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BIOANTHROPOLOGY

FROM THE ARCHAEOLOGICAL CONTEXTS TO THE ANTHROPOLOGICAL ANALYSIS AND BEYOND. OSTEOBIOGRAPHY OF AN EARLY MEDIEVAL INHUMATION

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When we study skeletons and mummies dating back hundreds or thousands of years, we focus our attention on individual lives made of experiences that are partly comparable to ours, as well as unique and unrepeatable, as part of their historical and cultural context. This perspective has spread widely in recent years thanks to the refinement in analytical methods in Anthropology and Paleopathology, increasing our ability to interpret ancient human remains within their cultural contexts. This presentation examines the case study of an 8th century AD skeleton buried within a privileged funerary area and shows the importance and the scientific benefits of the anthropological approach, following the investigations from the excavation of human remains to the lab analysis and catalogue.

ETHICS AND HUMAN REMAINS

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The relationship between ethics and science constitutes the key point of moral reflection which is emblematically expressed in the question: have we the right to do whatever we are able to do? This problem arises in particularly critical terms when it is confronted with a "matter" that is no longer living. Certainly, human remains play an important role in understanding anatomy, culture, and human history. However, the question is: can we freely treat human remains belonged to a person died hundreds or thousands of years ago? Is it permissible to pose an ethical problem on finds without identity? The answer to these questions requires preliminary to identify reasons that can justify a moral attention towards a deceased person or towards human remains, in opposition to the thesis that death determines the cessation of personal rights of personality of the subject. Indeed, an ethical reflection on material is no longer alive seems to be justified in relation to a plurality of reasons. Human remains, often equated with any other naturalistic remains (at least for some aspects inherent to their conservation) are characterized and significantly differentiate from the latter for the complex symbolic, anthropological, cultural, psychological and religious values that they are able to evoke on individuals and/or communities. These values cannot be ignored or trivialized and call for a precise moral responsibility. Far from being solely concerned with archaeologists, anthropologists, ethnographers and museum curators, the management of human remains calls attention to issues that involve a wider community. Approaching death and "after us" means not only knowing and making known one of the most intimate aspects of man, but also dealing with anthropological problems and psychological obstacles that require us to critically reflect on our intrinsic precariousness as well as on the network of the links between man and the whole world around us. At the same time, in a profoundly changed cultural, social and economic context, there is a critical review of the role of the museum institution, today very far from the one in which the display of human finds was an expression of colonial policy. The result is a physical and symbolic place which, while deals with the violence of a past of oppression and prejudices, intend to play a new critical role and to tell our story, our identity, our mistakes, our aspirations and become a future memory of the values that represent us today.

ST. MERCURIALIS, THE FIRST BISHOP OF FORLÌ: SCIENTIFIC RECOGNITION AND PALAEOPATHOLOGICAL ANALYSIS

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Historical information related to the life of St. Mercurialis are very scarce, everything we currently know, we owe to its *legenda*, contained within the manuscript Casanatense 718 dated back to the 12th century. The only certain historical information concerns the ordination of one of his successors, Grato, which took place in Ravenna during the 5th century. The relics of St. Mercurialis, preserved inside the homonymous abbey, in the Cathedral of Santa Croce and in the Santissima Trinità church, during 2018 were object of the sixth canonical recognition. Preliminary studies were performed by direct anthropological and radiological analyses by CT scan, FTIR analysis, ancient DNA, radiocarbon dating, stable isotope analyses and trace elements. St. Mercurialis, was about 1.60 meters tall, the age at death is 45-50 years, it was not particularly robust, even if marked by repeated musculoskeletal stress probably linked to habitual activities such as walking and weightlifting. He suffered from osteoporosis and perhaps had some discomfort with the shoulder girdle. He did not suffer from osteoarthritis and he had no particular indicators about deficiencies suffered during the first and last period of life. From the results obtained for carbon, compared with those of other medieval Italian and European populations, it can be deduced that the diet was based on superior cereals such as wheat (C3 plants). Furthermore, based on the isotopic values of C and N, it can be said that the proteins of the individual's diet came mainly from terrestrial animals. Based on the isotope data of oxygen in bioapatite, it is deduced that the individual in question did not live in the same place in childhood and in adulthood. He probably came from a place located in an area on average warmer than the city where he spent the last years of his life. Radiocarbon dating and accelerometry mass spectrometry (AMS) dated the relics to the I-III century AD, an interesting date that is chronologically before to the only historical indication, we have about the life of St. Mercurialis and which collocates his episcopate into the first stages of evangelization

of the Emilia Romagna Region. The analyses of ancient DNA were targeted on the hypervariable region 1 (HVR-1) of the mitochondrial DNA (mtDNA) and on Short Tandem Repeats (STRs) of the Y-chromosome and highlighted a rather pronounced diagenesis of the DNA. These analyses will be targeted to the capture of the entire mtDNA, coupled with Next Generation Sequencing. Spectroscopic bone analyses reveal significant osseous diagenesis not only due to the natural relics aging but in addition caused by a pathological condition that can be identified as osteoporosis. FTIR measurements on the sand of the burial site reflect the typical geo-lithological composition of the soil of Forlì land, rich in illite, kaolinite, smectite and quartz.

STATURAL DIFFERENCES IN MALARIAL AND HEALTHY AREAS IN THE 19TH CENTURY IN ITALY

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Human populations of short stature owe this condition to the natural selective pressure that has allowed them to adapt better, for example, to tropical forests where the heat is oppressive and food is scarce (Jarvis *et al.*, 2012). A factor that could have contributed to the maintenance of a lower stature than that of other populations would seem to be the presence of malaria. In the literature there are no large-scale works that verify this hypothesis. The goal of this project was to try to understand the role of malaria in the expression of the statural character, at least as regards the populations living in some of the main ex-malarial territories, now reclaimed, in Italy. The starting point is the verification that in Italian ex-malarial areas the stature of indigenous individuals is less than that of conscripts originating in adjacent areas, free from the disease ("healthy" areas). As previously noted (Scanferla, 2016), the populations should be characterized by shorter stature than those of the neighboring non-malarial areas. Surname, which are transmitted by patrilineal way, can be considered real genetic markers, similar to allelic forms with high polymorphism on the Y chromosome. For this reason, they are used for the typing of different populations, as well as defining a biological identity and cultural that characterizes the group to which they belong. Even the "autochthonous" surnames present in the ex-malarial areas and in the adjacent "healthy" ones should be different from each other, as they typify lines of individuals lived and selected in different environments. The conclusions reached by analyzing the data are the following: i) The former malarial areas inhabited for a long time have municipal populations characterized by a different surname structure compared to that of the neighboring healthy areas; even the stature are shorter. ii) In the recently colonized areas, the short stature has not had the opportunity to establish itself and the structure of the surnames has a geographical characterization. iii) The areas with lower malaria incidence have been subjected to less selective pressure and fewer constraints linked to the population of the areas themselves, therefore the characterization linked to the structure of the surnames follows the criterion of geographic diffusion. There are statural differences between ex-malarial areas (shorter individuals) and healthy ones (taller individuals). We gratefully thank to the Accademia Ligure di Scienze e Lettere for financial support by funding the Luigi Brian Scholarship.

PAIR-MATCHING ILIAC BONES: PRELIMINARY RESULTS FROM STEREOPHOTOGRAMMETRIC ANALYSIS FOR SORTING COMMINGLED REMAINS

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Sorting commingled human skeletal remains is among the most demanding assignments anthropologists are tasked with. Yet, it is of paramount importance to carry out the biological profile and to start the personal identification procedures. Currently, the segregation of mixed bones into single individuals is performed by morphological and osteometric methods, which have been extensively used in the resolution of archaeological and forensic commingled settings. Nevertheless, these two approaches present several limitations related to the dimension and features of the assemblage (*e.g.*, number of involved individuals, homogeneity of the sample). Recent works suggested a three-dimensional approach for pair-matching homologous bones (*i.e.*, right and left humeri, temporal bones, phalanges, clavicles), where 3D digital models of the bones are acquired and superimposed. Root Mean Square (RMS) values are then used as a proxy to discern matches and mismatches. This presentation will review the state of art about the 3D approach for pair-matching commingled bones and will describe the preliminary results on pair-matching iliac bones. For this pilot study, a sample of 10 male individuals was selected from the Collezione Antropologica LABANOF (CAL), a documented skeletal collection. The anterior surface of 20 innominate bones underwent image acquisition by stereophotogrammetry (Vectra-3D®) and was elaborated by VAM software® to isolate the ilium. A superimposition protocol was designed, and the 3D surfaces were superimposed two at once in 10 matches and 50 mismatches. Finally, the RMS point-to-point distance of one model from the other one was calculated; this value was used to assess the performance of the method for sorting matches and mismatches. The resulting data indicate that RMS values between matches and mismatches are significantly different ($p<0.001$) and that a given RMS threshold of 2.9 mm can discern the pairs of iliac bones with a sensitivity rate of 100% and a specificity rate of 60%. The results will be discussed focusing on factors influencing the success rates, such as the postmortem damage. Although this approach is time-consuming, it provides a numerical value (*i.e.*, RMS value) that indicates tentative matches and mismatches and could be employed as an effective screening test, followed by in-depth analyses. Further research will include increasing the accuracy of the results and testing the technique on unresolved commingled assemblages, such as the thousand skeletal remains of the sepulchre of the Ospedale Maggiore of Milan (Ca' Granda) and those of the shipwreck that occurred on April 18, 2015.

EXPERIMENTAL STUDY ON ANTHROPOGENIC MUMMIFICATION WITH LIME

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The use of lime for treating bodies at burial has been occasionally described in hagiographies. In the case of the Blessed Jean Bassand (c. 1360-1445), medieval sources state that lime was placed inside the casket to cover up any bad smells, and to "consume the flesh of the saint" quickly. The discovery of the still-intact body 18 years later, despite the destructive action of lime, explained the miracle of the imperishability of the flesh, in itself a topos of the recognition of sanctity. An account of a similar miracle concerning the body of Saint Francis Xavier was transmitted by Jesuit sources from the late 16th century onwards. Albeit not regarding a saint, the use of lime is also documented in a source referring to the body of Pope Clement VI (1342-1352); it provided rapid excarnation so that he could be reburied elsewhere to respect his testamentary dispositions. In addition to covering bad smells of putrefaction fluids, the referred treatment may have caused spontaneously enhanced mummification rather than "consuming the flesh". In order to ascertain the effects of lime on corpses buried within empty spaces, a dead laboratory mouse was treated (TM) by covering it with quicklime in a plastic jar for 2 months. A control mouse was left untreated (UM) for the same period in the same conditions. Both mice were checked at weekly intervals, and finally fixed in formalin to be sliced and submitted to histology. Slides were stained with hematoxylin/eosin, Masson's trichrome, PAS, and Grocott stains; an additional slide was immunostained using anti-cytokeratin monoclonal antibody (AE1/AE3). TM did not release unpleasant smell during the whole process, whereas UM started to emit repugnant scent after one week. The volume of UM was greater than TM, due to inner cavities inflation by gases. The microbial flora of TM was composed by fungal hyphae and spores, whereas UM showed a great variety of maturing forms. TM showed larger amounts of bacteria (cocci) than UM. In both cases, skin was composed mainly by the dermis, more basophilic in TM also with calcium deposits. Collagen fibers were diffusely stained blue with Masson's trichrome but multifocal areas were metachromatically red. Adipocere transformation of subcutaneous fat was observed in TM. Focal CK+ remnants were observed in both mice, along with aberrant, cytoplasmic positivity for CK in skeletal muscles of UM. Our results demonstrate that lime treatment of the body surface inside an empty space fosters tissue dehydration rather than destruction, and hinders fungal maturation. The treatment has a limited effect also on internal organs, and may heavily affect histochemical and immunohistochemical staining of dehydrated tissue.

COVID-19

EFFECT OF SARS-CoV-2 ACCESSORY PROTEINS ON IMMUNE RESPONSE IN A549-ACE2 LUNG CELLS

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) belongs to a highly diverse family of enveloped, positive-sense single-stranded RNA viruses. They infect many different animals and can cause mild to severe respiratory infections in humans. Phylogenetic analysis of the full-length genome showed that, RaTG13 is the closest relative of 2019-nCoV and they form a distinct lineage from other CoVs (1). SARS CoV-2 genome encodes 16 nonstructural proteins, 4 structural proteins (spike; envelop; membrane; nucleocapsid) and 8 accessory proteins (ORF3, ORF3a, ORF6, ORF7a, ORF7b, ORF8, ORF9 and ORF10). As these sequences may contribute to host adaptation and the modulation of immune responses, we applied a multidisciplinary approach to verify SARS-CoV-2 and BatCoV RaTG13 accessory protein effect on specific cellular pathways involved in the antiviral and immune response. Thus, 48-hours post viral accessory protein transfection in A549-ACE2 lung cells (lipofectamine) we analysed: 1) the release of 27 soluble factors including caspases and cytokines in the supernatants (Multiplex ELISA); 2) Gene expression profiles of 80 genes involved in the immune and antiviral response (QuantiGene Plex assays); 3) Activation of the inflammasome by intracellular ASC protein staining (AMNIS). Simultaneously, A549-ACE2 lung cells were *in vitro* infected with 0.5 MOI of SARS-CoV-2. Preliminary results show that: following SARS-CoV-2 infection the expression of inflammatory genes (IL-8, TNF, CSF2, IL-18) significantly increased. The same trend was observed in ORF8, ORF3c and ORF7b, A549-ACE2 transfected cells (IL1β, IL-2, IL-6, IL-8, IL-18, CCL2) and was accompanied by a higher expression of activation markers (CD14, CD38, HLADR) as well as interferon stimulated genes (IFITM1, IFITM3). Likewise, A549-ACE2 transfected and infected cells showed an increase of proinflammatory factors in their secretome. Notably, we found a stronger response in the cells transfected with BatCoV-ORF-expressing plasmid than SARS-CoV-2- ORF-ones. Results on inflammasome activation are currently under investigation. Though preliminary, these results suggest that coronavirus accessory proteins contribute to the activation of immune response at different levels. More deepen researches are needed to disclose their effect on the pathogenesis of COVID19, and to identify potential therapeutic molecules that target these proteins.

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PLACENTAL PATHOLOGY AND SARS-CoV-2 INFECTION: THE EXPERIENCE AND THE MANAGEMENT OF A REGIONAL REFERRAL CENTER

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Several studies have been conducted about how SARS CoV-2 infection affected the respiratory or cardiovascular system but also about placental histopathology and pregnancy outcome in pregnant affected women. SARS-Cov-2 outbreak progression in Italy showed two different peaks, with different regions distributions: in Liguria, starting from the first outbreak peak, all placentas of positive mothers were collected at San Martino hospital while Istituto Gaslini collected placentas from the rest of the region. In the period from February 2020 to February 2021, 74 placentas were collected in San Martino hospital while 25 placentas were sent to Istituto Giannina Gaslini. All the placentas were examined for gross and histologic findings and the reports are now available for Istituto Superiore di Sanità, in association with clinical information of pregnant women. The aim of this multidisciplinary project consists of describing placental findings in women with SARS-CoV-2 infection and correlate them with eventual vertical transmission. Although many reports of COVID-19 in pregnancy describe complications, such as preterm birth (1), our data do not confirm any evidence suggestive for an acute event: moreover, the majority of the study population had a third trimester gestational age, demonstrating that SARS CoV-2 infection doesn't increase the risk of preterm birth. Vertical transmission is apparently rare (2) and this may be related to the presence of a placental barrier (3). A hypothetic way of transmission may be represented by intervillous hemorrhage, a situation characterized by maternal and fetal blood mixture. Interdisciplinary collaboration between gynecologists and pathologists is really important to having a global knowledge about health pregnant women, in particular about SARS-CoV-2 infection timing: generally, placental lesions need of months to develop and produce significant effects. This proposed multidisciplinary collaboration may help pathologists to comprehend the true nature and severity of placental lesions detected and try to understand even more the hypothetic mechanisms and prevalence of vertical transmission of SARS CoV-2.

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NEW PROTOCOLS FOR REGENERATIVE MEDICINE IN POST COVID-19 TREATMENT CONFIGURED ON THE PERSONALIZED USE OF OXYGEN AND OZONE-BASED THERAPIES

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Resolving the pulmonary and somatic consequences of COVID-19 infection have created a new milestone for regenerative medicine. Our research group, which has been dealing with therapies based on the use of Oxygen-Ozone (O_2-O_3) for more than 20 years, is proceeding in biomedical research both from a molecular and a clinical point of view. In addition to COVID-19, a growing body of scientific findings supports the existence of what is known as post-COVID syndrome, also referred to as long COVID (long haul COVID fighters), a pathologic entity, which involves persistent physical, medical, and cognitive sequelae following COVID-19. It is thus needed also for this condition a targeted treatment. The correct line to follow for an effective post COVID-19 treatment consists first of all in the personalization of the therapy, adapting it to each individual patient. The personalization passes through a modeling of the subject that starts from the evaluation of blood tests and from the genetic background, obtaining organized datawarehouse sorted according to temporal parameters. The datawarehouse, native and derived from algorithmic processing (control and statistics), constitute the "virtual subject" on which the treatment simulations are applied, and the verifications are performed through the prediction algorithms. The protocol linked to the simulation is used to personalize the therapy on the real patient. The data deriving from each application will enrich both the specific database of the single patient and the general database, linked to post COVID-19 therapy. In this study, a group of age-sex matched post-COVID patients was analyzed. Although considered "resolved", they continued to show symptoms like Chronic Fatigue Syndrome, such as fatigue, generalized pain, dyspnea and difficulty in concentration. We propose for the first time, the implementation of new specific procedures and protocols for a personalized O_2-O_3 therapy, with the aim to intervene quickly and specifically on post COVID-19 tissue recovery. Thanks to the activation of the "virtual subject", we want to maximize the efficacy of the O_2-O_3 therapy, starting from the widely scientific demonstrations on the regenerative, immunomodulatory, anti-inflammatory and antioxidant properties of O_3.

PNPLA3 AND TLL-1 POLYMORPHISMS AFFECT DISEASE SEVERITY IN PATIENTS WITH COVID-19

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Albeit the pathogenetic mechanism of COVID-19 is yet partly unclear, the course and outcome seem to be significantly influenced by host factors supporting a strong proinflammatory response and inducing a massive release of cytokines leading to a "cytokine storm" ultimately causing severe alveolar damage but also multiorgan failure. It is thus conceivable that the complexity of host genetic background in terms of polymorphisms in genes involved in SARS-CoV-2 receptor-dependent endocytosis, antiviral responses and modulation of cell infection and reinfection, inflammation, or immune stimulation may play a key role in pathogenesis and outcome of COVID-19. To probe the possible effects of host's genetic polymorphism in different segments of the innate antiviral response, we aimed to assess some specific functional Single

Nucleotide Polymorphisms (SNPs) of genes involved in control of viral infection by induction of inflammation (IFNL3/IFNL4 rs1297860/rs368234815), in macrophage polarization (MERTK rs4374383), in tissue and systemic inflammation (PNPLA3 rs738409). In addition, we evaluated a SNP of Tolloid Like-1 (TLL-1 rs17047200), a secreted protease capable of activating complement through the C1q pathway and also potentially able to activate the Spike protein of SARS-CoV-2. We studied in the early phase of pandemia consecutive patients (N=383) with SARS-CoV-2 infection, whose subsequent clinical course was classified as mild or severe, the latter being characterised by admission to intensive therapy unit or death. The genotyping was assessed by using whole nucleic acids extracted from nasopharyngeal swabs. Specific protease cleavage sites of TLL-1 on the SARS-CoV-2 Spike protein were predicted *in silico*. Male subjects and older patients were significantly at higher risk for a severe outcome (p=0.02 and p<0.001, respectively). By considering patients ≤ 65 years, after adjusting for potential confounding due to sex, an increased risk of severe outcome was found in subjects with the GG genotype of PNPLA3 (adj-OR: 4.69; 95% CI= 1.01-22.04) or TT genotype of TLL-1 (adj-OR=9.1; 95% CI= 1.45-57.3). Subjects carrying a GG genotype for PNPLA3 might have a constitutive upregulation of the NLRP3 inflammosome and be more prone to tissue damage when infected by SARS-CoV-2. The TT genotype for TLL-1 might affect its protease activity on the SARS-CoV-2 Spike protein, enhancing the ability to infect or re-infect host's cells. The untoward effect of these variants on disease course is evident in younger patients due to the relative absence of comorbidities as determinants of prognosis.

MEDITERRANEAN DIET, PROBIOTICS AND SARS-CoV-2: COMPLEMENTARY STRATEGIES

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Respiratory infections have been a serious public health problem and the leading cause of death from sepsis in the last 20 years; moreover, the continuous and sudden displacement of the population for leisure or work reasons it could put populations in front of new pandemic risks. The SARS-CoV-2 pandemic although, from current data, it has shown a low lethality, has put the hospital system and the economic structure of nations in crisis with a significant impact on the elderly population and with pathologies. In addition, in hospitalized patients, as suggested by recent research, have been demonstrated profound alteration of the microbiota and nutritional deficiencies. For this reason, we can no longer underestimate these aspects and it is necessary to combine pharmacological resources with complementary strategies, which derive mainly from the relationships between immunenutrition, microbiota and viruses. Recent researches have shown that the microbiota, and in particular some probiotic strains, are an extraordinary driver for immune homeostasis; diet, probiotics, prebiotics and postbiotics therefore represent evidence-based strategies to modulate the gut microbiota. Very interesting is the role of SCFA (Short-Chain Fatty Acids) on the metabolism of Treg lymphocytes, their ability to use fatty acids for energy purposes and for the presence of FFAR2 (Free Fatty Acid Receptor 2) receptors on the wall of a multiplicity of immune cells. SCFA come from bacterial metabolism as a result of a diet rich in fiber and polyphenols. In this regard it has been shown that the Mediterranean Diet contributes to the development of a eubiotic microbiota ensuring the intake of soluble fibers, micronutrients and, as recently demonstrated, also flavonoids such as naringenin, quercetin and resveratrol that have demonstrated an antiviral action against Covid-19. In conclusion, it is considered appropriate a greater commitment to this front by implementing information campaigns on the population.

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MOLECULAR MIMICRY MAY EXPLAIN MULTI-ORGAN DAMAGE IN COVID-19

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) induced disease (COVID-19) is a planetary emergency that is urging many research groups to redirect their efforts and to channel their experience towards understanding its pathogenesis. Viruses can generate molecular mimicry phenomena within their hosts. Why should severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) not be considered one of these?. Here, we propose that the virus induces antibodies and that some of them may crossreact with host's antigens, thus eliciting autoimmune phenomena with devasting consequences in various tissues and organs (1-3). We performed an exhaustive search of all contiguous segments of SARS-CoV-2 proteins with an exact identity to human protein segments. Human and SARS-Cov-2 protein sequence files were downloaded from UniProt database. Only segments with a length of six amino acids or more were considered. Further analyses were performed using the Immune Epitope Database and analysis resource (IEDB, https://www.iedb.org/), a database of experimentally validated epitopes and a tool to predict T cell and B cell epitopes. Sequence analysis of 20,365 human proteins showed that 3781 share peptides of at least six amino acids (≥6 mer) with SARS-CoV-2 proteins. In particular, among them we identified molecular chaperones, neuronal receptors, endothelial and blood cells proteins. If confirmed, by *in vitro* and *in vivo* tests, these results could drive researchers to find effective treatments against the virus.

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HISTOLOGICAL AND IMMUNOMORPHOLOGICAL FEATURES IN LUNG OF COVID19 SUBJECTS

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The Coronavirus Disease 2019 (COVID 19) is a complex disease caused by coronavirus SARS-Cov-2, which, in a short time, has become pandemic. This pathology is mainly characterized by an acute respiratory manifestation but can also affect other organs simultaneously or individually. In our study we performed a histological analysis and an immunomorphological evaluation on lung samples from subjects who died from COVID 19. The histological evaluation carried out on hematoxylin-eosin stained sections, showed a significantly modified lung parenchyma architecture. It was evident a marked congestion, microthrombi of the small vessels, hemorrhage and detachment of the alveolar lining with desquamation of pneumocytes within the alveolar space. Immunomorphological evaluations were performed with immunohistochemistry using, as primary antibody, cytoketatins (CKAE1AE3 and CK7) to better highlight pneumocyte hyperplasia and to demonstrate their tendency to form aggregates. We have also conduct-ed immunohistochemistry experiments to characterize the abnormal cells with large, irregular and monstrous nuclei observed in the lung parenchyma. The data obtained showed that these cells are megakaryocytes (CD61 positive) and are in line with the thrombotic trend of the disease. Finally, our immunomorphological evaluation was performed, also, to verify the tissue expression of two molecular chaperones, Hsp60 and Hsp90, hypothesizing their role in the complex pathogenesis of this disease. We observed a higher tissue expression of both Hsp60 and Hsp90 in the COVID19 lungs compared with control samples. It was demonstrated that some human molecular chaperones such as Hsp60 and Hsp90, have been shown to share parts of immunogenic epitopes with SARS-Cov-2. This phenomenon could induce an autoimmune response by molecule mimicry.

The data obtained suggest the involvement of these molecular chaperones in the development of COVID19.

UV IRRADIATION AND SARS-CoV-2: A FOCUS ON UVA/UVB/UVC-INACTIVATION ON VIRAL REPLICATION

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UV radiation is one of the most reliable and recognised microbicide approach, whose application has been widely widespread since the last century. Recent publications reported the UV-C dose (254 nm) necessary to inactivate completely SARS-CoV-2, confirming the highly virucidal effect of such radiation [(1)]. Moreover, there are clues that SARS CoV 2 effectiveness is strongly reduced during summer season in many populous cities of the world, indicating that sunlight could play a role in the occurrence, spread rate and duration of coronavirus pandemics [(2)]. Since solar UV-C light is filtered out by the ozone layer of the stratosphere, the possible virucidal effect could be caused by UV light in the range 290-320 nm (UV-B) and 320-400 nm (UV-A) [(3)]. Herein, we report the effect of different quasi-monochromatic UV-A/B/C irradiation on SARS-CoV-2. Experiments were conducted using a custom-designed lamp based on 273 nm (UV-C), 308 nm (UV-B) and 366 nm (UV-A) LEDs, which has been characterized and calibrated in terms of irradiance, temporal stability and spatial uniformity over the illumination area. According to the irradiance at the different wavelengths, three exposure times were set to provide three different UV-doses. Vero E6 cells were infected with UV-irradiated SARS-CoV-2 (5 MOI). Cells were observed daily for cytopathic effect while cell culture supernatants were harvested at 24, 48, 72 hours and virus titers were measured by absolute copy number quantification (qRT-PCR). The results show that an UV-A dose of 4000 mJ/cm^2 and of 200 mJ/cm^2 for the UV-B are sufficient to inhibit the replication of SARS-CoV-2. As expected, a much lower UV-C dose (2 mJ/cm^2) was necessary to achieve the same effect. Data were confirmed by analysing SARS-CoV-2-induced cytopathic effect. These outcomes are crucial for the development of novel sterilizing methods based on UV-C technology to contain SARS-CoV-2 infection and could contemporary contribute to the explanation of seasonal fluctuations in COVID-19 epidemiological trends. In addition, this project opens the door to future studies to better understand the molecular mechanisms that lead to viral inactivation following UV irradiation.

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CYCLOSPORINE A INHIBITS SARS-CoV-2 INFECTION *IN-VITRO*

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In December 2019, SARS-CoV-2 spread worldwide causing the coronavirus disease 2019 (COVID-19) pandemic. Patients affected by this pathology are characterised by several different clinical manifestations, which all have in common the hyper-activation of the immune system. This is triggered by the so-called cytokine storm, and it is associated with progression of disease severity and COVID-19-related deaths. Cyclosporine A (CsA), a well-known inhibitor of cyclophilins, interferes with viral infection and/or replication

via sequestration of cyclophilin A (CyPA) from binding to viral accessory proteins of different viral strains, *i.e.* VSV, HBV, HCV, VV, HIV-1, and SARS-CoV [1-2]. Moreover, CsA is known to be a potent immunosuppressor [3]. Thus, we investigated the *in vitro* effects of CsA treatment on SARS-CoV-2 infection. CaLu3 pulmonary cells were treated with 10 μM CsA both before and after infection with SARS-CoV-2, and samples were collected 48 hours post infection. Viral and cellular RNA were quantified by droplet digital PCR (ddPCR) or RT-PCR, respectively. Protein concentration and localization was evaluated by western blot and immunofluorescence analysis. Finally, infectious virus particle titration was evaluated by $TCID_{50}$. Our findings show that: *i)* CsA-treated cells, either before or after SARS-CoV-2 infection, express significantly lower levels of SARS-CoV-2 Spike protein (S) as compared to control cells, whereas the levels of expression of the entry receptors, ACE2 and CD147, were unaffected; *ii)* the RNA levels of nucleocapside (N1) were significantly decreased in cells treated with either CsA treatment; *iii)* CsA treatment dampens the number of released infectious viral particles (evaluated by analysing the levels of N1 RNA in the supernatant and by $TCID_{50}$) in both experimental conditions; *iv)* CsA dampens the virus-induced synthesis of cytokines (*i.e.* IL6, IL8, IL1α and TNFα), type I IFN-modulated restriction factors (IFITM3) and cholesterol 25-hydroxylase. Altogether, these results suggest that CsA is able to counteract *in vitro* viral replication and to dampen the subsequent induction of cytokines in a human pulmonary cell line model. Therefore, CsA might be considered for repositioning to timely treat severe COVID-19 patients.

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MICRO- AND NANOVESICLES IN BIOMEDICINE

EXTRACELLULAR VESICLES FROM HIGH GRADE GLIOMA: TOWARDS A SMART COMBINATION OF MICROSCOPIC AND PROTEOMIC ANALYSIS IN A HETEROGENEOUS LANDSCAPE

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Glioblastoma multiforme (GBM) is a devastating tumor with distinct hierarchical cell populations that shed large quantities of extracellular vesicles (EVs). Owing to their unique biology and key roles in cell-cell communications, EVs hold promise for the discovery of potential tumour biomarkers useful in clinical management of patients. GBM derived EVs cross the blood-brain-barrier (BBB) and into the circulation, so they can be sampled directly from the glioma microenviroment[1]. Isolating GBM-EVs from body fluids and screening their protein contents may serve as a complementary approach to assess the heterogeneous molecular landscape of GBM as tumours evolve[2]. While the EVs hold promise as potential biomarkers for the clinic diagnostic, their identification and quantification remain challenging. This study aimed the characterization of plasma EVs as a source of potential biomarkers, analyzing samples from pre-operative and post-operative GBM patients and controls. To optimise the experimental workflow, we used an EVs isolation method that isolates relatively pure EVs populations in a quickly, efficient, and scalable fashion, *i.e.*, size exclusion chromatography (SEC). This method could allow its adoption by hospital clinical pathology services. Size distributions and concentrations of plasma-EV fractions were measured by nanoparticle tracking analysis (NTA) and transmission electron microscopy (TEM). Isolated EVs were analyzed for the presence of EV-specific protein markers (as CD81, TSG101) by western blotting. A complete spectral library comprised of peptides derived from EVs released by GBM and controls samples was used to identify proteins detected in circulating-EVs by LC-MS/MS. Continuous acquisition of all theoretical fragment ion spectra mass spectrometry was used in combination with a target data extraction approach to comprehensively profile circulating-EVs isolated from plasma. Plasma-EV proteins reflect the state of the art of the tumor and have the potential to describe diagnostic, prognostic and predictive biomarker signatures. Forthcoming studies analyzing plasma-EV proteins in larger longitudinal cohorts of glioma patients may substantiate the markers determined in this study for the conceptualisation of a high grade glioma liquid biopsy.

