CONTROLLO

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Introduzione
Abbiamo pensato di effettuare uno studio che indagasse circa la presenza delle citochine nell'albuginea di soggetti affetti da malattia di La Peyronie. Le citochine, in quanto responsabili della comunicazione intercellulare, potrebbero essere coinvolte nella patogenesi della malattia [1,2]. L'individuazione di una o più citochine responsabili potrebbe, infatti, risultare utile nel trattamento farmacologico grazie ai c.d. farmaci biologici, in grado di interferire con le citochine [3].

Materiali e metodi
Per lo studio sono stati reclutati tra gennaio 2009 e dicembre 2010 presso l'Ambulatorio di Chirurgia Andrologica del Policlinico di Palermo 20 soggetti affetti da malattia di La Peyronie (Figura 1) e 8 soggetti affetti da *recurvatum penis* congenito, questi ultimi adoperati come controllo. Come criteri di inclusione allo studio abbiamo considerato i criteri di operabilità con corporoplastica secondo Nesbit. I prelievi bioptici ottenuti dalle losanghe escisse in corso di intervento chirurgico sono stati utilizzati per saggiare l'espressione genica, attraverso Real-Time PCR, di citochine pro-fibrotiche e pro-infiammatorie. Inoltre sono stati sottoposti dopo fissazione ad esame istologico con la colorazione ematossilina-eosina.

PZ	SEDE CURVATURA	DE	COITO	DURATA MALATTIA (mesi)	FATTORI DI RISCHIO
1	Laterale	no	impossibile	14	Ex fumatore, DM II, HCV+, TIA
2	Laterale	lieve	ok	36	DM II, sclerosi multipla
3	Dorsale	lieve	difficile	17	DM II, pregressa IPP
4	Ventrale	no	difficile	9	Ex fumatore, DM II
5	Dorsale	no	impossibile	12	ex fumatore, IMA
6	Laterale	moderato	impossibile	24	Ex fumatore, DM II
7	dorso-laterale	no	difficile	12	Ex fumatore, IA
8	dorso-laterale	no	difficile	19	Ex fumatore, IA
9	Dorsale	no	impossibile	24	IA, iperuricemia
10	Dorsale	lieve	ok	21	Ex fumatore, DM II
11	Ventrale	moderato	difficile	19	Ex fumatore, DM II
12	Dorsale	moderato	ok	30	Ex fumatore, DM II
13	Laterale	no	ok	23	Ex fumatore, DM II
14	Laterale	no	impossibile	12	Ex fumatore, DM II
15	Dorsale	lieve	impossibile	18	Ex fumatore, DM II
16	Dorsale	grave	difficile	24	Ex fumatore, IA
17	dorso-laterale	no	difficile	24	Ex fumatore, DM II
18	Laterale	grave	impossibile	10	Ex fumatore, IA
19	dorso-laterale	no	impossibile	15	Ex fumatore, DM II
20	Dorsale	moderato	difficile	24	Ex fumatore, IA

Figura 1: Caratteristiche dei pazienti affetti da Malattia di La Peyronie.

Risultati.

L'esame istologico ha rilevato l'assenza di cellule infiammatorie in tutti i pazienti recensiti per lo studio. L'analisi dell'espressione dei geni codificanti per IL-4 (Interleuchina-4), IL-6 (Interleuchina-6), IL-13(Interleuchina-13), TGF-β1 (Trasforming Growth Factor- β1), IL-2 (Interleuchina-2), IL-10 (Interleuchina-10), TNF-α (Tumor Necrosis Factor- α) e IFN-γ (Interferone- γ) ha evidenziato in tutti i campioni un livello molto basso di trascritti e in alcuni casi indosabili (Figura 2). Inoltre i livelli dei trascritti delle citochine prese in esame sono risultati minori nei campioni provenienti dagli individui affetti da malaltia di La Peyronie rispetto ai controlli.

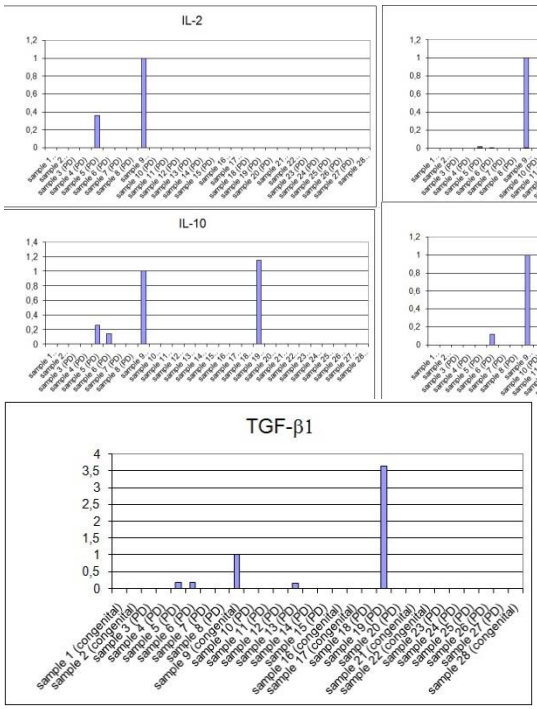

Figura 2: Livelli di trascritti delle citochine dosate

Conclusioni

Alla luce dei risultati ottenuti, l'utilizzo di farmaci biologici (anticorpi) contro le citochine non sembra essere applicabile nella fase stabile della malattia di La Peyronie.

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P80 - QUALITÀ DELLA VITA E LIVELLI DI CORTISOLO

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INTRODUZIONE

Lo stress psicologico è da un lato una condizione che potenzia le capacità reattive dell'individuo di fronte a condizioni di allarme e dall'altro un fattore di rischio per lo sviluppo e la progressione di numerose malattie [1]. È opinione condivisa dalla comunità scientifica che l'attività fisica sia in grado di indurre cambiamenti fisiologici a largo spettro, influendo positivamente anche suli livelli di stress [2]. Scopo del presente lavoro è stato quello di valutare se ed in che modo una particolare tecnica di rilassamento, nota come tecnica di respirazione profonda (deep breathing), fosse in grado di produrre miglioramenti fisiologici sui valori di cortisolo, su un campione di soggetti volontari.

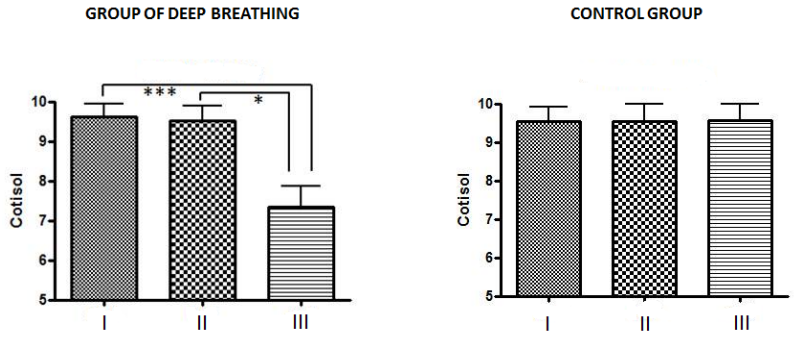
METODI

Hanno preso parte allo studio 38 soggetti volontari sani; 19 hanno costituito il gruppo di soggetti sui quali studiare gli effetti della respirazione profonda (deep breathing) e 19 il gruppo di controllo. Tutti i soggetti hanno firmato il consenso informato preparato sulla base delle indicazioni del Comitato Etico della nostra Università. I livelli di stress psicologico sono stati misurati sulla base dei livelli di cortisolo salivare [3, 4,5]. Il protocollo consisteva in 10 sedute di deep breating [6] della durata di un'ora e trenta minuti. Sono state eseguite le misurazioni dei livelli di cortisolo in tre tempi: la prima è stata all'inizio durante la prima sessione (I), la seconda durante la quinta sessione (II), la terza durante l'ultima sessione (III).

RISULTATI

I valori di cortisolo ottenuti durante la I sessione mostrano valori simili fra il gruppo di controllo ed il deep breathing group. La Figura 1 mostra a sinistra i valori di cortisolo misurati nei soggetti facenti parte del deep breathing group; è possibile osservare un miglioramento statisticamente significativo fra la I e la III sessione. La stessa figura mostra a destra i valori di cortisolo del gruppo di controllo che non mostrano modificazioni significative nelle diverse sedute.

Figura 1. Livelli di cortisolo salivare misurato nelle diverse sessioni nei soggetti che praticavano deep breathing (a destra) e nei controlli.



DISCUSSIONE E CONCLUSIONI

I presenti risultati confermano quelli di Martarelli e collaboratori [6] che hanno osservato come il rilassamento indotto da respirazione diaframmatica aumenti lo stato di difesa antiossidante negli atleti dopo esercizio esaustivo, e che questi effetti si correlano con la concomitante diminuzione del cortisolo. I risultati ottenuti dal presente lavoro mostrano un miglioramento significativo dei valori di cortisolo nel gruppo che ha praticato deep breathing. È possibile ipotizzare che praticare con regolarità e costanza la tecnica di respirazione profonda possa essere un facile e utile strumento per una buona qualità della vita, agendo indirettamente sulla gestione delle tensioni-stress che la vita di tutti i giorni costringe a seguire.

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P81 - VALUTAZIONE DEL COLORE DEL PISTACCHIO DI BRONTE

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I maggiori produttori mondiali di pistacchi al giorno d'oggi l'Iran con 100.000 t, pari al 40% della produzione mondiale, e gli Stati Uniti con il 32%, seguiti da Turchia ed Siria. L'Italia è in questa classifica nettamente distaccata con una produzione che si aggira intorno ai 30.000 q. l'anno. La maggior parte di questo prodotto proviene dalla Sicilia e da Bronte. Lo scopo di questa ricerca è stato pertanto di valutare la qualità del Pistacchio verde di Bronte attraverso la determinazione dei parametri cromatici e del contenuto in clorofilla. I campioni di pistacchi, provenienti dal comprensorio di Bronte, sono stati conservati a una temperatura di 10°C. Per la valutazione oggettiva del colore 50 g di pistacchi, sgusciati e privati del tegumento, sono stati macinati in Waring Blendor con giara raffreddata ad acqua. Il materiale così ottenuto è stato setacciato e per l'analisi è stata impiegata la frazione di pistacchio che passava attraverso un setaccio da 35 mesh, ma si raccoglieva in uno da 50 mesh. Il colore è stato valutato, secondo il sistema CIE-Lab. tramite il colorimetro a riflessione MINOLTA mod. METER CR-210 ver. 3.0 con area di misura pari ad un disco di 50 mm di diametro. Le letture dello stesso campione sono state ripetute 5 volte in punti diversi della capsula dopo aver rimescolato il contenuto della stessa. Per la determinazione della clorofilla è stato utilizzato il metodo AOAC Official Method 942.04 (1). Nelle tabelle 1 e 2 , sono riportati i risultati medi ottenuti dalla determinazione oggettiva del colore dei campioni prelevati presso una industria di trasformazione nel comune di Bronte.

Campioni	**Luminosità**	**a ***	**b ***
A1	60,62	-14,81	44,76
A2	64,58	-13,52	44,67
A3	68.61	-10,70	46,30
Media T0	64,60 ± 0,43	- 13,01 ± 0,08	45,24 ± 0,29
B1	63,54	-14,02	43,96
B2	63,60	-13,97	44,72
B3	67,36	-10,84	44,65
Media 3 mesi	64,83 ± 0,54	- 12,94 ± 0,07	44,45 ± 0,40

Tabella 1. Valori dei parametri CIE Lab rilevati sui pistacchi conservati in aria.

I valori della luminosità e di a* e b* che indicano le coordinate cromatiche in uno spazio tridimensionale (le coordinate appunto L, a, b) indicano che al variare del tempo si ha una graduale diminuzione della luminosità e contemporaneamente un aumento dei valori di a* e b* a cui corrisponde una sostanziale diminuzione del colore. Le analisi per la clorofilla, sono state condotte con l'obiettivo di valutare possibili variazioni di concentrazione della clorofilla totale e delle due forme *a* e *b* dei pistacchi conservati in aria. I dati sono riportati nelle tabelle 2.

Campioni	Clorofilla totale (mg/kg)	Clorofilla *a* (mg/kg)	Clorofilla *b* (mg/kg)	% Clorofilla *a*	% Clorofilla *b*
A1	3,98	1,85	0,84	69	31
A2	3,80	1,10	0,40	73	27
A3	2,28	0,60	0,31	66	34
Media T0	3,35 ± 0,03	1,18 ± 0,08	0,51 ± 0,09	61	39
B1	2,95	1,68	1,26	57	43
B2	2,67	1,38	1,28	52	48
B3	1,61	0,84	0,76	52	48
MEDIA 3 mese	2,40 ± 0,07	1,30 ± 0,01	1,10 ± 0,09	55	45

Tabella 2. Contenuto in clorofilla dei pistacchi conservati in aria.

I risultati ottenuti non sono di facile interpretazione perché riferiti ad un arco temporale troppo breve, i valori della clorofilla tendenzialmente diminuiscono al passare del tempo ma si dovranno attendere le successive analisi sui prossimi campioni per poter effettuare una migliore valutazione sulla evoluzione nel tempo del contenuto in clorofilla (2).

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P82 - RISCHI E VIRTU' DEL CONSUMO DI PESCE CRUDO

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Il consumo di pesce crudo in questo ultimo ventennio è notevolmente aumentato, ciò è dovuto non solo alle benefiche caratteristiche alimentari del pesce ma soprattutto alla moda di cui il consumatore è fortemente influenzato. Il pesce è una preziosa fonte di proteine, minerali e vitamine di alta qualità. Quello grasso, inoltre, è ricco di acidi grassi polinsaturi omega-3 (PUFA), le cui proprietà benefiche sono ampiamente riconosciute. Il consumo di pesce crudo è la base di una piccola parte della cucina mediterranea, ma è il principale protagonista di quella dall'estremo oriente (Giappone).
La salubrità del prodotto consumato è messa a rischio dall'espansione delle attività umane che ha determinato, in questi ultimi quarant'anni, profonde alterazioni negli ambienti naturali.
Secondo le direttive ufficiali dell'Organizzazione delle Nazioni Unite per inquinamento marino si intende l'immissione in mare, diretta o indiretta, di sostanze che producono effetti negativi sulla qualità delle acque, sulla salute umana e sulle risorse biologiche. In base al loro comportamento nell'acqua si possono distinguere quattro categorie principali di rifiuti: *biodegradabili*, *non conservativi*, *particellati* e *conservativi*.
I rifiuti biodegradabili sono costituiti da materiale organico proveniente da scarichi urbani, allevamenti zootecnici e terreni coltivati. Non conservative sono quelle sostanze, generalmente di origine industriale, che perdono rapidamente le loro proprietà una volta in acqua, con un effetto quindi localizzato all'area di scarico: sostanze acide o alcaline e acque di raffreddamento delle centrali elettriche. Per rifiuti particellati si intendono materiali inerti di grandezza variabile. Infine, i rifiuti conservativi sono i metalli pesanti ed i composti organo-alogenati, che permangono nelle acque senza subire degradazione, i quali tendono a concentrarsi negli organismi attraverso la catena alimentare [1]. I materiali organici provenienti dalle reti urbane e dagli allevamenti rappresentano una ricca fonte di microrganismi di origine intestinale. L'inquinamento dei mari, evidenzia una problematica legata al consumo del pescato, in particolare di tonno o pesce spada, nei cui tessuti le sostanze tossiche si possono accumulare.
La conservazione errata del pesce soprattutto di tonno porta allo sviluppo di pericoli per la salute del consumatore, come avvenuto nel mese di giugno del 2013 nell'hinterland di Palermo.
Le principali contaminazioni chimiche nei prodotti della pesca derivano per la maggior parte da metalli pesanti come l'arsenico, il cadmio, il piombo ed il mercurio, ma anche da residui di pesticidi. Il mercurio può essere riscontrato nei tessuti muscolari del tonno (*Thunnus spp.)* e del pescespada (*Xiphias gladius*). Importante è altresì l'impatto dovuto alle alterazioni microbiologiche del pesce.
La qualità del pesce diminuisce in seguito allo sviluppo di microrganismi alterativi ed alle reazioni biochimiche che avvengono durante la manipolazione e lo stoccaggio. I due fenomeni sono strettamente correlati. Tra i vari metodi disponibili per la determinazione della qualità del pesce durante lo stoccaggio, l'evoluzione della popolazione microbica [2] e la produzione di azoto volatile nel tempo [3] sono generalmente i più utilizzati e più precisi.
L'ammina biogena principalmente implicata in avvelenamenti da consumo di pesce è l'istamina ed i pesci comunemente associati a tali insorgenze sono pesci sgombroidi, soprattutto tonno e sgombro (CSPI, 2004) [4]. L'istamina nei pesci è formata dall'azione dell'enzima batterico istidina decarbossilasi (EC 4.1.1.22) che agisce sull'aminoacido istidina.

Il pesce crudo può essere contaminato da diversi batteri - come *Listeria*, *Escherichia coli*, *Salmonella* - che provocano problemi gastrointestinali. Ma il microrganismo che riscuote maggior interesse nell'ambito della sanità pubblica è in atto l'*Anisakis*, verme nematode parassita del pesce, come il tonno, sardina, acciuga, pesce spatola, anguilla e sgombro, chiamato in causa nell'insorgenza di manifestazioni allergiche sia in popolazione generale che professionalmente esposta [5]. Dal 1992 il Ministero della Sanità obbliga chi somministra pesce crudo o in salamoia a utilizzare pesce congelato, o a sottoporre a congelamento preventivo il pesce fresco da somministrare crudo: l'Anisakis e le sue larve muoiono infatti se sottoposti a 60 gradi di temperatura, oppure dopo 96 ore a -15° C, 60 ore a -20° C, 12 ore a –30° C, 9 ore a -40° C.
Le virtù del consumo del pesce crudo riguardano soprattutto aspetti nutrizionali, infatti il pesce è ricco in omega-3 e in proteine essenziali facilmente digeribili, di vitamine e sali minerali. I vantaggi del consumo di pesce crudo si possono riscontrare nella salute di quei popoli che hanno come base della loro cucina il pesce crudo. I giapponesi grazie al consumo anche del pesce crudo hanno ridotto i rischi di insorgenza della depressione e di malattie cardiovascolari.