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EFFECT OF MESENCHYMAL STEM CELL-DERIVED EXTRACELLULAR VESICLES ON DAMAGED HUMAN CORNEAL ENDOTHELIAL CELLS

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Introduction. Human Corneal Endothelial Cells (HCECs) are essential to visual function, however, since they have limited proliferative capacity *in vivo* and they are prone to corneal endothelial dysfunction. Recent studies highlight that stem cell–derived Extracellular Vesicles (EVs) play a relevant role in stem cell-induced regeneration by reprogramming injured cells and inducing pro-regenerative pathways. The aim of this work was to evaluate whether EVs derived from Mesenchymal Stem Cells (MSC-EVs) were able to promote regeneration of HCECs after exposing them to nutrient deprivation or tunicamycin-induced endoplasmic reticulum stress (ER-stress). Methods. We isolated HCECs from discarded corneas in patients undergoing corneal transplantation (n=23 patients). Human bone marrow Mesenchymal Stem Cells (MSCs) were obtained from Lonza, cultured and characterized. MSC-EVs were obtained from supernatants of MSCs. We evaluated the proliferation rate, apoptosis and migration after exposing them to nutrient deprivation or to tunicamycin treatment. We then evaluated the regulation of ER-stress-related genes, such as CHOP, ATF4, EIF2a, BIM, XBP1, BCLXL in presence or in absence of MSC-EVs and compared their effect with a different source of EVs, derived from patient blood serum (SER-EV). Results. In the serum deprivation conditions, the treatment with different doses of MSC-EVs resulted in a significantly higher proliferation rate of HCECs at all the tested concentrations of EVs and resulted in the decrease of total apoptotic cells and faster repair of the wound after 24 hours of serum-deprivation. We then observed an upregulation of the ER stress-related genes both depriving cells of serum and treating them with tunicamycin. ATF4, CHOP, BIM, XBP1 when HCECs were upregulated in both damage models, and a significant down-regulation of their expression after the treatment with MSC-EVs was observed. Summary and Conclusions. Our results highlight the well-known pro-regenerative potential of MSC-EVs and bring to light their ability to restore HCECs from ER-stress induced by nutrient deprivation and by treatment with tunicamycin.

DISSECTING THE ROLE OF EXTRACELLULAR VESICLES IN THE CROSS-TALK BETWEEN ADIPOSE TISSUE AND PROSTATE CANCER

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Prostate cancer (PCa) is the second leading cause of cancer-related deaths among men in Western countries. Interestingly, a progressive rise in the incidence of this tumor has been observed in the last two decades, accompanied by a parallel increase in the prevalence of obesity. While the association between the latter and PCa initiation is still a matter of debate, an increasing body of evidence suggests that an obese condition correlates with a more aggressive carcinoma and a limited therapeutic outcome. Extracellular vesicles (EVs) are nano-sized vesicles involved in cell-to-cell communication. They can be found in various body fluids, transferring proteins, RNAs and miRNAs from the originating cells to both neighboring and distant cells. EVs have been shown to modulate different tumorigenic processes, including cancer proliferation and migration, as well as tumor drug resistance. Moreover, they are implicated in the interactions between the tumor and other cells, including adipocytes. It is still unclear how these microvesicles can modulate the adipose tissue-mediated PCa progression. We demonstrated that 3T3-L1 adipocyte conditioned media can affect PC3 and DU145 cell features, inducing increased proliferation, associated with AKT phosphorylation, and invasion, correlated with MMP2 and 9 activation, E/N-cadherin switch and Snail upregulation. Moreover, PCa cells were found to accumulate lipid droplets and, more importantly, to undergo a neuroendocrine differentiation, accompanied by CD44 enhanced expression and docetaxel resistance. Notably, these results were confirmed in 3T3-L1 EV-treated PC3 and DU145 cells, where an increase in the glucose consumption, mitochondrial activity, ATP production and ROS generation was also observed, suggesting that adipocyte EVs can reprogram PCa metabolism and drive its aggressiveness. Further studies will be performed to identify the adipocyte EV molecular cargo responsible for the modulation of this dialog. Acknowledgements: F. F. was supported by an AIRC fellowship for Italy.

ISOLATION, CHARACTERIZATION AND DETECTION OF EXTRACELLULAR VESICLES FROM IMMORTALIZED MURINE MYOBLASTS (C2C12) AND H292 CELLS

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Understanding how intercellular communication occurs through the formation of extracellular vesicles (EVs) is essential to better understand both physiological and pathological conditions that occur during the exchange of information and material (proteins, RNA) in cellular crosstalk. In vitro studies are essential to evaluate the biogenesis, structural and functional properties of EVs. C2C12 cells, an immortalised line of mouse skeletal myoblasts that readily differentiate into contractile myotubes, have proven to be a good source for studying EVs. For characterization, we used exosomal markers such as CD81, ALIX. We also investigate the presence of heat shock proteins (Hsp) such as Hsp60, Hsp70, which may be involved in the inflammatory activity of muscle cells during the course of pathologies that can lead to muscle atrophy, cachexia, weight loss, which can affect the quality of life of patients and reduce the response to cancer therapy. To isolate the exosomes from the cell culture medium, we used different methods such as ultracentrifugation (UC) and Total exosomes isolation (TEI) kit from Invitrogen Thermofisher. The advantages of ultracentrifugation are that large volumes of biological samples can be used and there is no excessive shock other than gravity. We chose to use the TEI kit to avoid ultracentrifugation and to obtain the highest yield of vesicles for the same volume. However, it is necessary to perform some more experiments because in most of the experiments performed so far, this kit was used to isolate EVs from urine and plasma. The above techniques were used to isolate EVs from the culture medium of C2C12 cells naïve or transfected with the pCMV6-Entry- HSD1 overexpression plasmid, from which the patented product Physiactisome was obtained. This plasmid is able to overexpress Hsp60 with the DDK flag, which is used as a tag

to recognise transfected cells. The aim of the present work is to obtain the highest yield of vesicles from transfected C2C12 cells and of good quality to inject cachectic mice and test *in vivo* the effects of the new drug, Physiactisome, patented by our research group. Skeletal muscle Heat shock protein 60 increases after endurance training and induces peroxisome proliferator-activated receptor gamma coactivator 1 α1 expression.

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CITRAVES™ REDUCES LOW DENSITY LIPOPROTEIN-CHOLESTEROL AND WAIST CIRCUMFERENCE IN HEALTHY SUBJECTS AFTER 12 WEEKS: A PROSPECTIVE OPEN-LABEL STUDY

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Appropriate monitoring and control of modifiable risk factors, such as dyslipidemia, and level of low-density lipoprotein cholesterol (LDL-C) have an important role in the prevention of cardiovascular diseases (CVD). Recently, various nutraceuticals with lipid-lowering effects have gained attention. In addition to the properties of plant derived bioactive compounds, recent studies suggest that plant cells release small lipoproteic structures named extracellular vesicles (EVs) that, interacting with mammalian cells, could lead to beneficial effects such as anti-inflammatory and anti-oxidant activities. The present study aimed to investigate the role of a natural product, citraVes™, containing EVs from *Citrus limon (L.)* juice, on the modulation of different CV risk factors in healthy subjects. A cohort of 20 healthy volunteers was recruited in a prospective open-label study. All participants received citraVes™ supplement in a spray-dried formulation at a stable dose of 1000 mg/day for 3 months. Anthropometric and hematobiochemical parameters were analyzed at baseline and after the follow-up period, 1 and 3 months. We observed that citraVes™ reduces two key factors of cardiometabolic risk in healthy subjects. Significant decreases in waist circumference were found in women at 4 (85.4 [79.9,91.0] cm, p<0.0005) and 12 (85.0 [80.0, 90,0] cm, p<0.0005) weeks compared to baseline value (87.6 [81.7, 93.6] cm), while no differences were found in men (baseline: 100.3 [95.4, 105.2] cm; 4 weeks: 102.0 [95.7, 108.3] cm; 12 weeks: 100.0 [95.3, 104.7] cm). LDL-C decreased significantly at 12 weeks *versus* 4 weeks (p=0.0064). In conclusion, this is the first study evaluating the effects of a natural product, containing plant-derived EVs, on healthy volunteers. The results support the use of this extract as an effective tool to manage cardiometabolic risk factors.

EXPLORATION OF MICROALGAE-DERIVED EXTRACELLULAR VESICLES: CELLULAR UPTAKE OF NANOALGOSOMES

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Extracellular vesicles (EVs) are lipid membrane nano-sized vesicles secreted by various cell types for intercellular communication. EVs also constitute cross-species communication means and have been found in all kingdoms of life. The exploitation of the biotechnological potential of EVs as carriers of bioactive compounds, such as miRNA, siRNA, mRNA, lncRNA, proteins, peptides, lipids, synthetic drugs or other cargo, for different theranostic applications is of increasing interest. In this view, VES4US, an European project funded under the Horizon 2020 Future and Emerging Technology (FET), has developed an innovative platform for the efficient production of EVs from microalgae, a renewable and sustainable bioresource. Microalgae-derived bioactives are already used in many sectors, including the formulation of health supplements, cosmetic products or food ingredients. We demonstrate that microalgae are promising producers of EVs[1], which we named nanoalgosomes. A comprehensive physiochemical characterization of nanoalgosomes was performed according to MISEV-2018[2] guidelines. Then, after analyzing a number of species in different microalgae lineages, we identified the best nanoalgosomes-producing microalgae strains. Next, we evaluated the potential of nanoagosomes as next-generation biogenic nanocarriers of bioactive molecules. Specifically, here we focus on the cellular uptake of the nanoalgosomes, confirming that nanoalgosomes actively bypass cell membranes and that they were uptaken by mammalian cell lines through an energy dependent mechanism, confirming the cross kingdom communication potential of EVs. Funding. This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 801338.

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EFFECTS OF COLON CANCER-DERIVED SMALL EXTRACELLULAR VESICLES ON HEPATOCYTES: NEW INSIGHTS IN LIVER PRE-METASTATIC NICHE FORMATION

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The liver is the main metastatic site for patients with colorectal cancer (CRC) and represents the most frequent cause of death in cancer patients. Numerous studies have shown that in the liver the dissemination of cancer cells is the result of complex mechanisms based on two-way interactions between metastatic cancer cells and resident cells. Liver cell populations can differentially affect tumor expansion, often playing a divergent role on cancer cells ability to proliferate and colonize liver tissue. To date, data available in literature show the active multiple roles played by non-parenchymal cells (NPCs) in regulating both pre-metastatic niche formation and liver tumor cell colonization, while less is known about the role of parenchymal hepatocytes. The role of small extracellular vesicles (sEVs) released by cancer cells in regulating the cancer progression, through the creation of a permissive environment for the growth of metastasis (pre-metastatic niche) is deeply investigated by researchers. In the liver, the role of sEVs released by cancer cells in promoting the formation of the pre-metastatic niche has been studied by evaluating its action on the non-parenchymal liver component, while there are currently no data showing their effect on parenchymal hepatocytes, whose involvement has been exclusively described during the colonization phase. Since liver parenchyma represents the most structurally and functionally relevant part of the liver, to highlight its possible active role already in the pre-metastatic phase could provide new insights to better characterize the mechanisms that determine the formation of liver metastases. Our central hypothesis is that hepatocytes (Heps) may have a noticeable, and until now neglected role in modulating liver metastatic cascade already in the pre-metastatic phase, compromising the functionality of the liver even before the arrival of metastatic cells. Our research activity is concerned with studying the effects induced by CRC-derived sEVs on structural features and functional activities of healthy Heps in a *2D* and *3D* cell culture systems. Our data show for the first time that CRC-sEVs modulate the properties of hepatocytes inducing a de-differentiation process associated to an epithelial to mesenchymal transition (EMT) induced by sEVs-TGF-beta. This would highlight that the liver damage induced by CRC cells is not a conclusive event of the metastatic process, but rather represents an early event driven by CRC-sEVs already during the pre-metastatic niche formation and on which to act in a targeted and timely manner to limit the often-fatal clinical complications of CRC. The study of the cellular and molecular mechanisms that characterize the pre-metastatic niche formation can help to identify novel molecular targets for the development of new therapeutic approaches to achieve an early treatment of liver metastasis.

EXTRACELLULAR VESICLES IN THYROID CANCER: ASSESSING OF PREDECTIVE BIOMARKERS FOR THE EARLY DIAGNOSIS AND FOLLOW UP

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Thyroid cancers (TCs) are the most common endocrine malignancies and their incidence is increasing in many countries of the world. TCs account for 3-4% of all human cancers and mainly affects women between the ages of 40 and 60 (1). Fine-needle aspiration (FNA) biopsy is the most common diagnostic test in the initial evaluation of patients with a thyroid cancer, which is invasive, often inaccurate and can cause complications. Therefore, it is urgent the need to identify a novel non-invasive approach for the detection of diagnostic biomarkers for early tumor diagnosis, prognosis, and disease monitoring. Increasing evidence suggests that extracellular vesicles (EVs) could be considered a potential candidate as circulating biomarkers for cancer. EVs are lipid-bilayered nanoparticles with a specific cargo representative of the producing cell, including proteins, lipids, DNA, mRNAs and microRNAs (miRNAs) (2), and are secreted into surrounding body fluids, such as blood, urine, saliva, semen and breast milk. The cancer-derived EVs transfer their specific molecular cargo to the recipient cells, suggesting their relevant role in tumor initiation, growth, progression and metastasis (3). In this scenario, among the various molecular constituents of EVs, the most potential candidates as useful biomarkers for TCs diagnosis and prognosis are heat shock proteins (HSPs) and miRNAs, since they are a class of molecules dysregulated in a wide range of human cancers. The aim of the current study is to identify thyroid cancer-specific biomarkers within EVs. Blood samples were obtained from patients, initially diagnosed with thyroid disorders, before and after ablative surgery. Blood was collected from each patients and it was processed for serum isolation. At this point, EVs were isolated and characterized from plasma samples. EVs isolation and characterization was performed also from cell culture medium of a human papillary thyroid carcinoma cell line. Finally, the study will be focused on the detection of possible thyroid cancer biomarker candidates, such as proteins and miRNAs, within thyroid cancer-derived EVs from plasma samples. This work will provided information about different protein and miRNA expression levels between patients with thyroid cancer and a control group without thyroid cancer, with the purpose of detecting potential biomarkers for thyroid cancer.

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DEVELOPMENT OF A NEW ANTI-CACHECTIC DRUG BASED ON NANO-VESICLES

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Cachexia is a serious, still very underestimated and unrecognized medical condition resulting from several chronic diseases. The prevalence is high, reaching 5-15% of people affected by cardiac and respiratory diseases, and 60-80% in cases of advanced cancer. Cachexia is a metabolic syndrome characterized by muscle wasting, often associated with anorexia, inflammation, insulin resistance, and increased muscle protein breakdown. In developed countries, the United States, Europe, and Japan, the overall prevalence of cachexia caused by all diseases is estimated to be about 1%, affecting 9 million people [1]. To date, there is no specific treatment for cachexia, and the available therapeutic approaches only allow maintaining muscle mass and body weight in patients suffering from this condition, thus improving their activities of daily living [2]. We know from research that exercise improves the health and muscle recovery of cachectic individuals. Indeed, contractile activity during exercise promotes an increase in intracellular calcium (Ca2+), which in turn activates the Ca2+-dependent phos-

phatase calcineurin (CaN) and Ca2+-calmodulin-dependent kinase (CaMK), stimulating the expression of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) and mitochondrial biogenesis [3]. Physiactisome, from Physical Activity Exosomes, mimics the effects of physical exercise and for this reason may represent the first customizable anticachectic drug that acts on skeletal muscle and mimics physical exercise. Physiactisome consists of the secretion of muscle cells C2C12 engineered with a Heat Shock Protein 60 (HSP60) and activates molecular mechanisms typical of muscle regeneration [4]. Indeed, HSP60 is constitutively expressed in muscle in proportion to the content of mitochondria. Recently, Barone *et al.* [5] demonstrated a correlation between HSP60 overexpression and PCG-1α, highlighting a possible role of physical activity in the treatment of cachexia syndrome. Moreover, PGC-1α activation occurs only when HSP60 is released from muscle cells via specific extracellular vesicles [6]. Overall, this drug could improve the health status of cancer patients by giving them more time to undergo other oncological therapies that are often denied to them because they cannot bear them anymore. The global cancer cachexia market is currently in its infancy. Due to the promising clinical hotline and upcoming regulatory approvals, this market is expected to witness exceptional growth in the coming years.

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CHARACTERIZATION OF SURFACE MARKERS OF DIFFERENT MSC-EV POPULATIONS USING MACSPLEX, EXOVIEW AND NANOIMAGER

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Introduction. Mesenchymal stromal cells and their extracellular vesicles (MSCs-EVs) have been in the centre of regenerative research. Current field of EV-stem cell therapy focuses on the small-EVs fraction of EVs, as they have been implicated to ameliorate tissue injury. This project uses differential centrifugation to separate and fully investigate different fraction of MSC-EVs (small and large EVs) from different sources of MSCs (bone marrow, adipose tissue, and umbilical cord). The project is part of RenalToolBox consortium dedicated to the mechanisms by which stem cells can contribute to regenerative research in the field of kidney injury repair. Methods. MSCs are cultured until 80% confluency at 37°C in Alpha MEM medium with 10% of serum. To collect apoptotic EVs, apoptosis is induced using 500nM Anti-Fas antibody. Medium from overnight starved cells is collected then centrifuged at 1500g for 15mins to pellet apoptotic bodies. The supernatant is centrifuged further at 10,000g for 1h, to pellet large-size EVs, another ultracentrifugation step of 100,000g for 1h is applied to collect small-size EVs. EVs are pooled and kept at -80°C in medium with 0.1%DMSO. Nanosight is performed to analyse the concentration and size of EVs. Super-resolution microscopy is used to detect single vesicles and their markers. ExoView is used to detect the size and marker expression. MACSPlex, a semi quantitative kit aimed to assess EV surface markers is used to test each fraction of the MSC-EVs. Functional assays to assess the bio-function of MSCs-EVs in angiogenesis and fibrosis are ongoing. Results. The techniques used showed the EVs to be in their expected size ranges. The presence of tetraspanin (CD63, CD81, CD9) and mesenchymal (CD105, CD49e, CD44, CD29, CD146) markers were confirmed. In comparison with all fractions umbilical cord derived small-EVs showed a higher expression of CD63 and CD105. All large size EVs showed a higher expression of CD40 whereas the small size EVs of Annexin A1. Super resolution microscopy visually confirming EV populations. ExoView analysis further allowed us to determine the EV particle count and the size correlated with each concentration while, simultaneously showing positivity to exosome markers. Conclusions. This will generate insights for safe and effective application of stem cells and/or their bioproducts in clinical application and to ameliorate the life of people suffering with renal disease.

LEMON-DERIVED EXTRACELLULAR VESICLES EXERT ANTI-INFLAMMATORY EFFECTS BY INHIBITING THE ERK/NF-KB PATHWAY

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Inflammation can be the leading cause of several diseases, including cancer, diabetes, and ulcerative colitis; however, the existing anti-inflammatory drugs cause side effects. For these reasons, it is necessary to find new supportive therapeutic agents. In recent years, plant-derived extracellular vesicles (PDEVs) are gaining increasing interest in the scientific community as they have been found to possess a variety of biological properties; among those, the anti-inflammatory effects have been described by different groups that evaluated the effects of PDEVs in *in vitro* and *in vivo* models. We successfully isolated and characterized extracellular vesicles from *Citrus limon* juice (LEVs) and we studied their anti-inflammatory properties on *in vitro* and *ex-vivo* models: murine macrophages (RAW264.7) and immune cells isolated from healthy donors. LEVs were isolated by differential centrifugation followed by a final ultracentrifugation step. Further, biophysical analyses confirmed the size and morphology of LEVs. Also, through metabolomics analysis by means of HPLC-ESI-Q-ToF-MS, we characterized flavonoids, limonoids and lipids contained in the LEVs. Comparing the vesicle content with the total juice and LEVs-deprived juice, we observed an enrichment in the lipid component for LEVs. We then tested the toxicity of LEVs on target cells at different doses and time points and we observed that LEVs did not affect RAW and primary immune cell growth. To assess the role of EVs on inflammatory stimuli, we pre-treated both cell types for 24h with LEVs and subsequently stimulated them with LPS. Through RT-PCR, ELISA and FACS analysis, we saw that the pre-treatment with LEVs decreased the gene and protein expression levels of the pro-inflammatory cytokines IL-6, IL-1β, and TNFα. Besides, we observed a reduction in the gene expression of prostaglandin COX-2, compared to LPS alone. Similarly, pre-treatment with LEVs followed by stimulation with LPS in human

monocytes, down-regulated TNFα levels compared to LPS alone. Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) is a key pro-inflammatory pathway that can be activated by the ERK 1/2 signalling cascade. By confocal analysis and western blot, we observed that the pre-treatment with LEVs reduced the nuclear translocation of NF-kB in RAW stimulated with LPS as well as its phosphorylation. NF-kB modulation is associated with the decrease of ERK phosphorylation. In conclusion, LEVs showed promising anti-inflammatory properties, both *in vitro* and *ex vivo*, by reducing the production of pro-inflammatory cytokines through the ERK/NF-kB pathway.

EXTRACELLULAR VESICLES FROM PLANT EXTRACT: ISOLATION AND CHARACTERIZATION. POTENTIAL USE IN BIOMEDICINE

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Extracellular vesicles (EVs) are a heterogeneous group of nanoscale membranous vesicles involved in many cellular processes, both under physiological and pathological conditions. EVs are present in human body fluids such as blood, urine, breast milk, saliva, bronchoalveolar lavage, cerebrospinal, ascitic, and amniotic fluids. In addition, plant extracts are known to contain a variety of EVs and, due to their significant importance in intercellular communication, in recent years attention has been focused on them to study their effects on mammalian cells. Plant extract-derived EVs show a molecular profile similar to mammalian cells, including lipids, proteins, and nucleic acids. The aim of this study is to evaluate the effect of plant extract-derived EVs on tumor cells, using *in vitro* and *in vivo* models. In fact, numerous evidences suggests that plant EVs derived are able to interact with mammalian cells and mediate regulation of gene expression, involved in apoptosis activation, inflammatory response, and tumor microenvironment modulation. For these important features, plant extract-derived EVs may find application in cancer therapy. For instance, *Citrus limon*-derived EVs have been shown anti-tumor effect both *in vivo* and *in vitro* system by regulating a TRAIL-mediated apoptotic mechanism. It also has been observed that the orally administered Zingiber officinale-derived EVs exhibited important anti-inflammatory and antiproliferative activity by regulating proinflammatory cytokines, by suppressing tumorigenesis. Moreover, Panax ginseng-derived EVs are able to modify the tumor microenvironment by inducing the differentiation of tumor-associated macrophages towards the tumoricidal M1 phenotype in melanoma. This and other evidence suggest the considerable importance and potential of plant extract-derived EVs in inter-species communication for biomedicine applications, also because of their high efficiency in cell targeting and absence of toxicity. The first step of this project was to assess isolation and characterization methods of plant extract-derived EVs.

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NATURE AND HEALTH IN THE EURO-MEDITERRANEAN AREA

GREEN SYNTHESIS OF *Artemisia* SILVER NANOPARTICLES AND THEIR ANTIMALARIAL EFFECT

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Malaria is one of the most common diseases caused by mosquito bites. The infection results from the introduction into the host's bloodstream of a parasite of the *Plasmodium* species, which requires two successive hosts to complete its biological cycle: man and mosquito. With multi-drug resistant strains emerging in Southeast Asia and rates of infection beginning to increase again, interest in finding new cures for the disease is escalating. Indeed, according to the World Health Organization, $2.7 billion was invested in malaria research in 2018 alone. In this context, nanoparticles represent an interesting field of research. Already widely used in the development of diagnostic tests and as contrast agents in some instrumental examinations, they could be introduced as a vehicle for drug delivery or designed as particles active against a specific pathogen. Silver nanoparticles possess a broad spectrum of antibacterial, antifungal and antiviral properties. Silver nanoparticles have the ability to penetrate bacterial cell walls, changing the structure of cell membranes and even resulting in cell death. Their efficacy is due not only to their nanoscale size but also to their large ratio of surface area to volume. In our study "green" silver nanoparticles (AgNPs) have been prepared from two *Artemisia* species (*A. abrotanum* and *A. arborescens*), and their hemocompatibility and antimalarial activity have been studied in *Plasmodium falciparum* cultures in *in vitro* experiments. The antiplasmodial effect has been assessed using increasing doses of AgNPs (0.6 to 7.5 μg/mL) on parasitized red blood cells (pRBCs). The method of synthesis has allowed to create small size nanoparticles useful for biomedical applications connected to an effective action against *P. falciparum* parasite.

EFFECTS OF IRON ON THE TRANSCRIPTION OF STRESS-RELATED GENES IN THE SOLITARY ASCIDIAN *Ciona robusta*

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Heavy metal pollution of aquatic environments is an increasingly widespread problem affecting the survival of living organisms. Due to its frequent presence and impact on marine ecosystems, iron has recently attracted the interest of researchers involved in ecotoxicological studies. Despite its being an essential metal required for various catalytic functions of living cells, iron excess leads to the generation of free radical species through the Fenton/Haber–Weiss reaction, resulting in oxidative stress (Vajayavel *et al.*, 2012). In this study we aim to investigate the sub-lethal effects of iron chloride (10 μM), in *Ciona robusta*, an invertebrate chordate from the Lagoon of Venice, during 5 days of exposure. We measured the transcription of a series of oxidative genes for anti-stress proteins, such as super-

oxide dismutase (SOD), glutathione peroxidase-7 (GPX7), peroxiredoxin-6 (PRDX6), glutathione synthase (GS) and metallothionein (MT), the role of which in ascidians detoxification was previously demonstrated in our laboratory (Ferro *et al.*, 2018). We also considered two important protein components of stress granules (SGs), TIA-1 related nucleolysin (TIAR) and tristetraprolin (TTP), which are cytoplasmic foci operating in anti-stress protein mRNA preservation (Drago *et al.*, 2021). The obtained results show a modulation of expression of stress-related genes and the activation of stress granules in response to iron-stress. This highlights the importance of SGs as new biomarkers of heavy metal pollution.

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EFFECTS OF POLYSTIRENE NANOPLASTICS ON THE BRITTLE STAR *Ophiactis virens*

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Plastic pollution has become a major environmental problem due to its presence in all types of environments and its degradation into smaller long-lasting micro and nano particles. While microplastics have been widely studied in terms of both environmental concentrations on wildlife, few data are available on nanoplastics. Nanoplastics (NPs) are particles produced by the degradation and manufacture of plastic objects, within the size range from 1 to 1000 nm. The aim of the present study was to investigate the effects of polystyrene nanoplastic on *Ophiactis virens* (Echinodermata, Ophiuroidea), a filter-feeding and fissiparous brittle star widely distributed in the Atlantic-Mediterranean region and particularly abundant in the La Spezia Gulf (Ligurian Sea).The experimental animals were exposed to increasing concentrations(0,05 µg/mL; 0,5 µg/mL; 5 µg/mL) of fluorescent polystyrene NP beads (50 nm diameter)over a period of 2 weeks.Every other day a complete change of NP solution was made and food was provided to the animals. Several parameters, at different levels of biological organization, were taken into account: tissue distribution and accumulation of NPs, effects on animal behaviour (tipping time) as well on arm regeneration.NPs mainly remained stick to the surface of the animal's body or occasionally penetrated the first cell layer (epidermis) without entering into the deeper tissue layers. Most NPs were observed on arm stump surface rather than over the regenerate.Moreover, at high concentrations it was possible to observe the presence of NPs inside the first tract of the brittle star buccal cavity, thus suggesting their possible ingestion by the brittles star.The time of the animal's tipping showed a slight increase in the average value, although no statistically significant difference was registered.No evident effects were observed on arm regeneration in terms of both developmental anomalies and regenerate length.In conclusion, the selected NPs concentrations do not remarkably affect the considered parameters over a short exposure period. Indeed, the epithelia seem to perform at best their barrier function, preventing the deep entry of NPs;the main way of access into the animal is apparently the mouth where they can be actually accumulated.Nevertheless,long-term effects cannot be excluded, particularly related to NPs ingestion and subsequent feeding impairment or to a compromised cutaneous respiration which in turn can cause systemic stress conditions (*e.g.* oxidative stress).

DIGITAL TECHNOLOGIES APPLIED TO CONTROL THE DECARBOXYLATION PROCESS OF *Cannabis* OIL EXTRACTION

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In recent years, *Cannabis* for medical uses has grown in popularity for its increasing number of therapeutic indications. The most interesting extemporaneous galenic formulations of *Cannabis* are based on the extraction of its lipophilic active ingredients from female inflorescences using vegetal oils. The plant contains cannabinoids in the acidic form, which as such are not psychoactive compounds and have limited therapeutic effects. In fact, to be pharmacologically active, the cannabinoids need to be heat decarboxylated. Furthermore, a complex of volatile substances, namely terpenes, which seem to strongly contribute to the therapeutic effects of the extract through a synergy mechanism known as the "entourage effect" are also contained. Until today, numerous studies have been proposed for the correct oily extraction of the active components from the plants and their characterization by using several methods. Among them, in 2016 the *Società Italiana Farmacisti Preparatori* (SIFAP) developed and proposed a method for preparing *Cannabis* oil galenic extracts characterized by high yields. Nevertheless, this method uses traditional heating systems, which do not allow controlling the entire decarboxylation process of cannabinoids and is susceptible to changes due to the operator. Moreover, the method is described for the preparation of a small oily extract batch, specifically 5 g of inflorescences extracted in 50 mL of olive oil F.U. (Italian Pharmacopoeia). To our knowledge, all the reported *Cannabis* oil extraction methods do not guarantee standardized oleolites so far, especially if applied to larger quantities of plant material producing extracts with different yields. This limit is reflected with the difficulty in comparing clinical trials, in which extracts with different characteristics are often used. For these reasons, the development of a fully automatized method characterized by high standardization and reproducibility from batch to batch is a particularly important issue. This study investigates an innovative technique for the oily extraction of active ingredients from *Cannabis sativa L.* plant material to be used for the preparation of high quality, effective and safe products for pharmaceutical or nutraceutical applications. The proposed extraction method, patented and branded as Pharmagear, offers several advantages including: repeatability, standardization through self-regulation of the temperature according to the mass to be heated, reduction of oxidative stress of the oil, preservation of terpenes, remote control of the entire procedure and prevention of human errors. Lastly, since the heat decarboxylation process of acidic cannabinoids involves the loss of CO_2, the method allows to quantitatively monitoring the entire extraction procedure.

THE PROBLEM OF INDOOR AIR MANAGEMENT IN A CRITICAL PERIOD: WE CAN LEARN FROM TERMITES

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Currently, about 2200 species of termites have been recorded, compared to 3100 varieties of amphibians or 4100 of mammals. It is one of the smallest orders of insects and, at the same time, one of the most ingenious. Most of the termites live in tropical areas and some species populate the temperate zones. All termites feed on cellulose in its various forms and do not digest it completely, swallowing it and transferring it to areas of the digestive system predisposed to fermentation where the microorganisms continue to demolish macromolecules into glucose. The most evolved species of termites present an upgrade related to the feeding strategy that allows them to produce not only food but also real building material. A typical termite mound of an evolved species consists of a royal chamber, several nurseries, gardens, waste dumps, drinking water wells and, above all, a ventilation system. In fact, these buildings satisfy a common need: to dissipate the dangerous levels of heat and carbon dioxide that accumulate in a building containing several hundreds of thousands of individuals and, at the same time, fungal colonies that consume the equivalent (to scale) of 40 billion kilograms of fertilizer. In freer-structured nests, each room can be equipped with its own chimney. Some larger chambers may include a cylinder made of earth and cardboard that protrudes up to 50 cm above the surface. To understand the principle by which the networks of ducts are developed, our research group first made plaster casts in the termite mounds. Once the cardboard with which the termite mound was built was crumbled, it was possible to observe the detail of the main chimneys and of the radial connections that went up from the central cylinders to the peripheral ones as well as of the return pipes that connected the upper external part with the lower external part. From this plastic model a digital model was obtained in which the individual conditions were applied. When this humid air, charged with carbon dioxide, comes out of the vent chimneys, it draws in fresh air coming from the underground part of the nest, where it then begins to diffuse into the various chambers. According to the "design" of the nest, the warmer air can be simply ventilated by the chimneys or it can circulate along the peripheral buttresses. These are riddled with very small holes but sufficient to allow warm, stale air to spread outside, while cooler air filters inside. The "filter" cavities placed immediately after the access of air from outside to inside were also observed, measured and analyzed: in addition to being excellent filters for particulate matter, they contain part of the digestive biome of termites which in this case it acts as a real biological filter. The research continues both in the analysis of the different air exchange systems of the termite mounds, and in the algorithmic modeling and design of *Homo sapiens* in civil areas.

ACUTE TOXICITY OF PROMETHAZINE HYDROCHLORIDE ON *Artemia salina* NAUPLII

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In the last years, there has been a worrying increase in the pollution of the aquatic ecosystem caused by residues a lot of emerging contaminants (ECs), such as, pesticides, pharmaceuticals, personal care products and endocrine disruptors compounds that have been detected in wastewater effluent discharges (Grassi *et al.*, 2014; Zhang *et al.*, 2018). Traces of ECs in waters are present in relatively low concentrations but with high toxicity and they can lead negative effects on the health of non-target organisms and the environment. Antihistamines are a class of drugs widely used, whose active ingredients or metabolites are globally widespread in surface waters and effluents, particularly in Europe and North America (Kristofco and Brooks, 2017). Some studies have evaluated the toxicity of antihistamines on model organisms such as *Daphnia magna* (Furuhagen *et al.*, 2014) and *Amphibalanus amphitrite* (Jin *et al.*, 2014). These works show that the antihistamines test on *Dafnia magna* can detect toxicity effects at low concentrations, while the studies on *Amphibalanus amphitrite* have been evaluating the antihistamines as possible use in products antifouling, as some non-toxic concentrations would still allow the larvae to metamorphose in the adult stage. We have evaluated the toxicity of Promethazine hydrochloride (99% pure), an antihistamine widely used and found in the analysis of wastewaters (Kostich *et al.*, 2008). The aim of this study was to evaluate the effects caused by acute exposure (24h-48h; six concentrations range from 2,5 µg to 40 µg) on nauplii of *Artemia salina*, saltwater microcrustacean, widely used as a model organism to evaluate the impact of many contaminants (Pecoraro *et al.*, 2020). The results showed a high percentage of mortality and morphological alterations of nauplii. These results allow us to compare endpoint values on different model organisms.

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POLYHYDROXYNAPHTOQUINONE PIGMENTS FROM SEA URCHINS WASTE: STRATEGIES FOR BIOMASS VALORIZATION

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In the last decades, the demand for sea urchins from food industry, is increasing[1]. Most of them come from natural stocks, thus resulting in large environmental impact. In this framework, the CIRCULAr and BRITEs projects aim to fully reuse wastes from edible sea urchin industry to convert them, according to the logic of circular economy, into products with high added-value, including materials for biomedical applications and feed supplements to sustain sea urchin aquaculture, a valid alternative to overcome their overexploitation. For this purpose, sea urchins wastes from some Milan's restaurants were finely grinded to produce a powder that could be a valuable additive to the feed for animals requiring high doses of carbonates, like hens and

sea urchins themselves. The powder was characterized in its mineral and pigments content. In fact, pigments contained in *Paracentrotus lividus*, the species under investigation, are of high interest. They belong to the family of polyhydroxynaphthoquinones, a class of small polyphenols, and are natural antioxidants with potential health benefits. Pigments were obtained from sea urchin powder by means of solvent-based extraction procedures, performing treatments with aqueous acidic solution to decompose the carbonates matrix, and then a counter-extraction with selective organic solvents[2]. The presence of high amount of polyphenols in the extract was confirmed with Folin-Ciocalteau assay and its antioxidant activity was assessed by ABTS assay (2,2'-azino-bis(3-ethylnenzothiazoline-6-sulfonic acid), being comparable to the one of Trolox®, used as reference antioxidant in the literature. The extract was then characterized by Ultraperformance Liquid Chromatography coupled to Mass Spectrometry, and the presence of Spinochrome A and Spinochrome B was confirmed. Furthermore, having in mind future biomedical applications, cytotoxicity of the pigments was tested *in vitro* with MTT assay using human dermal fibroblasts. No evidence of cytotoxicity was observed. The developed extraction strategy allows to obtain a product of high added value useful to be employed as feed additive or in other health sectors.

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EFFECTS OF THE ENVIRONMENT ACIDIFICATION: TARDIGRADE EXPOSURE TO INCREASING ACID CONCENTRATIONS

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Acid rains [ARs] are a global environmental threat caused by dissolution in rainwater of SO_2 and NO_x, pollutant gasses emitted by domestic heating, industries, and vehicles, especially present in high industrialised areas. In the past 20 years, environmental protection strategies have tried to mitigate pollutions. However, ARs still cause effects on water and soil environments. Despite these effects have been largely studied, we still ignore soil microfauna sensibility almost completely. In this study, we tested a moss-dwelling tardigrade species to acid resistance. *Macrobiotus* cf. *hufelandi* was sampled in Modena (N44.631049, E10.942341; Po Plain, Northern Italy), a city characterised by stagnant air, high pollutions, and where ARs reached pH=4.5. Firstly, we exposed tardigrades to synthetic acid rainwater [SAR] at different pH ($3H_2SO_4$:$1HNO_3$ v/v; pHs 4.0, 4.5, 5.0, 5.5) at 12°C to assess loss of animal viability in response to ARs. For each of 4 pHs and control (distilled water; pH=6.2), 40 animals were singularly tested and their mobility monitored constantly for 7.5h, then after 12, 24, and 48h. Secondly, we exposed further tardigrades to SAR, organic (CH_3COOH), and inorganic (HCl, H_2SO_4, HNO_3) acids (pH=4.5) at 12°C to test whether effects on viability were related to pH or to acids nature and to assess sensibility of calcium stylets of the feeding apparatus [FAS-Ca] to acid exposure. For each treatment, 16 animals were singularly tested. Their mobility was monitored constantly for 6h and after 24h. Then, the effect on FAS-Ca was checked after 1h of recovery in culturing water. Linear regression, ANOVA, and probit (lognormal) were run to calculate median effective concentration [EC_{50}] and to T-test regression slopes. The negative effects of SAR on *M.* cf. *hufelandi* started at pH<5.0 ($p<0.01$) and were related to pH decrease and exposure time. EC_{50} in SAR was 1.7h at pH 4.0 and 4h at pH 4.5. Differences among tested acids were not found ($p<0.05$), therefore the effects follow pH variation only. Acids caused dissolution of FAS-Ca of dead animals, but FAS-Ca of alive animals resulted not affected. The study reveals the harmfulness of acid rains in a moss micrometazoan. ARs seem to not directly menace tardigrades survival in short exposure, but they affect tardigrades mobility possibly leading to starvation and death after longer exposure. We planned further studies on acid resistance on more tardigrade species from different environments and latitude to highlight species adaptations differences.

PRESENCE OF MELANOMACROPHAGE CENTERS IN THE MALE GONADS OF TWO SPECIES OF MULLIDAE AS INDICATORS OF EXPOSURE TO ENVIRONMENTAL CONTAMINANTS

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Melanomacrophage centers (MMCs) are distinctive clusters of pigment-containing cells, generally found in the reticulum-endothelial matrix of hematopoietic tissues. They are most commonly found in fish liver and spleen, while they are occasionally present in the gills, brain and gonads[1]. According to the central role of MMCs as a metabolic dump for the transfer of dead or damaged cell debris[3], the MMCs increase in size and number as fish age increases and tissues degenerate[2]. In the male gonad their presence has been related to the degradation and reabsorption of sperm cells not regenerated during gonadal regression. Furthermore, they have also been suggested as possible biomarkers of environmental stress[4]. The aim of the present study is: i) to evaluate the presence of MMCs in the male gonads of two mullet species, *Mullus surmuletus* L., 1758 and *M. barbatus* L.,1758, collected from three different sampling sites along the east coast of Sicily, characterized by different anthropogenic impacts and ii) to detect a possible correlation to the chemical and ecological status found in the coastal marine waters sampling points. Two sites are located in a very polluted coastal area, with complex harbor structures (Riposto-Acitrezza) and a petrochemic center (Augusta).The third sampling site is between Avola and Portopalo di Capo Passero, an area of high naturalistic interest affected by agricultural activities. The reproductive stages of 50 male individuals of *M. barbatus* and 50 of *M. surmuletus* were defined through histological analysis. The results showed that in all the areas examined, the *M. barbatus* samples present many MMCs even in the early reproductive stages where they should not usually be present. In contrast, the specimens of *M. surmuletus* present a more significant number of MMCs in the final stages of the reproductive period and only in the Riposto-Acitrezza area. Our results suggest that *M. barbatus* could be considered as a good bioindicator of the quality of the marine environment.

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FISH WASTE, NOT A PROBLEM BUT A POSSIBLE SOLUTION: CHITOSAN EXTRACTS AND NEW PACKAGING POSSIBILITIES

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The fishing industry is one of the oldest industries in Europe and also one of the most important economic resources in the Mediterranean area. However, to date, in addition to creating considerable economic wealth, the various sectors (markets, restaurants, shops, etc.) also create significant levels of fish waste. If not processed or recycled adequately, these waste streams can lead to environmental degradation and severe pollution (Gao *et al.*, 2018). Although today fish waste is considered a potential resource to generate high-added value, current practices in the fishing industry do not fully exploit its biological potential. Amongst the various possibilities for reuse, the production of biodegradable polymers emerges as a point of considerable interest for the food industry in the development of packaging materials and in order to improve the quality and appearance of food products (Elsabee and Abdou, 2013). In this context, chitosan, a polysaccharide of natural origin, has been experimentally used to produce edible packaging films due to its antimicrobial activity and physical-mechanical properties (Peng and Li, 2014). It is obtained from chitin, one of the most abundant natural polysaccharides and a typical component of the exoskeleton of crustaceans, molluscs and insects, and fungal cell walls (Sivaramakrishna *et al.*, 2020). Although good progress has been made in the production of chitosan biofilms, the type of packaging that can be obtained still needs to be perfected. This study, supported by the EU Interreg Italia-Malta V-A project *Bythos – Biotechnologies for Human Health and Blue Growth (C1-1.1-9)*, shows the preliminary results from the production of chitosan biofilms and the potential for improvement. For the first time, chitosan biofilms were produced from the head part of the exoskeleton of *Aristaeomorpha foliacea*, a species of considerable economic interest in the Sicilian fishing sector. Methods of extraction and purification of chitosan were developed, and the biological activities of the biofilms were evaluated. The biofilms demonstrated an antibacterial potential against Gram-positive and Gram-negative indicator strains, thus constituting a possible resource against microbial and chemical contamination in the food industries. Although further research is needed to identify new packaging methods and natural compounds that could improve the physical properties and antibacterial/antioxidant activities of the films, the possibility to reuse waste from this leading species in the Sicilian economy plays a significant role from the perspective of sustainable development and green economy.

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SIGNALS OF STRUCTURING FROM MITOCHONDRIAL DNA FOR *Mullus surmuletus* IN THE MEDITERRANEAN SEA

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Striped red mullet (*M. surmuletus* L.) is a very important target species for small-scale fisheries in the Mediterranean Sea. The genetic structure of this demersal fish has been studied using different types of molecular marker such as allozymes, RAPD, RFLP of mitochondrial genes (Mamuris *et al.* 2001, Apostolidis *et al.* 2009) microsatellites (Maggio *et al.* 2009, Galarza *et al.* 2009, Mati -Skoko *et al.* 2018). However, the results of these investigations have not been resolutive to understand the complex pattern of structuring of this species. In this study, new sequences of Citochrome oxidase I (*COI*) (N=72) and cytochrome b (*Cytb*) (N= 65) genes have been obtained from populations of *M. surmuletus* sampled in the Ionian Sea (Riposto and Portopalo di Capo Passero) in the Strait of Sicily (Lampedusa) and in the Balearic Sea (Grao de Castellon). Sequences of both genes obtained from GenBank have been added to the dataset to perform data analyses. The ML tree obtained from the COI sequences, indicates the presence of two clusters without any geographic structure. Each cluster includes haplotypes from all populations. However, some subclusters supported by high bootstrap values, encompasse the haplotypes of Grao de Castellon. This population shows the higher haplotype diversity (*h*=0,9) among the examined ones. The two main clusters and subclusters before detected were not observed in the *cytb* ML tree. Previous investigations on the genetic structure of the striped red mullet provided contrasting results probably deriving from the different molecular markers used and the adopted sampling plan. Our results demonstrate that local differentiation of the populations occurs suggesting that a comprehensive study should be done taking into account a high number of populations to capture the subtle level of structuring of the species, in the light also of the climatic change occurring in the Mediterranean sea.

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CIRCULAR ECONOMY: CHARACTERIZATION OF COLLAGEN PEPTIDES FROM SEA URCHIN WASTE

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The concept of Circular Economy is based on the recycling and exploitation of wastes that can be used as a source of "secondary resources". In this framework, we have recently started two research projects (CIRCULAr and BRITEs) addressed to the recycling of food wastes of the sea urchin industry and their valorization in different products, including collagen-based biomaterials for tissue regeneration applications. In previous works we developed and characterized these innovative sea urchin-derived medical device prototypes in terms of structure, mechanical performances, efficacy and *in vitro* cytocompatibility. In the present work we further characterized them in terms of collagen aminoacidic composition, *in vitro* degradation rates in physiological (PBS) and enzymatic (collagenase) conditions, preliminary antioxidant activity as well *in vitro* cytotoxicity (human fibroblasts) of the biomaterial degradation products *i.e.* collagen peptides. These peptides could in turn become "bioactive molecules" useful in the regenerating tissue microenvironment. Amino acid profiling confirmed that sea urchin collagen is mainly composed of glycine, hydroxyproline and proline and its overall aa composition is similar to that of human collagen, with a few small exceptions for alanine, arginine, methionine and glutamic acid. Degradation test showed that in collagenase scaffolds are degraded by more than 50% after 48 hours and Integra, a commercially available bovine collagen membrane used as a control, by less than 10%. Preliminary evaluation of antioxidant activity suggest that the collagen peptides might have a potential role as radical scavenger and therefore anti-inflammatory biomolecules. Finally, the cytotoxicity showed that in a short time (24h) the higher concentrations of peptides seem to have a "beneficial" effect, favoring the vitality (and indirectly the proliferation) of human dermal fibroblasts. Overall, in the present work we provided further evidence of the suitability and potential usefulness of our innovative and ecofriendly biomaterial for biomedical applications. As expected, this has good biodegradability rates which can be useful *in vivo* allowing the progressive substitution and regeneration of the new tissue. Further *in vivo* study will provide information on the actual efficacy of this novel biomaterial.

ABSENCE OF LARVAL REGENERATION IN HIGHLY REGENERATIVE CRINOID *Antedon mediterranea*

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Regeneration is a fundamental mechanism among metazoan, being present in most existing phyla. Echinoderms are marine deuterostomes, therefore phylogenetically close to Vertebrates, and they are characterized by extraordinary regenerative abilities, both in adults and larval stages. Larval regeneration is well documented for all echinoderm classes, except Crinoidea, the most basal taxon. Therefore, the aim of this work was to assess if the larval stage (doliolaria) of the crinoid *Antedon mediterranea*, whose adults are able to regenerate, can actually regenerate. In normal conditions, free-swimming *A. mediterranea* doliolaria attaches to the substratum and produces a stalked stage (pentacrinoid) with an apical calix. Adult spicemens of *A. mediterranea* were collected at Le Grazie (La Spezia Gulf, SP, Liguria). After hatching, doliolaria larvae were collected and bisected with surgical blades, thus obtaining anterior and posterior halves. The fragments were monitored for 2-3 weeks and the survival rate was registered and compared to non-bisected doliolariae. We defined different "developmental" stages (seven for the anterior fragments and five for the posterior ones) through which both fragments go during the days post-amputation. Each stage was characterized by stereomicroscopy and Scanning Electron Microscopy (SEM). Results indicate that less than 50% of the bisected larvae survived after 3 weeks, thus suggesting that the amputation represents a stressful event for them. None of the halves was able to regenerate: each of them passed through a wound-healing phase without reforming the other half and then continued its pre-determined development. The post-metamorphic stage lacked structures deriving from the missing half: anterior fragments generally originated a stalk without the calix whereas the posterior halves usually produced a calix without a stalk. These data suggest that doliolaria cells are strictly committed to their original fate and 1) they cannot be redirected towards other fates, 2) doliolariae lack stem cells or they are in a "stand-by" state. Considering the basal phylogenetic position of Crinoidea these results are particularly significant to better understand the evolutionary trajectories which led to gain or loss of (larval) regenerative abilities. More studies could also shed light on the correlation between regeneration in adults and regeneration in early stages.

ANTIMICROBIAL ACTIVITY FROM POLYPEPTIDE-RICH EXTRACTS OF THE SEAGRASS *Posidonia oceanica*

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The present study was carried out to assess the antibacterial, antifungal and antibiofilm properties of polypeptide-rich extracts isolated from green leaves and rhizomes of Mediterranean seagrass *Posidonia oceanica* (L. Delile) (Posidoniaceae). The seagrass was collected, washed with freshwater, grinded with liquid nitrogen in order to obtain fine powders that were exposed to extraction by acetic acid and antiproteases. The crude extracts isolated from leaves and rhizomes of *P. oceanica* were subjected to microbiological assays to evaluate the antibacterial, antifungal and antibiofilm activity of polypeptide fraction against two reference bacterial strains *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 15442, and the fungus *Candida albicans* ATCC 10231. The antimicrobial and antifungal activity of the extracts were evaluated starting from 50% v/v concentration of each sample and the results are expressed in terms of Minimum inhibitory concentration (MIC), with the values detailed in percentage v/v and in concentration mg/ml of protein content. The most interesting result has been deduced from rhizomes that showed a MIC of 12.5% v/v, corresponding to 3.37 mg/ml of protein content, against three selected pathogens compared to the sample from the leaves that revealed a MIC of 25% v/v, corresponding to a protein concentration of 4.25 mg/ml. The crude extracts isolated from *P. oceanica* were also active to combat the biofilm formation at sub-MIC concentration. The inhibition was evident with crude extracts from rhizomes in *S. aureus* ATCC 25923 at IC_{50} of 0.54 mg/ml, compared to

a value of IC_{50} of 0.74mg/ml with the extracts from leaves. Moreover, the polypeptide fraction of leaves of *P. oceanica* was also able to inhibit the biofilm formation in *C. albicans* ATCC 10231 at IC_{50} = 0.58 mg/ml. Peptide fractions displaying antimicrobial activity were further investigated by by High-Pressure Liquid Chromatography/nano-Electrospray Ionization tandem Mass Spectrometry (RP-HPLC/nESI-MS/MS) and database search. Database search allowed the characterization of fourteen peptides, one of them is related to a Viridiplantae-derived protein, whereas the others are attributable to bacterial proteins present in the investigated database. Moreover, most of identified peptides showed similarities with already described antimicrobial peptides (AMPs) from bacteria, animals and plants.

VITELLOGENIN AS A BIOMARKER OF EXPOSURE TO ENDOCRINE DISTRUPTORS IN TWO SPECIES OF MULLIDAE, *Mullus barbatus* AND *Mullus surmuletus*

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The use of biomarkers in environmental contamination studies is a widespread practice[1]. The advantages are the low cost compared to classic direct abiotic chemical analyses and the additional information on the organisms' health status. One of the most commonly used biomarkers is vitellogenin (VTG), an estradiol (E2) inducible female-specific protein in oviparous species. VTG is the precursor of egg yolk, and it is synthesized in the liver and transported through the blood to the growing oocytes. VTG is naturally absent in males. Thus, the detection of VTG in males' blood can be considered an indicator of exposure to endocrine disruptors (ECDs), whether they are exogenous E2 or estrogen-like contaminant[2]. The aim of this study was to evaluate the validity of using plasma VTG analysis, by ELISA, as a biomarker in two species of *Mullus*, *M. surmuletus* L., 1758, and *M. barbatus* L., 1758, and to correlate the response of fish to their reproductive stage, determined by histological analysis of gonads and plasma levels of the primary sex steroids. Fish used in the study were sampled on the Sicilian east coast, specifically in three areas characterized by different anthropogenic impacts, 1) urban, 2) petrochemical industry and, 3) agriculture. As expected, VTG was detected in plasma of females from both species, although at different levels and was correlated with the stage of gonad development and plasma levels of E2, testosterone (T), 11-ketotestosterone (11KT) and the maturation inducing steroid 17α,20β-dihydroxypregnenone (DHP). On another hand, VTG detected in the plasma of males in both species, indicating potential presence of EDCs in the sampling areas. In particular, VTG positive males were found in the area with a strong urban impact, suggesting a correlation with wastewater discharge and emerging contaminants such as medicines. Also, VTG positive males were found in the petrochemical industry area, where metals have persisted for decades and may play a role as ECDs. In conclusion, the results of this study show that VTG analysis might be used as a biomarker for these two *Mullus* species and that some differences can be observed depending on the species and their stage of reproductive development.

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EFFECTS OF HERBICIDE EXPOSURE ON HAEMOCYTE MORPHOLOGY IN A GROUND BEETLE

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Persistence of agrochemical residues has become a serious threat for human health. Especially herbicides usage, which is considered a fundamental practice to maximize crop productivity, leads to soil and trophic web contamination [1]. Suitable tools and markers are required to predict and prevent risks for humans, animals and environment. Insects provide useful services in agroecosystems, such as pollination and pest control. Carabid beetles, in particular, are considered beneficial organisms inhabiting cropland, acting as biocontrol agents and being well known bioindicators. In this study, we investigated the variations of haemocyte morphology in *Harpalus (Pseudoophonus) rufipes* (De Geer 1774) in response to a pendimethalin-based herbicide (PND) exposure. PND is one of the most commonly used herbicides in agriculture, and the circulating haemocytes represent the principal effectors of cellular immune response in insects [2]. Beetles were field collected, and treated in laboratory, simulating the exposure to PND at the recommended field rate (4L per ha). To assess cytological and ultrastructure analyses of haemocytes, withdrawals were performed at different time points after the initial exposure (0, 2, 7 and 21 days). PND was found to cause cytotoxic effects on the circulating haemocytes mainly during the first week of exposure. Cells appeared irregular in shape, featured by vacuolization of the cytoplasm and condensation of periferic chromatin. Swelling of membranes occurs in different organelles, suggesting an altered permeability due to PND and necrotic, apoptotic cells and autophagic bodies were recorded in the haemolymph. A recovery was monitored at 21 days after exposure, in agreement with information on PND soil degradation. Our results showed that sublethal doses of PND severely alter morphology and functionality of haemocytes with consequences for animals' immunocompetence and potential effects on vulnerability to pathogens. This study demonstrates *H. rufipes* being a suitable model and a useful bioindicator in ecotoxicological studies. Moreover, variations in haemocytes morphology represent a good marker of exposure to pesticides. This information could improve the knowledge of herbicide effects on non-target species, in order to preserve beneficial insects and biodiversity in agrosystems.

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NEUROSCIENCE

SOCIAL BEHAVIOR AND VASOPRESSIN SYSTEM ARE ALTERED BY CHRONIC EXPOSURE TO BISPHENOL A IN PREGNANT FEMALE MICE

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Bisphenol A (BPA), an organic synthetic compound found in some plastics and epoxyresins, is one of the best known and most studied Endocrine Disrupting Chemicals (EDCs,): exogenous chemicals that can interfere with any aspect of hormone action. Exposure to BPA is especially dangerous if it occurs during specific "critical periods" of life, such as intrauterine, perinatal, juvenile or puberty periods, when organisms are more sensitive to hormonal changes. In this study, we focused, in particular, on the effects of exposure to BPA during pregnancy, which represents a highly sensitive period for both the fetus and the mother. We treated C57BL/6J dams (n=10/group) orally with BPA (4µg/kg body weight/day, *i.e.*, the EFSA Tolerable Daily Intake dose) dissolved in corn oil or with the vehicle, starting from mating and continuing for 20 weeks. The dams were monitored for variations of the body weight and of food intake. During the last two weeks of treatment, we monitored also the estrous cycle and, due to the observation that BPA-treated dams exhibited aggressive behavior towards males during mating compared to the controls, we also performed the Three-Chamber Test to assess sociability. This test revealed that the time spent within the chamber of the male non-tester mouse, decreased in BPA-treated females compared to the controls. Moreover, other behavioral differences were found in the treated group, such as increase of movement speed when the animal was alone in the apparatus and, even more, in the presence of the non-tester male. Therefore, we decided to analyze the vasopressin system (a peptidergic system particularly sensitive to endogenous gonadal hormones, and involved in the control of social behaviors), measuring, when possible, both fractional area and number of cells, in paraventricular, supraoptic and suprachiasmatic nuclei of the hypothalamus and in the medial amygdala. We observed a decrease of the fractional area and of the number of cells in the vasopressin system of BPA-treated dams, which could be partially linked to their behavioral alterations. These results suggest that exposure to BPA may pose a risk even in adulthood, particularly when it occurs during delicate periods such as pregnancy.

EXPOSURE TO BISPHENOL A OR S DURING PREGNANCY AND LACTATION ALTERS MATERNAL BEHAVIOR IN MICE

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Maternal behavior is a complex variety of peculiar behaviors which depend on a multitude of factors, including the environmental ones. Among these factors, Endocrine Disrupting Chemicals (EDCs, *i.e.* exogenous chemicals, or mixture of chemicals, that can interfere with any aspect of hormone action) are increasingly attracting attention. In particular, bisphenol A (BPA), an organic synthetic compound largely used for the production of plastic, is already known to exerts some negative effects in the displaying of maternal behavior. Interestingly, one of the proposed, and already used, BPA-substitutes, bisphenol S (BPS), seems to have the same, or even worse, endocrine disrupting properties as the BPA. Despite this, potential effects of BPS on maternal behavior are still poorly investigated. In this study we focused on the analysis of the effects of BPA and BPS exposure during pregnancy and lactation in mice. We treated 3-months old C57BL/6J females (n=10/group) orally with a dose of 4µg/kg body weight/day of BPA (*i.e.* EFSA Tolerable Daily Intake dose) or BPS dissolved in corn oil or with vehicle, starting from mating and continuing during lactation until the weaning of the offspring (postnatal day 28, PND28). We monitored both dams and offspring, assessing the number of pups at birth, the female-to-male *ratio* and the percentage of death in each litter. The spontaneous maternal behavior was recorded during the first postnatal week (PND1-7). At P0, pups from BPA-treated dams had lower female-to-male *ratio* compared to control group, while we observed the opposite among the pups from BPS-treated dams (higher female-to-male sex ratio compared to controls). Besides, the percentage of dead pups was higher in the bisphenol-treated dams' litters, compared to the controls' ones. Remarkably, the offspring mortality impacted differentially BPA and BPS litters, with more female pups found dead in the BPA litters, while more male pups found dead in the BPS litters, sharpening the difference in the female-to-male *ratio*. Both BPA- and BPS-treated dams displayed alterations in spontaneous maternal behavior compared to the controls. In fact, both BP-treated groups spent less time performing pups-related behaviors than controls. It is important to recall that alterations in maternal care, along with the treatment itself, may affect, later in life, physiology and behavior of the offspring. In conclusion, present data indicate that the exposure to bisphenols, particularly when it occurs during sensitive developmental periods, such as pregnancy and lactation, represents a risk for both dams and offspring, even at low doses, through the functional alteration of neural circuits controlling fundamental behaviors. Present results support the idea that new and more specific strategies are necessary in order to reduce and contain the impact of environmental bisphenols on public health.