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P83 - OXIDATIVE STRESS MARKERS ASSOCIATED WITH MIDDLE DISTANCE RUNNING PERFORMANCE

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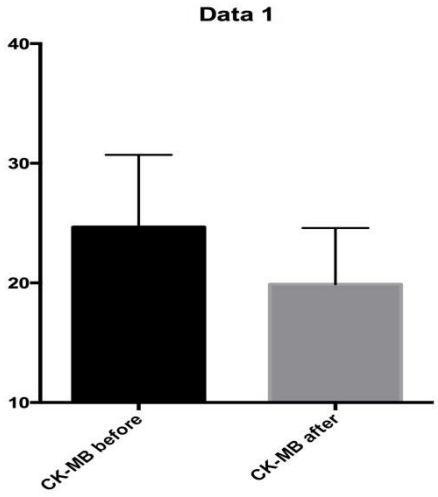
The aim of this study was to understand the mechanism underlying the physiological adaptation of a kind purely aerobic workout. Particular attention has also been given to the analysis of oxidative stress by detecting some enzymatic blood parameters.
We investigate the effect of 2 months of training on middle distance running (800 meters and 1500 meters) agonistic athletes; nine active specifically trained males of mean (± SD) age 19,9 years.
The physiological characteristics of middle runners are different from those sprinters and long distance runners because include a variety of aerobic and anaerobic capabilities.
Two weeks prior to the 2 months period of exercise, subjects were tested for VO_{2max} during a graded, treadmill test with the Cosmed FitMate metabolic device (Cosmed, Italy). Antropometric parameters were detected used a Bioimpedance analyzer (BF 302 Ormon BIA). A blood sample was collected in the morning and were collected by a clinical specialized center to analyze: triglycerides, total cholesterol, G.O.T. and G.P.T. transaminase, γ-GT, CPK and CK-MB, as well as lipid profile.
All these tests were performed before and after the two months of training period.
The results obtained suggest that the endurance training, as it is high oxygen consumption, should increase reactive oxygen species (ROS), but it has been shown that exercise leads to increased activation of antioxidant defenses. Infact, serum levels of gamma-glutamyltransferase (GGT) enzyme, which plays a key role in the metabolism of extracellular reduced glutathione (2) was not increased. However, a classic cardiac biomarkers (1), CK-MB as well as total CK was analyzed and while the total CK after two months of training increased, the CK-MB isoform decrease, in a significant statistical way.
Even the emathological parameters were analyzed and there were the variations overall on neutrophils and monocytes value. These two cell type are involved in the infection respons.
An antropometric parameter that changed after two months of training, was the weight. Infact after statistical analysis, the P value was < 0,0001, considered extremely significant (59,3±5,4 Kg before training; 58,1±5,2 Kg after training).
Finally, put together all the results, we can say that middle distance runners are subject at not high oxidative and biological stress; infact there were no change in γ-GT value, that usually is involved in the oxidative stress as well as a decrease of CK-MB value, that usually associated with cardiac injury. These are preliminary results that need to confirm with other studies using a larger sample of subjects or comparing with the runners that are involved in a different running distance (sprinters or long distance runners).



Tab1
Changes in CK-Mb value after two months of training

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P84 - LA COMUNITÀ INUIT DI IGLOOLIK (CANADA) NELLA SECONDA META' DEL NOVECENTO: UNA RICERCA PIONIERISTICA DI CITOGENETICA PER RACCONTARE LA STORIA DI UN POPOLO

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Negli anni 1971-1972 nell'ambito dell'*International Biological Programme: Human Adaptability Project*, l'UNESCO promosse un progetto di ricerca sull'insediamento esquimese di Igloolik (Canada). Il programma di ricerca, coordinato dal Prof. Brunetto Chiarelli, già ordinario di Antropologia presso l'Università degli Studi di Firenze, aveva come obiettivo lo studio delle caratteristiche genetiche della popolazione eschimese a confronto con le caratteristiche delle popolazioni urbanizzate. Lo spunto per questo studio nasceva dall'ipotesi che le popolazioni isolate, non esposte ai vari agenti mutageni del mondo industrializzato, avrebbero dovuto presentare una percentuale molto bassa di aberrazioni cromosomiche rispetto alle altre popolazioni. Gli Eschimesi o meglio gli "Inuit", per le loro caratteristiche di popolazione adattata ad un ambiente estremo e isolata biologicamente, si rivelarono un campione di studio adatto per sperimentare le tecniche della moderna citogenetica. Agli inizi degli anni Settanta la disciplina aveva già conosciuto la grande rivoluzione metodologica rappresentata dalla tecnica del bandeggio che consente di produrre bande orizzontali di differente intensità di colorazione su tutti i cromosomi del corredo rendendo possibile così una facile identificazione e un preciso accoppiamento. Lo studio condotto negli anni Settanta sugli abitanti di Igloolik rappresentò la prima applicazione delle tecniche di bandeggiamento dei cromosomi su popolazioni artiche.
Il nostro contributo ha lo scopo di ripercorrere la storia di questo studio pionieristico e del contesto storico-geografico che lo ispirò alla luce anche delle nuove metodologie di indagine, tuttora in continuo perfezionamento. Vogliamo, inoltre, sottolineare che gli strumenti e le tecniche del passato possono rappresentare un patrimonio di conoscenze con valenze museali quando sono associati alle testimonianze materiali del popolo studiato.

P85 - PROCIANIDINA B2 ED (–)-EPICATECHINA PRESENTI NELLE MELE: STUDIO PRELIMINARE SUL LORO EFFETTO SUI MECCANISMI DI AGGREGAZIONE E DI INIBIZIONE DI PROTEINE AMILOIDI

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I polifenoli estratti da diversi frutti, come mele, pompelmi, arance, limoni o melograni, sono noti per le loro azioni benefiche sulla salute dell'uomo, per la loro attività antiossidante [1], antitumorale [2] e anti-infiammatoria [3]. È stato, inoltre, riscontrato un loro effetto neuroprotettivo e anti-amiloidogenico *in vitro* [4, 5]. Studi recenti hanno infatti dimostrato che alcuni polifenoli, come l'acido gallico, l'epicatechina gallate e l'epigallocatechina gallate esercitano un'azione neuroprotettiva nei confronti della tossicità indotta dalla proteina β-amiloide (peptide Aβ), coinvolta nella patologia dell'Alzheimer [4]. In particolare è stato dimostrato come polifenoli estratti dalle mele, con alto contenuto di procianidine, siano in grado di inibire *in vitro* l'aggregazione del peptide Aβ [6].
La malattia di Alzheimer (AD) è la forma più comune di demenza neurodegenerativa invalidante ad esordio prevalentemente senile. Colpisce le più alte funzioni cerebrali come la memoria e le funzioni cognitive e ciò si ripercuote sulle capacità intersociali dei malati di tale patologia causando stati confusionali, cambiamenti di umore e disorientamento spazio-temporale. Osservazioni eseguite tramite impiego di tecniche di immagine, indicano che i pazienti affetti da AD mostrano, a livello neurologico macroscopico, una diminuzione del peso e del volume cerebrale per atrofia corticale, con allargamento dei solchi e corrispondente appiattimento delle circonvoluzioni. Nei pazienti con AD si osservano deposizioni a livello dell'ippocampo e della corteccia cerebrale chiamate placche senili, costituite da aggregati proteici di un peptide di 40-42 amminoacidi, il peptide β-amiloide. Il peptide Aβ deriva da uno specifico *pathway* proteolitico di una glicoproteina di transmembrana ubiquitariamente espressa, nota come proteina precursore dell'amiloide le cui funzioni non sono ancora conosciute, anche se recenti studi suggeriscono una sua implicazione nella formazione delle sinapsi, nella plasticità neuronale e nell'esporto di ferro. L'accumulo del peptide Aβ è ipotizzato come inizio di una cascata patogenetica che eventualmente porta alla malattia [7]. In condizioni fisiologiche il rapporto tra Aβ-42 e Aβ-40 è circa 1:10. In letteratura, l'aggregazione del peptide amiloide è descritta secondo una tipica cinetica di nucleazione-polimerizzazione, in cui ognuna delle fasi è caratterizzata da specifici intermedi strutturali che presentano diverse dimensioni, morfologie e potenziale citotossico [8].
Il presente lavoro è finalizzato allo studio dell'effetto di alcuni composti naturali, quali le procianidine, sull'inibizione del processo di formazione di fibre amiloidi *in vitro*.
Mediante cromatografia liquida (HPLC-UV/Vis) si è provveduto inizialmente all'identificazione ed alla quantificazione di polifenoli in mele della cultivar 'Gala', coltivate in Sicilia in regime di agricoltura biologica. Studi bibliografici individuano cinque principali gruppi polifenolici in diverse varietà di mele: acidi idrossicinnamici, procianidine, antocianine, flavonoli e diidrocalconi. Le procianidine sono i maggiori componenti del profilo polifenolico, presenti per il 59,7% nella buccia e per il 55,7% nella polpa [9]. Dopo aver verificato la presenza di Procianidina B2 e del monomero (–)-Epicatechina tra le procianidine presenti in queste mele, e' stato valutato mediante spettroscopia di fluorescenza ("Test della Tioflavina T") il loro effetto sulle proprietà di aggregazione di una proteina modello, la k-caseina, che, analogamente al

peptide Aβ, forma fibre amiloidi a partire da un monomero intrinsecamente disordinato che contribuisce al core fibrillare con un *double strand* β. Le misure di fluorescenza mostrano che sia il dimero che il monomero, se pur in misura diversa, inibiscono in maniera dose-dipendente l'aggregazione della k-caseina. Misure di dicroismo circolare (CD) hanno anche permesso di valutare la variazione della struttura secondaria di aggregati formati in assenza ed in presenza di Procianidina B2 ed (–)-Epicatechina. Dagli spettri CD risulta che l'(–)-Epicatechina (65µg/ml) non influenza la conversione strutturale della proteina a foglietti β ordinati, caratteristici delle specie amiloidi. Infatti dopo incubazione a 37°C per 44 ore si assiste allo *shift* del minimo CD da 205nm (valore di pre-incubazione) verso lunghezze d'onda maggiori, analogamente a quanto riscontrato nel campione controllo (k-caseina nativa). I risultati ottenuti costituiscono la base metodologica-sperimentale per la successiva analisi dell'effetto di tali polifenoli sull'aggregazione e tossicità del peptide Aβ coinvolto nel morbo di Alzheimer e per l'indagine sul loro meccanismo di azione ai fini di un potenziale impiego terapeutico.

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P86 - COMPARATIVE ANALYSIS OF HSP10 AND HSP90 IN LARGE BOWEL HEALTHY MUCOSA AND ADENOCARCINOMAS

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Background: Heat shock proteins (Hsps) are an important class of molecules with various functions. Their classic role is to assist other proteins in folding and re-folding and, when proteins are defective or irreversibly misfolded, to drive their degradation. For this reason, some Hsps are also named molecular chaperones. During evolution, this class of proteins has also acquired "extrachaperoning" roles such as participation in immune system regulation, cell differentiation, programmed cell death and carcinogenesis. Hsp10 is a partner of Hsp60 in the Hsp60/10 folding machine, but numerous scientific studies have shown that Hsp10 may also play other roles. In fact, Hsp10 seems to have an immunomodulatory activity and a role in tumor progression. Hsp90 regulates late-stage maturation, activation and stability of a range of "client" proteins, such as HER2, EGFR and BRAF, some of which are involved in signal transduction and other key pathways important for malignancy in several cancers, including large bowel carcinomas. The aim of the present study was to evaluate levels and expression of Hsp10 and Hsp90 in a series of samples of large bowel mucosa obtained from healthy controls and patients with adenocarcinomas.
Methods: Twenty samples of large bowel human mucosa from healthy control subjects and twenty samples of large bowel adenocarcinomas with moderate grade of differentiation, were obtained from the DICHIRONS Department of the University of Palermo, Italy. RT-PCR and Western Blotting analyses were performed on these samples in order to study gene and protein expression of Hsp10 and Hsp90 (both Hsp90α and Hsp90β isoforms). Moreover, an immunohistochemical study for Hsp10 and Hsp90 was performed to evaluate the localization of these proteins in both the epithelium and the lamina propria.
Results: RT-PCR analysis showed a higher gene expression of Hsp10 and Hsp90 in adenocarcinoma samples compared to healthy mucosa. The Western Blotting analysis confirmed a greater amount of Hsp10 and Hsp90 proteins in the samples of adenocarcinoma of large bowel compared to healthy mucosa. Finally, levels of Hsp10 were higher in adenocarcinoma compared to normal mucosa in both the epithelium and in the lamina propria, as revealed by immunohistochemistry. By contrast, Hsp90 levels were not significantly different in the epithelium, while they were higher in the lamina propria of adenocarcinoma samples compared to normal mucosa.
Conclusion: These data suggest that Hsp10 and Hsp90 may be involved in the carcinogenesis of the large bowel by different molecular mechanisms.

P87 - NUTRIZIONE, OBESITÀ E SINDROME METABOLICA IN UN CAMPIONE DI ANZIANI DELLA VAL CENISCHIA

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In un campione di 189 persone con più di 60 anni appartenenti alle comunità della Val Cenischia (Piemonte) e originari della valle da almeno tre generazioni è stata valutata la prevalenza di sindrome metabolica secondo i criteri del NCEP ATPIII [1]. A questo fine ad ogni partecipante è stato effettuato un prelievo venoso a digiuno per la determinazione della glicemia, della trigliceridemia e della colesterolemia totale e HDL, è stata misurata la pressione arteriosa, sono state rilevate alcune misure antropometriche (peso, altezza e circonferenza addominale) ed è stata somministrata un'intervista standardizzata volta ad indagare le abitudini alimentari, il livello di attività fisica ed altri aspetti dello stile di vita.
Nel campione, composto da 104 donne (età media: 73,6 ± 8,4 anni) e 85 uomini (età media: 73,0 ± 7,4 anni) la sindrome metabolica ha una prevalenza del 42,1% (F=43,2%; M=40,8%). Lo stato di sovrappeso (definito da un BMI compreso tra 24,9 e 29,9 kg/mq) ha una prevalenza del 39,7% (F=40,2%; M=39,1%), mentre l'obesità (definita da un BMI≥30,0 kg/mq) del 23,8% (F=22,5%; M=25,3%).
Dall'analisi dei dati clinici e dei dati nutrizionali è emersa una associazione significativa tra l'apporto nutrizionale della prima colazione e uno stato di obesità/sovrappeso. In particolare è stata riscontrata la presenza di una correlazione negativa tra il valore energetico della prima colazione e il BMI (R=-0,227, P=0,002). Le donne che consumano una colazione non adeguata (cioè inferiore al 15,0% dell'apporto energetico quotidiano) hanno un BMI medio significativamente superiore rispetto a coloro che consumano una colazione adeguata (27,6 vs 25,6; T=2,669, DF=102, P=0,009) e lo stesso avviene per gli uomini (31,0 vs 26,7; T=3,298, DF=83, P=0,001).
Inoltre, è emersa un'associazione significativa anche tra assunzione di quantità eccessive di alcool (>20 g/dì per le donne e >40 g/dì per gli uomini) e obesità addominale (definita da una circonferenza addominale >88 cm per le donne e >102 cm per gli uomini) in entrambi i sessi (donne: χ^2=4,989; DF=1; p=0,025; O.R.=2,706 [IC95%: 1,115 - 6,569]; uomini: χ^2=7,167; DF=1; p=0,007; O.R.=3,525 [IC95%: 1,363 - 9,119]).
Questo lavoro fa parte di un progetto di ricerca più ampio svolto in collaborazione con il dipartimento di Neuroscienze dell'Università di Torino e con il Dipartimento di Scienze Biologiche, Geologiche e Ambientali dell'Università di Bologna volto a studiare il decadimento cognitivo legato all'invecchiamento in relazione a fattori di rischio cardiovascolari di tipo clinico, genetico e comportamentale e ha ricevuto l'approvazione del Comitato di Bioetica dell'Università di Torino.

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P88 - PLASMA SEROTONIN IN HORSES: COMPARISON BETWEEN TWO DIFFERENT MANAGEMENT CONDITIONS

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Recent reports of new and important roles for serotonin in the periphery have served to increase interest in circulating serotonin (5-HT). The much smaller pool of free (extraplatelet) plasma 5-HT is accessible to sites of action and receptors, and may be important in many processes. Assessing this extraplatelet plasma pool could be very difficult also because many factors could influence 5-HT levels[1]. Horses kept in stalls are deprived of opportunities for social interactions and the performance of natural behaviors is limited. The hypothesis of this study was that stalling horses results in a negative effect on their welfare. As marker of poor welfare we evaluate plasma 5-HT and its precursor tryptophan (TRP) in 14 adult horses heterogeneous for sex, breed and age (13±7 years). In previous studies lower levels of plasma 5-HT were found in horses with cribbing behaviour[2] and in subjects feed with high levels in concentrates[3]. Horses of this study were divided in two groups: Stall (S) Group and Pasture (P) Group. Group S (n.6 horses) was maintained in individual box under a natural photoperiod (sunrise at 06:06, sunset at 18:49) and natural indoor temperature (19–21°C) from the day before the experiment to the afternoon of follow day. Horses were fasted overnights (12-14 hours) and then feed with hay that was provided at 08:30 and 12:30, water was available at *libitum*. Group P (n.8 horses) was maintained at the same condition of Group S until 8:30 then it was transferred from box stalls to pasture. Blood samples were obtained from the jugular vein at 08:00, 12:00 and 16:00 and collected into EDTA-containing tubes. Within 30 minutes from the venipuncture, sample tubes were centrifuged at 1350 x g for 10 minutes to obtain the fraction defined as platelet poor plasma. One hundred µl of plasma were then supplemented with an equal volume of an internal standard represented by N-methylserotonin and treated with 100 µl of a precipitating reagent to ensure protein removal. Samples were the vortex-mixed for 30 seconds, allow to stand for 10 minutes at 4°C and centrifuged in a top-bench centrifuge at the maximal speed. The resulting clear supernatants were stored at -20°C and analysed within one week for the HPLC quantification of 5-HT and TRP according to protocols earlier described[4]. All the results obtained were expressed as mean values ± standard deviation (SD). One-way repeated measure analysis of variance (ANOVA) was performed to determine the statistically significance and Bonferroni's test was applied as post hoc comparison test. Mann Whitney test was used to compare differences between groups. The data were analysed using the software STATISTICA 8 (Stat Soft Inc.). Results for 5-HT and TRP are shown on table 1 and figure 1 respectively. The influence of time evaluate by ANOVA was significant in both Groups ($P<0.001$) with levels significantly higher at 12:00 and at 16:00 compared to levels at 08:00. 5-HT levels were significantly higher in Group P compared to Group S at 12:00 ($P<0.01$) and 16:00 ($P<0.001$). Also TRP levels were significantly influenced by time in both Groups ($P<0.001$) with higher levels at 12:00 and at 16:00 compared to levels at 08:00. No difference between Groups were found for TRP concentrations. The pattern of 5-HT and TRP levels confirmed previous results on equine daily rhythms for these parameters[5]. The lower levels of 5-HT measured at 12:00 and at 16:00 in Group S could indicate that factors as absence of exercise and isolation could influence 5-HT levels. Regardless of this, we recognize that, in addition to differences in exercise and social interaction there are confounding factors between treatment groups including nutrition rate and exposure to sunlight. However, these same confounding factors would be

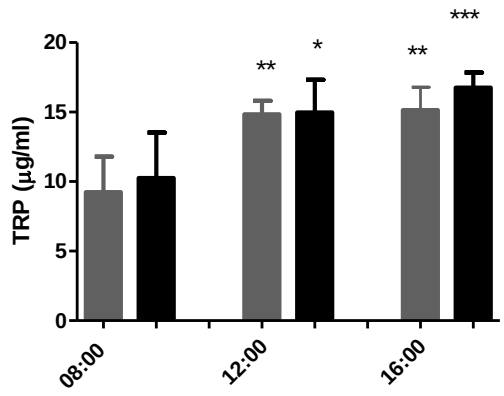
present in any operation where a decision has to be made as to whether to stall horses or provide access to pasture. In conclusion obtained results in the present study showed the modulation of plasma 5-HT by two different management conditions. Our suggestion is to improve the knowledge about factors that can increase plasma equine 5-HT levels in order to guarantee the animal welfare.

Table 1. Patterns of mean values (±SD) of plasma 5-HT (ng/ml) from 08:00 to 16:00 in horses maintained in individual box (08:00 both groups, 12:00 and 16:00 Group S) and at pasture (Group P: 12:00 and 16:00).