PLEIOTROPIC ROLE OF THE TRANSCRIPTIONAL REGULATOR NR2F1/COUP-TFI IN THE ADULT MOUSE HIPPOCAMPUS: FROM THE CONTROL OF NEURAL STEM CELL BEHAVIOR TO MITOCHONDRIAL FUNCTION IN NEURONS

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The strong transcriptional regulator COUP-TFI, also known as Nr2f1, is emerging as a disease gene in humans. Its mutations cause the Bosch-Boonstra-Schaaf optic atrophy-intellectu-

al syndrome (BBSOAS; OMIM:615722), a rare disorder characterized by multiple clinical features including intellectual disability, optic nerve atrophy, seizures and autism spectrum disorder[1]. Recently, alterations in mitochondrial energy supply in muscle have been reported in two BBSOAS patients[2,3]. However, if and how Nr2f1 can directly regulate mitochondria function in neural cells is still unknown, and understanding the mechanisms by which Nr2f1 loss-of-function triggers mitochondrial dysfunction in BBSOAS patients will greatly benefit from novel discoveries on mouse models. We have recently discovered that Nr2f1 is highly expressed in the neurogenic niche of the mouse hippocampal dentate gyrus (DG), where neurogenesis persists into adulthood thanks to adult neural stem/progenitor cells (aNSPCs) and whose occurrence is crucial for learning and memory. In this system, we showed that Nr2f1 ensures proper neurogenesis by driving aNSPCs toward a neuronal lineage inhibiting an astroglial fate both in physiological and neuroinflammatory conditions[4]. By using the same system, we are now dissecting the mitochondrial features affected by manipulation of Nr2f1 in neurons, as their development from aNSPCs is accompanied by extensive changes in mitochondrial mass, distribution and shape. Interestingly, by combining multiple approaches ranging from gold-standard neuroanatomical approaches to genome-wide and *in silico* analyses, we discovered that Nr2f1 sculpts mitochondrial architecture in adult-born DG neurons by directly controlling the expression of genes encoding mitochondrial protein crucial for mitochondrial dynamics and function. Moreover, we found that impaired mitochondrial network in adult-born DG neurons leads to defective expression of mitochondrial respiratory chain components that could ultimately contribute to the neuronal death observed after long-term Nr2f1 loss in the DG neurogenic niche. Overall, our study provides the first evidence of a direct involvement in the mitochondrial phenotype upon Nr2f1 manipulation and paves the way for future development of novel therapeutical approaches for the human disease BBSOAS.

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THE ROLE OF PREMOTOR AREAS IN GABAERGIC INHIBITION IN JOINT ACTION

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Behavioural coordination, during Joint Action (JA) interaction, requires sensorimotor communication between agents to smoothly negotiate and adjust actions in time and space (D'Ausilio *et al.*, 2012; Sebanz *et al.*, 2006). Inhibitory motor control must also play a key role when the motor system needs to integrate inferences about others' action (Cardellicchio *et al.*, 2020b). Very little research has been carried out on the contribution of motor inhibition in JA tasks and on the contribution of different cortical areas. Recent studies suggest that ventral (PMv) and dorsal (PMd) premotor cortex might differently sculpt movements according to the socio-interactive context. We used an interactive task in which subjects were required to open a bottle with one hand, as in a previous experiment (Cardellicchio *et al.*, 2020a). The bottle was held and stabilized by a co-actor (JA) or by a mechanical holder (vice clamp, no-JA). To analyse the contribution of premotor areas we have inhibited, by means of a continuous Theta Burst Stimulation (cTBS) the PMd, the PMv or Vertex (as control area). We collected, by Transcranial Magnetic Stimulation (TMS), cortical silent period (cSP) and short-interval intracortical inhibition (sICI), during the reaching phase of the task. These two indices respectively reflect slow corticospinal (GABAb-mediated) and fast intracortical (GABAa-mediated) inhibition. Vertex stimulation confirm results of our previous study: an increment of the cSP and a downregulation of intracortical inhibition during JA condition. Analyses on sICI confirm a reduction of intracortical inhibition in JA condition aspecific for the site of stimulation. cSP seems to be reduced in the JA conditions in both PMd and PMv stimulation sites. Moreover, we found a significant difference of the cSP between PMd and PMv stimulation in noJA condition. These results, showing a dissociation between fast and slow inhibition during JA, shed light on the different inhibitory contribution of the two premotor areas.

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PARALLEL FAST AND SLOW MOTOR INHIBITION IN JOINT ACTION

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Sensorimotor communication is central to cooperative behaviors in which two or more agents coordinate their actions in time and space, to achieve a common goal. These interactions, defined Joint Actions (JA), are based on online inter-individual mutual motor adaptations and on shared cognitive representation of a given task (D'Ausilio *et al.*, 2012; Sebanz *et al.*, 2006). Inhibitory motor control may play a key role during JA tasks, where the motor system has to further integrate inferences about others' action. This integration process might be reflected in modulation of corticospinal inhibitory mechanisms (Cardellicchio *et al.*, 2020a). Yet, very little research has been carried out on the contribution of motor inhibition in JA tasks. To explore this issue, we used a novel experimental task in which subjects were required to open a bottle with one hand. The bottle was held and stabilized by a co-actor (JA) or by a mechanical holder (vice clamp, no-JA). A first motion capture study characterized the reaching and grasping kinematics of the two conditions. In a second study, by means of Transcranial Magnetic Stimulation (TMS), we measured neural inhibition, that is regulated by GABAergic neuromodulation which alters polarization of neural membranes via fast acting $GABA_a$ receptors and slow acting $GABA_b$ receptors. We recorded (i) corticospinal excitability (CSE), (ii) cortical silent period (cSP) and (iii) short-interval intracortical inhibition (sICI), during the reaching phase of the task. These latter two indexes respectively reflect GABAb and GABAa-mediated inhibition. We found no modulation for CSE, while cSP was increased and intracortical inhibition was downregulated during JA. Interestingly, the cSP correlated

with partners' predictability as a whole and with partners' behaviour in the previous trial. We provide evidence for two parallel modes of inhibition acting during JA and possibly indexing complementary phenomena (Cardellicchio *et al.*, 2020b). The first one, the sICI, seems to reflect an unspecific preparation to coordinate, while the results obtained in cSP might underline the fact that participants build predictive models of their partner to improve interaction success.

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MECHANICAL STIMULI INDUCE PHENOTYPIC CHANGES IN PERIPHERAL NERVES RELATED WITH PAIN RELIEF

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The selective repeated tension of the Peripheral Nervous System (PNS) also known as neurodynamic treatment (NDT) is successful in pain modulation of patients affected by nerve related chronic and acute back and neck pain, the main cause of disability worldwide. Even if NDT reduces pain and disability the biological effects involved are still unknown and no standard protocol is available. The study aims to assess the effects of NDT on PNS cells in order to develop a standardized protocol, to define any dose response changes in PNS cells and even any side effects of NDT. We adopted *ex vivo* model of rat Dorsal Root Ganglia (DRG) and *in vivo* models of median end ulnar nerve crush in adult rats. Protocols of repeated mechanical stimuli were tested starting from those reported in literature and refined by previous trial results. *ex vivo* experiments were performed in triplicates seeding cells on pre-coated silicone membranes and repeated tension protocols were administered using a bioreactor. Taking advantage of DRG results *in vivo* protocols were administered. Morphological, Gene and Protein expression analysis were performed and comparted to behavioral tests on motor and sensory tasks. A standardized protocol of NDT was possible to be defined. NDT protocols are able to induce dose response changes in DRGs explants and in DRGs from animals after 3 weeks of daily treatments. No side effects were possible to be detected. In particular NDT is able to promote cell differentiation and promotes neurites outgrowth. Interestingly, the NDT do significantly affect the expression of PIEZO1 and TACAN, that are genes linked to receptors transducing respectively mechanical non painful stimuli and mechanical painful stimuli involved in pain modulation. Those results suggest that NDT promotes the regeneration processes in sensory neurons with anti-allodynic effect. Notably, our results describe a key-role of the DRG in neuropathic pain modulation suggesting an effective treatment dosage of non-pharmacological treatment to be effective in mechanical allodynia modulation. Even if *ex vivo* and *in vivo* rodent models looks distant from clinical practice the cell subpopulations and their behavior are similar to human PNS cells. Also, all variables on which the NDT protocol was defined (amplitude of elongation, number of repetition, speed etc.) still be very suitable to be translated in clinical settings.

PACAP NEUROPROTECTIVE EFFECTS: FOCUS ON ANTIINVASIVE ROLE EXERTED IN GLIOBLASTOMA MULTIFORME

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Glioblastoma (GBM) is the most common and aggressive brain cancer affecting adult patients, characterized by high rate of cells migration and invasion. Recent findings have demonstrated that the pleiotropic neuropeptide known as pituitary adenylyl cyclase-activating peptide (PACAP) is involved GBM progression. This solid tumor is characterized by extensive hypoxic areas inducing hypoxia-inducible factors (HIFs) transcription. The hypoxic signaling cascade is the main responsible of uncontrolled cells proliferation as well as neovascularization. In accord, the hypoxic factors induction triggers a signaling cascade that conduces to epidermal growth factor receptor (EGFR) overexpression and vascular endothelial growth factor (VEGF) release, two factors exerting a crucial role in growth and aggressiveness of GBM. The aim of our studies was investigated the molecular mechanisms underlying the anti-invasive effect of PACAP on GBM cells in hypoxic microenvironment as well as the peptide involvement in glioma's aberrant angiogenesis. Our data have showed that PACAP counteracts GBM cell migration under hypoxia by modulating HIFs and EGFR expression through the inhibition of PI3K/Akt and MAPK/ERK signaling pathways. Moreover, PACAP affects the expression levels of VEGF, by inhibiting the formation of vessel-like structures. Overall, our findings contributed to elucidate the modulatory role exerted by PACAP in GBM malignancy.

MUTATED ION CHANNELS-ENCODING GENES COULD IMPAIR RETINAL NEUROTRANSMISSION: NEW INSIGHTS ON RETINAL DEGENERATIONS

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Ion channels are molecular machines acting as major integrating and regulatory devices for controlling cellular excitability. Although ion channels are essential in many physiological processes and represent a key class of drug targets, much is still unknown about their function and possible breakdowns that lead to diseases. One of the most recent research field regards signal transmission and visual processing. A wide range of mutations have been reported in ion channels and their interacting subunit coding genes, which contribute significantly to a wide spectrum of eye-related diseases collectively called channelopathies, a sub-group of inherited retinal dystrophies (IRDs). Such mutations result in either a loss- or gain-of channel func-

tions affecting the structure, assembly, trafficking, and localization of channel proteins. We present the results produced from whole exome sequencing of different Italian and Egyptian families, affected by orphan forms of IRDs. We investigated new mutated candidate genes, basing on patients' common neurotransmission impairments. We found 4 mutated genes, whose variants might alter important ligand binding sites, differently distributed through all considered patients. The use of computational methods based on artificial intelligence (more precisely machine learning) and molecular dynamics approaches, complementary to chemical-biological-based experimental methods, permitted to better understand ion channels alteration consequences. Furthermore, such genes encoding for ion channels or their regulatory proteins resulted strictly interacted with known causative genes, also sharing with them synaptic-related pathways. Considering several limitations that will be resolved by further experiments, we believe that our exploratory investigation will help scientists to provide a new promising paradigm for personalized diagnosis of IRDs to facilitate the development of innovative treatments.

COMPARISON OF DECELLULARIZATION PROTOCOLS TO GENERATE PERIPHERAL NERVE GRAFTS: A STUDY ON RAT SCIATIC NERVES

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New methods for repairing long gap associated peripheral nerve injuries and obtaining an optimal functional recovery are still needed. Autografts are the current gold standard repairing technique. Their use is accompanied by some limitations; as the need of a second intervention for donor nerve harvesting, donor site morbidity and eventual neuromas formation. The use of nerve allografts offers wide availability of donor nerves that are matching in size, calibre and type. Allografts use are limited by the need of a concomitant immunosuppressant treatment to preclude the possible provocation of an adverse immune response and consequent graft rejection. To overcome this problem the idea of decellularization technique was developed. Decellularization is based on removing all cells and their surface expressed antigens while preserving the natural tissue extracellular matrix[1]. In this study we were interested to investigate the use of two detergents (TBP and PAA) that have never been applied in nerve decellularization but are successfully used to decellularize tendons. For this study two protocols were applied on rat sciatic nerves DN-P1 (originally developed for tendons)[2] and DN-P2 (previously published protocol for nerves)[3]. Rat decellularized sciatic nerves were extensively tested *in vitro*. DN-P2 had demonstrated better cellular components removal. Immunohistochemical staining of different extracellular matrix components collagen, proteoglycans and laminin showed an adequate conservation level in both protocols. DN-P1 demonstrated superior results in SEM analysis, where a high conservation of endoneurial tubes was detected. TEM analysis has shown a high conservation of collagen fibres in DN-P1 as well. Further *in vivo* analysis is needed to evaluate the efficiency of these decellularized nerves (DN-P1) in repairing long rat sciatic nerve injuries.

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RHEUMATOID ARTHRITIS INDUCED BY NTRA-ARTICULAR INJECTION OF FREUND'S ADJUVANT ENHANCES THE EXPRESSION OF VARIOUS HSPS IN SENSORY NEURONS AND GLIAL CELLS IN THE DORSAL HORN OF THE SPINAL CORD

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Rheumatoid Arthritis (RA) is a chronic inflammatory and autoimmune disease characterized by the attack of its own joint lining. This disease also engages an increase release of inflammatory mediators, which alters the function of sensory neurons by exerting a positive feedback on sensory nerve endings to enhance this release. Small-diameter primary afferent fibers (C fibers) are the major players involved in this process, which simultaneously release glutamate, Substance P (SP) and Calcitonin gene-related peptide (CGRP) the main peptides responsible for pain, the severe symptoms of RA and the persistent neurogenic inflammation. Peripherally, these substances act on post-capillary venules rendering them leaky, resulting in plasmic extravasation and vasodilatation. Centrally, glutamate and neuropeptides have been shown to either activate or modify glutamate receptors on post-synaptic cells in the dorsal horn of the spinal cord. More importantly, HSPs (27, 60, and 90) have been demonstrated to possess an important protective role in both neuronal protection and peripheral nerve injury. In this study, we hypothesize that RA induced by intra-articular injection of Freund's adjuvant will enhance the expression of various HSPs in sensory neurons and glial cells in the dorsal horn of the spinal cord. This increased expression will be mediated through glutamate receptors activation. This study was conducted both at University of Palermo and in the Comprehensive Neuroscience Lab at American University of Beirut. Rats received the care in an animal house with suitable environment. The rats were randomly divided into 2 groups of equal numbers. The first one as control group, with the rats received only intra-articular injection of saline. The second group was subjected to complete Freund Adjuvant injections in the synovial cavity of the knee joints. To assess the effects of HSP release on sensory functions in inflamed rats, behavioral tests were conducted. Immunofluorescence and Western Blot were conducted to examine the expression of HSPs in the neurons and glial cells of the spinal cord. These results encourage that HSPs play a protective role in alleviating the progression of RA. NMDA and non-NMDA glutamate receptors antagonists will be used to determine the role of these receptors in HSP release.

ESTROGENS INHIBIT A -MEDIATED PHF-LIKE CONFORMATION OF TAU THROUGH ANTIOXIDANT ACTIVITY AND miRNA-218 REGULATION IN hTAU MICE

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Alzheimer's disease (AD) is the most common form of dementia and it's characterized by amyloid plaques, neurofibrillary tangles and neuroinflammation in the brain. Sex differences in the prevalence, risk, and severity in AD patients have been demonstrated in numerous studies, which revealed that women are more susceptible to developing AD than men. This could be due to estradiol levels, which seem to be inversely correlated with miRNA218 expression. The overexpression of ERα increases miR-218 expression and Tau phosphorylation. In this study we follow the hypothesis that biological sex influences the effect of Aβ42 monomers on pathological tau conformational change. On these bases, we observed miR-218 expression in mild cognitive impairment (MCI) and AD patient's liquor analysing the differences between male and female. The data revealed that miR-218 is more expressed in MCI and AD patients in both sexes, but miRNA levels are double in AD women patients than their gender control. In order to investigate the role of estrogen on Tau alterations we treated 2 months-old hTau mice with Aβ peptides via intracerebroventricular injection. Two groups of female mice underwent to ovariectomy, one of these groups and also a group of male mice were treated with 17β-estradiol. We performed western blot analysis to see the hyperphosphorylation and the pathological conformation of tau, and ELISA assay to observe the antioxidant effect of estradiol and its effect on miRNA-218 expression through RT-PCR. Our data revealed that conformational changes and hyperphosphorylation of Tau mediated by Aβ42 monomers occurs significantly in male or ovariectomized female mice but not in control female. Interestingly, the estradiol treatment in ovariectomized females is able to rescue from tau alterations and moreover, its mechanism of action could be due both by an antioxidant activity and its ability to modulate the expression of miRNA-218 linked to tau phosphorylation. Our study indicates that 17β-estradiol has protective effects on mice model of AD and that miRNA-218 could have an important role in the mechanism of action of estrogens. Our future studies are oriented to observe the effect of the injection of an antimiRNA of miR-218 in hTau mice and contemporary to analyse miR-218 levels also in AD, MCI and CVD patients' blood, which is less invasive than liquor, in order to identify an evidence of a possible future triggering of the disease and the best therapeutically approach in prevention of AD.

EXPLORING HUMAN BRAIN VISUO-MOTOR PLASTICITY WITH A NEW PAIRED ASSOCIATIVE STIMULATION PROTOCOL

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Background and aims. Hebbian learning, and Hebbian associative plasticity, its neurophysiological substrate, has been implied in the formation of the association between sensory and motor representations of actions in the *action observation network* (AON). At date, such inductor role of Hebbian plasticity still needs empirical support[1]. To address this issue, we have assessed whether a paired associative stimulation (PAS) protocol, a protocol known to activate Hebbian learning[2], can induce the formation of atypical (*i.e.*, absent in normal conditions), visuo-motor associations, in turn reshaping motor resonance. Materials and Methods. Forty healthy participants underwent our novel mirror-PAS (m-PAS) protocol during which they were exposed to 180 repeated pairings of transcranial magnetic stimulation (TMS) pulses, applied over the right primary motor cortex (M1), time-locked with the view of index-finger movements of the right (ipsilateral) hand at a frequency of 0.2 Hz. In a first experiment, the *inter-stimulus interval* (ISI) between the onset of the visual action stimulus and TMS pulse was varied following the chronometry of motor control (25 ms) or that of AON activation (250 ms). In a second experiment, a control condition was introduced where the visual stimulus of the PAS depicted a non-biological movement. In every experiment, before and after each PAS session, motor resonance was assessed by recording Motor Evoked Potentials (MEPs) induced by single-pulse TMS applied to the right M1, during the observation of both contralateral (left) and ipsilateral (right) index-finger movements or static hands. Results. As expected from literature[3], before m-PAS, a facilitation of cortico-spinal excitability (*i.e.*, MEPs) occurred only during the view of left, contralateral (with respect to the TMS side) index-finger movements. Importantly, m-PAS successfully induced new ipsilateral motor resonance responses, indexed by an atypical facilitation of cortico-spinal excitability by the view of ipsilateral (*i.e.*, right) hand movements. Crucially, this effect occurred only if the associative stimulation followed the chronometry of motor control (ISI of 25 ms) and the visual stimulus depicted a biological movement. Discussion and Conclusions. The present findings provide empirical evidence that Hebbian learning shapes the visuo-motor matching properties of the AON, which could be modulable by PAS. The m-PAS represents a promising non-invasive protocol to shed light on the neurofunctional bases of the human AON and, in turn, plastic properties of mirror neurons.

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IMAGING THE DEVELOPING AUDITORY CORTEX IN MOUSE

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During early postnatal development, sensory experience (*e.g.*, parental ultrasound vocalizations, USVs) shapes neural connections and individuals acquire memory of these cues. In adult age, these memories drive several ethologically relevant behaviours such as social and individual recognition and mate choice. Among the variety of sensory stimuli at which newborn pups are exposed during development, USVs have been shown to play an important role in forming memories of conspecifics

that are recalled in adult age to drive mate choice. However, how USVs are encoded in cortical auditory circuits and how they influence the functional development of these networks is unknown. Here, we performed *in vivo* two-photon functional imaging and characterized spontaneous activity of layer 2/3 (L2/3) neurons in the mouse primary auditory cortex at different postnatal ages. P0 mice were stereotaxically injected in auditory cortex with adeno-associated viral vectors expressing GCaMP7 (AAV1hSynGCaMP7) and the spontaneous activity was imaged at three developmental windows: *i)* P8-P10: when the external auditory canal was closed; *ii)* P11-P15: when the onset of hearing occurred; *iii)* P16-P21: during the critical period for hearing. Rhodamine was injected in the center of the craniotomy at the end of the recording to confirm the anatomical location corresponding to the imaged region *a posteriori*. We found that, at P8, L2/3 neurons of primary auditory cortex displayed spontaneous synchronous and highly correlated transients recruiting large neuronal ensembles. Starting from P11, when the onset of hearing occurred, the firing of L2/3 auditory neurons was less synchronous and the correlation between cell activity decreased. At P16-21 recordings displayed progressively increasing asynchronous calcium transients and decorrelated neuronal activity. These data are consistent with previous reports in the developing mouse barrel cortex. Taken together, our findings show that the transition from synchronous to asynchronous activity occurs at hearing onset and they suggest that this process may be influenced by the different stimuli received during postnatal development.

MOTOR NEURON VULNERABILITY AND RESISTANCE IN AMYOTROPHIC LATERAL SCLEROSIS: THE ROLE OF PITUITARY ADENYLATE CYCLASE-ACTIVATING POLYPEPTIDE

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Amyotrophic lateral sclerosis (ALS) is a progressive and fatal neurodegenerative disease caused by the death of upper and lower motor neurons (MNs) in the brain and spinal cord. Some lower MNs are relatively resistant to degeneration, for instance, neurons of the oculomotor nucleus, controlling eye movements, is more resistant as compared to those of hypoglossal nucleus. By analyzing postmortem samples from ALS patients, it was found a differential genomic pattern between the two nuclei. Among identified genes, adenylate cyclase activating polypeptide 1 (ADCYAP1) gene, encoding for pituitary adenylate cyclase-activating polypeptide (PACAP), was over-expressed in the oculomotor *versus* hypoglossal nucleus, suggesting that PACAP could plays a role on MNs in ALS. The purpose of the present study was to analyze the effect of PACAP to counteract MNs degeneration, by using the neuroblastoma-spinal cord-34 (NSC-34) cell line, stably expressing human wild type or mutant SOD1 G93A, representing a well characterized *in vitro* model of a familial form of ALS. Our results showed that the peptide increases cell viability following serum deprivation, via EGFR transactivation mediated by protein kinase A stimulation. Furthermore, PACAP significantly reduced hypoxia-induced mutant SOD1 accumulation by regulating the autophagy process through the activation of the MAPK/ERK survival signaling pathway. In conclusion, these findings indicate the protective role played by the peptide in ALS, suggesting that the different resilience of some cranial nerve motor nuclei could be due to differential expression of PACAP and its receptors in MNs.

α-SYNUCLEIN AS A COMMON KEY PLAYER IN NEURODEGENERATION AND EPILEPTOGENESIS

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Epilepsy is a common neurological disease, whose hallmark is the presence of recurring seizures, *i.e.* transient events of abnormal neuronal activity in the brain. The etiological factors can be structural, genetic, infectious, metabolic or neurodegenerative. In order to develop new therapeutic strategies against epileptogenesis, proteins involved in neurodegenerative disease, such as α-synuclein (α-syn), have recently gained increased attention. α-Syn is a protein widely expressed in brain tissue, mainly in presynaptic terminals. Its involvement in neurological diseases, such as Parkinson disease (PD) and dementia with Lewy bodies (DLB), defined them synucleinopathies. However, both PD and DLB, besides α-syn accumulation, showed sleep dysfunction and EEG alterations, frequently becoming epileptic seizures. Moreover, previous clinical and pre-clinical experimental studies underlined the alteration of α-syn expression both in epileptic human and animal model brains. Therefore, our aim was to analyze α-syn synaptic expression in a murine model of a genetic sleep-related epilepsy and in human epileptic brain. In particular, in wild type and transgenic (TG) mice we analyzed α-syn localization in two different types of synaptic terminals, identified by means of the relative vesicular neurotransmitter transporters (VGLUT1, VGAT), in cortical and striatal areas. Our results revealed an imbalance of α-syn expression in TG mice. Moreover, dorsal striatum displayed an increase of VGAT immunopositive synaptic terminals expressing α-syn in TG mice, suggesting an alteration of GABAergic circuit. Subsequently, post-mortem human brain sections were used to set the experimental protocols for the detection of α-syn both in control subjects and in patients affected by PD, revealing the synaptic localization of α-syn in controls and the presence of Lewy bodies and neurites, as expected, in PD patients. On these bases, we set up experiments to investigate its expression in post-surgical human tissues from patients with Temporal Lobe Epilepsy due to Hippocampal Sclerosis (TLE-HS), the most prevalent form of chronic focal epilepsy. Preliminary results showed: i) a severe loss of α-syn staining in sclerotic hippocampi, due to the synaptic density reduction; ii) PD, TLE-HS and control show different α-syn expression and immunolocalization in two different types of synaptic terminals. The present study provides novel insights to understand with a completely new perspective the pathogenesis and/or the histopathological consequences of different types of epilepsy.

MODULATION OF FORMYL PEPTIDE RECEPTOR 1 INDUCED BY RESVERATROL IN AN LPS INDUCED NEUROINFLAMMATORY ANIMAL MODEL

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The search of novel therapeutic agents for the management of neurodegenerative diseases has become an appealing research interest in recent years. Neuroinflammatory response within the brain or spinal cord is the production of several mediators, including cytokines, chemokines, reactive oxygen species and secondary messengers, released by resident glia cells. Although it may represent a neuroprotective mechanism a sustained neuroinflammation can induce neurotoxicity and is related to neurodegeneration [Cianciulli A. *et al.* 2020]. The invading pathogens and their components are recognized by pattern recognition receptors (PRR) including the G-protein coupled formyl peptide receptors (FPRs), which are expressed by immune cells [Bianchi M.E. 2007]. The murine FPR gene family includes at least six members in contrast to the only three in humans. The two most important members are the Fpr1 and Fpr2. Fpr1 encodes murine FPR1, which is considered the murine orthologue of human FPR. Resveratrol, a non-flavonoid polyphenol of the red wine and grapes, apart its beneficial health effects and anti-inflammatory properties, has been reported to reduce neuroinflammation in various neurodegenerative disease models. The anti-inflammatory responses of Resveratrol involve the activation of the protein deacetylase sirtuin 1 (SIRT1) gene. In this research we have investigated in an LPS based murine model of neuroinflammation the role of FPR1, examining not only if this receptor undergoes a reduction of its expression during neuroinflammation, but also whether treatment with Resveratrol was able to modulate its expression leading to an amelioration of neuroinflammatory picture. We have demonstrated that FPR1 together with SIRT1 resulted upregulated by Resveratrol treatment and that this increase is associated to a to reduction of neuroinflammatory responses. In this respect, different pro-inflammatory hallmarks such as IL-1β, IL-1βR, TNFα, TNFα-RI resulted downregulated by treatment of Resveratrol. In conclusion, the modulation of the FPR1 expression by Resveratrol may be evaluated not only to propose a novel anti-inflammatory and pro-resolving therapeutic target for the reduction of the detrimental effects of neuroinflammation in course of neurodegenerative diseases but also as promising approach to promote human health by a diet rich of antioxidative bioactive compounds.

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UNVEILING A NOVEL ROLE FOR CYTOPLASMIC HDAC4 IN DMD

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Histone deacetylase 4 (HDAC4) is a member of class II HDACs which, by cooperating with class I HDACs, deacetylates proteins, thereby mediating the response to different stimuli in skeletal muscle. Recently its protective, essential role in maintaining skeletal muscle homeostasis after long-term denervation (1) or in Amyotrophic Lateral Sclerosis (2) and in satellite cell proliferation and differentiation (3) has been clarified. Further, HDAC4 is crucial for skeletal muscle regeneration by mediating soluble factors that influence muscle-derived cell proliferation and differentiation after injury (4). Duchenne Muscular Dystrophy (DMD) is a fatal inherited muscle-wasting disease, caused by mutations in the dystrophin gene and characterized by progressive muscle weakness and degeneration. The membrane repair response is enhanced to actively maintain membrane integrity in DMD (5). The pan-HDAC inhibitor givinostat is presently in phase III clinical trial for the treatment of DMD, despite no efficacy in skeletal muscle function has been registered in DMD patients (6), highlighting the needs to study the HDAC functions in skeletal muscle in DMD further. While the function of class I HDACs in DMD has been partially elucidated, little is known about the role of class II HDACs. To shed light on additional functions of HDAC4 in skeletal muscle, we are currently studying the role of HDAC4 in DMD by using a genetic approach. We generated DMD mice with HDAC4 deleted in skeletal muscle (mdx;HDAC4mKO), by crossing mice with a skeletal muscle-specific deletion of HDAC4 (HDAC4 mKO) with mdx mice, a mouse model of DMD. To determine HDAC4 functions, muscular dystrophy progression has been analyzed over time, by histological and functional analyses. Deletion of HDAC4 in skeletal muscles exacerbates muscle degeneration and decreases muscle regeneration and functionality over time. Further investigations have highlighted an impaired satellite cell differentiation and membrane repair mechanism in mdx;HDAC4mKO mice that may underpin the more pronounced progression of the pathology. Ectopic expression of cytoplasmic HDAC4 in mdx;HDAC4mKO rescues the phenotype both *in vitro* and *in vivo*, revealing a yet uncharacterized role of HDAC4 in DMD. From our results, we conclude that HDAC4 is important for maintaining skeletal muscle integrity in DMD mice. Ongoing studies are necessary to define the molecular signaling modulated by cytoplasmic HDAC4 in DMD in order to provide the experimental basis for a more efficacious pharmacological therapy.

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MUSCULAR BIOPSY EXAMINATION AND IN SILICO ANALYSIS OF A NOVEL GENETIC VARIANT OF CCT5 RELATED TO MOTOR NEUROPATHY: A NOVEL APPROACH TO STUDY INHERITED NEUROMUSCULAR DISEASES

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The identification, characterization, and classification of genetic neuromuscular diseases is becoming increasingly complex. Different genetic modifications occurring in a single gene can lead to distinct phenotypes and clinical examination alone is insufficient to define the pathological grade, *e.g.*, how, and how much, the skeletal muscle tissue is involved. The etiologic-pathogenic role of chaperonopathies in neuromyopathies is prevalent in many cases and must always be investigated. We have recently identified a novel homozygous c.670C>G p.(Leu224Val) variant in the gene encoding the subunit 5 of the chaperone CCT (CCT5), which is associated with severe neuromyopathy in an Italian girl. The phenotype is characterized by early onset, demyelinating neuropathy, and severe motor disability, *i.e.*, the phenotype is strikingly different from that previously observed in subjects affected by another variant, p.(His147Arg), in the same gene. In the present study of the patient bearing the novel variant p.(Leu224Val), we show histopathological impairment of myofibers and an incorrect organization of sarcomeric proteins in skeletal muscle. We also demonstrate by in silico 3D-structure analysis and bioinformatics that the Leu224Val mutation occurs within the intermediate domain of the CCT5 molecule but affects the conformation of the apical domain, a phenomenon of "mutation resonance-effect" on the molecular structure of the subunit. The data provide insight into the molecular mechanisms underpinning the effects of the mutation on the structure-function of CCT5 and on the impact of the chaperonopathy on nerves and muscles. Our combined approach to the study of this type of chaperonopathies holds promise for clinical applications in early diagnosis (including pre-natal), genetic counselling, and also for investigating the molecular basis of the tissue lesions and, thus, obtain clues useful for developing specific therapies.

MUTATIONS AT IMPRINTED LOCI AS MOLECULAR MECHANISM IN PEDIATRIC BRAIN ARTERIOVENOUS MALFORMATIONS

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Pediatric brain arteriovenous malformation (bAVM, OMIM #108010) represents about 3% of all bAVMs. BAVM is a congenital defect involving brain microvasculature. Lesions exhibit the direct shunt from arterioles to venules, lacking of the normal capillary bed. Feeding arteries and draining veins show impaired expression of vessel differentiation markers leading to loss of endothelial cells properties and increased permeability of the affected vessels. The high pressure of blood perfusing from arteries increases risk of lesion rupture, resulting in intracerebral haemorrhage. In children, bAVM is more prone to rupture, anticipating the age of symptom onset. Only a few dozen of familial cases have been described, inherited as autosomal dominant character. The high incidence of sporadic pediatric bAVM is attributed to defects during embryo vasculogenesis. Moreover, the severe lesion remodelling rate leads to consider bAVM a "dynamic" lesion resulting by continuous endogenous stimuli driven by genetic factors. These stimuli can include inherited germline and *de novo* genetic variants or epigenetic modifications occurring during embryo development. In order to increase knowledge about signalling cascades perturbed in pediatric bAVM patients, we performed whole exome sequencing on a small cohort of affected children. Following bioinformatic analysis, germline variants showing minor allele frequency < 0.01 were considered. By the ClueGo plugin of the Cytoscape platform, loci affected by these variants were functionally clustered in pathways, resulting related to NOTCH and TGFβR signalling, microtubule assembly, cell motility and adhesion, and ion conductance. In this context, we considered bAVM as the results of post-zygotic combination of mutated parental alleles. However, this mechanism might not be the only one or sufficient to explain lesion development. Lesions, indeed, arises in children, in pediatric age, despite the healthy parents. Therefore, we decided to focus our attention on mutations affecting imprinted genes. Among the wide spectrum of genes carrying variants in each patient, we found that 19 mutations occur in loci underwent to differential expression due to imprinting mechanism. Disrupting effect of these mutations on the protein structure was *in-silico* predicted. Most of mutated loci were functionally annotated to pathways related to transport and homeostasis of cations within the cell, in the regulation of angiogenic process. Then, we are proceeding by genotyping the parents of the patients in order to decipher the inheritance pattern of these mutations.

MITOCHONDRIAL MORPHO-FUNCTIONAL DYSFUNCTIONS IN SPINAL MUSCULAR ATROPHY: FOCUS ON ACONITASE2

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Spinal Muscular Atrophy (SMA) is due to a mutation/deletion of the Survival Motor Neuron 1 (*SMN1*) gene which

affects motor neurons (MNs) in children and young adults following a decrease in the levels of functional SMN protein; this results in motor impairment, muscle atrophy and premature death. Although the genetic cause of SMA has been identified, many aspects of its pathogenesis remain elusive and novel biological targets are investigated to develop new therapeutics and to monitor the efficacy of existing treatments. Indeed, the current experimental therapies aim at restoring SMN protein levels; however, their long term effects are still under evaluation, especially in adults. We focus on mitochondria since already at early stages in SMA their function, number, area and transport are significantly altered in axons of spinal MNs. We characterized subcellular and mitochondrial alterations (size, amount, area, cristae length and density) by TEM in MNs from the SMA mouse model (SMNdelta7). Moreover, in order to understand the mechanisms underpinning mitochondrial dysfunctions, we isolated pure mitochondria from the spinal cord of mice. After 2D gel and MALDI-TOF mass spectrometry, we identified differentially expressed proteins among which Aconitase2 (Aco2) was significantly altered. Aco2 turned out to be also dysfunctional in SMA MNs by enzymatic assays. Aco2 is the responsible for the isomerization of citrate to isocitrate in the Krebs Cycle and in mitochondria it is a sensitive redox sensor of reactive oxygen and nitrogen species. Interestingly, its levels and activity are altered also in other neurodegenerations such as Parkinson's and Huntington's disease. In order to study Aco2 as a new target and read-out of therapies efficacy in peripheral non-invasive tissues, we cultured primary fibroblasts (MEFs). By MitoTracker staining and multidimensional analysis by MiNA toolset from ImageJ, we studied the number of mitochondria, their distribution and trafficking. We also measured Aco2 activity by enzymatic assay. Currently, we are performing these investigations in fibroblasts derived from patients and aged-matched controls. Altogether, we found that mitochondrial networks, anatomical structures, dynamics and activity are affected in spinal cord and MEFs of SMA mice. Since mitochondria take part in a plethora of processes to preserve cellular homeostasis and genomic integrity, Aco2 could represent a potential new target to implement *SMN*-dependent therapies for SMA but also a target for other neurodegenerative diseases.

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IMPACT OF HONEY REGULAR INTAKE ON NEURODEGENERATION IN AN ANIMAL MODEL WITH DIET-INDUCED OBESITY

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The use of natural honey as a nutraceutical agent is associated with nutritional benefits and therapeutic promises. Honey flavonoids and phenolic acids can play a key role on health, due to the high antioxidant and anti-inflammatory properties. Up to date, it is unclear the effect of consumption of honey in obesity–related disorders, including neurodegeneration. The aim of the present study was to analyse the preventive effects of sicilian black bee chestnut honey daily intake on glucose dysmetabolism and neurodegeneration in mice fed high-fat diet (HFD). Three groups of mice were fed with standard diet (STD), HFD or HFD supplemented with honey (HFD-H) for 16 weeks. Glucose metabolism parameters, neuronal apoptosis (TUNEL assay and brain genes expression of Fas-L, Bim and P27) and central insulin resistance (cerebral cortex protein expression of pAKT, pERK and pGSK3 and microarray analysis) were analysed and compared between the different groups of animals. Fasting glucose, insulin levels, glucose tolerance and insulin sensitivity were significantly ameliorated in HFD-H mice compared to HFD, although the values were different from STD mice. Honey intake significantly reduced the HOMA index, which indeed was increased in HFD mice, suggesting a beneficial effect on insulin resistance. In addition, HFD mice showed a reduction in brain weight/body weight ratio, a significantly higher number of apoptotic nuclei in cerebral cortex, a higher gene expression of Fas-L, Bim and P27 (marker of neuronal apoptosis) in comparison with STD- and HFD-H mice, providing evidence for honey neuroprotective action. Moreover, honey intake significantly improved brain insulin resistance as demonstrated by PCR-array analysis showing upregulation of genes involved in insulin signalling (InsR, AdipoR and Irs1) and downregulation of genes involved in pro-inflammatory response (Rbp4, Cd36 and Stat3). In addition, in HFD-H mouse cortex, p-AKT and p-ERK protein expression was increased, while p-GSK3 was reduced in comparison with HFD cortex suggesting the ability of honey regular intake of protecting brain neurons from insulin resistance. In conclusion, the present results suggest a beneficial effect of the sicilian chestnut honey regular consumption on central nervous system in obesity conditions, by preventing onset of neurodegeneration and central insulin resistance.

HEREDITARY SPASTIC PARAPLEGIA AS NOVEL GENETIC NEURO-CHAPERONOPATHY

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Hereditary spastic paraplegia (HSP) is a term used to indicate a large group of clinically and genetically heterogeneous neurodegenerative disorders, involving the corticospinal tracts and characterized by slowly progressive spasticity and weakness in the lower limbs, associated with additional neurologic signs in complicated forms [1]. In recent years, an increasing number of missense mutations in the *HSPD1* gene, encoding for the mitochondrial chaperonin Hsp60, have been linked to the development of different forms of Spastic Paraplegia, making it as sortable in the vast and heterogeneous group of genetic neuro-chaperonopathies [2]. Among these, one of the most studied is the p.Val98Ile missense mutation, responsible for the onset of Hereditary Spastic Paraplegia 13 [3]. Other two disease-associated variants listed in public databases are the p.Glu129Lys and the p.Val287Ile. However, in current literature, there are no data about the molecular mechanisms underpinning the disease onset following these aminoacidic

replacements. A deep computational analysis, based on the use of the recently resolved crystal structure of the Hsp60-Hsp10 football-shaped complex [4], could explain how these missense mutations impact protein structure/function and unveil their pathological significance. As noticed, Glutamic Acid 129 forms a salt-bridge with the Lysine 133 of a monomer belonging to the opposite ring in the double-ring tetrameric complex. Thus, its replacement with a lysine could prevent the formation of this important inter-ring contact, impairing the formation and/or the stability of the macromolecular complex. Valine 287 is located at the apical domain near those residues directly involved in the interaction with the co-chaperonin Hsp10. Its replacement with an isoleucine, which has a higher molecular mass, could create steric bulk and alter the proper interaction with the co-chaperonin, compromising, also in this case, the formation of the macromolecular complex [5]. This first *in silico* analysis can drive further experimental analysis *in vitro*, required to validate the hypothesis inferred from the solved crystal structure, and to verify the real impact of these missense mutations on protein structure/function, mitochondrial activity and cells viability, in order to understand the induced molecular mechanisms responsible for the disease onset.

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NUTRITION AND METABOLISM

MEDICINAL MUSHROOMS AS INGREDIENTS IN SUPERFOODS, PREBIOTIC EFFECT AND ROLE ON HUMAN HEALTH

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Since ancient times mushrooms were considered a source of biological compounds (Venturella *et al.*, 2021). People appreciated mushrooms for their culinary properties and nutritional value, after the discovery of their medical properties they start to be used as food supplements and in the mycotherapy (Jayachandran *et al.*, 2017). These properties were imputable to different molecules with biological activities included in mycelia and fruiting bodies. The main bioactive compounds are the indigestible oligosaccharides and polysaccharides like chitin, hemicellulose, α and β -glucans, pleuran, lentinan, schizophyllan, mannans, xylans, and galactans, that show high effects on modulation and stimulation of gastrointestinal tract of microbiota, thus acting as prebiotic (Venturella *et al.*, 2021; Singdevsachan *et al.*, 2016). The International Scientific Association for Probiotics and Prebiotics (ISAPP) describe the prebiotic as *"a substrate that is selectively utilized by host microorganisms conferring a health benefit"* (Gibson *et al.*, 2017). They improve the growth of probiotic bacteria (in particular lactic acid bacteria) in the human gut and express the opposite effect on pathogen bacteria (clostridia, *Escherichia coli*, and *Salmonella*), which limits the growth, ensuring the health of intestinal microbiota (Synytsya *et al.*, 2009; Singdevsachan *et al.*, 2016). The studies on mushrooms showed that they carry out several functions to contrast disease like atherosclerosis, cancer, hypersensitivity, vascular diseases and help to minimize the damage that carries out by bacterial and viral infection (Gargano *et al.*, 2017; Jayachandran *et al.*, 2017). Mushrooms prebiotic compounds can normalize intestinal dysbiosis (reduce Firmicutes/Bacteroidetes ratio) with reduction of metabolic disorders, such as insulin resistance and hypercholesterolemia, determining body weight loss and anti-obesity effects (Meneses *et al.*, 2016). For the reasons given above, the industrial interest in prebiotics increased in recent times due to the possibility of their use as functional ingredients in the production of different types of foods (Davila *et al.*, 2019). In particular, mushrooms are considered a very versatile ingredient. They are used as fresh, dried (powdered), or as extracts, to obtain different products in which the previously described properties are added or increased. Authors investigated different species of medicinal mushrooms for improving the functional properties of meat-based foods (Kurt and Gençcelep, 2018), bakery products (Gaglio *et al.*, 2019), cheese (Ribeiro *et al.*, 2015), cheese-like foods (Okamura-Matsui *et al.*, 2001) and beer (Leskosek-Cukalovic *et al.*, 2010). The interest of the industry for healthy superfoods using prebiotic fortification using medicinal mushrooms is pointed out.

ADIPOSE-DERIVED STEM CELL DIFFERENTIATION, BEIGE PHENOTYPE AND INFLAMMATION

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Adipose-derived stem cells (ADSCs) are one of the most promising sources for regenerative medicine. Adipose tissue is essential in the regulation of whole-body metabolism, insulin sensitivity and thermogenesis. Dysregulation in stem cell recruitment and in preadipocyte differentiation in adipose tissue leads to secretion of pro-inflammatory cytokines, metabolic stress, insulin resistance, cardiovascular disease and obesity. Alterations in ADSC differentiation and adipose tissue physiology are linked to a state of chronic low-grade inflammation and macrophage infiltration inside the tissue. Obesity also inhibits cell autophagy, a process maintaining healthy cells by removing damaged components. Any alteration in this process is related to the development of insulin resistance. We previously identified specific conditioned media able to counteract the appearance of an adipogenic phenotype, by enhancing vitamin D metabolism, acting on CYP27B1 and CYP3A4. Here, we evaluated the role of metformin and vitamin D, alone or in combination, in modulating inflammation and authophagy of ADSCs during adipogenic commitment. Cells were cultured for 21 days in the presence of a specific adipogenic differentiation medium, together with metformin, vitamin D or both. We then analyzed the expression of specific adipogenic-related genes aP2, LPL and ACOT2, and Heat Shock Proteins (HSP). We also evaluated the gene and protein expression of the thermogenic protein UCP1, and the secretion of proinflammatory cytokines IL-6 and TNF-a by ELISA. Autophagy was also assessed by western blot. Our results showed the ability of the conditioned media to modulate adipogenic differentiation by inducing the appearance of a "brown-like" phenotype, finely tuning the inflammatory response and autophagy. Taken together, our findings suggest the possible application oh these molecules in clinical practice, to counteract uncontrolled lipogenesis and prevent obesity and obesity-related metabolic disorders.

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MORPHO-PHYSIOLOGICAL CORRELATIONS IN PRESENCE OF AN EXCESS OF NUTRIENTS IN A Caco2/ HT-29 CO-CULTURE

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The study of the interactions between nutrients and intestine requires *in vitro* models mimicking as close as possible both the morphology and the physiology of the human intestinal epithelium. Many experimental difficulties hampered in establishing a physiological long-term experimental model starting from primary cultures of normal small intestinal and colon cells. For this reason, a Caco2/HT-29 (70/30) co-culture was set up in our lab starting from the differentiated parental cell populations. Both cell lines originate from a human colon adeno carcinoma but, when differentiated, Caco2 cells are mostly absorptive and do not secrete mucus, while HT-29 cells are a heterogeneous population, comprising scattered enterocyte elements and mucus secreting cells. The co-culture represents an *in vitro* model of the small human intestine as it concerns final digestion and absorption of digested foods. This experimental setting allowed also studying the effects induced by an excess of nutrients by changing the frequency of the medium administration. Two parallel experimental groups were cultured: the standard group (ST) and the excess group (EX). In ST group the culture medium was changed every four days, whilst in EX group on alternate days from confluence (T0). Co-culture was harvested at T0 and at 3, 7, and 15 days post-confluence (T3, T7, and T15, respectively). In comparison with the ST group, the EX group revealed a maintenance in the number of microvilli, an increase in follicle like-structures and mucus production, and a decrease in the number of tight junction. The specific activity of Alkaline Phosphatase, Aminopeptidase N, and of Dipeptidyl Peptidase-IV, known markers of intestinal and enterocyte differentiation, progressively increased. At T15 in the EX group an increased permeability to large molecules evidenced by the Transepithelial Electrical Resistance and the Lucifer yellow permeability was evident. At the same time, the level of ROS and NO production increased as well as of interleukin-6 and interleukin-8, all markers representing a low grade of inflammation. These results agree with the morpho-functional features associated to the intestine of overweight/obese animals. The two *in vitro* intestinal models represent the possibility to study the patho-physiology of the interactions between nutrients and human gut. Next step will be the completeness of the co-culture models with the microbiota.

MAGNESIUM DEFICIENCY INDUCES LIPID ACCUMULATION BY UPREGULATING EDF-1 AND PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA

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Vascular endothelial cells (ECs) constitute the inner surface of blood vessels and are fundamental to maintain the integrity of the vascular wall and the homeostasis of the entire organism. ECs control the traffic of molecules between the blood and the neighbouring tissues and regulate blood fluidity, vascular tone, leukocyte trafficking and the immune response. A functional dysregulation of ECs is usually associated with the acquisition of a pro-inflammatory and pro-oxidant phenotype, which triggers events leading to atherosclerosis. It is known that low magnesium (Mg) concentrations promote inflammation and oxidative stress, contributing to cardiovascular diseases. Indeed, low Mg induces the acquisition of a pro-atherogenic phenotype in ECs both *in vivo* and *in vitro*. Endothelial Differentiation-Related Factor 1 (EDF-1) is expressed both in the cytosol, where it is involved in the modulation of endothelial Nitric Oxide Synthase (eNOS), and in the nucleus, where it acts as transcriptional co-activator for proteins required for lipid metabolism. One of its target genes is the Peroxisome Proliferator-Activated Receptor gamma (PPARγ) that is involved in the modulation of different pathways, such as cytokines production, energy metabolism and apoptosis in ECs. Since both magnesium homeostasis and EDF-1 with its target have crucial roles in shaping endothelial function, we investigated the modulation of these proteins as well the deposition of lipids in human ECs cultured in different concentrations of magnesium. Human endothelial cells from the umbilical vein (HUVEC) were cultured in medium containing from 0.1 to 5 mM Mg. 24h after culture, the levels of EDF-

1 and PPARγ were analyzed by Western Blot and the pro-oxidant state of ECs was assessed exploiting 2'-7'-dichlorofluorescein diacetate (DCFDA) to detect the intracellular accumulation of reactive oxygen species (ROS). Moreover, the alteration of fatty acids metabolism was investigated. After 24h of culture in low extracellular magnesium concentrations, we observed an upregulation of both EDF-1 and PPARγ in association with ROS accumulation. Finally, we found an accumulation of intracellular neutral lipids in the cells cultured in low Mg, suggesting that an alternation of Mg homeostasis might affect ECs metabolism.

UNDERSTANDING THE POTENTIAL HEALTH BENEFITS OF PLANT FOODS PROTEINS BEYOND THEIR NUTRITIVE ROLE

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New dietary approaches, ethical and environmental sustainability concerns attract the interest in plant proteins alternative to animal proteins. Beyond the nutritive role, recent research pointed out the potential health benefits of foods proteins and peptides that may exert bioactivities such as antioxidant, antilipidemic, antimicrobial and immunomodulating. There is even now a scarcity of information about the potential effects of pseudocereal proteins and their derived peptides, on chronic inflammation and oxidative stresses, that are considered major causes of age-related diseases and of some forms of cancer. This work aims to help filling these gaps. The immune-modulatory, the antioxidant and the trypsin-inhibitor activities of proteins from quinoa (*Chenopodium quinoa* Willd.), amaranth (*Amaranthus retroflexus* L.) and buckwheat (*Fagopyrum esculentum* Moench) seeds have been assessed *in vitro*, after purification and separation in different fractions. The three biological effects considered in this work are closely connected to each other for the maintaining of human well-being. Proteins have been tested as such and after simulated gastrointestinal digestion. The immune-modulation capacity of protein fraction and peptides was evaluated in undifferentiated Caco-2 cells under stimulation with the pro-inflammatory cytokine IL-1β and assessing NF-kB pathway activation. All proteins showed a capacity to decrease the inflammatory response, but at different percentages. The antioxidant activity seems to be related to the amino acids composition of the isolated fractions. Our results indicate that the protein fractions with higher trypsin inhibitor (TI) activity also possess radical scavenging and immune modulating properties, supporting the hypothesis of the involvement of TI in protection against oxidation and inflammation. The results showed in most cases a lower activity after proteolysis, but with some exceptions. In addition, the immunomodulation capacity of chenopodin, the major protein of quinoa seeds, was further investigated in order to deep on the molecular mechanisms of action at the basis of the observed effects. The interaction with possible target molecules has been studied *in vivo* using Caco-2cell models and with in silico structural predictions. Chenopodin mechanism of action involves a competitive inhibition tuning of the inflammatory effect of IL-1β based on its structural features. In conclusion, our findings lay the basis for possible uses as nutraceutical molecules of proteins from pseudocereal seeds.

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THE GUT-BRAIN AXIS: PROTECTIVE ROLE OF *Lactobacillus fermentum* IN A MOUSE MODEL OF ETHANOL-INDUCED OXIDATIVE STRESS DAMAGE

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Chronic alcohol consumption alters the composition of the intestinal microbiota favouring intestinal permeability (1). Endotoxin and non-metabolized alcohol reach the systemic circulation and other organs, including the central nervous system (CNS), leading to neurodegeneration (2,3). Probiotics could play a key role in protection from alcohol damage, modulating the gut microbiota (4). On these premises, the aim of this study was centred on the protective effects of the probiotic *Lactobacillus fermentum* (*L. fermentum*) against oxidative stress damage and inflammation induced by ethanol (EtOH). Female 12-month-old mice (BALB/cAnNHsd) were orally given EtOH (96%) alone (n=5) or in combination with *L. fermentum* (10^9 CFU) (n=5), every day for twelve weeks (12 EtOH and 12 EtOH-P, respectively). Both groups were compared with mice (n=5) fed with the standard diet (CN) (5). Immunomorphological analysis was conducted to look for molecular chaperones (Hsps) and glial markers in the small intestine and cerebellum, since they are typically implicated in mechanisms pertaining to the effects of EtOH. Hematoxylin-eosin staining of small intestine slices showed a leaky gut in the mice treated with EtOH, characterized by altered barrier structure and loss of the normal morphology of Purkinje cells. Since glial cells participate in the regulation of inflammatory mechanisms, immunohistochemistry was conducted to identify GFAP (glial fibrillary acid protein), S100β (S100 calcium binding protein B) and FABP7 (fatty acid binding protein-7) in small intestine slices. The glial markers (GFAP, and S100β) were increased after EtOH consumption, probably as a consequence of dysbiosis. GFAP, S100β, and FABP7 were also increased in cerebellum slices, probably as a consequence of astrogliosis. The 12 EtOH-P group showed a decreased amount of GFAP, S100β, and FABP7, suggesting a protective role of *L. fermentum* against inflammation. Hsp60 and Hsp90 levels, considered markers of oxidative stress, were increased in both small intestine and cerebellum of 12EtOH-P, suggesting a protective effect of *L. fermentum* against oxidative stress damage. The results indicate that probiotics might help in counteracting the deleterious effect of alcohol consumption.

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VITAMIN D PREVENTS TRIGLYCERIDES ACCUMULATION IN ENDOTHELIAL CELLS EXPOSED TO HIGH GLUCOSE

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A hallmark of diabetes is the insufficient levels of Vitamin D_3 (VitD) that, besides its canonical role in intestinal calcium absorption and bone mineralization, protects against atherosclerosis by inhibiting the conversion of macrophages into foam cells and by enhancing cholesterol efflux. Since macrovascular complications are the leading cause of mortality associated with diabetes, this study aims to test the effects of VitD on macrovascular endothelial cells (HUVEC) exposed to high glucose concentrations. HUVEC were cultured for 24h in medium containing physiological (5.5 mM) or high levels (11.1 mM and 30 mM) of D-glucose in the presence or not of VitD (20 nM). Extracellular oxygen consumption, lactate production and triglycerides (TG) accumulation were measured. Glutathione activity was quantified using the luminescence-based GSH/GSSG-Glo assay. Endothelial permeability was evaluated by Transwell Permeability assay using fluorescent albumin and ROS production was measured through DCFH fluorescent probe. The expression of PPAR-γ, EDF-1 and TXNIP was analysed by Western Blot. siRNA targeting *TXNIP* was used to evaluate a potential correlation between high glucose-induced ROS production and the accumulation of neutral lipids that is proposed to be a trigger of endothelial dysfunction. Upon culture in high glucose, oxidative stress occurs because of the upregulation of TXNIP. The overexpression of TXNIP is responsible for increased endothelial permeability, boosted oxygen consumption, enhanced lactate production and TG accumulation. In according with this last evidence, PPAR-γ and EDF-1, key markers of lipogenesis, are overexpressed in cells cultured in high glucose. Interestingly, VitD mimics the effect of *TXNIP* silencing. VitD blocks the ROS increase by suppressing TXNIP expression and simultaneously strengthens the antioxidant GSH activity. VitD also restores the normal lactate production, decreases TG accumulation and downregulates PPAR-γ and EDF-1. In conclusion, it is feasible to establish a nutritional/dietetic regimen that includes the tailored supplementation of VitD in hyperglycaemic patients to ameliorate the high glucose-induced alterations of endothelium.

IODINE BIOFORTIFICATION IN GROWN CURLY ENDIVE AS A NOVEL FUNCTIONAL FOOD: PILOT NUTRITIONAL INTERVENTION IN HEALTHY YOUNG INDIVIDUALS

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Globally, 2 billion people suffer from "hidden hunger" which is a deficiency of micronutrients such as iron, selenium, zinc, iodine and many others. In relation to Iodine, it is estimated that a third of the world s population lives in areas where Iodine is scarce and Iodine deficiency is responsible for many related disorders as such as goiter, reproductive failure, hearing loss, growth impairment, cretinism, and numerous kinds of brain injury. It is quite clear, that mineral deficiencies can be overcome through careful dietary diversification and mineral supplementation. An alternative or even complementary way is represented by the intake of bio-fortified foods, which can tackle this lack of micronutrients. Several researches aimed to Iodine enrichment in various fruiting and leafy vegetable crops, such as lettuce, spinach and tomato. Starting from the aforesaid evidences, the current study aims to assess the effect of human nutritional intervention with Iodine biofortified curly endive on a population of healthy individuals and its possible effect on haematological and chemical blood parameters. In this study Iodine-enrichment was made by supplying iodine in form of potassium iodate provided through foliar spray during the period of growth. After the harvesting season, the crops were provided for human consumption in a selected population. In details: 100 gr of Curly endive was consumed by ten individuals (5 men and 5 woman, aged 25-50) and blood and urine samples were obtained at the beginning and after 14 days of consumption. With regard to Iodine determination, the iodine content in leaves tissues and urine samples were assessed via inductively coupled plasma mass spectrometry (ICP-MS). The results confirmed the increased of iodine presence in urine samples after the 14 days of I-enriched crop consumption.

MAGNESIUM DEFICIENCY IMPACTS ON SKELETAL MUSCLE REGENERATION BY INFLUENCING MEMBRANE FUSION

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Magnesium (Mg^{2+}) deficiency is the most underestimated electrolyte imbalance in Western countries and it is related to many dysfunctions. Mg^{2+} is essential to all vital processes and in skeletal muscle, which contains 25% of body Mg^{2+}, the cation is crucial for myofibers relaxation. A low-Mg^{2+} status results in cramps and weakness and, if underestimated, can contribute to age-related sarcopenia. Although the importance of Mg^{2+} for skeletal muscle health is widely recognized, little is known about the molecular effects of Mg^{2+} depletion on myogenesis, the process responsible for myofibers regeneration and skeletal muscle plasticity. We used C2C12 murine myoblasts that, under serum depletion, differentiate to multinucleated myotubes with contractile capacity. We reproduced the myogenic process *in vitro* e we studied the effects of Mg^{2+} depletion both in early myogenesis, when myoblasts fuse together forming small myotubes, and late myogenesis, when myoblasts fuse with the already formed myotubes to generate mature myofibers. To study the early phase, cells were induced to differentiate for 6 days in the presence of low (0.1 mM) or physiologic (1 mM) Mg^{2+} concentrations. To study the effect of low Mg on late myogenesis, the myotubes obtained after 6 days of serum deprivation were exposed to 0.1 mM or 1 mM Mg^{2+} for other 6 days. Both in early and late myogenesis, cells differentiated in 0.1 mM Mg^{2+} presented a different morphology and a strong downregulation of the contractile protein Myosin Heavy Chain compared to the physiological condition. Since we observed a significant reduction in fusion index values (nuclei in myotubes *vs.* total nuclei) both in early and in late myogenesis, we evaluated the expression of proteins involved in membrane fusion such as Caveolin-3

and the newly discovered fusogenic peptide Myomixer and we found them significantly decreased in Mg^{2+}-deficient cells. We then focused on cellular stress parameters and we showed that Mg^{2+} deficiency induces an increase of reactive oxygen species which are directly responsible for the inhibition of membrane fusion and therefore for the impairment of myogenesis. With the data obtained in our model, we suggest that a correct Mg homeostasis is essential to maintain myofibers regenerative capacity.

SPORT AND PHYSICAL EXERCISE SCIENCE AND MEDICINE

EFFECT OF LONG-TERM SUPERJUMP® TRAINING ON BONE REMODELING AND METABOLISM IN WOMEN

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Physical exercise is recommended to prevent fractures and osteoporosis especially in women. Superjump® is a new workout activity performed on a modified elastic mini trampoline. It mixes jumping with music and total body exercises. The aim of the study was to investigate the potential preventive effects Superjump® on bone remodeling and metabolism in eumenorrheic women. Twelve women were involved in the study. They performed Superjump® activity three times a week, each session lasting 60 minutes for a total of twenty weeks. Blood samples were collected and anthropometric measurements and bioelectrical impedance analysis (BIA) was performed at baseline and at the end of the twenty weeks of training. The biomarkers of bone resorption (c-terminal telopeptide region of collagen type 1, CTX), bone formation (osteocalcin), bone metabolism such as calcitonin, parathyroid hormone (PTH), vitamin D, albumin adjusted calcium (aAcalcium), phosphorus, potassium, were analyzed. The marker of bone resorption CTX was reduced along with PTH while the markers of bone formation osteocalcin was increased along with aACalcuim after 20 weeks of Superjump® training in the woman. The training with the mini elastic trampoline did not affect calcitonin, vitamin D and phosphorus. In conclusion the findings suggest that Superjump® training may be a valuable intervention to prevent osteoporosis in women. In fact, the 20-weeks of training with the elastic mini trampoline had a high impact in reducing bone resorption and increasing bone formation by positively influencing bone metabolism.