Time	Experimental condition			ANOVA
	08:00	12:00	16:00	
5-HT ng/ml (Group S)	29.4 ±10.0	110.9±27.1[c]	121.7±31.2[c]	$F_{2,21}$=33.66; P<0.001
5-HT ng/ml (Group P)	23.0±4.78	42.04±6.8[b*]	68.13±11.2[c**]	$F_{2,15}$=47.81; P<0.001

Bonferroni post-hoc comparison: vs 08:00 [c] P<0.001; [b] P<0.01
Mann-Whitney test: vs Group S * P<0.01; **P<0.001

Figure 1**.** Patterns of mean values (±SD) of plasma TRP in Group S (grey bar) and in Group P (dark bar) from 08:00 to 16:00.



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P89 - EVALUATION OF SOME OXIDATIVE STRESS MARKERS IN OVIS ARIES DURING DIFFERENT EXPERIMENTAL CONDITIONS

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Oxidative stress can be regarded as an imbalance between prooxidant/free radical production and opposing antioxidant defenses. There is growing evidence that oxidative stress (OS) significantly impairs organic function and plays a major role in the aetiology and pathogenesis of several metabolic diseases in veterinary medicine [(1)]. In view of this, we evaluate some oxidative stress markers in ten Comisana ewes (3 years old, clinically healthy) at different experimental conditions: shearing, road transportation, different reproductive status. Moreover we evaluate daily rhythms of oxidative stress markers. On each subject, blood samples were collected by jugular venipuncture to assess oxidative stress markers during: shearing (before, after shearing and 8h, 1d, 2d, 3d, 4d, 5d post- shearing); road transportation for 6 h over a distance about 490 km with an average speed of 80 km/h (before, after transportation and 8, 24, 48 h post-transport); different reproductive status (days 1, 40 and 200 of lactation and during dry period); and circadian rhythm under natural photoperiod -sunrise 5:10, sunset 20:45- (every 3 h over a 24 h period, starting at 8:00 on day 1 and finishing at 8:00 on day 2). All blood samples were collected into vacutainer tubes with no additive and were centrifuged at 3000 × g for 20 min. The sera obtained were immediately analyzed by means of a UV spectrophotometer (Slim SEAC, Firenze, Italy) for the assessment of dROMs, Oxy-adsorbent and SHp. These techniques are based on the "spin traps" system, molecules which react with free radicals, creating complexes revealed by spectrophotometry. During milking and dry period was also evaluate daily rhythm of oxidative stress markers (mean level, amplitude, acrophase and robustness) applying a trigonometric statistical model on mean values. All the results obtained were expressed as mean values ± standard deviation (SD). Data were normally distributed ($P < 0.05$, Kolmogorov–Smirnov test). One-way repeated measure analysis of variance (ANOVA) was performed to determine the statistically significance and Bonferroni's test was applied as post hoc comparison test. The data were analyzed using the software STATISTICA 8 (Stat Soft Inc.).
The lowest values of dROMs observed before shearing and a their subsequent increase, could be due to the energy deficiency that occurs in ewes after shearing. Ambient temperature, relative humidity and shearing can influence thermoregulatory mechanisms and the productive performance and welfare of ewes. The high values of oxy-adsorbent and SHp values further demonstrate the compensatory response of the organism to the increase of free radicals (dROMs) inducing oxidative stress after shearing (Table1). Our results indicate that shearing causes a change in the ewes homeostatic balance that leads to oxidative stress. The road transportation in ewes appears to have an influence on the increase of catabolic reactions that leads to the onset of oxidative stress. The results of our study (Table1) showed an increase of dROMs, Oxy-adsorbent and SHp values in ewes after the road transportation in comparison with basal level ($P < 0.05$). The pattern of dROMs, characterized by a significant increase during post transport respect to, is in agreement with other researchers [(2)]. The increase of dROMs after 48 h rest time corresponds to approximately 95.85% and represents the energetic deficiency that occurs in ewes during transport period. The high values of Oxy-adsorbent and SHp during rest time further demonstrated the compensatory response of the organism to the increase of dROMs inducing oxidative stress successively to road transportation. During the different reproductive periods, lowest values of dROMs at the start of experimental period could be due to the energetic

deficiency which occurs in ewes during the last period of pregnancy. The significant increase of dROMs on days 40 and 200 of lactation and during dry period compared to values obtained on day 1 of lactation shows high oxidative processes which occur during lactation in ewes. The pattern of dROMs characterized by low values at the beginning of lactation and by a significant increase at the mid-point of lactation was previously observed in lactating ewes [3,4]. The high values of Oxy-adsorbent and SHp at the end of lactation document the compensative response of the organism to oxidative stress. Oxidative processes increased ($P<0.05$) at the end of milking period (Figure1), together with a compensative response of the organism to this stress and suggest the important role of oxidative status in dairy ewes. Our result showed that the high energetic requirements of milking period in ewes are directly proportional to free radicals formation and oxidative stress. The trigonometric statistical model of the single cosinor procedure indicated the existence of daily rhythm of dROMs, Oxy-ads and SHp in ewes with a nocturnal acrophase (dROMs=21:00; Oxy-ads.=21:30; SHp=21:15). All three markers showed a rhythm robustness above significance line (dROMs=81.5%; Oxy-ads.=79.1%; SHp=81.70%). In conclusion we can claim that there is a synergism between oxidative stress markers and the circadian rhythm of anti-oxidant power in ewes. The right zootechnics management and the formulation of ration that totally satisfy the requirements of the lactating subjects, seem o be essential in order to guarantee the productive status and the animal welfare.

Figure 1. Mean values with relative standard deviations and statistical significance of daily patterns of dROMs, Oxy-ads. and SHp observed during different reproductive status.

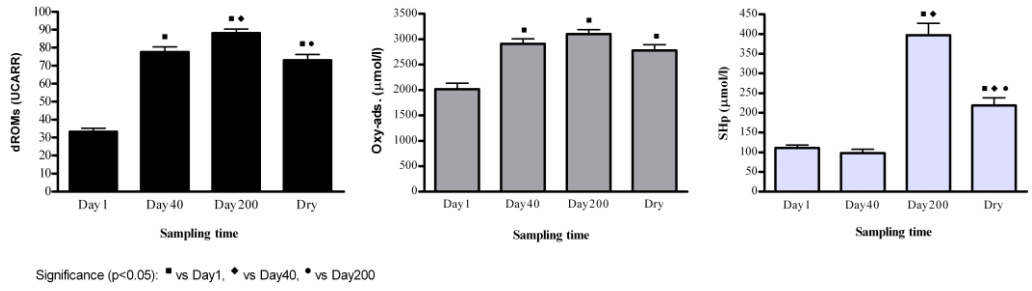

Table 1.

Patterns of mean values (±SD) of dROMs, Oxy-ads. and SHp and statistical significance changes about sampling time from pre-treatments.

Sampling time	**Parameters**					
	dROMs (UCARR)		Oxy-ads. (µmol/l)		SHp (µmol/l)	
	Shearing	Transport	Shearing	Transport	Shearing	Transport
before	31.80 ± 2.20	31.20 ± 2.03	1.930 ± 0.04	2.015 ± 0.14	102.70 ± 3.82	112.60 ± 2.58
after	33.80 ± 2.05	34.90 ± 2.09	1.939 ± 0.06	2.158 ± 0.18	104.80 ± 3.04	114.50 ± 2.25
after 8h	34.60 ± 1.93	36.40 ± 2.50■	1.941 ± 0.05	2.289 ± 0.18■	105.70 ± 5.06	125.70 ± 1.96■
after 1d	36.00 ± 1.74	48.10 ± 3.01■	1.957 ± 0.04	2.732 ± 0.16■	107.60 ± 3.32	146.30 ± 2.22■
after 2d	42.00 ± 1.49■	62.00 ± 2.94■	2.035 ± 0.04	2.892 ± 0.13■	117.80 ± 3.55	169.10 ± 2.95■
after 3d	45.60 ± 1.81■▲		2.179 ± 0.06■		156.60 ± 4.76■▲	
after 4d	55.00 ± 1.91■▲		2.335 ± 0.08■▲		210.60 ± 6.35■▲	
after 5d	70.20 ± 2.42■▲•		2.882 ± 0.08■▲		343.60 ± 4.72■▲	

Significance ($p<0.05$): ■ vs before; ▲ vs after, after 8h and after 1d; ♦ vs after 2d and after 3d; • vs after 4d.

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P90 - A 3D TOTALLY ASSORBABLE SYNTHETIC MESH IN ANTIREFLUX SURGERY: GORE BIO-A TISSUE REINFORCEMENT FOR HIATAL HERNIA REPAIRING

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Introduction
Hiatal hernia, defined as "transitory or stable dislocation of a part of the stomach in mediastinum through the diaphragmatic crura delimiting esophageal hiatus". Its appearance presupposes anatomic anomalies or weakening of structures and mechanisms able to maintain esophago-gastric junction and stomach in the abdominal cavity [1]. Classically hiatal hernia was classified in four types using Hill's classification: Type 1 hiatal hernia is associated with GERD in 50-90% of cases, in facts its presence gradually compromises esophago-gastric junction's continence favouriting the backwater of acid secretion and its reflux in contact with esophageal mucosa during transient relaxations of the LES and also reducing clearing systems overall for large hiatal hernias [2, 3]. Several randomized controlled trials with long-term follow-up comparing surgical with medical therapy for the treatment of GERD, strongly support surgery as an effective alternative to medical therapy [4]. Fundoplication has also been demonstrated to lead to improved or at least comparable quality of life to that of medically treated patients and it is associated with high patients satisfactions rate [5]. A laparoscopic total fundoplication is considered today the procedure of choice increasing the resting pressure and length of the lower esophageal sphincter, decreasing the number of transient LES relaxations and improving quality of esophageal peristalsis and follow-up demonstrates complete symptoms control in 80-90% of patients 10 years later [6]. However primary laparoscopic hiatal hernia repair is associated with up 42% recurrence rate [7]. Several level data suggest that mesh reinforcement of the crural closure for hiatal hernia repair decreases the recurrence of hernia, but can lead to esophageal erosion and stenosis or disphagya, above all non-absorbable mesh [8, 9]. For this clinical case, we experiment a new totally absorbable Gore Bio-A® mesh [10].

Materials and methods: Clinical Case
Female patient; 65-year old; 6-year classic history of GERD (regurgitation, belching, bloating, "acid in the throat" treated for several years by multiple proton pump inhibitors); BMI 22. An EGDS revealed a > 3 cm hiatal hernia, grade B Los Angeles esophagitis. 24-hour pH study was positive for acid reflux and esophageal manometry revealed LES intrathoracic dislocation. With laparoscopic 5-trocars approach, the hiatal hernia defect was identified and primarily repaired, by crural closure, with size 0 permanent suture (ETHIBOND). GORE BIO-A® Tissue Reinforcement was trimmed to fit the defect with a "U" shape cutout to accommodate the esophagus. It was secured using two absorbable sutures (VICYRL). At least A Nissen fundoplication was performed without incident. Result: Gore BIO-A® mesh was easily placed through a 10-12 mm trocar. It had good handling characteristics laparoscopically, and no pre-operative preparation was required of the prosthetic. It can be cut and tailored intraoperatively to an optimal adaptation. There were no short-term complications from the mesh. The patient had not significant post-operative sequelae.

Conclusion
Crural closure reinforcement during hiatal hernia repair can be done readily with this new totally absorbable Gore Bio A Tissue Reinforcement: it is a 3D web of completely absorbable synthetic

polymers replaced by soft tissue over six months; it is a mix of glycolic acid and trimethylene carbonate and its function consistes in stimulating collagens deposition and ingrowth of new connective soft tissue [11]. It was demonstrated that Gore Bio-A increases cellular in-growth in 7-30 days more and more previously than biologics mesh; it also increases new blood vessels formation in 7-14 days reaching the greatest vascular in-growth. Instead the biologic meshes gore BIO-A seems to induce the least inflammatory infiltrate. Gore BIO-A tissue reinforcement seems to have all the best characteristics to hernia hiatal laparoscopic repair reducing both recurrence rates and post-operative mesh-related complications, even if several other cases and studies are necessary. However further data and studies are needed to evaluate long-term efficacy and complications associated with its use.

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P91 - INVESTIGATING THE GENESIS OF INGUINAL HERNIA TO FINALIZE A NEW CONCEPT OF REGENERATIVE 3D SCAFFOLD FOR PROSTHETIC REPAIR

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The genesis of inguinal hernia still represents a dilemma. The unacceptable high rate of complications and recurrence seems to be a consequence of the lacking knowledge in the underlying genesis of inguinal hernia which, to date, remains undefined. Surprisingly, in the literature very few reports deal with the structure damages of the inguinal region affected by groin protrusion.[1, 2, 3, 4] To fill this lack, was organized an extensive study focused to the detection of histological changes of the herniated inguinal wall. Following a specific method, tissue samples were excised for histological study in 30 fresh male cadavers with inguinal hernias, from structures close to the hernia orifice. 15 cadavers without hernia served as control. The excised tissue samples demonstrated many histological damages. Among these, a wide-ranging inflammatory infiltrate composed by limpho-histiocytic and plasmacellular clusters. Structure damages also involved the vascular, nervous and muscolar components (Fig. 1). The impact of these injuries on the physiology and kinetics of the groin, suggests the following scenario to be a realistic one:

- Degenerative damages of motor nerves and thickened myelin sheath could reduce motility, leading to muscle atrophy and to a weakened contractile response to visceral impact, when abdominal pressure arises.
- An additional weakening effect is consequent to artery sub-occlusion and obstruction, source of ischemic degeneration of the groin structures.
- Venous congestion, vein fibrosis and inflammatory infiltrate could embody the outcome of chronic compression exerted by the abdominal viscera, followed by tissue edema and impaired metabolism.
- Hyaline degeneration, fibrosis and fatty muscle dystrophy could represent the result of the damages of the vascular and nervous structures.

These multifactorial muscular injures are probably amplified by the direct compression of the viscera upon the lower abdominal wall. The weakening of the inguinal area and the consequent hernia protrusion are the effects. This knowledge resulted very useful in developing more physiologic hernia repair concept. Conventional prosthetics used for inguinal hernia repair are static and passive. They do not move in harmony with the dynamic elements of the groin structure and, as a result, induce the ingrowth of thin scar plates or shrinking regressive tissue that colonizes the implants. These characteristics may be a contributing factor for recurrences and patient discomfort [6, 7, 8]. Therefore, to improve results of hernia repair by respecting the physiology and kinetics of the inguinal region a new type of prosthesis was design. The intent was to fill the hernia defect by using a 3D polypropylene implant, which should induce a more structured tissue ingrowth similar to the natural biologic components of the abdominal wall. This prosthetic device was specifically designed to be placed with no point fixation. A secondary benefit of this “dynamic” design is that the implant moves in a three-dimensional way in unison with the movements of the myotendineal structures of the groin. The implant was tested in porcine experimental model, delivered in the groin of pigs and then removed 1, 2, 4 weeks and 3, 6 and 8 month after placement. To detect the quality of the ingrown tissue, the excised implants were histologically studied. The results showed that the three-dimensional structure of the device not only acted as a suitable scaffold for a full thickness ingrowth of a tissue barrier but also seemed to induce an ordered, supple, elastic tissue,which allows for neorevascularization and neoneural growth (Fig. 2). Concluding, under the perspective of the pathogenesis the study

confirms that addressing the genesis of a disease is essential for developing a suitable therapeutic strategy. In addition, the depicted experimental experience with the newly developed prosthetic device demonstrates that a different arrangement of the prosthetic design,even if same polypropylene material is used, totally modifies the response. In few words, changing the design and the attitude of the implant, from flat to 3D and from static to dynamic responsive, is sufficient to achieve a regenerative biologic scaffold instead of a shrunken stiff fibrotic scar plate that impairs the groin movements, causing discomfort and pain.

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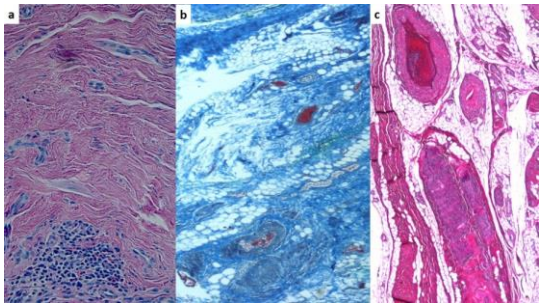

Figure 1 - Degenerative damages of the inguinal tissue close to the hernia opening excised in cadavers. a: wide-ranging muscle hyalinosis and fibrosis in a surround of noteworthy inflammatory infiltrate composed by lymphohistiocytic clusters. Hex20 – b: Severe muscle dystrophy and stromal fibroadipousdystrophy. The media of the arteries are evidently thickened. Venous congestion. Azan-Mallory x 2.5 - c: Large nervous trunk showing clear degenerative fibrotic dystrophy and fatty substitution of the axons in a surround of adipous dystrophy of the muscle fibers. Artery with endoluminal thrombus, arterial sub-occlusion due to media hyperplasia. Hex10

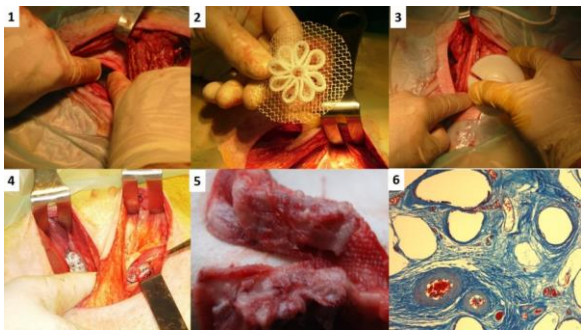

Figure 2 - Photographic sequence of the experimental attempt in porcine model. – 1: Inducing an inguinal hernia in the animal groin through fingerforced dilation of the whole inguinal floor. - 2: The 3-D polypropylene implantwith its multilamellar structure, which includes a preperitonealflat disk. – 3: Delivering the dynamicimplant during the experimental trial by means of a specific delivery device. – 4: Dynamic implants delivered within the both groins of the animal. Of note the self-retaining feature of the prosthesis. – 5: the implant excised after 8 months. Macroscopically the prosthesis appears fully colonized by soft, viable and well-vascularized incorporation tissue. – 6: The histology confirms the ingrowth and slack well-hydrated connective. A great amount of mature vascular structures is clearly detectable. Azan-Mallory x 10

P92 - MECHANICAL DEFLECTION OF MACROVIBRISSAE INDUCES IN RATS ACTIVATION OF TRIGEMINAL MESENCEPHALIC NUCLEUS NEURONS

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The sensory system of rodent vibrissae has been extensively analysed to understand how these animals may successfully explore the nearby environment, detect objects and orient their behaviour using the macrovibrissae. Recent data (1) showed that besides the Gasser's ganglia neurons also the trigeminal mesencephalic nucleus (Me5) appears significantly involved in the sensory perception of the whisker pad structures. In fact: i) Tracer injection into mystacial pad of the rats significantly labelled the Me5 neurons and ii) electrophysiological experiments showed that Me5 neurons are responsive to spontaneous movement of the macrovibrissae. It has been proposed that Me5 neurons may be involved in relaying kinetic information to the CNS.
The present study was performed to better clarify the functional role of the Me5 sensory innervation of the whisker pad structures by analysing the electrophysiological responses of Me5 neurons to mechanical deflection of the macrovibrissae.
The spontaneous electrical activity of the Me5 neurons, identified by their responses to the masseter muscle stretch, was extracellularly recorded using tungsten-in-glass microelectrodes (impedance 700-900 KΩ) carefully advanced into the Me5 by an electronic microdrive (David Kopf). The electrical signals were relayed to conventional preamplifiers connected to oscilloscopes and then to computers for the specific analysis (Tecfen computerscope analysis ISC-16 software, and PowerLab 4/30 Chart 5, V 5.4.2 software). The spontaneous electrical activity of the Me5 neurons multiunit activity (MUA) was continuously monitored and recorded under resting conditions (i.e. vibrissae motionless), during the masseter muscle stretch, and during/after the mechanical deflection of the vibrissae bundle, which was performed in four principal directions (forward/backward, backward/forward, up/down and down/up) using a delicate glass rod connected to a craft-made electronic drive.
The electrophysiological results demonstrated that mechanical deflection of the macrovibrissae induced a significant increase in the spontaneous electrical activity of the Me5 neurons as well as significant changes in the spontaneous firing pattern. It appeared that Me5 neurons could be activated in response to macrovibrissae deflection in specific directions.
The results of the present and previous experiments, allow us to conclude that in addition to the neurons connected to the masticatory muscles (2) and those connected to the periodontal ligament (3), the Me5 enclose primary neurons specifically dedicated to encoding kinetic information related to vibrissae movements.
It is known that the central terminals of Me5 neurons join the brain stem trigeminal sensory nuclei, which in turn receive, by the primary Gasser's ganglia neurons, tactile information from macrovibrissae receptors. If so, it is possible to hypothesize that the brain stem trigeminal nuclei may deduce higher-order information by combining touch information from macrovibrissae receptors, and carried out by afferents from the Gasser's neurons, with kinetic information arising from the Me5 neurons. The integrated information can be then relayed to more rostral SNC structures to allow a central reconstruction and representation of the spatial displacement of the macrovibrissae.