PERIPHERAL MODULATION OF CENTRAL FATIGUE DEVELOPMENT DURING PHYSICAL EXERCISE: FINDINGS FROM EQUINE ATHLETE MODEL

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Physical exercise induces various stress responses leading to a disturbance of homeostasis and, a number of regulatory systems are called upon to return the body to a new level of equilibrium. This study aimed to investigate whether peripheral modulators of serotoninergic function and neurohumoral factors change in athletic horses during an official jumping competition, and to evaluate their relationship with the physical performance of competing horses. Seven Italian Saddle mares (6-9 years; mean body weight 440 ± 15 kg) were enrolled in the study. Horses took part in an outdoor jumping competition. After a warm-up on the flat and a

warm-up jumps, horses competed in the same jumping course (total length, 500 m) with the following technical specifications: obstacle height, 140 cm; total efforts, 15 (9 verticals, 6 oxer, 1 double combination, 1 triple combination). The competition stage included four phases: inbound (waiting time inside the arena before competing), course (time of the jumping phase), outbound (time lapse between the end of the course and the exit from the arena), end (time lapse between the exit and the arrival to the stable). In order to evaluate the workload during the competition, each horse was equipped with equine Heart Rate (HR) monitors (Polar Horse Trainer, S 610, Polar Electro Europe BV, Switzerland) to record HR during each step of warm-up and competition. Rectal temperature (RT) measurement, blood lactate and glucose, serum tryptophan, leucine, valine, the tryptophan/branched-chain amino-acids ratio (Try/BCAAs), dopamine, prolactin and non-esterified fatty acids (NEFAs) were assessed before the exercise event (T0), at the end of competition stage (5 min ± 10 sec following the cessation of the exercise, TPOST5), and 30 min after the end of competition (TPOST30). Highest HR values were recorded during the course and at the outbound ($P<0.0001$); blood lactate and RT increased after exercise respect to T0 ($P<0.0001$). Lower leucine and valine levels ($P<0.01$), and higher tryptophan, Try/BCAAs ratio and NEFAs values were found at TPOST5 and TPOST30 respect to T0 ($P<0.0001$). Higher prolactin concentration was found after exercise than rest condition ($P<0.0001$), whereas dopamine showed decreased values after exercise compared to T0 ($P<0.0001$). Significant correlations among the peripheral indices of serotoninergic function, neurohumoral factors and athletic performance were found. The findings suggest that the serotoninergic system may be involved in fatigue during jumper exercise under stressful situation as competition, in which, in addition to physical effort, athletic horses experience emotional reactivity and mental stress.

SEX-BASED DIFFERENCES OF SKELETAL MUSCLE AFTER A SINGLE BOUT OF ENDURANCE EXERCISE

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The gender dimorphism of skeletal muscle can be stated in the of mass, diameter, metabolism, and fatigue differences of the individual fibers, as well as it can be associated with the distinct hormone levels in males and females. The aim of our study was to show the different distribution of skeletal muscle fibers based on the mRNA and protein expression of the myosin heavy chain (MHC) isoforms in the soleus and extensor digitorum longus (EDL) muscles of adult BALB/c mice. Additional differences between males and females were identified in response to a single bout of endurance exercise. Skeletal muscle adaptation was reflected on the mRNA levels of transcription factor Peroxisome proliferation-activated receptor-γ (PPAR-γ) coactivator-1α (PGC1α), as well as the mRNA levels of Heat shock protein 60 (Hsp60), and interleukin 6 (IL-6). Our analysis focused on gender differences showed that the aerobic muscle soleus in females is rich in type I fibers, while the male one in type IIa fibers. Otherwise, the glycolytic muscle EDL in males showed a high mRNA and protein expression of type IIb fibers, that in females displayed only high mRNA levels of MHC type IIa fibers. The muscle response to a single bout of endurance exercise resulted in an increase expression of genes involved in mitochondrial biogenesis such as PGC1 α1, Hsp60 and IL-6 in the soleus muscle of males, while the expression of PGC1 α2 and α3 was increased in the EDL muscle of both animal sexes. Although these genes are important in the physiological response to exercise, we strongly believe that our data are crucial for the future studies involved in the characterization of fiber distribution of other muscles with different anatomical function and location.

PATTERNS OF RAPID WEIGHT LOSS IN ELITE SAMBO ATHLETES

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Rapid weight loss (RWL) is commonly practiced in combat sports. Both magnitude and methods used to induce RWL are largely similar among combat sports, but currently there is no data on RWL methodology used by sambo athletes. Therefore, the aim of this study was to determine RWL procedures sambo athletes apply to lose weight rapidly. The sample consisted of 199 participants, of which 132 males and 67 females who participated in the World Sambo Championship 2020 held in Novi Sad, Serbia. Each participant received a RWL questionnaire that was available in multiple languages, and each participant was instructed how to fill it out. Almost 87% of sambo participants declared to have intentionally cut their weight prior to the competition, whereby 5.27 kg (SD: ±7.57) was lost. Gradual dieting, sauna use and skipping meals were the most dominant methods used to reduce weight prior to competition while more extreme methods of RWL such as the use of laxatives, diuretics, diet pills and vomiting were also implemented but by much smaller fragment of the participants involved. Findings from our study largely match with previously conducted RWL studies in terms of prevalence, magnitude and methods used by combat sport athletes, especially in judo and wrestling. Knowing the hazardous consequences of RWL, alternative methods of sustainable weight loss should be considered.

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THE IMPACT OF COVID-19 LOCKDOWN ON PHYSICAL ACTIVITY PRACTICE AND SEDENTARY BEHAVIORS IN YOUNG SICILIAN POPULATION

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The first wave of the COVID-19 pandemic has led Italy, as well as almost all countries around the world, to adopt confinement measures. In Italy, the lockdown lasted 69 days (from 9 March to 18 May 2020), requiring changes in the lifestyle of the entire population, including the limitation to the practice of physical activity. Since the crucial role of physical activity in youth is widely recognized, the aim of this study was to evaluate any changes in the practice of physical activity in the young Sicilian population during the lockdown. In particular, we analysed the differences during the last 7 days of lockdown and the usual week of the following parameters: 1) energy expenditure (MET–minute/week) of each physical activity intensity; 2) time spent (minutes) in sedentary behaviours. 333 young Sicilian participants (female=197; male=136; age range: 11-25) completed a questionnaire constructed based on the IPAQ-SF which was used to investigate the days/week and the minutes/day spent in vigorous activities, moderate activities, walking and sedentary behaviours. Based on these data we computed the energy expenditure of each intensity of physical activity using the IPAQ scoring protocol. Results showed a significant decrease in each intensity of physical activity and an increase in sedentary behaviours during the lockdown. In detail, young Sicilian participants reduced the level of vigorous-intensity physical activity by 24% (1996.73 to 1517.57 MET-min/wk, $p<0.01$); they reported a decrease of 16.79% in moderate-intensity physical activity (819.65 to 682.04 MET-min/wk, $p<0.01$); and they had a reduction of 73.87% in energy expenditure of walking activities (826.65 to 216.00 MET-min/wk, $p<0.01$). On the contrary, there was a 63.91% increase in time spent in sedentary behaviours (175.77 to 288.12 min, $p<0.01$). As expected, our outcomes detected a negative impact of the lockdown on the practice of physical activity in the young Sicilian population. These findings suggest the importance of planning adapted strategies for maintaining an active lifestyle in young people during emergency situations, such as any future confinement measures.

INDUCTION OF ARTICULAR CARTILAGE REGENERATION BY A CELL-FREE COLLAGEN TYPE I-BASED SCAFFOLD

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The management of chondral defects represents a big challenge because of the limited self-healing capacity of cartilage. Many approaches in this field obtained partial satisfactory results. Cartilage tissue engineering, combining innovative scaffolds and stem cells from different sources, emerges as a promising strategy for cartilage regeneration. The aim of this study was to evaluate the capability of a cell-free collagen I-based scaffold to promote cartilaginous repair after orthotopic implantation *in vivo*. Articular cartilage lesions (ACL) were created at the femoropatellar groove in rat knees and cell free collagen I-based scaffolds (S) were then implanted into right knee defect for the ACL-S group. No scaffold was implanted for the ACL group. At 4-, 8- and 16-weeks post-transplantation, degrees of cartilage repair were evaluated by morphological, histochemical and gene expression analyses. Histological analysis shows the formation of fibrous tissue, at 4-weeks replaced by a tissue resembling the calcified one at 16-weeks in the ACL group. In the ACL-S group, progressive replacement of the scaffold with the newly formed cartilage-like tissue is shown, as confirmed by Alcian Blue staining. Immunohistochemical and quantitative real-time PCR (qRT-PCR) analyses display the expression of typical cartilage markers, such as collagen type I and II (ColI and ColII), Aggrecan and Sox9. The results of this study display that the collagen I-based scaffold is highly biocompatible and able to recruit host cells from the surrounding joint tissues to promote cartilaginous repair of articular defects, suggesting its use as a potential approach for cartilage tissue regeneration.

THE EFFECT OF EXERCISE TRAINING ON CACHECTIC TUMOR-BEARING MICE

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Cachexia is a complex syndrome involving organs in the late stage of different chronic clinical conditions such as AIDS, chronic obstructive pulmonary disease, congestive heart failure, multiple sclerosis, tuberculosis, and cancer. The main characterizing feature consists in an involuntary weight loss. In particular, it has been reported a structural and functional skeletal muscle impairment with underlying altered balance between pro, and anti-inflammatory cytokines, as well as, anabolic and catabolic hormones. Recent studies, both in human cancer patients and in animal models, highlighted the health effects of exercise training. Interestingly, the data reported showed a decreasing trend in skeletal muscle degradation, body weight loss, oxidative stress and inflammation [1,2]. Based on this finding, the present study is focused on the effects of exercise training on skeletal muscle of C26 tumor-bearing mice. The experiment was carried out on one hundred and fifty 3-month-old male mice (BALB/c AnNHsd), subcutaneous inoculated with a fresh fragment of C26 colon carcinoma. The mice were divided into four different groups: sedentary-inoculated-sedentary (SED/I/SED), sedentary-inoculated-training progressive (SED/I/TR_P), training progressive-inoculated-training low intensity (TR_P/I/TR_L) and training progressive-inoculated-training high intensity (TR_P/I/TR_H). In these mice the survival curve and cachexia onset were evaluated. Immunofluorescence analysis was performed on gastrocnemius, plantaris, and soleus muscles slices to detect Hsp60 and Isolectin expression as a marker of oxidative stress and vascularization, respectively. The results showed a significant increasing in survival's average of the TR_P/I/TR_H group comparing to the other groups, and a significant difference

between the $TR_P/I/TR_H$ and the $SED/I/TR_P$ in cachexia onset. Among the different muscle analysed, Hsp60 immunoreactivity was more increased in soleus muscle compared with plantaris and gastrocnemius muscles, and Hsp60 signal was increased in the trained groups compared with sedentary counterparts. Taken together, these findings suggest a role of the exercise training in slowing down the cachexia onset. This would suggests a promising role of exercise training in improving the quality of life in clinical condition and in containing the health care cost.

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INFLUENCE OF THE FELDENKRAIS METHOD ON SELF-PERCEPTION OF ANXIETY, DEPRESSION AND FATIGUE IN FIBROMYALGIA PATIENTS: PRELIMINARY RESULTS

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The Feldenkrais Method® (FM) is based on the deep integration of movement, sensation, feeling and thought, and thus makes a concrete and effective contribution to man's quest for well-being, balance, flexibility and mobility (Ambrosio F., 2004). Fatigue, Anxiety and depression are among of the most frequent complaints of fibromyalgic adults and are strongly associated with loss of independence, decreased physical activity and functional decay (Sarzi-Puttini P., 2020). The aim of present study was to examine whether FM® could alleviate the self-perception of fatigue, anxiety and depression in individuals with fibromyalgia. The research design is a randomized pre/post study, with an experimental group who performs a specific Feldenkrais Method® training protocol. Twelve patients affected by fibromyalgia since 13.25±11.64 years participated in this study (age 56±8 years old; weight 78.58±15.7 Kg; height 157.5±5.85 cm; BMI 31.6±5,46 Kg/m^2). The length of FM® intervention was of 24 weeks, with 2 ATM (Awareness Through Movement) group lessons of 1 hour per week, conducted by certified Feldenkrais teacher. The lessons were aimed at developing self-awareness and perception to make everyday gestures with less effort and fatigue, releasing muscle tension and integrating breathing into movements. The self-reported fatigue, anxiety and depression were assessed at baseline and at the end of 24-weeks training period with the Functional Assessment of Chronic Illness Therapy Fatigue subscale (FACIT-F), Zung Self-Rating Anxiety Scale (SAS) and Zung Self-Rating Depression Scale (SDS), respectively. The significant difference between pre- and post-intervention was analysed with paired T-test and set with $p<0.05$. We found a significant increase ($p=0.0083$) in FACIT-F score after 24-week training period compared with baseline condition (20.83±8.69 *vs.* 28.58±8.81); while a decrease ($p=0.33$) in SDS score in 24-week training period respect to baseline level (47.33±8.64 *vs.* 44.17±7.83). Moreover, SAS score enhanced ($p=0.45$) in response to 24-weeks training period compared with baseline (46.33±10.29 *vs.* 48.33±11.71). In conclusion, our preliminary results show that the Feldenkrais Method® seems to be effective in decreasing fatigue and depression in patients with fibromyalgia and could contribute to improve their quality of life.

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ROLE OF SKELETAL MUSCLE PERICYTES IN THE ANGIOGENIC AND MYOGENIC RESPONSE TO EXERCISE IN YOUNG AND OLDER ADULTS

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Aging induces an overall decline in muscle quality and function and impaired tissue regenerative capacity. This physiological process advances slowly with healthy aging and can be rushed by concurrent diseases resulting in risk of developing disability, frailty and institutionalization in later life. There is, therefore, a critical need to define the correct strategy to promote muscle recovery and accretion in older adults. Several studies show that resistance exercise (RET) is a valid approach to neutralize sarcopenia, but the mechanisms are not yet well understood. Recently, capillaries have taken on a leading role on the hypertrophic response to RET, both in young and older individuals. Indeed, blood vessels deliver oxygen and nutrients to muscle tissue, supporting its growth. Unfortunately, aging is associated with a decrease in capillary density and function. Our preliminary results show that older individuals with greater capillary density have a more robust hypertrophic response to RET, without a concomitant increase in their muscle capillarization. Pericytes are perivascular cells wrapped around capillaries, with important roles to support endothelial cell growth and proliferation as well as promoting skeletal muscle regeneration and maintenance of the satellite cell pool. Thus, the aim of this study was to indagate the role of pericytes in the modulation of muscle hypertrophy following RET in a group of young and older adults. 17 young adults (age: 25.2±1.1 years) and 19 healthy older adults (age: 71.1±4.3 years) underwent to 12 weeks of RET. Muscle biopsies from vastus lateralis were obtained before and after 12 week of training. Immunohistochemical analysis was used to quantify myosin heavy chain isoform expression, cross-sectional area (CSA), satellite cell abundance, capillary and pericytes content. Subjects where then retrospectively divided into a LOW or HIGH group, based on their pre-RET capillary-to-fiber perimeter exchange index (CFPE) to determine the effect of basal capillarization on muscle response to training. Following RET, both young and older adults increase fiber CSA. Young subjects in the LOW group underwent hypertrophy with significant improvements in fiber type I, whilst those in the HIGH group increase fiber type II size. In older adults, subjects in the LOW group presented an increase in muscle capillarization, with no improvement in fiber hypertrophy; whilst subjects in the HIGH group increase muscle fiber size without altering their capillarization. Pericytes counts correlated with the increase in capillaries density, however the hypothesis that, with training, pericytes would differentiate into satellite cells to support the anabolic response to the RET was not confirmed.

PHYSICAL ACTIVITY TO PREVENT KNEE OSTEOARTHRITIS "THE SYNOVIUM THEORY"

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The purpose of this study was to investigate the influence of moderate physical activity (MPA) on the expression of osteoarthritis (OA)-related (IL-1, IL-6, TNF-α, MMP-13) and anti-inflammatory and chondroprotective (IL-4, IL-10, lubricin) biomarkers in the synovium of an OA-induced rat model. The MPA-based approach may support joint tribology and synovial lubrication, leading to improved joint function and pain relief. In addition, in pathologic conditions, synoviocytes type A secrete cathepsins, MMPs, and pro-inflammatory cytokines/chemokines into the extracellular matrix, triggering tissue damage. A total of 32 rats were divided into four groups: Control rats (Group 1); rats performing MPA (Group 2); anterior cruciate ligament transection (ACLT)-rats with OA (Group 3); and, ACLT-rats performing MPA (Group 4). Early OA was induced through the anterior cruciate ligament transection (ACLT) technique. Analyses were performed using Hematoxylin & Eosin staining, histomorphometry and immunohistochemistry. In Group 3, OA biomarkers were significantly increased, whereas, IL-4, IL-10, and lubricin were significantly lower than in the other groups. The results from MPA experimental group (Group 4) highlighted the decreased expression of OA-related biomarkers (IL-1, TNF-α, MMP-13) and the increased expression of chondroprotective ones (IL-4, IL-10, and lubricin). We hypothesize that MPA might partake in rescuing type B synoviocyte dysfunction at the early stages of OA, delaying the progression of the disease and finally postponing the need for joint replacement.

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MORPHOLOGICAL ADAPTATIONS OF SKELETAL MUSCLE FIBERS AFTER PLYOMETRIC EXERCISE

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Plyometrics refers to exercise that exploits the stretch-shortening cycle, which proceeds with the rapid stretch of a muscle (eccentric phase), followed by rapid shortening of the same muscle (concentric phase), which results in increased force and power output of the activated muscles. This type of training has been successfully used in different sporting contexts to improve strength, muscle power, coordination, and athletic performance. Studies investigating the effect of an acute bout of plyometric exercise or the regular plyometric training on muscle morphology are limited. Skeletal muscle biopsies of healthy untrained volunteers have been observed for structural and ultrastructural change induced by an acute bout of plyometric exercise (10 × 10 squat-jumps, 1-min rest) or plyometric training (3 times per week for 8 weeks). The results indicate that an acute bout of plyometric exercise mainly affected the fast-twitch muscle fibers (Type II muscle fibers), damaging both the sarcolemma and the sarcomere at the site of the Z-disk. While the plyometric exercise in persons accustomed to eccentric exercise (trained for 8 weeks) does not damage the sarcolemma and the sarcomere at the site of the Z-disk. Athletic trainers should avoid prescribing high-volume plyometric exercise bouts within quick succession or after other forms of high-intensity exercise that are known to stress the fast-twitch muscle fibers, so that athletes have sufficient time to regenerate damaged fibers.

AEROBIC EXERCISE AND PHARMACOLOGICAL TREATMENTS COUNTERACT CACHEXIA BY MODULATING AUTOPHAGY IN COLON CANCER

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Physical activity has been clearly correlated with a better prognosis in cachectic patients, although the underlying mechanisms are not yet understood. To study the pathways involved in the physical activity-mediated rescue of skeletal muscle mass and function, we investigated the effects of voluntary exercise on cachexia in colon carcinoma (C26)-bearing mice. Voluntary exercise prevented loss of muscle mass and function, ultimately increasing survival of C26-bearing mice. We found that the autophagic flux is overloaded in skeletal muscle of both colon carcinoma murine models and patients, but not in running C26-bearing mice, thus suggesting that exercise may release the autophagic flux and ultimately rescue muscle homeostasis. Treatment of C26-bearing mice with either AICAR or rapamycin, two drugs that trigger the autophagic flux, also rescued muscle mass and prevented induction of genes crucial for muscle proteolysis. Similar effects were reproduced on myotubes *in vitro*, which displayed atrophy following exposure to C26-conditioned medium, a phenomenon that was rescued by AICAR or rapamycin treatment and relies on autophagosome-lysosome fusion (inhibited by chloroquine). Since AICAR, rapamycin and exercise equally affect the autophagic system and counteract cachexia, we believe autophagy-triggering drugs may be exploited to treat cachexia in conditions in which exercise cannot be prescribed.

IS HYPOXIA-INDUCIBLE FACTOR (HIF-1) ALPHA INVOLVED IN TARAVANA SYNDROME?

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Breath holding diving (BH) is a discipline practiced by an increasing number of people. BH-divers are exposed to extreme environmental conditions such as: increased hyperbaric pressure and low temperature that caused change in arterial blood gases (1-2) and induced an human diving response which includes bradycardia, reduced cardiac output, increased arterial blood pressure and peripheral vasoconstriction (3). Some of these adaptations are still not fully understood, so we decided to investigate some biomarkers that should be involved in this disorders starting to analyse Hypoxia-inducible factor 1 alpha (HIF-1α). We enrolled in our study 31 healthy free divers from different part of Sicily, aged 41,86 ± 11,41, weight 77,52 ± 11,30 kg, height 175,19 ± 6,13 cm, who practiced this activity for more than 5 years. We asked them to perform 5 consecutive dives at 20 meters, without permanence on the bottom, with free recovery between the different dives. A baseline venous blood sample was taken immediately after surfacing from the 5th dive (less than 3 minutes). The samples were stored to -80 until the analysis were done using ELISA method. The results showed that comparing HIF-1α value before and after the dives, there were an increase in the concentration that started from 158,43 pg/ml and be at end 246,06 pg/ml. In conclusion, the Hypoxia-inducible factor 1 alpha can be considered co-responsible for various phenomena, certainly not with pathological destinies, but rather working with "adaptation" phenomena to this type of activity. These are just the preliminary results that need further investigations to be confirmed.

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COMPUTATIONAL MODELLING OF HMSCS/SCAFFOLD CONSTRUCT ADAPTATIONS IN RESPONSE TO MECHANICALLY INDUCED CHONDROGENESIS

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Mesenchymal stem cells (MSCs) are largely investigated as candidate cells for tissue-engineering approaches in regenerative medicine. One of the most challenging ambitions in this field is to restore damaged articular cartilage, distinctive feature of osteoarthritis. MSCs can be directed towards chondrogenic differentiation, in the absence of any growth factors, through exposure to a multi-axial mechanical loads in order to mimic the physiological joint environment. To this purpose, a large type of scaffolds and bioreactors are used. A better understanding of the complexity of this process could benefit from the use of computational techniques to speed the rate of improvement. We developed an agent-based model (ABM), with Netlogo software program, of hMSC, seeded into fibrin-poly(ester-urethane) scaffolds, subjected to a combination of shear and compression loading in a bioreactor. Combining empirical data, deriving both from previous immunohistochemistry analysis and literature, we created a dynamic platform of simulation and prediction. In silico successful adherence to *in vitro* phenomenon, allowed us to conduct a series of simulations aimed at identifying the critical biophysical parameters mediating hMSC differentiation. The model carried out the differences in hyaline cartilage biomarkers expression following cycles of shear or compression modules in asymmetrically seeded scaffolds. In vitro analysis confirmed the predictions provided by the computational simulations, suggesting that a combination of both shear and compression stimuli is the ideal mechanical protocol to follow in order to stimulate chondogenesis. This work led to the design of a prediction tool, which may have important technical implications in MSCs-based therapy. It could give the opportunity to study the dynamics in the long-term and also sifting through many different conditions. The framework generated is able to create a solid network of information that will speed the understanding of the *in vivo* complex environment, refining further *in vitro* analysis and reducing animals testing.

BIOMECHANICS CHANGES IN NON-PROFESSIONAL RUNNERS THROUGH STEP FREQUENCY AND METRONOME TRAINING

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Runner's postural biomechanics is a qualitative aspect of sports performance that must be perfected to increase performance and reduce joint trauma incidence. Specifically, a motor pattern that prefers the rearfoot run over the forefoot is common among western runners. From a dynamic point of view, the heel generates a ground reaction force of about 1.5-3 of the bodyweight in 50 ms, affecting the lower limb joints. In the forefoot run, the ground reaction force develops more slowly, in about 200 ms, to reduce the shock absorbed by the joints. The purpose of this study is twofold: to investigate the biomechanical aspects of non-professional runners and to understand if, following a training based on the acquisition of a correct forefoot posture in conjunction with the learning of the correct running frequency set at 180 steps per minute (spm), they can improve sports and physical performance. The study involved 30 non-professional runners, average age 45.7 ± 6.7, and with the support of a motion analysis system, a treadmill with a markerless 3D

camera, the joint biomechanical relationship of running posture was evaluated. A specific 4-week training protocol was then administered to a pilot group whose aim was to induce the correct lower limb kinematic, learn forefoot running posture, train muscles through the isoinertial system, and run on the correct step frequency at 180 spm with a digital metronome aid. Results report standard values in which it is possible to identify joint biomechanical relationships of non-professional runners. The 4-week training instead showed the change in joint values, such as the foot's dorsiflexion from 26° to 2.7°, and spatio-temporal parameters related to running, such as contact time from 0.26 sec to 0.23 sec. The data attest that a 4-week training based on posture management, a step frequency training, and targeted muscle training can induce significant changes in non-professional runners whose running posture is rearfoot.

RAPID WEIGHT LOSS INDUCES MOOD STATE OSCILLATIONS IN MALE JUDOKAS

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Judokas commonly engage in rapid weight loss (RWL) before competition in order to gain advantage over their lighter opponents. Although causes of psychological perturbations detected in previous studies that examined the effects of RWL on mood states are not entirely clear, athletes have consistently reported mood alterations. On the basis of previous findings, the aim of the study was to determine the oscillations in mood state of judokas affected by RWL. Eighteen judokas (25.3 ± 5.4 yrs. ,85.3 ± 8.1 kg, 179 ± 6.7 cm) participated in the study. Each participant had at least five years of competitive experience at a national level. Participants also had to have experience in performing RWL procedures for at least two competitive seasons. This crossover study included two phases: weight maintenance phase (3 days) and RWL phase (3 days). Self-selected RWL procedures were conducted by judokas during the last three consecutive days. Profile of Mood State questionnaire was used during all 6 days of the study to determine the mood state among judokas. Repeated measures ANOVA was used to determine the oscillations in mood state. Within-subjects effects showed that state of anxiety increased over a period of time as judokas were reducing body weight (p=0.016). Along with the anxiety, the feeling of bad temper has significantly raised (p=0.029). Additionally, participants appeared to be significantly more exhausted and worried as they were losing weight over a RWL period (p=0.049 and p=0.044, respectively). It seems that RWL can affect the psychological state in male judokas. Although RWL methods are often used in combat sports, mood oscillations that were detected can reduce the performance of athletes and further affect their competitive success. Having in mind that participants in this study used their own weight loss methods, it would be advisable for coaches to provide and implement more sustainable weight loss methods that will prioritize health of the athletes.

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ANALYSIS ON THE ROLE OF AEROBIC EXERCISE IN DIFFERENT TYPES OF DIABETES MELLITUS

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Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels. This disease is classified by etiology. The most frequent is type 2 diabetes, usually in adults, occurring when organism becomes resistant to insulin; it is followed by type 1 diabetes, once known as juvenile diabetes or insulin-dependent diabetes, a chronic autoimmune condition in which pancreas produces little or no insulin because of the insulitis. Other types of less common diabetes are LADA (Latent Autoimmune Diabetes of Adult), MODY (Maturity Onset Diabetes of the Young) and gestational diabetes. +In the past three decades the prevalence of diabetes (especially type 2) has risen dramatically in developed countries because of the increase of metabolic syndrome and the decrease of attitude to physical exercises. According to Saeedi *et al.*, the global diabetes prevalence in 2019 is estimated to be 9.3% (463 million people), rising to 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045 [1]. In spite of the different types of diabetes, healthy food and physical exercises can make the difference in prevention and treatment. ìAccording to Villareal *et al.*, combined aerobic and resistance exercises are suggested in association with weight loss programs, to improve physical function and reduction of frailty with relative preservation of lean mass [2]. Resistance trainings include a different kind of exercises that foresee muscles contraction against an external resistance with the expectation of increases in strength, tone, mass, and/or endurance. Otherwise, aerobic exercises are any type of cardiovascular conditioning. They are composed by 4 steps: warmup, cardiovascular, muscular conditioning and cool down. During this training, optimal cardiac frequency and VO2 max are recommended. In type 1 diabetes is important to evaluate, before, during and after the activities, the risk of hypoglycemia, controlled by a different setting of insuline-dose or introducing more carbohydrates [3]. The aim of our review is to describe benefits of physical activities in the prevention and treatment of diabetes.

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STRETCHING BEYOND FLEXIBILITY

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Several speculations over the years have been posited regarding the effects of muscles stretching. Among these, primarily range of movement improvement but also injury prevention, performance enhancement and performance decline, pain reduction and gait improvement. Leading to a debate with the proposal to de-emphasize stretching as a major component of physical fitness and therefore, reconsider the structure of physical testing batteries. With this work we would like to discuss recent evidence with the aim to understand if stretching can be considered as a form of exercise with effects beyond those on flexibility and range of movement improvement. Three systematic reviews were conducted in order to identify the effects of stretching on the cardiovascular system, the effects of stretching on peripheral nerves and finally to identify a protocol for optimal range of movement improvement. 15 articles were retrieved investigating the effects of heart rate (HR), blood pressure, pulse wave velocity (PWV of which baPWV for brachial-ankle and cfPWV for carotid-femoral waveforms), heart rate variability and endothelial vascular function. 10 articles were retrieved investigating the effects of nerve stiffness, nerve displacement and pain pressure thresholds. Finally, 23 articles were reviewed considering stretching modalities and protocols for optimal range of movement improvement. Regarding cardiovascular effects, stretching was able to significantly decrease HR (d = 0.38), baPWV (d = 2.04) and cfPWV (d = 0.46). Nerve stiffness (-15.6%) and pain pressure thresholds (-1.9kg) also decreased. Nerve displacements on each movement plane for all the considered nerves and nerve deformation were also frequently observed. Static stretching for at least 5 minutes a week, performed 5 days a week seems to be the most beneficial approach to increase range of movement. Our results highlight that a daily approach to stretching may be beneficial not only for increased range of movement but also as a therapeutic form of exercise in order to reduce nerve and vascular stiffness and decrease pain pressure thresholds. Clinical outcomes related to degenerative vascular pathologies or symptomatic populations still need to be elucidated.

STRESS

QUANTITATIVE PATTERNS OF THE CHAPERONES HSP27 AND HSP60 IN TUMORS OF THE HUMAN SUBMANDIBULAR SALIVARY GLAND: DIFFERENCES WITH NORMAL TISSUE AND PATHOGENIC IMPLICATIONS

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Advances in diagnosis, prognostication, and understanding tumorigenesis are currently linked to progress in the knowledge of the chaperone system (CS). It is increasingly clear that the CS is involved in various ways in the initiation, growth, dissemination, and response to treatment of diverse cancers. It is, therefore, of the essence to study the components of the CS in all organs in which tumors develop. We have undertaken the study of the CS and its changes in tumors of the human salivary glands, for which pertinent information is scarce, thus pathologists, clinicians, and scientists in practice and research have few firm tenets on to which base their work. Consequently, patient care is wanting and needs rapid improvement. Here, we present recent results pertaining to the chaperones Hsp27 and Hsp60 in the submandibular gland (SMG). Quantitative evaluations of both chaperones with immunohistochemistry (IHC) and immunofluorescence-confocal microscopy (IF-CM) showed marked differences between different tumors and between tumoral and normal tissue. Thus, the data offer a promising platform for differential diagnosis and suggest diverse avenues for investigating the role of these chaperones in carcinogenic mechanisms in the submandibular salivary gland. Noteworthy also is the agreement between results obtained with IHC and IF-CM. This is of great practical importance because while IHC typically relies on the experience and training of the pathologist counting the cells bearing the chaperones and estimating their quantity in each cell, IF-CM measures the chaperones instrumentally, without the need of specifically trained pathologists to obtain and interpret the results and, therefore, avoiding human error/biases.

GOLD NANOFIBERS AND VITAMIN D: A NOVEL FUNCTIONALIZED NANODEVICE SUPPORTING SKIN REGENERATION

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Gold (Au) has been used since ancient times for beauty topic treatments. Gold facial masks are currently commercialised to improve skin elasticity and blood circulation helping in maintaining a young skin reducing wrinkles appearance. Despite the fact that gold could represent a useful therapeutic treatment to counteract inflammatory diseases, the high incidence of side effects has limited gold use till the discover of nanomaterials, able to reduce the toxicity of this compound. Nanofibers can properly vehiculate molecules of interest through the skin helping stem cells and fibroblasts in maintaining tissue homeostasis. Skin stem cells (SSCs) are able to replace damaged elements after injuries as burns and wound healing restoring tissue homeostasis. Moreover, during wound healing also fibroblasts play a crucial role in the repairing processes. Bioactive molecules, properly delivered, can implement the regenerative process supported by skin cell populations. Vitamin D is typically described for its role in bone health, however in the last decades further Vitamin D effects on cell differentiation and wound healing processes have been largely described. In particular a role of Vitamin D in wound healing have been defined in human Diabetic foot ulcer, a major complication of diabetes mellitus. Aim of the present study was to evaluate the effect of Polycaprolactone (PCL) nanofibers loaded with gold and vitamin D (D-Gold nanofibers) on wound healing *in vitro*. The results of MTT and BrdU assays, show the ability of D-Gold nanofibers to increase skin fibroblasts and SSCs viability and proliferation. Nevertheless, D-Gold nanofibers accelerate wound repair *in vitro* in both skin fibroblasts and SSCs cultures, also modulating the expression of genes involved in the regenerative process. All together our results suggest a possible application of D-Gold nanofibers in ameliorating skin regeneration process after injuries.

BINGE ALCOHOL DRINKING DURING ADOLESCENCE ALTERS STRESS RESPONSE IN THE NUCLEUS ACCUMBENS AND DECREASES SOCIAL RESILIENCE IN RATS

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Binge alcohol drinking - *i.e.* episodic heavy alcohol consumption - is increasingly common among adolescents and may negatively impact on the underdeveloped neural substrates that process reward and stress response. As socially stressful events pose a particular challenge for vulnerable individuals, including altered neuroplasticity in brain regions key to reward processing, such as the nucleus accumbens (NAc), this research aimed at exploring the consequences of binge alcohol drinking during adolescence on: I) dopamine- and glutamate-related neuroplasticity in the NAc following social stress exposure; II) social stress coping strategies at early adulthood. Adolescent male rats underwent binge-like alcohol exposure - or water administration, over the three weeks of adolescence. Binge- and water-exposed rats were subjected to the resident-intruder paradigm, an ethologically relevant animal model of social stress, and evaluated for defensive coping strategy and serum corticosterone level. Then, pre- and postsynaptic markers of dopamine- and glutamate signaling were evaluated in the NAc. Our results show that binge alcohol exposure did not modify the stress-induced effects on dopamine input to the NAc, while it exerted opposite effects on dopamine postsynaptic response and glutamate-related markers; at the behavioural level, binge alcohol exposure during adolescence increased passive social stress coping and induced an abnormal stress response, with respect to water-exposed rats. Binge alcohol drinking during adolescence perturbed the stress induced-neuroplastic chances in the NAc microcircuits and decreased social resilience in young adulthood, inducing a locus minoris resistentiae for stress-induced pathologies later in life.

CIRCULATING IMMUNE-CHECKPOINTS AND LYMPHOCYTE MICRORNAs IN RENAL CELL CARCINOMA AS A NOVEL BIOMARKERS OF IMMUNOTHERAPY RESPONSE

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Renal cell carcinoma (RCC) represents a heterogeneous group of cancers where understanding of genetic and molecular drivers, clinical behavior and responses to therapy have evolved over the past years, changing the clinical landscape and the natural history of the disease. Despite immunotherapy has revolutionized the treatment of metastatic RCC (mRCC), predicting which patient will benefit from the treatment still remain an issue. Since previous findings showed that the epigenetic modifications are a key feature of RCC, investigating the epigenetic reprogramming, such as microRNA (miRNA)-mediated regulation of tumor suppressor genes, could represent a step toward a real-time monitoring of the dynamic changes during cancer evolution and treatment. Also, since immunotherapy inhibits the PD-1/PD-L1 interaction, PD-L1 expression should be a valid predictive biomarker to anti-PD-(L)1 immunotherapies. We performed a prospective study including a cohort of 56 RCC patients treated with immunotherapy: 21 metastatic patients, 15 with localized disease and a validation cohort of 20 mccRCC. miRNAs were isolated from peripheral lymphocytes of metastatic patients. The blood samples were collected before starting treatment (T0) and after 4 weeks (T1). The expression profile of 377 lymphocyte miRNAs was analyzed, with a cut off of FC >2 for up-regulated and <0.3 for down-regulated miRNAs. Patients with complete response more than 12 months showed a subset of 8 miRNAs specifically induced by treatment. These miRNAs result to be silenced or downregulated in RCC. This could explain the exceptional up-regulation in long-responders patients, evident already after 4 weeks of treatment. The plasma sPD-1, sPD-L1 and pan-sBTN3A, sBTN3A1 and sBTN2A1 levels were measured by specific ELISA assays. In the sub-population of long-responders patients (PFS>18 months), T0 levels of sPD-1 and sPD-L1 were higher respect to the patients with shorter PFS (<6 months); conversely, the T0

levels of sBTN2A1 were lower than short-responders. Particularly, the median PFS of patients with sPD1<8.05 ng/ml measured at T0 was 5.8 months whereas in patients with sPD1>8.05 the median PFS was 17.5 months. An exploratory analysis in metastatic *versus* localized ccRCC patients showed that the concentrations of sPD-1 and sPD-L1 were elevated in the plasma of metastatic in comparison with localized RCC patients. These findings could help to identify patient's subgroups for immune checkpoint treatment and novel predictive biomarkers, urgently needed to guide therapeutic choice and monitoring the patient's response.

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Hsp60 QUANTIFICATION IN GASTRIC MUCOSA SHOWS DIFFERENCES BETWEEN PATHOLOGIES WITH VARIOUS DEGREES OF PROLIFERATION AND MALIGNANCY GRADE AND CAN BE A DECIDING CRITERION FOR DIFFERENTIAL DIAGNOSIS

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Stomach diseases are an important sector of gastroenterology, including proliferative benign, premalignant, and malignant pathologies of the gastric mucosa, such as gastritis, hyperplastic polyps, metaplasia, dysplasia, and adenocarcinoma. There are data showing quantitative changes of chaperone system (CS) components in inflammatory pathologies and tumorigenesis, but their roles are poorly understood and information pertaining to the stomach is scarce. The interest in this molecular chaperone arose due to it is direct involvement in the maintenance of cellular homeostasis, in the loss of cell differentiation and in the consequent organ remodeling. Immuno-morphological assessments of Hsp60 levels in the epithelium have shown that this protein significantly increases in gastric carcinoma samples compared to normal mucosa, mild-moderate and severe gastritis, hyperplastic polyp and intestinal metaplasia samples. The immunohistochemical chaperone quantification in the gastric mucosa and found the following percentages of Hsp60-positive epithelial cells: 32.8 in normal mucosa; 33.5 in mild-to-moderate gastritis; 51.8 in severe gastritis; 58.5 in hyperplastic polyps; 67 in intestinal metaplasia; 89.4 in gastric dysplasia; and 92.5 in adenocarcinomas. Noteworthy were (i) the difference between dysplasia and adenocarcinoma with the other pathologies; (ii) the progressive increase of Hsp60 from gastritis to hyperplastic polyp, gastric dysplasia, and gastric carcinoma; and (iii) the correlation of Hsp60 levels with histological patterns of cell proliferation and, specially, with tissue malignancy grades. The increase in Hsp60 levels in the gastric mucosa during the progression from hyperplasia to carcinoma, passing through dysplasia, possibly reflects the cells mounting need for the CS, including Hsp60, to deal with their escalating metabolism and proliferation as they become malignant. The Hsp60 positivity in mild to moderate gastritis is the same as that in normal mucosa, but in severe gastritis it reaches higher levels, suggesting that also in inflammatory processes the chaperonin is involved to some extent, paralleling the rate of cell proliferation typical of these processes. Here, the non-canonical functions of Hsp60, for example stimulation of production of pro-inflammatory cytokines may be at play, an issue that deserves further investigation.

OXIDATIVE STRESS INDUCED BY THE NOVEL COMPOUND TRIBUTYLTIN(IV) FERULATE PROMOTES ER STRESS AND AUTOPHAGY IN COLON CANCER CELLS

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Organotin compounds display anticancer activity due to their apoptosis inducing properties. We recently synthesized the novel organotin ferulic acid derivative: tributyltin(IV) ferulate (TBT-F) that exerts an anti-tumor action in colon cancer cells, which was clearly consequent to autophagic cell death induction (1). TBT-F-induced autophagy was correlated with an early production of reactive oxygen species (ROS). The addition of the antioxidant N-acetylcysteine (NAC) almost completely prevented the effect of TBT-F on cell viability, lactate dehydrogenase release and clonogenic growth of colon cancer cells. Moreover, NAC markedly prevented the formation of autophagic vacuoles as well as the increase in the levels of autophagic markers LC3-II and p62. As a consequence of ROS production, TBT-F induced an antioxidant pro-survival response that involved Keap1/Nrf2 pathway and expression of antioxidant enzymes such as catalase and SOD2. Nrf2 silencing by RNA interference markedly increased the anti-tumor efficacy of TBT-F and reduced the levels of p62. TBT-F also increased the levels of Grp78 and CHOP, two markers of endoplasmic reticulum (ER) stress, an event that was also related to the pro-oxidative action of TBT-F. Interestingly, the levels of both LC3 and p62 were significantly reduced in Grp78 silenced cells, while only p62 decreased in CHOP-silenced cells thus indicating a possible relationship between ER stress and autophagy. These results corroborate the evidence that TBT-F-induced oxidative stress promotes ER stress and autophagy leading to cell death and suggest that TBT-F can be considered a good candidate for colon cancer treatment.

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EVALUATION OF THE PROERYPTOTIC ACTIVITY OF MONOSODIUM GLUTAMATE AT NUTRITIONALLY RELEVANT CONCENTRATIONS

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Thanks to its pleasant taste, the monosodium salt of glutamic acid (MSG) is widely used as a flavouring enhancer in processed food. Plasma levels of MSG usually vary in the range between 25 μM and 300 μM depending on the type of diet. Specifically, while plasma concentrations of MSG associated with healthy diets are around 25 μM, hyperproteic/oriental-style diet patterns are able to increase this value up to 300 μM. Eryptosis is a coordinated, programmed cell death, eventually leading to disposal of erythrocytes (RBC) without disruption of cell membrane and release of intracellular oxidative and pro-inflammatory milieu. While providing a convenient form of death for RBC, dysregulated eryptosis may result in a series of detrimental and harmful pathological consequences such as anemias, cytostatic-induced malignancies, sepsis, psychosis, malaria and cardiovascular diseases. The aim of the present work was to evaluate if, and to what extent, MSG could trigger the activation of the eryptotic process at concentrations of nutritional interest. To this end, RBC from blood samples of healthy volunteers were incubated either in the absence or in the presence of MSG in the range between 25 and 300 M for 24 h. Eryptosis, evaluated as phosphatidylserine (PS) externalization, cytosolic Ca^{2+} concentration and intracellular reactive oxygen and nitrogen species were assessed by flow-cytometry, while haemolysis by spectrophotometric assay. Our results demonstrate that the MSG at 25 μM is not able to induce eryptosis. Conversely, the salt exerts significant proeryptotic effects in a concentration-dependent manner, within the range between 50 and 300 M. From a mechanistic perspective, our evidences show that the eryptotic effect of MSG is related to the increase of Ca^{2+} intracellular concentration and does not depend on variations of the endocellular redox milieu. In conclusion, while ruling out any proeryptotic effect for MSG at concentrations compatible with a healthy diet, we here demonstrate that the salt can trigger eryptosis at higher concentrations, consistent with hyperproteic/oriental-style diets. Provided that eryptosis plays a pivotal role in the etiopathogenesis of vascular diseases, our work may pave the way for other studies on the dysfunctional effects of MSG-induced eryptotic RBC on the vascular endothelium.

APHAMAX® ATTENUATES INFLAMMATORY AND OXIDATIVE STRESS IN 2, 4-DINITROBENZENE SULFONIC ACID-INDUCED COLITIS IN RAT AMELIORATING INTESTINAL FUNCTIONALITY

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Accumulating evidences indicate that inflammatory and oxidative stress play an essential role in the pathogenesis and progression of inflammatory bowel disease (IBD). In IBD the excessive production of reactive oxygen species (ROS) and nitrogen metabolites contribute to tissue injury and could have also a profound impact on gut functions, including motility. We characterised the inflammatory and oxidative condition and the impact on colon motility in an experimental rat model of colitis, the 2, 4-dinitrobenzene sulfonic acid (DNBS)-induced colitis, and we evaluated if oral treatment with a natural extract of Aphanizomenon flos-aquae (AFA) AphaMax®, containing concentrated quantity of AFA-phycocyanins(PCs), compound with a significantly higher antioxidant power than other PCs, can attenuate the inflammatory and oxidative stress and help to recover intestinal motor functionality. Inflamed preparations from DNBS induced colitis rats showed an increase of different inflammatory markers, as MPO activity, a biochemical index for neutrophil infiltration, an increase in the expression of pro inflammatory cytokines and in the levels of marker of oxidative stress as ROS and Nitrites. Inflamed preparations showed also macroscopic and microscopic tissue damages and a marked hypocontractility, as recorded *in vitro* in colonic longitudinal muscle. AphaMax® downregulated in a dose-dependent manner colonic expression of cytokines, IL-1β, IL-6 and iNOS, the activity of MPO and improved anti-oxidant system, inhibiting also NF-κB activation. The colon injury and the colonic contractility in inflamed tissues were also improved by AphaMax® treatment. Our data reveal that in DNBS-rat model an intense oxidative insult would contribute to tissue damage during chronic intestinal inflammation and that AphaMax®- treatment was able to modulate the redox status by scavenging ROS and reducing the severity of the colitis, contributing also to the recovery of colonic muscle functionality.

POLYPHENOLS ANTIOXIDANT EFFECTS ON DIFFERENT CELL LINES

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Ultraviolet radiation and the numerous contaminants present in the environment gradually can be induced skin damage over the years. Several studies have shown that oxidative stress produced by the action of free radicals and their derivatives is responsible for disturbances in redox homeostasis [1]. It is also a major factor involved in the development of chronic ailments, aging or cancer. Reactive oxygen species (ROS) are molecules that can be generated in all cells during normal physiological and biochemical processes. Excessive ROS production can cause cell and tissue damage [2] with a negative effect mainly on organelles and cell membranes indeed can damage DNA, membranes and protein buildings. Phenolic compounds, the biggest group of natural antioxidants, have a known and wide variety of biological activities, as well as health effects [3,4]. They have an efficent antioxidant action and a key role in the control of several inflammation-associated processes, also in improving antioxidant defense system. In our recent study, the action of polyphenols extracted from red wine has been investigated in different cell lines exposed to UVB radiations. We have analyzed the effect of polyphenol mix contained in various kind of wines. The antioxidant and antiapoptotic effects have been evaluated by means of morpho-functional analyses, which revealed that red wine polyphenols, strongly prevent mitochondrial damage and apoptotic cell death. Thus, the considered extracts show

strong antioxidant properties and the ability to prevent apoptosis. In conclusion, these findings suggest, for these compounds, a potential role in all pathological conditions where the body antioxidant system is overwhelmed.

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WHAT ARE THE MULTIGENIC FINGERPRINT HALLMARKS IN TRIPLE-NEGATIVE BREAST CANCER?

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Nowadays, Next Generation Sequencing (NGS) data are detecting a distinctive mutational portrait of germline and somatic alterations in Triple-Negative Breast Cancer (TNBC) the most heterogeneous and aggressive BC subtype (1) (2). The genomic disease-driving scars highlights the clinical utility of sequencing the major susceptibility *BRCA1/2* genes and other involved in Homologous Recombination DNA repair pathway (HR) (3). The aim of this research is to describe molecular and clinical TNBC data *versus* the other subtypes focusing on germline Pathogenic/Likely Pathogenic variants (PVs/LPVs) by using a 22 genes cancer panel, including the risk genes *CDH1, PALB2, PTEN, STK11, TP53, ATM, CHEK2, BARD1, BRIP1,* RAD51C, RAD51D. Data of 696 BC patients have been collected from October 2016 and January 2021 after an accurate selection for patients with eligibility criteria during the onco-genetic counseling at the "Sicilian Regional Center for the Prevention, Diagnosis and Treatment of Rare and Heredo-Familial Tumors" of the Section of Medical Oncology of University Hospital Policlinico "P. Giaccone" of Palermo ". At all 83 (11.9%) BC patients harbored a *BRCA1/2* PVs/LPVs, while 24 (3.4%) had PVs/LPVs in other susceptibility genes. 186 (26.7%) TNBC patients have been enrolled and 34 of these (18.3%) resulted *BRCA1/2* PVs/LPVs carriers (mean age at diagnosis: 43.7 years). Specifically, 28 TNBC patients (15.1%) showed a *BRCA1* gene variant and 6 (3.2%) a *BRCA2* gene variant. 3 TNBC (1.6%) had PVs/LPVs in other genes, such as c.758_759insT, *PALB2*; c.1026+5_1026+7del, *RAD51C*; c.2T>C, *PMS2*. Analysis have been performed using IonS5 NGS system and confirmed by Sanger Sequencing. Our results underline that *BRCA1*-related tumors often have a TNBC profile, while *BRCA2*-associated tumors have a luminary BC profile, especially Luminal B. The PVs/LPVs detected in TNBC were not largely overlapping with the alterations of other subtypes. The most frequent PVs were the c.514del, *BRCA1* (5/34) among *BRCA1/2* PVs/LPVs TNBC carriers and the c.1238del, *BRCA2* (8/46) among Luminal B *BRCA1/2* PVs/LPVs carrier patients. One Large Genomic Rearrangement (LGR) c.-232_4675del, *BRCA1* has been identified in a TNBC patient. Significant clinico-pathological differences between carriers and non-carriers BC patients were observed. Using the Multi-Gene cancer panel to define a broader molecular profile is useful to evaluate the hereditary risk of HBOC and to develop novel therapeutic strategies. Further analysis exploring the different molecular fingerprints of BC subtypes are needed.

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ADIPOSE DERIVED STEM CELL POTENCY AND MCF7

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Adipose tissue represents an important source of stem cells (ADSCs), which can be easily employed in regenerative medicine. They are widely used thanks to their features, being capable to accelerate healing processes, and tissue regeneration. ADSCs are also widely used also in the field of reconstructive and aesthetic surgery, being able to improve wound healing and skin regeneration, giving a natural effect by restoring volumes, as in breast reconstruction after mastectomy. Stimuli from the environmental milieu are able to influence the fate and the behavior of these cells. In this work, we treated stem cells isolated from adipose tissue (ADSCs) with an exhausted medium from breast cancer cell line (MCF-7), to evaluate whether factors released by these cells are able to induce changes in ADSCs behavior. ADSCs were exposed to the medium, then RNA extraction was performed and the expression of stemness-related genes (Oct-4; Sox 2 Nanog, c-Myc), the cell-cycle regulators p21 (WAF1/CIP1) and p53 and Heat Shock Proteins was evaluated. The results indicate that soluble factors from the MCF-7 exhausted medium can influence different cellular mechanisms as the expression of stemness genes and cell cycle. From these results we can assume that the factors contained in the exhausted medium from MCF 7 are able to influence stem cell behavior. Increased expression of stemness-related genes was clear. We also observed a related increase in cell proliferation. On the whole our results seem to indicate a switch from an ADSC stemness toward a dangerous highly proliferative phenotype.

DISSECTING THE ROLE OF IL-4 AND IL-13 USING A 3D MODEL OF NORMAL HUMAN SKIN: AN INNOVATIVE EXPERIMENTAL APPROACH

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The identification of the specific cellular events underlying and/or triggering a diffuse chronic inflammatory skin disorder as atopic dermatitis (AD) is more and more challenging. In particular, the peculiar role played by interleukin (IL)-4 and IL-13 in promoting and supporting AD etiopathogenesis is still unclear. The aim of the present study is to dissect the early, specific, and direct effects of these pro-inflammatory cytokines

in a 3D model of organotypic cultures of normal human skin standardized in our lab, strictly mimicking the physiological condition. Bioptic skin fragments obtained after aesthetic surgery of healthy women (n=7) were cultured in a Transwell system with either IL-4 (50 ng/ml) or IL-13 (50 ng/ml) and harvested after 24 and 48 hours, with parallel control groups. All samples were processed for light microscopy (LM), immunofluorescence (IF), and transmission electron microscopy (TEM) analysis. As biomarkers of terminal differentiation, keratin (K)14 and K10, respectively expressed in the basal and the suprabasal layers, and filaggrin, typically found in the granular layer, were analysed. K16 and K17, absent in normal skin, were assessed as skin alarmins. The molecular composition of tight junction (TJs) encompassed claudin-1 and zonula occludens (ZO)-1 expression. Quantitative analysis was performed for i) Langerhans cells (LCs) on blue toluidine-stained semithin sections, ii) keratin immunostaining after indirect IF, and iii) intercellular spaces by TEM observations. Both cytokines induced a time-dependent increase of LCs number, a K14 immunostaining weakening, and K17 expression in scattered suprabasal keratinocytes. At 24 hours, IL-13 elicited the dilation of intercellular spaces throughout the entire epidermal compartment and the restriction of K10 immunoreactivity from the medium spinous layer upwards. K16 induction and discontinuity of filaggrin immunostaining were always more evident in IL-13 than in IL-4 groups. Regarding TJs, the two cytokines inhibited similarly claudin-1 expression at both time points. Conversely, ZO-1 distribution was more affected by IL-13 than by IL-4. In our experimental conditions, IL-13 initially triggers the modulation of keratin expression and the molecular composition of TJs. Later, IL-4 sustains the existing pro-inflammatory milieu, with weaker effects than IL-13. Synergic or additional actions between IL-4 and IL-13 in AD progression will be evaluated.

SICILIAN MANGO PEEL INDUCES CELLULAR STRESS ACCOMPANIED TO MITOCHONDRIAL DYSFUNCTION IN COLON CANCER CELLS

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Currently, cellular stresses as the oxidative, metabolic and genotoxic stress are considered the cause of many different human pathologies as neurodegenerative diseases (*e.g.*, Alzheimer's, Parkinson's, amyotrophic lateral sclerosis), alcoholic liver disease, chronic obstructive pulmonary disease, and also cancer. Although the role of cellular stress has been largely debated in cancer, nowadays some therapies aim to target the intracellular pro-oxidant/anti-oxidant balance triggering the tumor commitment to cell death. Therefore, it has become more necessary an improved understanding of cancer response to cellular stress that could be advantageous to develop cancer tailored therapies. In this scenario, the present study shows how extracts of some fractions of Sicilian mango, a tropical fruit rich in phytochemicals with nutraceutical properties, are able to affect the cell viability of three colon cancer cell lines (HT29, Caco2 and HCT116) inducing cellular stress. By using hydro-alcoholic extracts of three different portions of the fruit (peel, pulp and kernel), we observed that mango peel extract (MPE) is the most effective in reducing cell viability, causing a remarkable LDH release and the death of all three cancer cells. The effect was accompanied by mitochondrial injury, dissipation of mitochondrial potential membrane and decrease in the level of proteins localized in the mitochondrial membrane such as voltage-dependent anion-selective channel (VDAC), mitofilin, and some members of Bcl-2 family proteins (Mcl-1, Bcl-2 and Bcl-XL). All these effects were accompanied by redox balance changes and upregulation in MnSOD, a mitochondrial scavenger enzyme able to modulate the cellular response against oxidative damage. The analysis of the effects exerted by the different phytochemicals present in MPE allowed to identify those molecules responsible for the observed anticancer effects sustaining their future employment as chemopreventive or therapeutic agents.

A NOVEL HYPOTETICAL MECHANISM OF ERYTHROCYTE SENESCENCE?

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The erythrocyte senescence mechanism and its subsequent spleen removal remain still unknown. The view that the aging of the red cell and its removal from the circulation result from a progressive series of events during the 120 days of its lifespan appears to be the most consistent with the available data. One of the best described phenomena that relate to erythrocyte senescence during the erythrocyte lifespan is the Gardos effect, in which red blood cells (RBCs) loss through this channel the KCl and gain NaCl and this lead the cells to dehydration, as well are involved the oxidative stress due to the reactive oxygen species (ROS) that can damage the RBCs function, vesiculation of the erythrocyte membrane, the exposure of the phosphatidylserine (PS) as a death signal for the RBCs. Taking into account all these changes and several other that occur and lead red blood cells to senescence, we proposed a new mechanism that could lead to senescence and its spleen removal. Our findings indicate that hemichromes possess a strong affinity for band 3 cytoplasmic domain and, following their binding, lead to band 3 oxidation and clusterization. Those band 3 clusters show increased affinity for NAbs which activate complement and finally trigger the phagocytosis of altered RBC. Persistent tyrosine phosphorylation of Band 3 protein produces extensive membrane destabilization leading to the loss of vesicles conteing hemichromes. Understanding this mechanism should provide insight into the critical membrane alterations in hereditary hematologic disorders and malaria that are characterized by tyrosine hyperphosphorylation of band 3.

BIOACTIVE TRITERPENES OF *Protium heptaphyllum* GUM RESIN EXTRACT DISPLAYED CHOLESTEROL-LOWERING POTENTIAL

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Hypercholesterolemia is one of the major causes of Cardio-Vascular Diseases, which risk is further increased if other forms of dyslipidemia occur. Current therapeutic strategies include changes in lifestyle coupled with drug administration. Statins represent the most common therapeutic approach, but in the most of cases the monotherapy with this drugs showed to be insufficient to achieve the recommended LDL-C level, also because of the onset of drug resistance mechanisms. In these cases, the monotherapy is replaced or accompanied by the administration of other drugs (such as fibrate, niacin, and ezetimibe) or non-pharmacological approaches. Among them, patients with a clinical picture of low hypercholesteremic severity prefer applying to phytotherapeutic ones, because they are perceived natural, safer, and free of side effects. For these reasons, in the last decades the research of new medicinal plants as alternative approach for the treatment of hypercholesterolemia is still under exploration. In particular, the attention is focused on the identification of new phytochemicals that may exert an antihypercholesterolemic action through a different mechanism of action from what reported for statins. In this work, we investigated the hypocholesterolemic potential of a new chemically characterized *Protium heptaphyllum* gum resin extract (PHE). With this purpose, we measured *in vitro* the effects on cholesterol biosynthesis in comparison with lovastatin. Moreover, we also evaluated if PHE may affects HMGCR, evaluating both changes in enzymatic activity and binding ability of the molecules identified in the extract. Finally, studies on the expression of genes related to cholesterol metabolism were performed. Studies on human hepatocytes revealed how PHE was able to reduce cholesterol production and regulate expression of proteins involved in its metabolism (HMGCR, PCSK9, LDLR, FXR, IDOL, and PPAR). Moreover, measuring the inhibitory activity of PHE against HMGR, a moderate inhibition was recorded in comparison to lovastatin. Chemical characterization via HPLC-APCI-HRMS^2 and GC-FID/MS identified 13 compounds mainly belonging to ursane, oleanane and tirucallane groups. Among them, molecular docking studies suggested that the acidic tetra- and pentacyclic triterpenoids could be the main responsible of observed biological activity. In conclusion, our study demonstrates how PHE may be a useful alternative to contrast hypercholesterolemia, highlighting its potential to become a sustainable multi-target natural extract for the nutraceutical industry and rapidly gain acceptance as health-promoting source.

Opuntia ficus indica METHANOLIC FRUIT EXTRACT COUNTERACTS OXIDIZED LDL-MEDIATED HUMAN ENDOTHELIAL CELL DYSFUNCTION THROUGH THE INHIBITION OF NF-κB ACTIVATION

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Oxidized low-density lipoproteins (oxLDL) play a crucial role in the etiopathogenesis of atherosclerosis through the activation of proinflammatory signalling events, eventually leading to endothelial dysfunction (ED). *Opuntia ficus indica (Opuntia)* fruit has been shown to contain several bioavailable phytochemicals with effective anti-oxidative and anti-inflammatory properties and, among these, the betalain pigment indicaxanthin. Along these lines, supplementation with *Opuntia* fruit has been demonstrated to counteract oxidative stress and low-grade inflammation in healthy humans. In the light of the strong interconnections between oxidative stress, inflammation and atherosclerosis, this work has evaluated the effects of a methanolic extract of *Opuntia* fruit (OFE), at nutritionally relevant concentrations, in an *in vitro* model of oxLDL-induced ED. To this end, human LDL were oxidated with 5 μM $CuSO_4$ for 24 h. Human umbilical vein cord cells (HUVEC) were preincubated for 1 h either in the absence or in the presence of OFE in a range between 5 and 20 M indicaxanthin equivalent. These concentrations are nutritionally relevant and consistent with indicaxanthin plasma level after a fruit meal. HUVEC were then stimulated for 16 h with oxLDL and the effects of OFE pre-treatment evaluated. Cytotoxicity of oxLDL was assessed by MTT test; oxLDL-induced ICAM-1, VCAM-1 and ELAM-1 over-expression by flow cytometry as well as endocellular reactive oxygen and nitrogen species levels; NF-kB activity by reporter assay. Our results clearly show that pre-treatment of HUVEC with OFE significantly and concentration-dependently inhibits oxLDL-induced both cytotoxicity and ICAM-1, VCAM-1, ELAM-1 over-expression. Interestingly enough and from a mechanistic perspective, our experimental data also demonstrate that these effects are redox-dependent and related to the ability of OFE to inhibit NF-κB transcriptional activity. In conclusion, we here show that OFE is able to exert remarkable protective vascular effects *in vitro* at nutritionally relevant concentrations.