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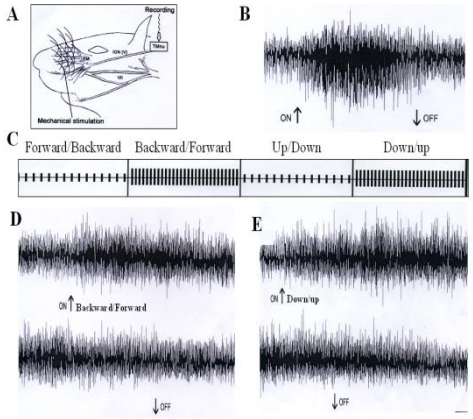

Fig. 1

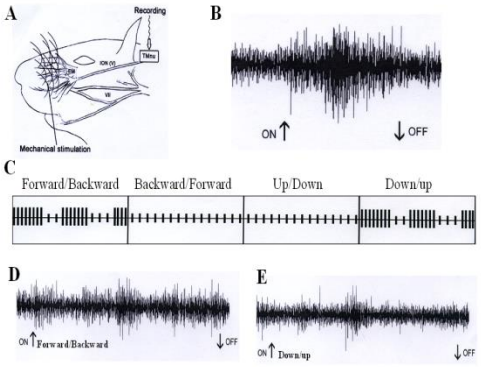

Fig.2

Figs. 1, 2 - Multiunit electrical activity (MUA) of trigeminal mesencephalic neurons (Me5) recorded in the medial-caudal part of the Me5 during mechanical deflection of the macrovibrissae. A: Schematic drawings of the experimental set up; B: specimens showing the Me5 neurons response to mechanical stretch of the masseter muscle to functionally identify the Me5; C: diagrams resuming the four principal directions used during mechanical deflection of the vibrissae bundle together with the schematic pattern of the correspondent Me5 MUA response. D, E: show the spontaneous electrical activity of the Me5 neurons during mechanical deflection of the macrovibrissae bundle.
Fig. 1- D: tonic increase of Me5 MUA, although with adaptation, to vibrissae deflection in back/forward direction. E: tonic increase of the same Me5 MUA to vibrissae deflection in down/up direction, the increased activity lasted throughout the stimulus application as well as several seconds after its end.
Fig. 2 - D: bursts of Me5 MUA, in response to vibrissae deflection in forward/backward direction. E: the same Me5 MUA responded with bursts, but at longer latency and rapid adaptation, to vibrissae deflection in down/up direction.
ON, OFF indicate the beginning and the end of vibrissae displacement. Horizontal calibration: 1 sec; vertical calibration: 1 mV.

P93 - COULD A WRONG CONSUMPTION OF CEREALS INFLUENCE PRETEENS OBESITY IN SICILY?

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Cereals are an important source of carbohydrates in the human contributing to a certain extent also to the need of protein. They constitute a rich source of both nonstarch polysaccharides (dietary fiber) and starch, which together comprise 70–77% of kernel.
The Guidelines for a Healthy Diet in Italy (1) define the opportunity to take, on a daily basis, at least 60 % of energy from carbohydrates, and particularly 45% from complex carbohydrates. In Italy, especially in Sicily, these nutrients are contained in traditional food as pasta and bread made starting from durum wheat as grow material. Recently, especially in the bigger towns, there is also widespread consumption of bread made with soft wheat (2) (3).
In the last decade, consumer behavior is changing in Italy (4) and it is clear a trend of decline in consumption of pasta and bread, instead there is an opposite trend for the consumption of products considered as a substitute for bread (brioches, crackers , biscuits, and other baked goods). It is evident how the nutritional profile of the latter products deviates from the bread due to the fat content, that ranges from 5 to 20 %. It is also known as the derivatives of durum wheat , preferably whole grain products , are also characterized by a lower glycemic index than products of soft wheat.
So cereals are a key component to set a proper diet. Some studies also report that influence of a regular and correct consumption of cereals may contribute to reach and to maintain " normal weight" (5).
In 2011 was carried out investigations with the aim to understand better the relationship and the presence of cereals in the diet of preteen. Moreover, it is known as in Sicily , as well as other areas of southern Europe, there is a serious problem of childhood obesity (6) (7) , mainly related to lifestyles considered incorrect and a lower level of education of the population than other European areas that do not registers this phenomenon .
The investigation involved a sample of 1335 subjects, aged between 10 and 13 years, identified through the involvement of 62 schools distributed throughout the region. The sample involved in the investigation was extrapolated according to a stratification that has taken into account the size of the population under study, age ,sex, and distribution by province. For each individual was recorded during the first half of December 2011, thanks to personnel appropriately trained to collect information in a standardized way, a 24-h recall questionnaire (noting the foods eaten in the last 24 hours from the individual). In addition to the parents were asked to complete a questionnaire on the frequency to record information on lifestyle, frequency of intake of cereals. For each individual was recorded weight (kg), height (cm) and body mass index (BMI, Body Mass Index), according to the standards proposed by the International Obesity Task Force (8).
About frequency of the conditions of the weight of the individuals in the sample, the results have confirmed what was already mentioned by other authors for Sicily (6) (7).
Results of the study shows as the population sample, in the various daily meals , have not a correct relationship with cereals, considering the models known for the Mediterranean population. In particular, it is clear the high frequency of subjects who have not took any kind of cereals at breakfast (n = 699, 52 %) or at morning-snack (n = 396 , 30 %) . Moreover 17% of individuals (n = 228) has neither taken any kind of breakfast cereal nor at morning-snack; these individuals have access to the primary source of complex carbohydrates, needed daily, only with lunch.

The individuals who regularly eat pasta are characterized by a lower Body Mass Index than those who do not consume habitually; the same is for those who regularly take breakfast, according as noted by other authors in specific studies conducted in the Mediterranean basin (9), and for individuals who have eaten at least one type of cereal for breakfast.
Regarding this survey it can be stated that further studies are needed to define the extent to which an erroneous presence and distribution in diets of cereals can affect overweight and obesity, particularly among younger age groups of the population. It is evident, however, that it is more necessary than ever to support an effective education campaign that fill the gaps on nutritional knowledge on cereals; contribute to implement pathways on enhancement of cereals mainly on nutritional point of view, with the aim to promoting correct lifestyles and diet patterns better oriented to limit the risks of occurrence in the population of chronic degenerative diseases related to obesity.

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P94 - AMINO ACID PROFILE OF FOUR VARIETIES OF DURUM WHEAT GROWN IN SICILY

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The major use of durum wheat is for pasta products , particularly in the European and North American countries, whereas in other areas (Middle East and North Africa) also it is used as couscous and for various types of bread. The durum wheat is therefore a staple food in the Mediterranean and is one of the commodities that characterize the dietary pattern of the "Mediterranean diet".
During the year 2012 were made in Ciminna (Palermo) in Sicily, four fields of experimental cultivation using four different varieties of durum wheat cultivated in Sicily: Simeto, Iride, Duilio and Saragolla . For each variety were set up two different kinds of cultivation tecniques, in order to verify the influence of the environment on the final results.
In particular, the technique "A" employed the same seeding density for the 4 varieties (2.4 quintals of seed per hectare), using the pre-sowing and coverage fertilization with respectively 30.6 and 47.25 units of nitrogen, distributed by the use of diammonium phosphate (18 /46) in pre-sowing and ammonium nitrate (27 %) in coverage; "technique B" employed a seeding density lower for 4 varieties (variable between 1.8 and 2.2 quintals of seed per hectare); fertilizing in pre-sowing was carried out with sodium superphosphate (that means zero units of nitrogen) and in coverage with 101.79 units of total nitrogen, distributed through the use of ammonium nitrate (27 %) used in two different moments after the crop raised.
The results show that the protein content of the different batches analyzed stood between 12.9 and 16.1 % D.M., higher than the average recorded for the Sicilian crops (1).
Investigations on the amino acid profile showed some differences between the different varieties and also between the different cultivation techniques (Table 1).
The most represented amino acid was glutamic acid which is also the one who showed greater variability among the four varieties. Even proline showed a variability in the context of the four varieties evaluated . The other amino acids showed slight or no differences among the four varieties evaluated .
The survey results are partially comparable with the data available in the literature for other varieties grown in Italy (2) (3) (4).

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Table 1. Average values and standard deviation in amino acids content in four varieties of durum wheat grown with two different cultivation techniques in Sicily.

For the determination of tryptophan was used the method EN ISO 13904: 2005 (Hydrolysis in a basic environment). For all other amino acids was used the method ISO 13903: 2005. The values are reported in g/100 g of protein (SD = standard deviation).

Amino acid	**Agronomic tecnique A**		**Agronomic tecnique B**	
	g/100 g of protein	**SD**	g/100 g of protein	**SD**
Alanine	**3,4**	0,06	**3,3**	0,07
Arginine	**4,5**	0,14	**4,4**	0,11
Aspartic Acid	**4,6**	0,12	**4,6**	0,14
Glutamic Acid	**27,1**	0,75	**27,4**	0,81
Glycine	**3,6**	0,04	**3,5**	0,04
Histidine	**2,3**	0,02	**2,3**	0,03
Isoleucine	**3,4**	0,05	**3,3**	0,04
Leucine	**6,6**	0,08	**6,6**	0,16
Lysine	**2,7**	0,08	**2,6**	0,09
Phenylalanine	**4,4**	0,13	**4,4**	0,10
Proline	**9,4**	0,30	**9,5**	0,28
Serine	**4,5**	0,12	**4,5**	0,05
Threonine	**2,7**	0,07	**2,7**	0,07
Thyrosine	**2,7**	0,07	**2,7**	0,07
Valine	**4,3**	0,12	**4,2**	0,11
Thryptophan (basic hydrolysis)	**1,3**	0,04	**1,2**	0,07
Cystine + Cysteine	**2,1**	0,07	**2,1**	0,09
Methionine	**1,7**	0,17	**1,8**	0,04

P95 - ANALYSIS OF INTERACTION BETWEEN MESSENGER RNA ENCODING H3.3 HISTONE VARIANT AND PIPPIN PROTEIN BY BIOLAYER INTERFEROMETRY

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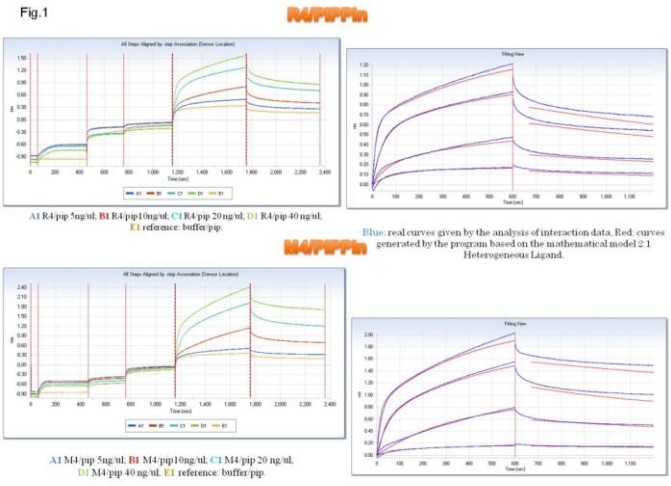
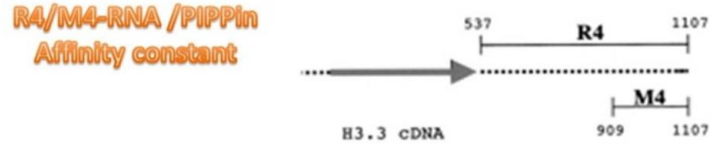
A combination of biochemical and cell biological studies have led to the current notion that each mRNA is bound by multiple RNA binding proteins (RBPs) (1) and that, conversely, individual RBPs have hundreds of mRNA targets (2). Furthermore, RBPs are often components of multi-protein complexes and bring additional proteins into messenger ribonucleoprotein particles (mRNPs) through protein-protein interaction (3). Among RBPs, the cold-shock domain (CSD)-containing (or Y-box) proteins, play a key role in controlling the recruitment of mRNAs to the translational machinery, in response to environmental cues, both in development and in differentiated cells.
PiPPin, highly enriched in the rat brain, is an RBP containing two putative double stranded RNA-binding domains (PIP1 and PIP2) and a central CSD. It binds with high specificity the mRNAs encoding H1° and H3.3 histone variants (4,5), undergoes thyroid hormone-dependent sumoylation (6), and has been recently demonstrated to interact with other RBPs (7).
The aim of this study was to confirm histone mRNA-PIPPin interaction and to describe binding properties trough streptavidin-biotin conjugation method. This method is based on the RNA aptamer sensor system, that uses a biotinylated RNA oligonucleotide on an Octet optical biosensor. Biolayer interferometry (BLI) is the underlying technique that measures changes in an interference pattern generated from visible light which is reflected from an optical layer and a biolayer containing proteins of interest. The BLI format is based on a disposable sensor that is immersed in 96-well or 384-well plates. BLI has been validated for small molecule detection and fragment screening with model systems and well-characterized targets where affinity constants and binding profiles are generally similar to those obtained with surface plasmon resonsance (SPR).
Here we report the data obtained in the case of H3.3 mRNA-PIPPin interaction (Fig 1), and the specific affinity constant for this binding (Fig 2). In order to identify RNA portions involved in binding, we used two RNA probes: **R4**, which corresponds to the whole 3'-untranslated region (3'-UTR) of the H3.3 mRNA, and **M4**, which corresponds to the last 198 nucleotides of the 3'-UTR of the same mRNA.

Fig.1



The real-time binding data shown in Fig 1 for R4/PIPPin- and M4/PIPPin- interaction, respectively, suggest that both the association and dissociation phases are biphasic processes: an initial short and steep increase in signal intensity is followed by a long flat increase in signal intensity. At the highest analyte concentrations steady-state is not reached. Binding curve fitting was done using the 2:1 heterogeneous ligand (hl) equations, which assume that there are at least two populations of immobilized ligands that differ in their ability to bind the analyte and/or heterogeneity of the ligand itself; as a consequence, the binding curves are described by two reactions with different rates, KD and KD2 for M4; KD and KD2 for R4 (Fig 2). Some of the factors that can cause deviations from pseudo-first order approximation of binding data include: mass transfer effects, immobilized ligand density, lack of homogeneity of immobilized ligand or soluble analyte, immobilization chemistry, and rebinding of dissociated analyte. Residual plots derived from curve fitting in Fig 1 show, in general, very small (less than 10% of the response) and random residuals, supporting the use of the 2:1 hl-fitting model for the data. Only a minor deviation from the experimental data were observed for the initial fast step of both the association and dissociation phases; however, *R*2 values were above 0.9 and $\chi 2$ values below 2.0 for all the fits. We used as negative controls PIPPin protein without mRNA and PIPPin protein with a control RNA, to better understand the real binding. In summary, we were able to confirm that PIPPin binds H3.3 mRNA with very high affinity (KD $2E^{-08}$, Fig2).

Fig.2



M4

	KD (M)	KD2
A1	1,81E-08	3,23E-09
B1	1,15E-08	2,74E-08
C1	1,07E-08	2,31E-08
D1	5,18E-08	1,78E-08

R4

	KD (M)	KD2
A1	8,37E-10	8,61E-09
B1	9,18E-10	9,69E-08
C1	6,26E-09	4,46E-08
D1	5,43E-09	1,37E-08

M4: **A1** M4/pip 5ng/ul; **B1** M4/pip10ng/ul; **C1** M4/pip 20 ng/ul; **D1** M4/pip 40 ng/ul; **E1** reference: buffer/pip.

R4: **A1** R4/pip 5ng/ul; **B1** R4/pip10ng/ul; **C1** R4/pip 20 ng/ul; **D1** R4/pip 40 ng/ul; **E1** reference: buffer/pip.

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P96 - EXTRACELLULAR VESICLES CAN SHUTTLE MOLECULES AMONG BRAIN CELLS

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Both tumor and normal brain cells release membrane vesicles (MVs) into the extracellular space (1-3). MVs shed by astrocytes and neurons contain FGF2 and VEGF, which could be responsible for inducing endothelial cells to form the blood-brain barrier (2,3). MVs shed from G26/24 oligodendroglioma cells, when added to primary cultures of rat cortical neurons, inhibit neurite outgrowth, and induce apoptosis in about 75% of the cells (1). The same amount of shed MVs induce apoptosis in about 40% of cultured astrocytes (4). The analysis of G26/24 vesicles demonstrated the presence of Fas Ligand and TRAIL, which could cooperate in inducing brain cell death (1,4). The horizontal transfer of labeled proteins from oligodendroglioma cells to astrocytes in culture was also demonstrated (4).
Since MVs were shown to transfer RNA, and, on the other hand, some RBPs are involved in tumorigenesis, we searched for RBPs in MVs. A preliminary analysis in A375 melanoma cells revealed the presence of at least three RBPs, with apparent MW of about 64, 50 and 36 kDa, respectively. These proteins are able to bind H1° mRNA.
In developing rat brain, the amount of histone H1° increases during neuronal differentiation, while the level of the corresponding mRNA decreases, suggesting that H1° gene expression is mainly regulated at the post-transcriptional level (5).
Similar results were obtained with cultured astrocytes, while G2624 maintain high levels of both H1° protein and mRNA. Moreover, oligodendroglioma cells, but not astrocytes, release H1° protein into the culture medium by shedding MVs (6). These findings suggest that oligodendroglioma cells can escape antiproliferative cues by discharging into the extracellular environment molecules expressed concomitant with differentiation, such as H1° histone.

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P97 - VALUTAZIONE DELLA TERMOPORAZIONE DINAMICA IRREVERSIBILE (DIT) COME STRUMENTO PER L'ABBATTIMENTO DELLA CARICA BATTERICA IN MATRICI ALIMENTARI

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Introduzione

La Termoporazione Dinamica Irreversibile (DIT) consiste in un nuovo tipo di tecnologia di pastorizzazione ideato da Koulik et al. [1] basato su un processo termico dinamico, caratterizzato da riscaldamenti molto rapidi (shock termici) a temperature finali non superiori a 70 °C.
L'obiettivo del nostro studio è stato la valutazione dell'influenza di parametri di processo quali la temperatura di partenza (T_1) del campione da trattare, la temperatura finale in corso di trattamento (T_2), la differenza tra temperatura iniziale e temperatura finale (ΔT), la velocità di riscaldamento (θ) e la durata del trattamento sull'abbattimento della carica batterica nella matrice alimentare.

Materiali e metodi

I test sono stati effettuati su ceppi di riferimento di microrganismi di possibile interesse alimentare: *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus hirae*, *Listeria innocua*. La sospensione test è stata preparata poco prima dell'esecuzione dei test con le seguenti composizioni: 10% sospensione microbica (10^8 CFU/ml) in acqua peptonata, da una coltura fresca (18-24 ore); 10% sostanza interferente (albumina bovina 0,3 g/L o saccarosio 10 g/L); 80% acqua dura (calcio e magnesio) [2]. I test DIT sono stati effettuati fissando per i parametri velocità di riscaldamento, temperatura iniziale e temperatura finale i seguenti valori:
DIT 1. θ 20°C/s ; T_1 30°C; T_2 60°C (ΔT 30°C)
DIT 2. θ 20°C/s; T_1 30°C; T_2 65°C (ΔT 35°C)
DIT 3. θ 30°C/s; T_1 30°C; T_2 60°C (ΔT 30°C)
DIT 4. θ 30°C/s; T_1 30°C; T_2 65°C (ΔT 35°C)
È stato anche saggiato l'effetto di un prolungato mantenimento della temperatura T_2. A fine trattamento, aliquote di ciascun campione sono state seminate in piastre di terreno solido. La lettura è stata effettuata dopo 24 e 48 ore di incubazione a 37°C. L'attività battericida è stata definita come la dimostrazione di una riduzione di almeno 5 log (logR>5) rispetto alla carica batterica iniziale.

Risultati

Le tabelle 1 e 2 riportano l'efficacia dei trattamenti DIT su ciascun microrganismo. I trattamenti con T_2 = 60°C e ΔT=30°C (DIT1 e DIT3) hanno dimostrato efficacia solo quando seguiti dal mantenimento della temperatura T_2. Questo risultato è stato osservato su *P. aeruginosa* e meno costantemente su *E. coli*. I trattamenti con T_2 = 65°C e ΔT=35°C (DIT2 e DIT4) sono risultati efficaci su tutti i microrganismi. Su *E. coli*, *L. innocua*, *P. aeruginosa e S. aureus* logR>5 è stata ottenuta anche senza mantenimento della temperatura T_2, e indipendentemente dalla sostanza interferente (Tab. 1). Diversamente, per *E. hirae* logR>5 è stata ottenuta solamente sui campioni trattati con DIT2 o DIT4 seguiti da un prolungato mantenimento di T_2 (Tab. 2). L'efficacia dei trattamenti è stata più marcata e costante per la sospensione contenente saccarosio.

Discussione

Il trattamento DIT è risultato efficace nel ridurre la carica batterica iniziale di almeno 5 log su tutti i ceppi batterici esaminati con almeno uno dei protocolli testati. Tra i parametri di processo valutati, la temperatura T_2 sembra avere influenzato notevolmente l'efficacia del trattamento, unitamente alla differenza tra temperatura iniziale e temperatura finale (ΔT) del processo DIT. Infatti, gli obiettivi DIT con T_2 = 60°C e ΔT=30°C (DIT1 e DIT3) non si sono dimostrati efficaci su nessuna delle specie batteriche testate; al contrario, gli obiettivi DIT con T_2 = 65°C e ΔT=35°C (DIT2 e DIT4), hanno determinato un abbattimento della carica batterica > 5 log per tutte le specie, ad eccezione di *E. hirae*, la cui resistenza ai trattamenti termici è nota in letteratura [3,4]. *E. hirae* è risultato sensibile ai trattamenti DIT 2 e DIT4 solo se seguiti dal mantenimento della temperatura T_2 e in presenza di saccarosio. Per questo microrganismo, in particolare, sarebbe opportuno ottimizzare il trattamento DIT 2 e 4 (ΔT=35°C) cercando di ridurre al massimo il tempo di mantenimento della T_2. La velocità di riscaldamento (θ) e le due sostanze interferenti utilizzate nella preparazione della sospensione test non hanno generalmente influenzato la suscettibilità delle specie batteriche ai trattamenti DIT.

In conclusione, questi test preliminari suggeriscono che il processo DIT può essere efficace nell'ottenere l'abbattimento della carica batterica in un substrato liquido. I parametri che influenzano maggiormente l'efficacia del processo sembrano essere quelli puramente termici: ΔT e T_2. Ulteriori sperimentazioni tenderanno ad adattare i parametri di processo alle esigenze della produzione a livello industriale.

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Tabella 1. Trattamenti DIT efficaci su 4 ceppi batterici di interesse alimentare. Numero di test efficaci/numero di test effettuati.

Microrganismo testato	**DIT1+mant.**		**DIT3+mant.**		**DIT2**		**DIT4**	
	A	**S**	**A**	**S**	**A**	**S**	**A**	**S**
E.coli	**1/4**	**1/4**	**1/4**	**0/4**	**4/4**	**4/4**	**4/4**	**4/4**
L.innocua	**-**	**-**	**-**	**-**	**4/4**	**4/4**	**4/4**	**4/4**
P.aeruginosa	**2/4**	**4/4**	**4/4**	**3/3**	**4/4**	**4/4**	**3/4**	**4/4**
S.aureus	**0/4**	**0/4**	**0/4**	**0/4**	**5/5**	**5/5**	**5/5**	**5/5**

A= albumina; S=saccarosio

Tabella 2. Trattamenti DIT efficaci su *E. hirae*. Numero di test efficaci/numero di test effettuati.

Microrganismo testato	**DIT2 + mant.**		**DIT4+ mant**	
	A	**S**	**A**	**S**
E.hirae	**0/4**	**3/4**	**1/4**	**4/4**

A= albumina; S=saccarosio

P98 - EFFECTS OF OVERLOADED STRENGTH TRAINING ON VERTICAL JUMPING PERFORMANCE IN YOUNG VOLLEYBALL PLAYERS

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The performance of vertical jump is one of the most commonly used parameters for the evaluation of the explosive force in volleyball. Vertical jumps are performed frequently by volleyball players in games and practices (1,2). Indeed, in a lot of defensive and offensive drills, volleyball players are required to jump vertically as high as they are capable. It is known in literature that volleyball players performe about 100/150 jumps in a match according to the sets played and their role (3). For these reasons strength training is a key factor to improve the physical performance and to prevent injuries in volleyball players. The purpose of our research was to study the acute effects of training with overloads in young female volleyball players (VP) on the vertical jumping performance. To determine performance characteristics of VP, test batteries were administered. Ten young female volleyball players (age: 14±1 yrs, BMI: 21.52±3.84, height: 1.65±0.03 m, weight: 58.33±10.25 kg) were included in our study. Before starting the experiment, all subjects were familiarised to test procedures and exercises included in the training program. After the familiarization period athletes were subjected to three experimental training sessions (ETS): ETS-A without overload, ETS-B with 10% of body weight (BW) overload and ETS-C with 20% of BW overload. A standard training protocol (exercises, recovery times, used technics, sets and repetitions) was used; however we increased the overload by 10% and 20% of BW in ETS-B and ETS-C respectively. We used ETS-A as control. All the evaluations were carried out before and after each ETS. Four recovery days were included between two performed ETS.
For the evaluation of the lower-body power, subjects were instructed to perform each jump with maximal effort. During the CMJ-AS, participants performed a single vertical jump using a countermovement from the standing upright with the arms down the sides. For the CMJ, subjects performed a single jump using a countermovement from stood upright with their hands on their hips. In the SJ, subjects started in a half squat position with their hands on their hips. For SJ and CMJ, subjects were instructed to keep their hands on their hips throughout the entire jumps (4). All jumps were executed on an optoelectronic platform consisting of 2 bars placed opposite to each other and connected directly to PC via serial port (Optojump, Microgate S.R.L., Italy). The optojump device transmitted an infrared light 1 to 2 mm above the floor and when the light was interrupted by feet, the units triggered a timer with a precision of 1 ms, which allowed the measurement of flight time and contact time.
We showed that subjects after each ETS decreased vertical jumping performace compared to baseline in SJ, CMJ and CMJ-AS. In particular we found a significant reduction of flight time in the studied subjects, in response to ETS-C in all jumping tests. All the three training sessions appear to be valid and usable at a young age. We showed that ETS B could be more effective than the other two studied ETS in agreement with Bosco's study (1985), that showed a higher recruitment of elastic component of muscles in response to a strength training program at low intensity (5).

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P99 - *N*- VALPROYL-AMINOACIDS AS NEW POTENTIAL ANTIEPILEPTIC DRUGS: SYNTHESIS, CHARACTERIZATION AND *IN VITRO* STUDIES ON STABILITY

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Epilepsy, affecting at least 50 million persons worldwide, is one of the most common neurological disorders.
Despite the significant advances in understanding epileptogenic mechanisms and in counteracting their pathological consequences, this clinical condition still has to be faced of treating more effectively the symptoms (epileptic seizures) and of preventing their unfavourable evolution. So far, research has been unsuccessful involved in developing effective antiepileptic drugs (AEDs) capable of preventing the development of the pathogenic process, set in motion by different etiological factors, that leads ultimately to chronic epilepsies .[1, 2]
So, a substantial need remains to develop new AEDs with better safety, less toxicity, and higher efficacy [3, 4].
Valproic acid, VPA, is one of the four most widely prescribed AEDs. Besides its wide use in both generalized and partial epilepsies, VPA has also gained widespread use in recent years for the treatment of bipolar disorders, neuropathic pain and for prophylactic treatment of migraine. [5,6]
However the use of VPA is limited by two rare but potentially life-threatening side effects, hepatotoxicity, induced from the formation of metabolite(s) with a terminal double bond, specifically 4-ene-VPA, [7] and teratogenicity, associated with the parent compound itself [8].
In a previous work we reported the synthesis of aminoacidic ester derivatives of VPA as resulted of chemical conjugation of VPA with esters of essential neutral aminoacids, with the aim of modifying the physicochemical properties relevant to bioavailability, such as solubility or lipophilicity, improving the efficacy and reducing unwanted side or toxic effects of VPA [9]. We had reported also the synthesis of N-valproyl-L-tryptophan, that has shown adequate physicochemical characteristics to permeate biological membranes and antiepileptic activity at lower concentration than VPA. [10,11]
In this paper, we focused our research on synthesis and characterization of new aminoacidic compounds with potential antiepileptic activity: N-Valproyl-L-Leucine (ValLeu), N-Valproyl-L-methionine (ValMet) and N-Valproyl-L-Histidine (ValHist).
The conjugation could consent to obtain VPA derivatives, lacking of structural characteristics usually implicated on VPA teratogenicity, and avoiding formation of possible hepatotoxic metabolites.
The aminoacidic derivatives of VPA was successfully obtained covalent linking carboxyl group of drug with aminic group of L-aminoacids, by synthesis involving two main steps. The first step, described in our previous work [9] was modified by adding DMAP as further coupling agent together with DCC. The structures of obtained compounds were assigned on the basis of respective analytical data-sets, FT-IR, MS and ^{1}H and ^{13}C-NMR spectral data.
Since the drug lipophilicity is an important factor conditioning brain uptake, the apparent partition coefficient (*P*app) could be used as simple descriptor of ability to cross the BBB: values of log *P*app within -0.2 to 1.3 have been described as optimal for cerebral transport; on the other hand higher values than these could reduce the rate of transport inside the membrane [12, 13].
Apparent partition coefficient (P_{app}) of ValLeu, ValMet and ValHist were determined in *n*-

octanol /phosphate buffer pH 7.4 solution and expressed as Log P_{app}. The determined Log P_{app} resulted
-0.11,- 1,02 and -1,61 respectively. The Log $D^{pH7.4}$ values indicate that ValLeu, ValMet are adequate to cross biological membranes and in particular BBB barrier while ValHist value is too low, probably due to the fact that was obtained as hydrochloride.
Compared to others drug administration routes, the oral one remains the most preferred as it implies ease of administration as well as high patient *compliance*. However, the transit through the gastrointestinal tract could constitute a limiting step to bioavailability as a consequence of degradation correlated to the environmental pH. In view of a possible administration of ValLeu, ValMet and ValHist by oral route, studies on their chemical stability were performed in simulated gastro-intestinal buffer (37°C, pH 1.2 to 8.0) and monitored by HPLC analysis. The experiments demonstrated that ValLeu, ValMet and ValHist remained unchanged up to 24 h, and didn't produce degradation products or potential metabolites. This behaviour indicates high stability at pH conditions of gastro-intestinal tract.
Since compounds containing amide functional group could be susceptible of hydrolysis by plasma and/or cerebral enzymes, our experiments were focused on the evaluation of stability of ValLeu, ValMet and ValHist in these biological environments. Otherwise, plasma stability of drug candidates plays an important role in drug discovery and development; it is essential for maintaining acceptable drug concentration and half-life in order to achieve desirable pharmacological effects [14].
Experimental data highlighted that ValLeu, ValMet and ValHist remained unmodified up to 24 h in plasma environment. In rat brain homogenate ValLeu, ValMet and ValHist didn't undergo cleavage after 24 h, indicating that synthetized compounds have also good stability to cerebral enzymes.

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P100 - ANTIFUNGAL SUSCEPTIBILITIES OF SPECIES OF THE SPOROTHRIX SCHENCKII COMPLEX ISOLATED IN ITALY

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Introduction

Recent molecular studies showed that the dimorphic fungus Sporothrix schenckii is no longer the only species able to cause sporotrichosis, a cutaneous lymphatic or systemic mycosis particularly frequent in certain geographical areas such as Mexico, Brazil, Peru, and India [1]. In fact, S. schenckii can now be recognized as a species complex comprising at least six sibling species: Sporothrix brasiliensis, Sporothrix globosa, Sporothrix luriei, Sporothrix mexicana, Sporothrix pallida (formerly Sporothrix albicans) and S. schenckii sensu stricto **[2, 3]**. Like S. schenckii, all these new species have been reported to cause diseases in humans and in other animals [1, 4, 5] although the extent of their impact on human infections is not yet completely known**.** However, infections due to *S. schenckii* have also been reported from other parts of the world, including Europe, where sporotrichosis is considered a rare disease [6]. Nevertheless, in recent years, several clinical autochthonous cases have been described in patients and animals that live in European countries, showing that this pathogenic fungus is more widespread than is now believed [1, 6].

At present, there are relatively few works that have evaluated the susceptibility of *S. schenckii* sensu lato to antifungal agents and the drugs tested so far have shown, in general, poor activity especially against *S. pallida*, *S. globosa*, and *S. mexicana*. Therefore, in this study we decided to evaluate the activities of a panel of antifungal drugs against all members of the *S. schenckii* complex with particular reference to Italian isolates.

To our knowledge this is the first study that evaluates in vitro activities of antifungal agents against a number of *Sporothrix* spp. isolates recovered from clinical and environmental samples in Italy.

Methods

Fourteen *Sporothrix* spp. were examined in this study (Table 1). Seven of them were environmental *S. pallida* isolates that have already been well characterized in our previous study [6]. The ***identity of each isolate was*** determined by partial amplification and sequencing of the calmodulin-encoding gene according to recent studies [2, 6]. Antifungal activity of seven drugs (Table 1) was evaluated by disk diffusion method according to the procedures reported in the National Committee for Clinical Laboratory Standards (NCCLS) document M44-A.

Table 1: Fungal strains, species and antifungal agents used in this study

Strain	Species	Antifungal agent tested (mean±SD)*:					
		FCN	FY	MZ	MCL	KCA	NY
SPO1	*S. schenckii*	R	R	R	32.50±1.11	29.00±1.22	14.00±2.35
CDM18	*S. schenckii*	R	R	R	22.00±1.35	25.00±1.05	07.00±1.51
SS40	*S. schenckii*	R	R	R	47.00±1,82	49.00±1.87	16.00±1.65
SPA1	*S. pallida*	R	R	R	21.00±1,24	ND	07.00±0.73
SPA2	*S. pallida*	R	R	R	22.00±1.08	ND	09.00±1.04
SPA8	*S. pallida*	R	R	R	19.00±0.86	ND	13.00±1.91
SAM1	*S. pallida*	R	R	R	27.00±1.46	ND	10.00±0.86
BG	*S. pallida*	R	R	R	23.00±1.71	ND	08.00±1.81
BG2	*S. pallida*	R	R	R	21.00±1.38	ND	07.00±1.52
BG6	*S. pallida*	R	R	R	23.00±1.02	ND	11.00±1.31
SS52	*S. brasiliensis*	R	R	R	21.00±1.29	28.70±1.21	09.00±0.50
SS49	*S. globosa*	R	R	R	23.00±1.61	ND	09.00±1.90
FMR9108	*S. mexicana*	R	R	R	22.00±1.10	25.75±2.01	14.00±1.11
KMU2787	*S. luriei*	R	R	R	25.00±1.71	ND	16.00±1.13

*mean values ± standard deviation (SD). FCN: Fluconazole; FY: Flucytosine; MZ: Metronidazole; MCL: Miconazole; KCA: Ketoconazole; NY: Nystatin. R = "Resistant" (No alone present); ND = Not Determined.

Res

ults

In this study, a total of 14 clinical and environmental *Sporothrix* spp. were examined to evaluate their susceptibility to a panel of antifungal agents. The resulting values of the in vitro susceptibility of *S. schenckii* sensu lato isolates are shown in Table 1.

All fungal species were resistant to fluconazole, flucytosine and metronidazole whereas were susceptible to nystatin. An excellent broad-spectrum antifungal activity of miconazole was observed against all examined strains. Regarding ketoconazole, different degree of susceptibility were observed. In particular this drug was active against *S. schenckii*, *S. brasiliensis* and *S. mexicana* but for *S. pallida*, *S. globosa* and *S. luriei* was not possible to measure the diameter of the zone of inhibition due to the presence of a high number of resistant colonies.

Discussion and Conclusion

The discovery of genetically different species within the *S. schenckii* population has generated considerable interest on different aspects of their biology including epidemiology, virulence and antifungal susceptibilities. Previous studies have clearly shown that the geographic distribution of members of the *S. schenckii* complex as well as their trends in antifungal susceptibilities are variable [1, 2, 7] and therefore more attention should be paid in the diagnosis and therapeutic treatment of infections caused by these species.

Throughout this work, miconazole, and to a lesser extent nystatin, showed a good activity against all *Sporothrix* species tested while all isolates were resistant to fluconazole, flucytosine and metronidazole which is in agreement with other previous studies [7]. The broad *in vitro* resistance to fluconazole in clinical isolates of the *S. schenckii* complex suggests an intrinsic resistance to this drug. This is an interesting topic for further study, because this drug is considered the second-line treatment for sporotrichosis.

One important result of this study is the excellent broad-spectrum antifungal activity displayed by miconazole, an synthetic imidazole antifungal agent which has never been tested against all members of the *S. schenckii* complex so far. Thus, based on our *in vitro* data, we believe that this drug may represent a very promising antifungal agent in the treatment of human and animal sporotrichosis.

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P101 - POSSIBILE RUOLO DI SCH1 NEL CROSSTALK TRA I DUE MAGGIORI PROAGING PATHWAYS

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Il lievito *Saccharomyces cerevisiae* è ampiamente utilizzato per la comprensione di molti meccanismi cellulari di base ed il suo impiego, come organismo modello nello studio dei processi che controllano l'invecchiamento, si è rivelato uno strumento di grande validità per l'identificazione delle due più importanti vie che modulano l'invecchiamento negli eucarioti. La prima è la via Ras-dipendente, che vede l'attivazione della proteina chinasi A e la conseguente inibizione dei fattori Msn2 e Msn4 che regolano la trascrizione di alcune heat shock proteins, della catalasi citoplasmatica e delle due superossidodismutasi (Sods). La delezione di RAS è infatti in grado di aumentare la sopravvivenza e tale fenotipo viene revertito dalla successiva delezione dei geni che codificano per Msn2, Msn4 e Sod2, mentre alleli attenuati dell'adenilato ciclasi, effettore positivo della chinasi A, aumentano la longevità delle cellule di lievito confermando il ruolo della chinasi A nell'invecchiamento e nella resistenza agli stress [1, 2, 3]. Nella seconda via "pro-aging" gioca un ruolo chiave la serina treonina chinasi Sch9, ortologa di Akt e S6K dei mammiferi, e attivata principalmente tramite TOR; ambedue le vie metaboliche convergono sulla proteina chinasi Rim15, quest'ultima quando è attiva impedisce l'espressione di geni coinvolti nella sopravvivenza e nella risposta agli stress [4, 5].
È molto interessante il fatto che i mediatori che fanno parte di queste vie cellulari "pro-aging" trovino i loro ortologhi funzionali o strutturali negli eucarioti superiori, ed è stato confermato che i meccanismi fin ora descritti sono sostanzialmente conservati dal lievito fino ai mammiferi [6].
La via di Ras e quella di Sch9 hanno molti elementi in comune, ad esempio stimolano la crescita e la glicolisi e rispondono entrambe ai nutrienti. Sch9 è stato peraltro isolato come soppressore multi copia di un allele termosensibile di Cdc25 il fattore di scambio del nucleotide legato a Ras [7]. Inoltre il carbossiterminale di questa proteina somiglia a una chinasi cAMP-dipendente. Infine, è stato osservato che l'aumento della durata della fase G1 di ceppi di lievito con la delezione di Sch9 può essere compensato da un'iperattivazione della chinasi A. Questi esperimenti suggeriscano una sovrapposizione funzionale delle due vie, tuttavia la contemporanea delezione di Ras2 e Sch9 ha un effetto molto più pronunciato rispetto alle rispettive singole delezioni e i punti di comunicazione o divergenza tra le due vie metaboliche non sono ancora affatto chiari.
È in questo contesto che si inserisce il nostro studio. È stato scoperto che l'unità trascrizionale di Sch9 contiene due ORFs, quella più grande che codifica per Sch9, e una a monte all'interno della regione 5' che può codificare per un peptide di 54 aminoacidi di cui non si conosce la funzione e che è stata chiamata Sch1 [8]. Nello studio è stato analizzato il ruolo della regione 5'UTR di Sch9 e il suo coinvolgimento potenziale nel processo d'invecchiamento.
Abbiamo dimostrato che l'overespressione di Sch1 aumenta la sopravvivenza e la resistenza agli stress nei ceppi deleti su Sch9, e che questo aumento di resistenza è revertito dalla parziale inattivazione della PKA, ciò fa supporre che Sch9 inibisca Sch1 che a sua volta inibisce la chinasi A rappresentando così un link tra la via di Sch9 e quella di Ras fin ora sconosciuto.

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P102 - SIMULTANEOUS SACCHARIFICATION AND FERMENTATION OF LIGNOCELLULOSIC WASTE MATERIAL FOR "SECOND GENERATION" ETHANOL PRODUCTION

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Currently, bioethanol is produced at industrial scale from sugar and starch; however, bioethanol production systems evidence several concerns about competition with food and feed supplies [1-2]. Alternatively, lignocellulosic biomasses such as agricultural wastes, woody biomasses, and lignocellulosic energy crops, today are expected to be the new "second generation" feedstock for bioethanol production because do not compete with food sources [3-4].
Nowadays, industrial bioethanol production is mainly focused on corn, wheat and sugarcane, as well as on highly abundant agricultural wastes. Lignocellulose-containing biomass is mainly composed of hemicellulose (five carbon polymers), cellulose (six carbon polymers) and lignin (phenol polymers) and therefore has to be pre-treated prior of its use in ethanol production by yeast. The use of residual biomass for bioethanol productions has the added advantage of transforming a waste material into a high-value product [5].
The hydrolysis of celluloses and hemicelluloses to hexoses and pentoses is generally achieved by the addition of several different enzymes such as cellulases and hemicellulases [6].
In this study pineapple wastes, a material rich in sugars and lignocellulosic components, were assayed with the purpose of obtaining a valuable product from the residues of the juice and canning industries.
Pineapple wastes, enclosing fruit skin and core, were homogenized in a fruit blender. The resulting homogenate, with a dry matter content of 14% (w/w), was diluted with water to a 9% dry matter in a working volume of 1.5L, and immediately treated at 100˚C for 10 min under continuous mixing to inactivate endogenous hydrolytic enzymes and reduce in the same time any microbial spoilage. No further sterilization procedure was adopted.
Simultaneous saccharification and fermentation (SSF) was carried out adding together a commercially-available cocktails of cell-wall degrading enzymes and active *Saccharomyces cerevisiae* NCYC 2826 inoculum (approximately 10^7 cells per ml) to the substrate. Fermentation parameters were: 30˚C, pH 4.5 and constant stirring at 200 rpm.
CO_2 evolution was measured during the fermentation and representative samples of the fermenting substrate were taken at regular intervals. For each sample, ethanol, glycerol, soluble and insoluble sugars were evaluated using GC and HPLC methods.
Moreover total protein determination by Kijeldahal method and Klason lignin were carried out.
Substrate initial fibers and soluble sugars were 23.9% and 42.2% respectively (Table 1); at the beginning of the fermentation Glucose and Xylose were the most abundant neutral monosaccharides followed by Galacturonic acid, Arabinose, Galactose and Mannose, with smaller proportions of Rhamnose and Fucose. The main sugars in the soluble fraction were Glucose and Mannose; only small amounts of Galacturonic acid and Galactose was detected.

By 21 hours soluble sugars and fiber utilization by the yeast, as well as ethanol production, stopped (Figure 1). The highest ethanol production was 3.7%, reaching a 96% of the Theoretical Yield (TY).
Data about fiber, soluble sugars, ethanol, glycerol, protein, lignin and ash are reported in Table 1.
Though the ethanol yield obtained appears rather low, due of course to the low sugar content in the starting material, SSF of pineapple wastes could be attractive since TY, calculated on dry matter loss, reached up 96%, making these wastes an excellent raw material for ethanol production by *S. cerevisiae* NCYC 2826.
Moreover substrate resulting from the fermentation process is enriched in protein and lignin, suitable, after separation, for feed and further fuel production respectively.

Figure 1. Fiber % (square), soluble sugar % (triangle) calculated on initial dry matter, EtOH % (diamond) and glycerol % (circle) in pineapple waste fermented by *Saccharomyces cerevisiae* NCYC 2826 during simultaneous saccharification and fermentation (SSF).

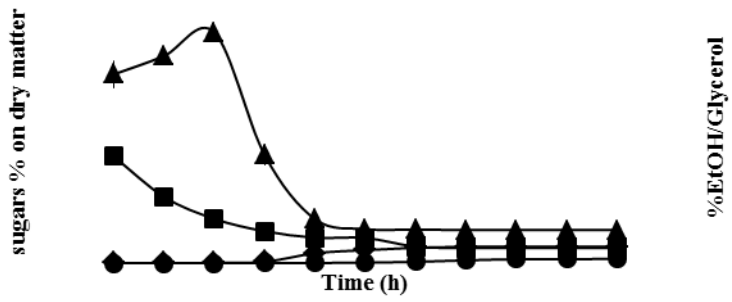

Table 1. Dry matter (DM), fiber and soluble sugars on dry matter, pH, EtOH amount and theoretical yield (TY*), glycerol, protein lignin and ash for SSF process.

	DM%	fiber in DM, %	soluble sugars in DM,%	pH	ethanol (V/V) %		Glycerol %	Protein %	Lignin %	Ash %
					amount	TY %				
Initial	**9.0**	**23.9±2.0**	**42.2±3.0**	**4.5**	**0.1± 0**	**0.0± 0**	**0.0± 0**	**4.1±0.2**	**3.6±1.0**	**0.5±0**
final	**1.7**	**3.4±0.5**	**7.5±1.1**	**3.3**	**3.7±0.1**	**96 ± 1**	**1.0±0.1**	**17.2±1.5**	**7.9±0.8**	**0.6±0**

* TY (theoretical yield represents the max ethanol yield: 0.511 g alcohol per 1.0 g glucose.

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P103 - AN HSF2-LIKE FACTOR IS PRESENT IN THE INVERTEBRATES: CHARACTERIZATION AND PURIFICATION IN SEA URCHIN EMBRYOS AND ITS LOCALIZATION IN PRIMARY MESENCHIME CELLS

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The cells respond to environmental, pathological and physiological stresses by inducing the synthesis of the heat shock proteins (HSP) which are highly conserved among all the organisms (1). The stress response is a common cellular defence mechanism against extracellular stress stimuli. The responsible for the stress-regulated synthesis is the transcription factors (HSF) which activate the transcription of the heat shock genes with a rapid synthesis of their encoded proteins (HSPs). The heat shock proteins are classified into different families on the basis of molecular mass, and one the most conserved during the evolution is HSP70 that is the most abundant and the most reacting HSP to both physiological and environmental stresses. The HSP70 and their cognate proteins (HSCs) function as molecular chaperones to protect cells by binding to partially denatured proteins and dissociating protein aggregates (2). Single genes for HSF have been cloned from yeast (3), fruit flies (*Drosophila*) (4), and frogs functionally homologous to mammalian HSF1. Four HSFs have been identified in mammalian and of these, HSF1 and HSF2, are ubiquitously expressed and conserved (5). HSF1 functions as a classical stress-responsive factor, HSF2 is active during specific development processes and it has been proposed to have a role in developmental processes. Although HSFs are best known as stress-inducible transcriptional regulators, they are also important for physiological processes. HSF functions are from the heat shock response to development, metabolism, disease, especially cancer and neurodegenerative disorders (6). HSFs contribute to multiple normal physiological processes and pathologies through direct regulation of their target genes. Since reproduction, the immune response and aging are the processes that are affected by the HSF activities an hypothesis would be that these new functions have been recruited during evolution in order to coordinate these processes (6). In order to verified this hypothesis we investigated whether HSF2-like factor in addition to HSF1 is present in one invertebrate which precedes chordates in evolution. To this aim we demonstrated that in sea urchin *Paracentrotus lividus* embryos are present HSF1 and also HSF2. After characterization and purification we found two HSF2 isoforms located both in the nucleus and in the cytoplasm. α and β sea urchin isoforms seems to be similar to those present in mouse and their expression pattern varies during embryo development, similarly to those of the mammalian HSFs, which are developmentally regulated in a stage-specific manner. In sea urchin the β isoform has greater DNA-binding activity than the α isoform. Moreover, in non-stress conditions the HSE-HSF complex present in early developmental stage embryos is composed predominantly of HSF2, whereas the late developmental stage binding activity is due to HSF1. Studies on territorial localization demonstrate that sea urchin HSF2 is maternal and that during embryo development, until gastrula, is more concentrated in primary mesenchyme cells (PMCs) (FIG 1). Interestingly, Hsp70 distribution shows no spatial correlation with HSF2 expression in non stressed conditions. However, in sea urchin embryos the particular HSF2 localization does not seem to be related to development, because the block of its function, by anti-HSF2 antibody microinjection in eggs, does not disturb the morphogenetic processes after fertilization. It is possible that at its

appearance HSF2 did not have any role related to development and this may have been achieved later in evolution.

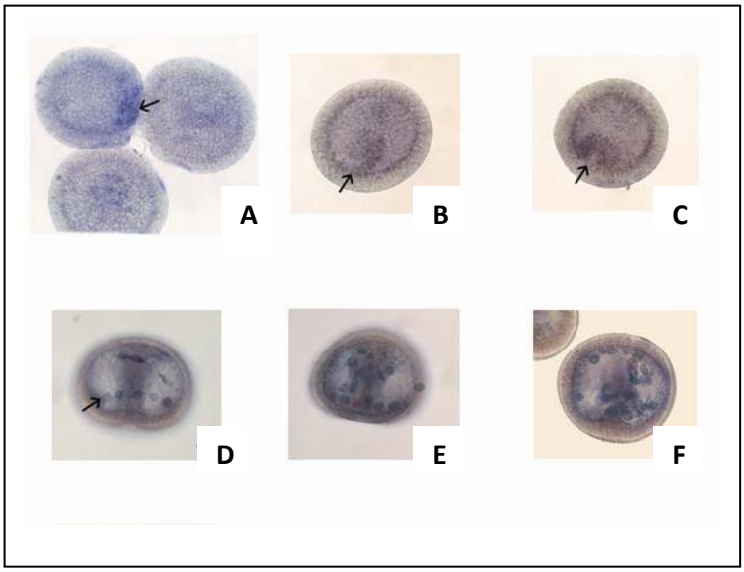

FIG 1. HSF2 localization during embryo development. (A-C) Blastula embryos. Arrows indicate the ingressing cells. (D-F) Gastrula embryos. Arrow indicate the PMC.

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P104 - FUNCTIONAL CHARACTERIZATION OF P65(-1), A NEW ISOFORM OF P65 FROM NF-KB COMPLEX

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Nuclear Factor-κB (NF-κB) are ubiquitous transcription factors that in mammals regulate many biological process including inflammation, immunoregulation, apoptosis, cell growth and cell proliferation (1).
Many studies show that NF-κB is overexpressed in solid and haematological tumours. These data suggest that a deregulated expression of NF-κB pathway is closely related with oncogenic phenotype (2).
NF-κB family members include *RelA* (p65), c-Rel, RelB, p50 and p52. These proteins exert their functions by binding as homodimers or heterodimers to specific DNA target sites (κB consensus).
The p65/p50 heterodimer is the most abundant and investigated form of NF-κB (3). A new isoform of p65, named p65(-1), have been discovered in human and mouse.
This isoform contains an unknown exon (named exon –1) located upstream to the first known exon of *RelA*, coding for p65. Transcription of the exon -1 leads to an alternative splicing between exon -1 and exon 1, thus skipping exon 0. By consequence p65(-1) has a smaller RHD than p65.
Previous evidences show that p65(-1), compared to p65, has different biochemical properties in some cellular mechanism like transcriptional activity on κB consensus, apoptosis and regulation of the glucocorticoid receptor (GR) activation (4).
In this study we investigated the function of p65(-1) by an *in vitro*, *ex vivo* and an *in vivo* approach.
In order to test the transcriptional role of p65(-1) we have analysed the transactivation of p65(-1) using both artificial and natural promoter regions, linked with the pathway of NF-κB. We have performed luciferase assays with: NF-κB-Luc (nuclear factor κB) CRE-Luc (cAMP response element), AP1-Luc (AP1 response element), SRE-Luc (serum response element), HSE-Luc (heat shock protein response element), pANXA-1-Luc (annexina 1) e pIL-6-Luc (interleukin 6) to study the activity of p65(-1) and we have also analyzed p65(-1) activity with p65 or p50 under the same conditions.
Our data suggest that p65(-1) has a central role during the regulation of pro and anti-inflammatory responses through a specific transcriptional activation using different partners according to the cellular requirements.
We have also studied p65(-1) expression on human peripheral blood mononuclear cells (PBMC); it is shown that the expression of mRNA is always present in the analyzed samples. Further, our data demonstrate different expression profiles between individuals considered.
The detection of additional factors belonging to NF-κB complex enhances the hypothesis of tuning responses.
According with central role of NF-κB in the biological responses we propose to investigate the function of p65(-1) and its network of interaction with other transcriptional factors, in many others cellular processes like apoptosis and cell proliferation.

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P105 - LA PEROSSIDAZIONE LIPIDICA COME ESEMPIO DI STRESS OSSIDATIVO

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Tra i fattori verosimilmente più implicati nel processo d'invecchiamento (in particolare nell'invecchiamento senza successo, caratterizzato dalla comparsa delle malattie correlate all'età e da disabilità), emerge il ruolo chiave dei radicali liberi, molecole o frammenti molecolari contenenti uno o più elettroni spaiati, e di conseguenza dotati di reattività elevata, e dello stress ossidativo (inteso come condizione ossidante, derivante da uno squilibrio tra fattori ossidanti ed anti-ossidanti a favore dei primi), capaci di indurre infiammazione e danno tessutale[1].
Lo stress ossidativo induce alterazione del bilancio redox delle cellule, con la conseguente attivazione di vie di trasduzione del segnale e di fattori di trascrizioni redox sensibili, capaci di evocare uno stato infiammatorio. Si ha, di conseguenza, un'attivazione continua e prolungata, che determina il rilascio di notevoli quantità di mediatori infiammatori, responsabili della conseguente evocazione di uno stato infiammatorio cronico. L'accumulo con l'età di molecole danneggiate a tutti i livelli (lipidi, proteine e DNA) è indubbiamente uno degli attori principali nei processi d'invecchiamento e di malattie, in quanto può essere responsabile di senescenza cellulare, infiammazione e cancerogenesi[2]. I radicali dell'ossigeno (ROS) e dell'azoto (RNS) appaiono quindi importanti mediatori della risposta infiammatoria da una parte ma responsabili pure del danneggiamento delle cellule dall'altra[3].
Attualmente nei paesi occidentali l'uso di cibi addizionati da integratori antiossidanti sta ricevendo una crescente attenzione e sta per essere sempre più adottato. Un migliore approccio terapeutico e una migliore informazione potrebbe derivare dalla corretta valutazione globale dello stress ossidativo mediante test diagnostici ove le due componenti contrapposte, quella pro- ed anti-ossidante possono essere valutate distintamente[4].
Lo studio si è proposto di valutare in uno studio longitudinale l'effetto dell'assunzione dell'integratore alimentare su 50 soggetti, ugualmente suddivisi per genere in un range di età 50-70 anni. L'integratore contiene tra l'altro polifenoli come; acido Gallico, catechine/epicatechine (monomeri), procianidine dimeri (B1-B4) epolifenoli identificati min. 35%. I biomarcatori valutati nell'ambito dello stress lipidico sono stati gli isoprostani nelle urine e le LDL ossidate nel siero.
Ulteriormente, solo nella popolazione maschile è stato valutato il livello di testosterone.
In questo studio di coorte prospettico l'integratore alimentare a base di estratti di acido Gallico, catechine/epicatechine (monomeri), procianidine dimeri (B1-B4)ed epolifenoli si è dimostrato capace in esperimenti ex vivo di: Influenzare positivamente la concentrazione di LDL ossidate in maniera statisticamente significativa, di avere potenzialità come stimolante nella produzione di testosterone nel genere e di decrementare in maniera statisticamente significativa la concentrazioni di isoprostani nelle urine.

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P106 - PREPARATION OF SOLID LIPID NANOPARTICLES FOR IBUPROFEN DELIVERY

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Ibuprofen (IBU), a non steroidal anti-inflammatory drug (NSAID), is largely and currently used for the treatment of several conditions, such as pain, fever and inflammatory reactions. Its therapeutic effects occur through the inhibition of prostaglandin H synthase (PGHS) and cyclooxygenase enzymes (COX-1, COX-2) responsible for the production of prostanoids e.g. prostaglandins [1]. However, IBU exhibits low aqueous solubility, short plasma half-life time of elimination ($t_{1/2}$) and rapid systemic removal, leading to slow dissolution and inadequate tissue absorption with subsequent short duration of its action and poor bioavailability [2]. Thus, current research trends focus on the development of potential delivery systems able to overcome these drawbacks. Notably lipid nanoparticles formulations have been identified as an approach to enhance the rate and extent of drug absorption, as well as, to improve IBU bioavailability [3].
Dynamic colloidal drug carriers based on solid lipid nanoparticles (SLN) may be promising for IBU delivery. SLN consist in a lipid core matrix, which should be solid both at room and body temperatures, with size within 50-1000 nm, stabilized by surfactants in order to prevent size growth during storage [4]. Their main advantages include: small size, ability to penetrate through small capillaries and be taken up by cells allowing the specific drug delivery and targeting, improving the therapeutic effects and reducing toxicity and adverse side effects [5, 6]. In addition they show high drug loading capacity, sustained and controlled drug release, long shelf-life, ability to protect the drug from chemical and enzymatic degradation, possibility to be administered by various routes (parenteral, oral, topical, ocular, brain, pulmonary), and they are biocompatible and biodegradable since they are prepared by physiologically well tolerated lipids [7].
The aim of the present work is the one to develop and characterize SLN formulations containing IBU (IBU-SLN). The production process includes a first step based on the lipid screening, performed by using a water bath and temperature above lipid melting point/below drug melting point (≈ 64°C), in order to select the most suitable lipid accommodating the drug and to evaluate the highest theoretical percentage of drug loading. The next step is based on the choice of adequate surfactants and their concentrations, taking into account their hydrophilic-hydrophobic balance (HLB). After that, the production of the blank formulation is carried on by mean hot high pressure homogenization (HPH) method. In these phases, the choice of the right solid lipid concentration, pressure value and number of cycles are pivotal. The last production step is related to the introduction of the drug in the formulation. The second part of this work is based on the characterization of IBU-SLN through drug entrapment efficiency evaluation, drug release test in sink condition, stability assessment at both room (25ºC) and low (4°C) temperatures, pH and conductivity measurements. The entrapment efficiency (EE) is studied using the indirect method with the construction of a previously determined calibration curve by UV-spectrophotometer at 222 nm. By the results of this work, reported in Table 1, what emerges is that Suppocire DM and Witepsol E85 are good lipid candidates for the development of IBU-SLN, stabilized by Phospholipon® 80H and Poloxamer 407 as surfactants. Optical images obtained by microscope (100x magnification) reveal spherical shape particles as shown in Fig.1.

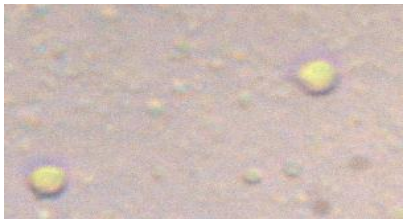

Fig. 1 Picture of ibuprofen-loaded SLN obtained by optical microscope.

pH measurements indicate their potential application for skin; parenteral administration becomes possible only with the increase of pH value e.g. by adding NaOH. In fact, it is well-known desirable physical-chemical features depend on the specific considered route of administration. In addition, these formulations show high EE values and they are stable for over 1 month in terms of absence of aggregation and phase separation.
However, further investigations are necessary to obtain information about polydispersity index, zeta potential and cytotoxicity in order to allow the application of these formulations for therapeutic purpose in future.

Table 1 SLN composition and the respective pH, conductivity and encapsulation efficiency of the obtained formulations.

SOLID LIPIDS	**SURFACTANTS**	**LOADED DRUG**	**pH (23°C)**	**CONDUCTIVITY**	**EE**
Suppocire DM (7%)	Poloxamer 407 (1%) Phospholipon® 80H (0.5%)	1%	4.64	129.04 mV	> 99%
Witepsol E85 (7%)	Poloxamer 407 (1%) Phospholipon® 80H (0.5%)	1%	4.68	124.97 mV	> 93%

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P107 - USO DELLE EPARINE A BASSO PESO MOLECOLARE IN CHIRURGIA GENERALE E D'URGENZA

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L'Eparina è un mucopolisaccaride estratto da alcuni organi quali il polmone, fegato e mucosa intestinale e si lega alla superficie delle cellule endoteliali.(fig.1) La sua attività biologica richiede la presenza di un cofattore plasmatico ad attività inibitrice proteasica [Antitrombina III], che inibisce i fattori proteasici della coagulazione formando con essi complessi stabili equimolari. In assenza di eparina, tali reazioni sono lente, mentre al contrario sono accelerate di quasi 1000 volte. Le molecole di Eparina sono altamente attive e si legano fortemente all'Antitrombina, determinando alterazioni conformazionali dell'inibitore: tali modifiche espongono il suo sito attivo per una più rapida interazione con le proteasi (i fattori attivati dalla coagulazione).L'eparina catalizza la reazione antitrombina-proteasi senza essere consumata e dopo la formazione del complesso, viene resa di nuovo disponibile per legarsi a nuova Antitrombina. Per comprenderne meglio l'azione occorre conoscere la genesi del trombo. Le eparine a basso peso molecolare vengono utilizzate per la prevenzione e la cura della malattia tromboembolica,si somministrano per inezione sottocutanea in specifiche aree del corpo in posizione distesa preferibilmente.
Tutte le eparine a basso molecolare non sono comunque uguali ,presso l'Azienda Ospedali riuniti Villa Sofia-Cervello per una questione aziendale e di manegevolezza sono state acquistate le seguenti molecole:nadroparina,enoxaparina,parnaparina,bemiparina.(fig .2)
Considerato il parere espresso dalla Commissione Tecnico - Scientifica (CTS) dell'AIFA nella seduta del 14 - 15 maggio 2013, che, dopo aver rivalutato le caratteristiche farmacocinetiche e farmacodinamiche delle eparine, limita l'applicazione del PHT per le eparine a basso peso molecolare e per l'eparina calcica alle sole indicazioni: "Profilassi della trombosi venosa profonda (TVP) e continuazione della terapia iniziata in ospedale sia dopo intervento ortopedico maggiore che dopo intervento di chirurgia generale maggiore", escludendo dal PHT tutte le altre indicazioni;
Le eparine a basso peso molecolare vengono utilizzate per la prevenzione e la cura della malattia tromboembolica,si somministrano per inezione sottocutanea in specifiche aree del corpo in posizione distesa preferibilmente. Su un campione di circa 80 pazienti ; con età compresa tra i 35-80anni, 50 sono di sesso femminile e 30 sono di sesso maschile; è stata somministrata l'eparina a basso peso molecolare a scopo profilattico alle ore 22.00 del giorno antecedente l'intervento allo scopo di allestire la migliore modalità di trattamento con minori effetti collaterali possibili. Qui di seguito viene riportata la media annuale di fiale utilizzate per molecola da cui si evince che due di esse sono le più utilizzate: la nadro e la enoxaparina; mediante l'osservazione diretta dei pazienti sono stati evidenziati i seguenti effetti collaterali per quanto riguarda la parnaparina e la bemiparina ;sanguinamento delle gengive durante il lavaggio dei denti maggiormente in soggetti anziani;mestruazioni particolarmente abbondanti; eccessiva perdita di sangue per tagli o piccole ferite ; ematomi in sede d'inezione. Non sono stati registrati effetti collaterali per le altre due molecole (Tab.1). Le evidenze rilevate da questo studio mostrano come con la somministrazione della nadroparina e della enoxaparina non si sono mai registrate alterazioni dell'attività A.P. e della P.T.T, con la somministrazione invece di parnaparina e di bemiparina osserviamo un comportamento di tipo fibrinolitico da parte di queste eparine ,diminuzione dell'A.P e un aumento della P.T.T

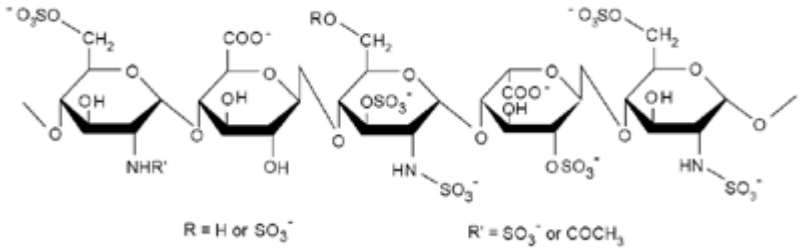
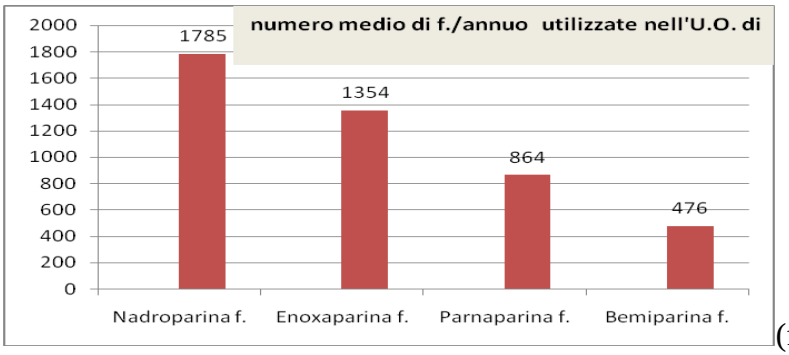


Fig.1



(fig .2)

	DONNE	UOMINI
EMATOMI SEDE DI INIEZIONE	50	30
SANGUINAMENTO FERITA	5	5
SANGUINAMENTO DENTI	3	
TURBE MESTRUALI	7	

ETA'	35-50aa.	35-60aa.	50-80aa.	
COMORBIDITA'	9/10	3/3	70/80	

(Tab.1)

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P108 - GLI EFFETTI DELL'ACAMPROSATO SULLA RIMODULAZIONE DELLA TRASMISSIONE GLUTAMMATERGICA ECCITATORIA ED IL SUO IMPIEGO NEL TRATTAMENTO DEL CRAVING DA ALCOLISMO NEL TERRITORIO DELL'A.S.P. 1 AG

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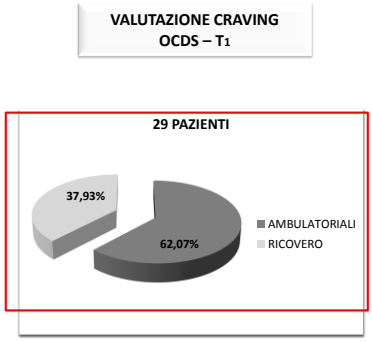
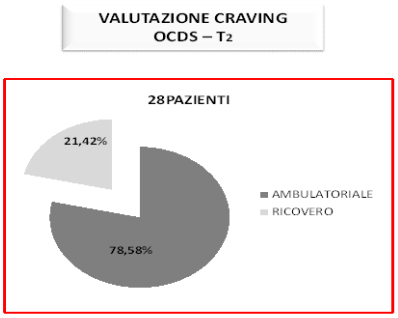
Secondo l'OMS definiamo l'alcolismo quel disturbo a genesi multifattoriale (bio-psico-sociale) associato all'assunzione episodica e/o cronica di bevande alcoliche con presenza o meno di dipendenza capace di determinare una sofferenza multidimensionale che si manifesta in maniera diversa da Soggetto a Soggetto. L'assunzione cronica di alcol modifica la normale attività neuronale attraverso il potenziamento dell'attività inibitoria del GABA e l'inibizione dell'effetto eccitatorio del Glutammato[(3)] che induce il neuro-adattamento attraverso una over-espressione dei recettori del glutammato in modo da ripristinare l'equilibrio del sistema in presenza di alcol[(4)]. Quando l'assunzione di alcol viene interrotta l'attività neuronale è caratterizzata sia da un aumento dell'eccitabilità dei recettori del glutammato[(3)] sia dall'attività dei recettori NMDA che rappresenta invece la causa dei caratteristici sintomi dell'astinenza: le convulsioni[(5)]. L'Acamprosato è un neuro modulatore specifico per il trattamento della dipendenza da alcol determinando il ripristino dell'equilibrio della trasmissione glutammatergica e l'inibizione dell'attività del glutammato agendo su due recettori[(3)]: NMDA e mGluR5 rispettivamente ionotropico e metabotropico. Contrastando l'iperattività glutammatergica l'Acamprosato riduce il craving negativo e conseguenzialmente diminuisce l'incidenza, la severità e la frequenza delle ricadute [(3)]. Nello studio clinico effettuato sono stati osservati 30 Pazienti reclutati nel territorio dell'A.S.P.1 di Agrigento suddivisi rispettivamente :
> 9 Pazienti di cui 2 donne e 7 uomini presso il Ser.T di Sciacca,
> 6 Pazienti di cui 1 donna e 5 uomini presso il Ser.T di Ribera,
> 7 Pazienti di cui 2 donne e 5 uomini presso il Ser.T di Agrigento,
> 8 Pazienti di cui 4 donne e 4 uomini presso il Ser.T di Canicattì.
Riportiamo i dati di alcuni Pazienti reclutati e seguiti ambulatorialmente presso i Ser.T che erano già stati sottoposti a precedenti trattamenti farmacologici con GHB:
* 4 Pazienti sui 30 – pari al 13,33% - esito negativo,
* 9 Pazienti sui 30 – pari al 30 % - esito positivo,
*17 Pazienti sui 30 – pari al 56,67 % - si sottoponevano per la prima volta alla terapia con Acamprosato. Ciascun Paziente è stato valutato mediante 2 questionari: OCDS (Obsessive Compulsive Drinking Scale) costituito da 14 item e SHORT SLEEP INDEX composto da 4 item. Tutti sono stati sottoposti a controlli seriati nel tempo che così abbiamo identificato: – $\mathbf{T_0}$ – prima dell'assunzione di Acamprosato; il primo follow –up al 4° mese – $\mathbf{T_1}$ - ed all'8 mese – $\mathbf{T_2}$ - il secondo. Abbiamo, negli esami laboratoristici effettuati, riscontrato i seguenti valori medi(Tab. 1)

VALORI EMATICI	To – INIZIO TRATTAM.	T_1 _ 4° MESE	T_2 _ 8° MESE
Hb	14,56 ± 1 g/dl	14,51± 1 g/dl	14,53± 1 g/dl
MCV	93,37± 1 fI/L	91,3 ± 1fI/L	90,8± 1 fI/L
CDT	1,57± 1	1,26± 1	1,06± 1
AST	58,3± 1 UI/L	37,59± 1 UI/L	32,93± 1 UI/L
ALT	48,73 ± 1UI/L	34,27± 1 UI/L	30,07 ± 1UI/L
GGT	209,47± 1 UI/L	128,76± 1 UI/L	77± 1 UI/L

Tab. 1

Per quanto riguarda la valutazione del craving si è osservato al T_0 una percentuale dell'86,67% ricovero ed una del 13,33% ambulatoriale. Dove per ricovero si intende la percentuale di Pazienti che, rispondendo alle domande del test, ha totalizzato un punteggio relativo al craving >22, valore che richiede un monitoraggio costante da parte del Medico Responsabile del Ser.T e contemporaneamente anche di un maggiore supporto psicologico. Con il termine ambulatoriale si indicano tutti quei Pazienti il cui grado di craving risulta al di sotto dei valori considerati a "rischio ricadute" e tali da permettere al Paziente di proseguire un trattamento esclusivamente diurno ma che prevede comunque l'adeguato supporto psicologico all'interno del Ser.T. Al T1 ed al T2 si osserva (Fig. 1, 2).





Al termine dell'odierno lavoro ed alla luce dei risultati ottenuti è innegabile l'efficacia dell'Acamprosato nel mantenimento dell'astinenza nei Soggetti dipendenti dall'alcol. Efficacia che si è manifestata riducendo il rischio di ricadute da un lato e, dall'altro per i ridotti effetti indesiderati registrati (la diarrea – il prurito) e per la notevole riduzione del craving negativo. Possiamo pertanto concludere dicendo che l'Acamprosato, associato ad un opportuno supporto psicologico, può senza dubbio rappresentare la terapia d'elezione che, certamente, in un futuro prossimo, sarà completata da altri supporti farmacologici-psicologici o altro per la riduzione/annientamento del problema: dipendenza dall'alcol.

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P109 - INCIDENZA DELLA MUTAZIONE RS12979860 DEL GENE DI IL28 IN UN GRUPPO DI SOGGETTI HCV POSITIVI DELL'U.O.C. DI MALATTIE INFETTIVE DEL P.O. CIVICO DI PALERMO

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L'infezione cronica da HCV colpisce 170 milioni di persone in tutto il mondo;su 100 persone infettate dal virus HCV il 15% non sviluppa alcuna patologia cronica ed elimina il virus, il restante 85% sviluppa forme croniche della malattia con evoluzione in cirrosi epatica e in carcinoma epato-cellulare
Il trattamento dell'epatite C cronica, che offre i maggiori vantaggi, è rappresentato da un ciclo di 24-48 settimane di PEG-Interferone-alfa-2a (Fig. 1) e Ribavirina. Il suo esito è influenzato da una serie di fattori tra cui il genotipo virale ed alcuni polimorfismi genetici dell'ospite
I pazienti infetti da HCV di genotipo 2 o 3 hanno probabilità 2-3 volte maggiore di rispondere alla terapia rispetto a coloro che sono infettati dal genotipo 1.
Nell'ospite le variazioni geniche dell' IL28 sono associate alla risposta virale al trattamento dell'HCV con PEG-Interferone-alfa-2a e Ribavirina. La presenza del polimorfismo SNP "rs 12979860" determina una sostituzione C/T nella sequenza del promotore a monte del gene umano che codifica per la citochina "IL28" localizzato sul cromosoma 19 la cui espressione è indotta ed attivata dalle infezioni virali. Il polimorfismo IL28 B non solo influenza direttamente la risposta del paziente al trattamento ma ne determina il dosaggio terapeutico in funzione del genotipo stesso. E' stato dimostrato come infatti vi siano genotipi favorevoli al trattamento (C-C) e genotipi meno favorevoli (C-T e T-T).
Nel nostro studio abbiamo valutato l'espressione delle mutazioni su un gruppo di 40 pazienti afferenti all'U.O.C. di Malattie infettive del P.O. Civico , affetti da infezione da HCV e candidati a terapia con IFN e RBV .La ricerca è stata effettuata con tecnica in real-time ed ha fornito i seguenti risultati : 12 pazienti postivi (30%) per genotipo CC suscettibili a terapia e 28 (70%) con genotipo meno favorevole (CT o TT) per i quali si dovrà valutare il potenziale impiego dei farmaci antivirali emergenti (Fig. 2).

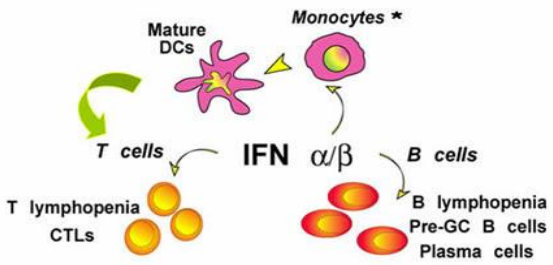

Fig. 1: Peg- Interferone-alfa-2a

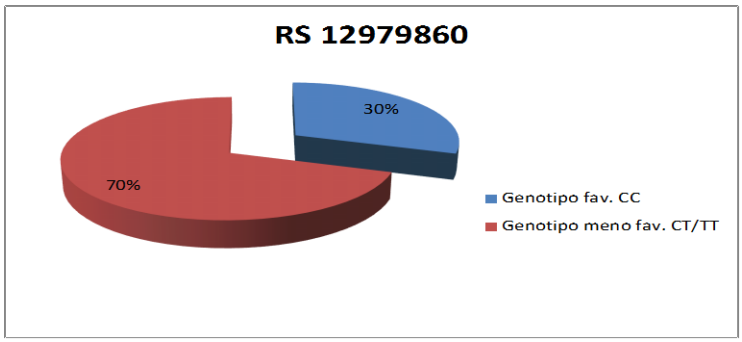

Fig.2: Percentuale di pz. Con genotipo favorevole e non

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P110 - EVALUATION OF BACTERIAL AND FUNGAL LOAD IN FRESH, FROZEN AND DRIED FOOD MUSHROOMS

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The analysis carried out by Doores et al. (1) and Venturini et al. (2) on the total bacterial load tested in wild and cultivated fresh mushrooms highlight values ranging between 3.7 and 9.3 log ufc/g. Due to the absence of pathogens, the microbiological quality of mushrooms analysed by the above-mentioned authors has been considered good.
On the basis of data reported in literature we have also extended the observations with frozen and dried mushrooms in order to evaluate the mesophilous bacterial and fungal load. In particular the presence/absence of *Escherichia coli* (Migula,1895) Castellani & Chalmers, 1919, *Salmonella* spp. and *Listeria monocytogenes* (Murray *et al.*1926) Pirie 1940 was analysed. The aim of this paper is also to evaluate the quality and safety of mushrooms daily consumption by consumers.
30 samples of mushrooms (10 cultivated fresh mushrooms identified with letter A 1-10; 10 dried mushrooms identified with letter B 1-10 and, 10 frozen mushrooms identified with letter C 1-10) were taken from large-scale distribution markets, mini-markets and small markets owned by migrants. The frozen mushrooms were contained in packages of *Agaricus bisporus* (J.E. Lange) Imbach 1946 and packages of *Boletus edulis* Bull. Besides mixed packages of *A. bisporus*, *Pholiota nameko* (T. Itô) S. Ito & S. Imai, *Boletus luteus* L., *Pleurotus ostreatus* (Jacq.) P. Kumm. and, *Lentinula edoses* (Berk.) Pegler were analysed. Other analyzed frozen mushrooms were a mixture of *A. bisporus*, *Agrocybe aegerita* (V. Brig.) Singer, *P. ostreatus* and, *P. cornucopiae* (Paulet) Rolland and a mixed package of *A. bisporus* and *P. ostreatus*.
The fresh mushrooms (*A. bisporus* and *Pleurotus ostreatus*) were purchaed from the grocery store. Dried mushrooms (*L. edodes*) were purchased from shops owned by Chinese migrants.
The microbiological analysis were carried out in the laboratory of the Center of Mycological Control belonging to the Sanitary Agency of the province of Palermo (southern Italy). The total bacterial load was analysed in 25 g of mushrooms (1:10 diluition) according to the rule ISO 4833:2004 (3). Moulds and yeasts were analysed according to the report ISTISAN 96/35 (4). *Salmonella* sp. was checked through the criteria of analysis ruled by UNI EN ISO 6579:2008 (5). The methodology of ISO 16649-2:2001 (6) was used to test the *Escherichia coli* positive beta-glucorinidase. *Listeria monocitogenes* was tested accordino to the rule ISO 11290-1:2005 (6). The total count in Petri dishes was made using the formula reported in ISO 7218:2007 (7). The cultura media were provided by the concern Lickson srl (Vicari, province of Palermo). The nomenclature follows the List of Prokaryotic Names with Standing in Nomenclature (LPSN).
L. monocitogenes and *Salmonella* spp. were not found in the analysed mushrooms. On the contrary a sample of fresh mushrooms from a supermarket of the town of Palermo was polluted by *E. coli*. The count of *E. coli* positive beta-glucorinidase correspond to 1.7×10^4 cfu/g.
The value of total bacterial count in all the mushrooms analysed varies from a minimum of 3.8×10^2 cfu/g found in dried mushrooms (*L. edodes*) to a maximum 2.6×10^8 ufc/g in a fresh sample

of mixed mushrooms (*A. bisporus* and *P. ostreatus*) in the supermarket. As regards moulds and yeasts the value varies from zero in dried mushrooms (*L. edodes*, *B. edulis*) to 4.4×10^4 ufc/g in fresh mushrooms (*A. bisporus*).
The results obtained showed that an analyzed sample of mushroom had a high pollutant load of *E. coli*. As known this bacterium is responsible of intestinal infections that can result in serious extra-intestinal infections. Besides *E. coli* is involved in drug resistance and thus have a significant impact on human health.
Since the packaging of fresh mushrooms polluted by *E. coli* was purchased from a supermarket belonging to the mass distribution highlights the need and importance of sanitary controls for the protection of the consumers.
The recent warning from the EFSA (European Food Safety Authority) that Italy is the second country in the EU as largest food borne diseases (especially salmonellosis) reinforces the need to respect to meet the parameters set out in Regulation 2073/2005 but also the checking of the proper handling of mushrooms during cultivation and packaging, including compliance with good hygienic practices by insiders.

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P111 - POSTNATAL MATURATION OF SEROTONIN SIGNALING SYSTEM IN MOUSE DUODENUM

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BACKGROUND AND AIMS: A plethora of study in animal neurodevelopmental models demonstrate that in central nervous system (CNS) temporal differences occur in the maturation of different neurotransmitter systems (Goldman-Rakic & Brown, 1982, Ehrlich et al., 2013). Although significant advances have been made in understanding the modifications in CNS, only primarily descriptive studies about the changes taking place in enteric nervous system (ENS), main regulator of gastrointestinal (GI) functions, have been underway.
As the other organ systems, digestive system is still developing and maturing after birth and thus it is possible to speculate that the changes in the chemical coding of ENS may occur with development. Studies about the postnatal maturation of enteric neurotransmitter systems could help to assess why drugs, able to modulate GI functions, may have diverse clinical effects at different ages. Serotonin (5-hydroxytryptamine or 5-HT), is an important enteric neurotransmitter in the GI tract (Gershon 2013). Neuronally-released 5-HT plays a crucial role in the regulation of several physiological functions, such as motility, secretion and visceral sensitivity (Gershon 2013; Baker 2005; Beattie & Smith 2008). Since serotoninergic system appears to undergo dramatic postnatal changes in CNS, in this study we aimed to assess if, in the enteric nervous system, 5-HT signalling may undergoes to postnatal maturation, using mouse duodenum as model.
METHODS: Using a pharmacological approach, we examined, *in vitro*, the role of 5-HT signalling in the regulation of duodenal contractility in neonatal mice (2 days old) compared to the adults.
RESULTS: 5-HT induced in both duodenal preparations a concentration-dependent muscular contraction, being its efficacy greater in duodenum from neonatal mice. Serotoninergic response was mediated by activation of muscular 5-HT receptors, antagonized by methysergide, nontarget 5-HT receptor antagonist, and of neural 5-HT_3 receptors, antagonized by ondasetron. In both preparations there was a major sensitivity of the postjunctional *vs* prejunctional receptors. In duodenum from neonatal mice pretreatment with atropine, muscarinic receptor antagonist, abolished neurally evoked serotoninergic contraction. Instead, in adult duodenum, in the presence of atropine, the response to 5-HT was converted in a muscular relaxation, abolished by L-NAME, a nitric oxide (NO) synthase inhibitor. L-NAME *per se* potentiated the 5-HT cholinergic contractile effects in adult preparations. Instead, L-NAME was ineffective in neonatal preparations.
CONCLUSIONS: In mouse duodenum, 5-HT signaling undergoes to age-related changes. In both preparations, 5-HT induces contractile effects via activation of muscular receptors and neural 5-HT_3 receptors on cholinergic nerves to induce acetylcholine release. Contractile response to 5HT is detectable from birth, but there is a gradual decrease in efficacy with age and a concurrent recruitment of inhibitory nitrergic nerves. These changes may contribute to gut motility adaptation to cope with the dietary changes at weaning.

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P112 - ANALISI DEI RISULTATI OTTENUTI IN SEGUITO ALLO SVILUPPO DI UN PROGETTO DI RICERCA NAZIONALE SU "SVILUPPO, APPLICAZIONE E VALIDAZIONE DI METODI: CHIMICI , FISICI E BIOLOGICI PER LA IDENTIFICAZIONE DI ALIMENTI IRRADIATI".

Di Noto A.M. [1], Cardamone C. [1] , Macaluso A.[1], Lo Cascio G.[1], Bartolotta A.[2], D'Oca M.C.[2] , Boniglia C.[3], Bortolin M.[3] , Giammarioli S.[3] , Benetti C.[4] , Pizzocchero V.[4] Chiaravalle E.[5], Rondini C.[6], Ruggeri F.M.[7] , Sangiorgi E.[8] , Scagliarini A [9].

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Nell'ambito della sicurezza alimentare, le contaminazioni di natura microbiologica rappresentano una problematica di notevole importanza e sono in continua evoluzione le strategie finalizzate a prevenire e a ridurre il rischio di contaminazione. A tal proposito l'irradiazione degli alimenti contro il rischio microbiologico rappresenta un approccio innovativo rispetto ai metodi tradizionali. In particolare, l'impiego delle radiazioni ionizzanti quali fotoni gamma o elettroni di energia tale da non produrre nell'alimento radioattività indotta, modificarne le proprietà nutrizionali né tanto meno provocare effetti tossici, coinvolge un numero sempre maggiore di Paesi e trova applicazione in diverse tipologie di alimenti. Attualmente in Europa il trattamento degli alimenti con radiazioni ionizzanti è disciplinato dalle direttive 1999/2/CE e 1999/3/CE recepite nel nostro Paese dal D.Lgs 30 gennaio 2001, n. 94. Tali direttive stabiliscono (per dare al consumatore una corretta informazione sulle caratteristiche del prodotto) che, tutti gli alimenti e/o ingredienti sottoposti al trattamento con radiazioni ionizzanti, debbano riportare in etichetta la dicitura "irradiato". Ogni stato membro, inoltre, deve effettuare controlli sugli alimenti presenti sul mercato al fine di individuare la correttezza dell'etichettatura. Pre-requisito per l'attuazione di tali controlli è la disponibilità di metodi atti ad identificare gli alimenti irradiati. A tal fine il CEN (Comitato di Normazione Europeo) ha standardizzato metodi di identificazione degli alimenti irradiati distinti in chimici, fisici e biologici in base alle diverse matrici alimentari da analizzare. Nell'ambito di questa problematica, il Ministero della Salute ha finanziato nel 2007 un progetto di ricerca nazionale che è stato successivamente sviluppato negli anni 2009-2012 dal titolo "Development, Validation and Application of Biological, Chemical and Physical Methods for Irradiated Food Identification and Evaluation of the original dose" che aveva lo scopo di studiare e validare i metodi di screening e/o di conferma per l'identificazione degli alimenti irradiati descritti in precedenza. In particolare lo scopo del progetto era quello di confrontare procedure di preparazione campioni e metodiche di misura, alla luce delle diverse caratteristiche degli alimenti, per la definizione di procedure standardizzate, semplici ed economiche da potere diffondere capillarmente ai diversi laboratori coinvolti nell'attività di controllo ufficiale degli alimenti e che erano unità operative del progetto stesso. Alle attività di ricerca hanno collaborato infatti i laboratori di diversi Istituti Zooprofilattici quali: l'Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, l' Istituto Zooprofilattico Sperimentale delle Venezie, l'Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna ,l'Istituto Zooprofilattico Sperimentale Umbria e Marche, l' Istituto Zooprofilattico Sperimentale della Sicilia oltre che l'Istituto Superiore di Sanità e ancora l'Università degli Studi di Bologna e l'Università degli Studi di Palermo.

Lo studio sperimentale ha permesso di sviluppare, applicare, validare e in taluni casi accreditare i seguenti metodi di identificazione degli alimenti irradiati: metodo Biologico di screening UNI EN 13784:2002 – Saggio Comet DNA per la ricerca di prodotti alimentari irraggiati, metodo Chimico di conferma UNI EN 1785:2004 - Ricerca di alimenti irraggiati contenenti grasso - Analisi mediante gascromatografia/spettrometria di massa dei 2-alchilciclobutanoni, metodo Fisico di conferma UNI EN 1786:1997 – Ricerca di alimenti irraggiati contenenti ossa: metodo per spettroscopia di risonanza di spin (ESR).

Il lavoro presenterà l'analisi critica dei risultati ottenuti al fine di contribuire alla diffusione delle esperienze maturate sulla tematica oggetto di studio.

I risultati ottenuti hanno confermato la necessità di rivolgere particolare attenzione alla scelta dei metodi in relazione alle diverse caratteristiche degli alimenti, quali: a) percentuale di grasso presente nel campione da analizzare, b) presenza o meno di ossa, c) stato di conservazione dell'alimento (refrigerato o congelato). La specificità del metodo in relazione alla tipologia di alimento risulta di notevole importanza per l'attività di controllo e allo stesso tempo per garantire il risultato analitico.

Da sottolineare inoltre che lo sviluppo di questa attività di ricerca ha permesso di accrescere la collaborazione scientifica tra tutti i soggetti partecipanti e ha contribuito ad aggiornare e formare personale tecnico esperto sull'uso di queste metodiche, per supportare piani di controllo e monitoraggio relative alle problematiche di sicurezza alimentare con particolare riferimento al trattamento degli alimenti con radiazioni ionizzanti.

P113 - METODI FISICO E BIOLOGICO DI IDENTIFICAZIONE DI ALIMENTI IRRADIATI CONTENTI CELLULOSA ATTRAVERSO L'USO DELLA DNA COMET ASSAY E DELLA SPETTROSCOPIA ESR

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Il trattamento a scopo conservativo degli alimenti tramite radiazioni ionizzanti prevede l'uso di dosi non superiori a 10 kGy, in grado di ridurre la flora microbica iniziale, inibire la germogliazione, aumentando così il grado di conservazione e di sicurezza dell'alimento. In Italia tale trattamento è applicabile solo ad aglio, cipolla, patata e spezie, ma si ritiene che possano essere introdotti nel mercato alimenti irradiati, provenienti da paesi in cui possono essere irradiati numerose tipologie di alimenti. Per tale ragione il Dl.vo n. 94 del 30.01.01, prescrive che tutti gli alimenti irradiati immessi sul mercato debbano riportare in etichetta la dicitura "irradiato" e che le autorità sanitarie debbano effettuare controlli sui prodotti in fase di commercializzazione. A tale scopo il Comitato Europeo di Normalizzazione ha emanato dei protocolli per l'utilizzo di metodi di identificazione di alimenti irradiati, distinti in metodi Fisici, Chimici e Biologici, a loro volta distinguibili in metodi di screening e di conferma.
Il presente lavoro riporta i risultati relativi alla messa a punto e validazione di un metodo di Screening "Metodo Biologico di screening UNI EN 13784:2002 -DNA Comet Assay-" e di un metodo di conferma " Metodo Fisico di conferma UNI EN 1787:2000 –Metodo per spettroscopia di risonanza di spin (ESR).
La matrice alimentare su cui sono state applicate ambo due le procedure sono alimenti vegetali contenti cellulosa, che possono essere presenti nel mercato nazionale sia come prodotti autoctoni sia come prodotti importati da paesi comunitari ed extracomunitari, ove possono vigere normative differenti nell'ambito dell'irragiamento alimentare, quindi di forte interesse nell'ambito dei controlli ufficiali da parte di enti autorizzati al controllo.
Nello specifico le matrici analizzate sono: pistacchi, paprica e fragole. E le analisi sono state condotte sia su campioni non irradiati, usati come bianco campione, che su campioni irradiati a dosi basse e medie.
Sono state effettuate prove di ripetibilità e riproducibilità del metodo sia sui campioni non irradiati che irradiati, che hanno permesso di standardizzare e quindi validare i due metodi di identificazione degli alimenti irradiati.
In figura 1a e 1b sono mostrate le immagini relative all'analisi di screening "DNA comet assay" applicata ad un campione di pistacchio prima e dopo irraggiamento a 5 kGy; tale tecnica si basa su un'analisi elettroforetica dei campioni. La presenza di una scia, appunto "cometa" nel campione irradiato (1b), è conferma dell'avvenuto trattamento radiante, dovuta alla rottura della membrana cellulare a causa delle radiazioni ionizzanti e quindi migrazione del fluido intracellulare tra i due poli della cella elettroforetica. Contrariamente nell'immagine del campione non irradiato (1b) si evidenzia nettamente la cellula intatta e quindi nessuna cometa.

In figura 2a e 2b sono mostrati gli Spettri ESR di un campione di pistacchio prima e dopo irraggiamento a 5 kGy. Tale tecnica permette di rilevare la presenza di molecole paramagnetiche, come i radicali radioindotti.
la figura 2a mostra il tipico segnale ESR di un campione di cellulosa non irradiato, caratterizzato da un singoletto, definito "endogeno", in quanto non dovuto al trattamento radiante bensì a caratteristiche paramagnetiche della molecola; la figura 2b invece mostra il tipico segnale della cellulosa irradiata, caratterizzato dalla comparsa di 2 linee satelliti rispetto al segnale endogeno alla distanza di 60 Gauss l'una dall'altra, che risulta essere l'indice dell'avvenuto irraggiamento del campione.

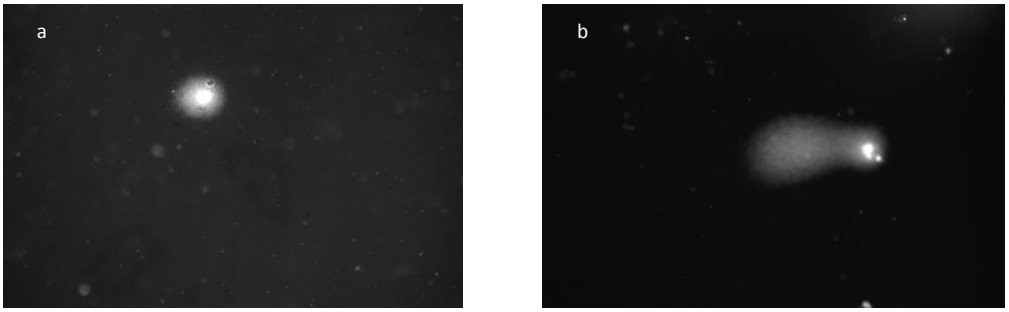

Fig: 1a e 1b immagini relative all'analisi di screening "DNA comet assay" applicata ad un campione di pistacchio prima (a) e dopo irraggiamento a 5 kGy (b)

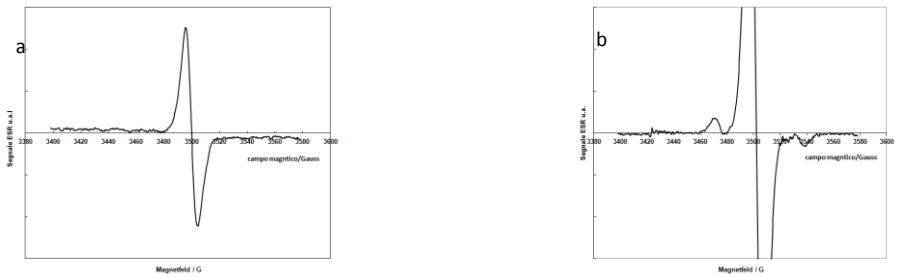

Fig.2a e 2b: Spettri ESR di un campione di pistacchio prima e dopo irraggiamento a 5 kGy

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