EFFECTS OF INDICAXANTHIN ON HUMAN MOTOR CORTICAL EXCITABILITY AND PLASTICITY

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The role of nutraceuticals has risen interest in the last decade for complex biological activities exerted on pathophysiological processes such as oxidative stress, inflammatory conditions and excitotoxicity. In this regard, the effects of nutraceuticals on basic functions of neuronal processes, such as cortical excitability and cortical plasticity are still to be unveiled. Translational studies performed on phytochemicals (PhC) with anti-inflammatory and antioxidant properties could trace the path for the possible modulation of neuronal excitability in humans, phenomena involved in neurodegenerative alterations and cell stress. In this context, *Opuntia ficus indica* is a mediterranean plant containing a betalaine pigment, indicaxanthin, that has been studied for its numerous activities, in particular the ability to cross the Blood-brain barrier (BBB) and to influence neuronal excitability in rats. On these bases we aimed to assess the effects of acute intake of indicaxanthin (IX) on human cortical excitability and plasticity. Eight healthy, right handed male subjects (mean age: 20-45 years) were recruited . No-one had a history or clinical signs of neurological diseases, brain trauma or use of drugs affecting cortical excitability as assessed by a clinical neurologist. Safety guidelines for Non Invasive Brain Stimulation (NIBS)

studies were rigidly applied for both stimulation and inclusion criteria. Intracortical facilitation (ICF), short-lasting intracortical inhibition (SICI) and test stimulus (TEST) were recorded (10 times each in a pseudo-randomized order) by means of a paired pulse Transcranial Magnetic Stimulation (TMS) stimulator (Magstim Co, Dyfed UK) before (PRE-TDCS) and after (POST-TDCS) 20' of anodal transcranial direct current stimulation (tDCS, Neuroconn, Ilmenau, Germany) delivered over right M1. The same data were recorded in baseline condition (T0) and 2 hours after intake of 400g of cactus pear fruit (T1) in separate sessions. IX significantly increased PRE-TDCS TEST ($p<0.01$), and PRE-TDCS ICF($p<0.05$) whereas PRE-TDCS SICI was unchanged, and significantly reduced POST-TDCS ICF ($p<0.0001$) and SICI ($p<0.001$). Our data support the hypothesis that IX is able to modulate human cortical excitability consistently with preclinical evaluations, and specifically to increase excitability of Human Motor Cortex. Moreover, the paradoxical effects after anodal tDCS seems to suggest that the excitatory drive exerted by IX is also able to induce homeostatic responses on motor cortical plasticity.

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THE IMPACT OF OXIDATIVE STRESS ON ANION EXCHANGE CAPABILITY OF BAND 3 PROTEIN

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The chloride/bicarbonate exchanger Band 3 protein (B3p) is the most abundant protein in the erythrocyte membrane, playing an important role in gas exchange and functioning as a point of attachment for cytoskeleton proteins, and which contributes to mechanical and osmotic stability. As cell homeostasis critically depends on the efficiency of membrane transport systems, rising attention has been devoted to the link between oxidative stress, ion transport, and diseases. Gaining knowledge about modulation of membrane transport systems by oxidizing conditions might allow to define novel targets for drug development. In addition, erythrocytes are constantly exposed to oxidating molecules within capillaries and they represent a good cell model to investigate the response to oxidative stress. In particular, B3p anion exchange capability, determined by measuring the rate constant for SO_4^{2-} uptake, is a suitable tool to study the impact of oxidative damage on erythrocyte homeostasis. On this basis, we summarize our recent findings about B3p's functioning under different *in vitro* oxidizing conditions gained by: 1) monitoring B3p anion exchange capacity in erythrocytes after incubation with H_2O_2, chosen as a model to induce increased oxidative stress levels *in vitro*; 2) monitoring B3p anion exchange capacity in erythrocytes after exposure to increasing concentrations of glucose and galactose, both sugars used to model *in vitro* diseases related to oxidative stress, such as diabetes and galactosemia. The present findings may be a useful basis to further focus on the impact of diseases associated to oxidative stress on erythrocytes homeostasis.

INTERNALIZATION OF ISOLATED RESPIRATION-COMPETENT MITOCHONDRIA AS THERAPEUTIC MODEL IN THE TREATMENT OF ACUTE KIDNEY INJURY

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In recent years, artificial mitochondrial transfer emerged as a promising therapeutic mechanism in the field of regenerative medicine [1]. Ischemia/reperfusion injury (IRI) is a rising pathology associated with poor clinical outcomes [2]. The disruption of mitochondria network in the early stage of IRI is known as pivotal for the progression of the pathology [3]. Considering that, we explored the transfer of fully-viable mitochondria in an *in vitro* model of IRI-induced renal tubular injury, and their possible rescue of injury, as a possible new therapeutic tool. IRI was induced in conditionally immortalized renal tubular cells (ciPTEC) by Antimycin A administration. We, then, co-incubated injured cells with viable mitochondria extracted from healthy ciPTEC tubular cells for 24 hours. The internalization of isolated mitochondria was demonstrated by optical evaluation using fluorescent mitochondrial dyer as well as with FACS flow cytometry. We demonstrated that mitochondria co-incubation was able to rescue ATP production in damaged cells. Furthermore, we observed a significant decrease in cytotoxicity compared with negative control, in parallel with the restoring of proliferation capacity. Taking together, these preliminary results demonstrated that isolated mitochondria obtained from differentiated renal tubular cells can be internalized by injured cells. Moreover, mitochondrial treatment was able to rescue injured renal tubular cells.

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CHEMICAL CHARACTERIZATION, RADICAL-SCAVENGING AND ANTIOXIDANT ACTIVITY OF FRUITS OF *Diospyros digyna* JACQ

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Epidemiological studies demonstrate how diets rich in plant foods are able to prevent several diseases. This functional role of plant foods is due to specific phytocomponents with peculiar biological activity that is beneficial to human health. Phytochemical bioactivity has been frequently related to the ability of these molecules to function as antioxidants thus protecting cells from oxidative stress phenomena (10.3390/nu12061748). In recent years, various studies

have evaluated the functional properties of several exotic fruits. On the other hand, in view of the growing market demand for those fruits, tropical plants are increasingly cultivated in areas of the world other than those of origin. In particular, tropical plants adapt well to the Mediterranean climate of Sicily, producing fruits of high quality, also in terms of nutraceutical value (10.1016/j.foodchem.2019.125515; 10.3390/molecules25112612). Sicilian production of mango, litchi, and avocado, has achieved a significant commercial success and the cultivation of other tropical species of lesser commercial importance is also being attempted. The black sapote (*Diospyros digyna* Jacq.), a species native to South America, is almost completely unknown in Europe. Its ripe fruits have green peel and a characteristic chocolate colored pulp which makes it deserve the name of black or chocolate khaki. Here we evaluated the nutraceutical value of *D. digyna*. fruits from plant grown in Sicily. Our results indicated that these fruits are an interesting source of antioxidant polyphenols, including proanthocyanidins, particularly concentrated in the fruit peel. Concerning the phytochemical profile, HPLC-DAD-ESI-MS/MS analysis identified 29 phenolic compounds, of which 12 are phenolic acids and the others belong to the flavonoid family. CAA assay showed that extracts from peel and pulp of these fruits prevent lipid peroxidation in HepG2 cells with higher efficacy than other common fruits. Moreover, our results suggest that the observed antioxidant protection involves both redox active properties of the black sapote components, as measured by in solution assays, and also upregulation of the genes coding for the antioxidant enzymes as evaluated by qRT-PCR. Overall, our data provide evidence on the functional properties of black sapote. In particular our results suggest that a moderate daily intake of these fruits may provide significant antioxidant protection and indicate the potential application of black sapote as raw material for the nutraceutical industry.

REDOX NANOPARTICLES: THE ROLE OF ION DOPING IN HEMOCOMPATIBILITY

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It is well known that nanoparticles have multiple applications in biomedicine, such as controlled drug administration and diagnostic applications. Also, nanotechnology science and nanoparticles (NPs) have been identified as a new approach to deal with bone tissue engineering due to their multitude of physicochemical and biological properties of ion doped mesoporous nanoparticles. They are attractive either as nanofillers in various scaffolding materials or as implants in bone cavities, thereby opening a wide field in bone tissue engineering. Considering that blood is not only the first contact for NPs administered intravenously, but also the gateway for all NPs, administered via other routes, to reach their target tissues or organs, our main scope is to identify their hemocompatible dosage range based to their ion doping. The aim of this study was the synthesis of various ion doped (Calcium, Copper, Cerium, Strontium) silica-based nanoparticles and the evaluation of their toxic effect on erythrocytes. Their synthesis was performed using the sol-gel method, by the progressive addition of Calcium, Copper, Cerium, Strontium ions on pure silica nanoparticles (SNs). The toxicity evaluation was based on hemolysis, lipid peroxidation, ROS, superoxide radicals, hydrogen peroxide species and antioxidant enzyme production. The addition of strontium in SNs presented the best hemocompatibility in compare with all the tested ions by protecting erythrocytes from oxidative stress. Considering the ongoing development of various NP formulations for different therapeutic applications, where their interactions with blood components is not only inevitable but also potentially perilous, hemocompatibility should be one of the foremost concerns in their design and development.

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HYPOXIA INDUCED MIR-675 CONTROLS CELL SURVIVAL BY MODULATING APOPTOSIS AND AUTOPHAGY

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Hypoxia is a hallmark feature of the tumour microenvironment and a key element in tumour progression. The reduced O_2 partial pressure, induced by the uncontrolled growing mass in tumour microenvironment, prompts tumour cell to activate specific pathways that, driven by the transcription factors complex HIF, induce hypermetabolism to favour glycolysis, neo-angiogenesis with an increase in local vasculature and tumour metastasis. All these strategies allow the cells to survive despite oxygen deprivation and, at the same time, are the cause of the chemoresistance observed in several tumors, including colorectal cancer. Recent studies associate hypoxia-induced chemoresistance with an activation of the autophagic pathway, however, the molecular mechanisms of this interaction are still to be defined. The characterization of the molecular mediators through which HIF controls tumor progression and chemoresistance will allow identifying new and more specific molecular targets for the treatment of cancer. Our recent data demonstrated that the hypoxia-induced long non-coding RNA H19 and its intragenic miR-675 are pivotal players in the establishment of hypoxic responses. Here, by silencing and over-expression experiments, we demonstrated that the miR-675 negatively regulates both apoptosis and autophagy, letting us hypothesize a combined use with conventional therapeutics to overcome the hypoxia-induced chemoresistance.

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THIRD SYMPOSIUM OF EXPERIMENTAL BIOLOGY APPLIED TO SEA AND ENVIRONMENT

EFFECTS OF MICROPLASTICS INGESTION ON DEVELOPMENT AND FEEDING BEHAVIOR IN BRINE SHRIMP (*Artemia salina*)

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The incidence of microplastics (MPs) uptake in marine organisms is a topic recently focused by scientific community. Despite this, the impacts of these pollutants still remain largely unknown, especially for zooplanktonic organisms. In the present study, it has been evaluated how within first 7 days of life cycle, 10 µm Polybead® Blue Dyed Microspheres can be ingested and egested by *Artemia salina* larvae, to comprehend the impact on health and development. Twelve *A. salina* larvae (instar I) groups were exposed in three replicates to different concentrations of MPs (0-1-10-100-1000-10000 MPs/ml), with and without *Dunaliella salina* food source. The results highlight that *A. salina* larvae ingest and swallows MPs in relation to the exposure times, in a dose-dependent manner, strongly influenced by food availability. *A. salina* larvae were already able to ingest and eliminate in feces MPs after 6 h post hatching (instar II). The lowest level of reported contamination was 0.2 MPs/individual at 1 MPs/ml exposure without food source; while the higher contamination found was 306.2 MPs/individual at 10000 MPs/ml exposure without food source. No MPs was found in presence of food source from 1 to 100 MPs/ml, while contamination was detected at all concentrations of MPs without food source. The effect of MPs exposure on the developmental stages within 7 days of exposure was evaluated, with the worse effect at the end of the exposure time (168h) at 10000 MPs/ml of microplastics whit and without food source, with a delay respectively of II and III instars compared to the positive control. Furthermore, microalgal feeding was significantly reduced about 50% in the presence of 10000 particles/ml microplastics. These results highlight that aquatic microplastics pollution could affect *A. salina* behavior and life cycle; more studies are in progress on the histopathological and metabolic effects.

ZEBRAFISH, DYSBIOSIS AND TOXICOLOGY EFFECTS

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It has been demonstrated that fish represent an excellent resource for research studies. In fact they are considered very useful model organisms for a comparative analyses to clarify the mechanisms involved in the physiological functions of organisms and their evolution. Notably, zebrafish is fast gaining popularity and consensus regarding its use in development, genetics, immunity, behaviour, nutrition and physiology research studies. Studies on xenobiotics have been increasing in recent decades and it has been shown that many of them have a possible toxic effect on human health[1]. In this regard, especially zebrafish represents an excellent vertebrate model, which is increasingly used to evaluate chemical toxicity and safety. Moreover, it is interesting the usage of the transgenic zebrafish as biosensors, which are employed to detect environmental pollutants[2]. In addition, particular attention is paid to gut microbiota. The latter is characterized by a multitude of microorganisms that populates intestine, maintaining a symbiotic relationship with the host, without underestimating its active part in metabolism and immunity. However intrinsic factors of the host or related to the surrounding environment are both involved in a possible alteration of symbiotic relationship.The imbalance in the intestinal microbiota leads to the alteration of the host's homeostasis causing the development of a phenomenon known as dysbiosis. Data in literature, show that changes in gut microbiota represent a good indicator of chemical exposure, since the toxicity of xenobiotics can be modulated after bacterial metabolism. Therefore the aim of our study is to underline the key role of zebrafish gut microbiota that could be an useful tool for evaluating the effect on exposure of various pollutants, including crude oil, microplastic, antibiotics, aliphatic and polycyclic aromatic hydrocarbons among others [3].

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OXIDATIVE STRESS AND LIPID METABOLISM RESPONSE IN *Sparus aurata* EXPOSED TO SUB-LETHAL DOSES MIXTURE OF EMERGING POLLUTANTS AND DRUGS

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Chemical contaminants, such as agricultural, industrial and urban by-products, pharmaceuticals, drugs metabolites and plastics, are continuously found in the oceans, affecting its quality and organism's welfare. Although these compounds are present at concentrations ranged ng/L at sub-lethal concentrations, there is an increasing concern about the potential adverse effects of the interactions among those substances

present, simultaneously, in a mixture. In this study, specimens of sea bream were exposed, by food, to a mixture of 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47), cadmium chloride ($CdCl_2$) and carbamazepine (CBZ) for 15 days, and further maintained for 2 weeks without contaminants. In the liver, lipid composition, reactive oxygen species and oxidative-stress related genes, were determined. Both markers resulted affected after the exposure showing only a partial degree of recovery. In addition, the study of the observed relationships between the above patterns of biomarkers could be adopted to monitoring the health of aquatic organisms and aquatic ecosystem.

EFFECTS OF THE ENRICHED-ORGANIC DIETS COMPOSITION ON EUROPEAN SEA BASS (*Dicentrarchus labrax*) NEW TOOLS FOR FISH WELFARE EVALUATION IN AQUACULTURE

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In recent years, concern for the welfare of animals, including fish, has a strong impact on public opinion, putting pressure not only on producers and the scientific community, but also on institution to guarantee adequate "standards" for animal welfare. The market today challenges the production capacity of aquaculture by asking for products with consistently high quality. Unlike other farming methods, there is a lack of scientific information on the welfare of fish reared under intensive aquaculture conditions. Among the various aspects that characterize aquaculture, the increase in fish welfare has an impact on public opinion also for economic reasons whereby is very important to keep animals in good health, determine a higher feed conversion rate, lower mortality, optimal growth rate and high quality of the animal raised. Whereas stress events, acute and chronic, are reflected on the flesh, due to the increase in muscle activity (generally associated with this condition). We have firstly demonstrated that accelerometer tags are useful tools for welfare monitoring, indeed they do not affect welfare and health of implanted fish and that tagged fish can be sampled during experiments and be considered as representative of population, by displaying similar growth and physiological parameters compared to untagged fish. Consequently, the purpose of this study is to investigate the effect of two organic diets (contain organic vegetables and a natural antioxidant compound) on the welfare of the European sea bass compared to one conventional. A holistic approach was adopted, including the measurement of the primary stress response (cortisol), secondary (*i.e.* lactate, glucose, haematological parameters, lysozyme) and tertiary (*i.e.* swimming performance, muscle activity and growth parameters) indicators. In parallel, we evaluated the enzymatic activities of 7-ethoxyresoruphine-O-deethylase and glutathione-S-transferase, as index of function of the microsomal hepatic system of mixed function oxygenase (MFO), to evaluate the possible effects of pollutant contamination through the diet. In this study, multiparameter analysis approaches were performed to bring a better understanding of the effectiveness of a holistic approach to quantify welfare in organic aquaculture. The multi-parameter approach has outlined a complete picture of the physiological state of the sea bass. The Principal Component Analysis and the Multi-Criteria-Decision-Analysis have proved to be useful tools for an integrated assessment of the well-being of the fish, highlighting that the best condition was achieved in the experimental group fed with the organic protein-rich diet.

CHEMICAL COMPOSITION DETERMINATION OF MARINE SEDIMENT BY NEAR INFRARED SPECTROSCOPY TO MONITOR AQUACULTURE ENVIRONMENTAL EFFECT

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Aquaculture is playing an increasingly important role in compensating for fishery inability to meet fish product demand, accounting for 46% of global production and 52% for human consumption. However, aquaculture expansion activity determines increasing concerns about its detrimental effects on water, sediments and biodiversity. Marine sediments are composed by inorganic and organic matrix with complex physical, chemical and biological characteristics. Their alterations can record in space and time effects of ecological processes and human activities in the surrounding systems. Characterization of their composition variations is used to monitor the effects of fish farming on marine environment. Chemical analyses utilized to assess marine sediments quality can be very expensive and time-consuming. Near Infrared Spectroscopy (NIRS), a fast instrumental technique with a high accuracy could be a valid alternative to standard analytical techniques to evaluate physical and chemical composition of marine sediments, reducing the cost of routine analysis, to predict and follow eventual effects on in-faunal biodiversity. Based on spectroscopy in the near-infrared range, from 700nm to 2500nm, correlating by complex statistics absorbance by covalent bonds between H, C, O, N and S, to concentration of protein, lipid, carboxylic, amide aminoacids and other molecules or groups. After calibration equations between analytical data and spectral results, by a single scan numerous parameters can be predicted. Being sediment drying the only manipulation required. The aim of this study was to evaluate the potential of NIRS to monitor the effects of fish farming on marine environment, through the evaluation chemical composition of sediments collected beneath a bluefin tuna farm and a sea bream farm, located in the Castellammare Gulf (Trapani, Italy). 140 sediment samples were collected from sampling stations at different distances from the cages and from control stations, not influenced by farming. Total Organic Matter, Carbohydrates, Lipids, Protein, Total Sulphides contents were analysed by standard methods. Near-infrared absorbances (700nm-2500nm) were recorded by NIRFlex Solids (BUCHI, Milan Italy). NIRS spectra and analytical data were compared through scatter plots. The regression lines obtained showed a high dependence relationship between the two variables (predicted NIRS and analytical data), with coefficient $r^2 > 0.7$ ($p<0.05$) for each analysed parameter. Our results showed that NIRS is able to estimate several properties in marine sediments simultaneously in a short period of time and without chemical laboratory analyses.

A SCANNING ELECTRON MICROSCOPY PROTOCOL FOR THE STUDY OF MARINE MICROPLASTICS

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Microplastics (MPs) are widespread all over the world, representing the main class of debris present in the marine environment. The effects of microplastics on the marine ecosystem needs to be addressed in order to plan effective actions at global scale. The aim of this study was to obtain a specific Scanning electron microscopy (SEM) protocol, in order to deeply understand MPs structure and characteristics and acquire the best tools to deal with it. MPs may function as sites for the colonization of microorganisms and the subsequent formation of associated biofilms (the so-named Plastisphere). In fact, several microorganisms (bacteria, archaea, pico-eukaryotes) as natural biodegrades and recyclers, could also play a role in breaking down chemical compounds of plastics. SEM was used to examine the surface of floating microplastics collected in Tyrrhenian Sea and the procedure has been adapted to preserve the microplastic structure as well as the organic biofilm associated. For this reason, instead of the common procedures for SEM which involved washing in CO_2 or undertaken the plastic fragments at high values of pressure/temperature (80bar/40°), the samples were fixed in 70% alcohol for 48 hours. Samples were dehydrated in a graded series of alcohol, 1h in each solution from 70 to 100°. The drying critical point, in which the microbial biofilm integrity can be compromised, has been avoided and replaced with a procedure at temperature of 28°C for 12h, in order to preserve both microplastics and biofilm structure. The SEM photographs highlighted the presence of microorganisms on the Low-Density Polyethylene (LDPE) microplastics sample, previously characterized. Moreover, the collected data showed that the use of this protocol did not produce any kind of damage on both plastics and biofilm. The presence of *Diatomae* sp. was recorded and filamentous bacteria and some bacteria shapes, rods and cocci, and fungal hyphae were evident. The SEM represent an extremely effective tool when it comes to analyzing various pollutants, particularly microplastics. Making practical use of automated feature analysis can lead to the development of straightforward and accurate techniques to perform rapid analysis for the classification of microplastic particles in water samples.

NEW DATA ON THE DIET AND FEEDING HABITS OF THE BLACKMOUTH CATSHARK, *Galeus melastomus* RAFINESQUE, 1810 FROM THE SOUTHERN TYRRHENIAN SEA

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The knowledge of feeding habits is important to understand the trophic status of fish stocks. Furthermore, the availability of data like these is of particular relevance for the conservation and management of elasmobranchs' populations. Sharks are top predators in marine environments. However, despite they cover a key role for marine ecosystems, sharks and elasmobranchs in general are threatened by several human activities, such as fishing, pollution and habitat degradation. In the present study, we present data on the diet of the Blackmouth catshark (*Galeus melastomus*) from southern and central Tyrrhenian Sea. The stomach content of a total of 154 individuals collected in 2018 and 2019 was analysed. *Galeus melastomus* is a benthic feeder and scavenger whose diet considerably varies with seasons and areas. Hence, we can consider it as an opportunistic feeder. Specimens were collected in spring, summer and autumn by the MEDITS bottom-trawl survey and by commercial landings of the fishing fleets CAMPBIOL survey. Specimens were frozen on board to prevent digestion of the stomach content and immediately transferred in laboratory after landing. Each individual was subsequently measured (total length, TL) to the nearest cm, weighed (total weigh, TW) to the nearest g, and sexed. They showed a size range between 14.5 and 52 cm of total length (TL). Each prey item was identified to the lowest taxonomic level possible, counted and weighed (g). The 13% of the total examined stomachs was empty. The main prey items were: cephalopods, fishes and decapods. The percentage of occurrence of the different prey items varies in relation with the total length of specimens. Our results showed that small specimens of *G. melastomus* feed mainly on Euphausiacea and small fishes, mainly Myctophidae (*Electrona risso, Diaphus holti*). On the other hand, big specimens of *G. melastomus* feed on large-sized cephalopods (*Todadorades sagittatus, Eledone sp., Scaeurgus unicirrhus),* decapods (*Pasiphaea sivado, Pasiphaea multidentata, Robustosergia robusta*) and fishes (*Ceratoscolpelus maderensis, Macroramphosus scolopax).* Results of stomach analysis also showed the scavenger habits of this species, with the presence of fisheries discard and offal, like fish head, and sea bird remains. The feeding strategy of *G. melastomus* could be the result of an adaptation to the oligotrophic deep environment. In conclusion, we can consider this shark as a generalist opportunistic feeder, able to exploit the most common trophic resources of its environment.

NEW INSIGHTS INTO OTOLITHS ECOMORPHOLOGY OF TWO CONGENERIC SEABREAMS SPECIES, *Pagellus erythrinus* (LINNAEUS, 1758) AND *Pagellus bogaraveo* (BRÜNNICH, 1768)

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Over the last decades, scientific interest for fish otolith morphology and morphometry increased significatively. These

are useful tools to study many aspects of teleost life in marine environment. Several factors can affect shape and microstructure of otoliths, such as environmental parameters, feeding habits, ontogeny, physiology and phylogeny. Among different types of otoliths, sagittae is the largest and the most variable, with a high taxonomic value. In the present study, attention was addressed to two high-values fish species in Mediterranean Sea, *Pagellus erythrinus* (Linnaeus, 1758) and *Pagellus bogaraveo* (Brünnich, 1768). The common pandora (*P. erythrinus*) is a demersal species that lives mainly on rocky and muddy-sandy bottoms, with a high frequency of occurrence at a depth between 20 and 300m. It is a generalist predator and a benthic feeder, and it shows protogynous hermaphroditism. The blackspot seabream (*P. bogaraveo*) lives above all substrata, near offshore banks, on seamounts and in cold-water reefs. It is a benthopelagic predator and a protandrous hermaphrodite. The juvenile stage lives near the coast, whereas the adults live on the slope down the 800m. A total of 44 otoliths of *P. bogaraveo* and 71 otoliths of *P. erythrinus* were collected from trawled specimens at south Tyrrhenian sea (GSA 10) between March and October 2019 (belonging to CAMP.BIOL.19 project). Sagittae were removed from otic capsule, cleaned from tissue and stored dry inside Eppendorf microtubes. Morphometry and microstructure of all samples were analysed. Digital images of each otolith samples were collected by using a stereomicroscope with a built-in digital camera and subsequently binarized for contour detection by ImageJ 1.48p software. Statistical and shape analysis were performed using open-source software packages that run on the R platform. Moreover, a total of 16 otoliths (12 of *P. bogaraveo* and 4 of *P. erythrinus*) were used for SEM analysis, for the first time for these species. The results showed inter-specific differences between the two congeneric seabreams, certainly due to habitats and diet of these species. The *P. bogaraveo* specimens showed an elliptical otolith shape with crenate margin while *P. erythrinus* specimens showed a pentagonal shape of otoliths. An interesting bilateral asymmetry was detected between left and right sagittae of this latter species. All these features represent the prove of the eco-morphological of sagitta and sulcus acusticus in life and adaptation to the environment of fish.

CONTRIBUTION OF THE PROCESSING TECHNOLOGIES TO THE INCREASE OF ENVIRONMENTAL AND ECONOMIC SUSTAINABILITY OF THE FISHERY VALUE-CHAIN: EFFECTS OF AN OZONIZED SLURRY ICE SYSTEM ON SHELF LIFE OF *Sardina pilchardus AND Engraulis encrasicolus*

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Innovative technologies are necessary to support the fishery value-chain, in order to meet the increasing global demand for fish products, due to the greater awareness of the beneficial effects of seafood on the human health and the increasing global population. Innovation aimed to improve competitiveness, safety, environmental and economic sustainability of the sector must be driven by the market and sustainability needs, leading to adaptation of the products to consumer preferences, product diversification and reduction of losses between landing and consumption, still amounting to 27% of the fish landed. Indeed, fish products are extremely perishable and appropriate processing and preservation techniques must be undertaken. Among the new innovative techniques related to the cold chain, great attention could be devoted to slurry ice or liquid ice, in preventing fish spoilage and product loss. It shows several characteristics that make it a suitable technique for maintaining the product freshness over time, faster cooling rates, allowing temperatures between -0.5 and -1.5°C, unlike traditional flake ice by which the temperature can only drop to temperatures just above 0 °C. The use of ozone, as an antimicrobial agent, combined with slurry-ice has proven to be an equally successful technique in prolonging the shelf-life of several fishery species such as turbots (*Lepidorhombus whiffiagonis, Psetta maxima*) and the sciaenid *Collichthys niveatus* compared to flake ice or slurry-ice without ozonation. To investigate the effectiveness of a combined slurry-ice/ozone system (SIO) on prolonging the shelf life of two fishery species, *i.e.* sardines (*Sardina pilchardus)* and anchovies *(Engraulis encrasicolus*), a multidisciplinary approach was used. As regards both fish species, preliminary results showed that specimens stored under SIO were better compared to the specimens stored under the traditional flake ice (FI) in terms of sensory aspects (Quality Index Method; $p < 0.05$). Moreover, results of the main biochemical parameters related to the shelf life (total volatile basic nitrogen and malondialdehyde contents) suggested a longer shelf life for samples stored in SIO. In addition, pH values were statistically lower ($p < 0.05$) in the SIO specimens over the shelf life period considered, leading to the assumption of an inhibiting effect of the preservation system on the bacterial growth. Further analyses in progress will clarify the effectiveness of this technique in the shelf-life extension of these species, that are among the most important for the economy of our region.

SEA SALT: INNOVATIVE TECHNOLOGIES FOR TRACEABILITY AND SECURITY – INDUSTRIAL APPLICATIONS

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Sea salt is a renewable natural resource considered as a paradigm of sustainability. Sea salt production process, in fact, is based on the use of renewable resources, such as seawater, sun and wind, using simple technologies with a various degrees of mechanization for harvesting and subsequent industrial treatments. However, it is worth stressing that this process, defined by centuries of experience, does not require substantial technological innovations. The aim of the study is to contribute to the improvement of the image, and therefore of the added value of the product, through the possibility of certifying its geographical origin. From a methodological point of view, the innovation of product traceability consists in analyzing the microbial composition of salt crystal community, which has been found to be peculiar to each salt work. Halotolerant and halophilic microorganisms develop in saline waters. Archaea and Bacteria growing in brine, during crystallization process, can be entrapped in salt crystals, where they can survive for very long periods. By means of metagenomic analysis with 16S rRNA

gene extracted from Archaea and Bacteria recovered from salt crystals, it is possible to characterize the microbial communities of salt crystals and associate them to saltwork where the salt crystals come from. Keeping in mind this possibility, we analyzed the diversity of viable archea recovered from salt crystals originating from six Mediterranean Saltworks. Results of metagenomic analysis utilizing 16S rRNA gene showed that salt crystals from Mediterranean salt work considered are characterized by the presence of phylogenetically diverse populations of halophiles that are peculiar and methodologically identifiable. This results can be useful to thus trace the geographical origin of the salt.

FROM BY-PRODUCTS TO A FUNCTIONAL BIOMOLECULE: ASTAXANTHIN, A CAROTENOID WITH POTENTIAL IN HUMAN AND ANIMAL HEALTH AND NUTRITION

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One of the biggest challenges, nowadays, is the production of functional biomolecules through the valorisation of agro-industrial by-products or food waste. To date, these represent a growing concern not only from an economic point of view but also from an environmental and public health one. In this work the possibility to obtain added value natural compounds from industry by-products, through a simple aerobic cultivation of microorganisms, such as astaxanthin from *Xanthophyllomyces dendrorhous*, was evaluated. Astaxanthin is a natural pigment, with a strong antioxidant activity, that has been shown to be substantially greater than β-carotene and about a thousand times more effective than vitamin E. This carotenoid offers numerous health benefits to humans and animals and, consequently, has several uses. The most conspicuous sector of astaxanthin applications is its use in foods and feeds, mostly in marine aquaculture. The growth and the carotenoid biosynthesis of the yeast *X. dendrorhous* (ATCC 24202) were studied initially by a batch cultivation on a complex medium in 2L Erlenmeyer flasks with a working volume of 1L. The working conditions were: 22°C, agitation at 300 rpm, aeration at 4 L/min and pH 5, without further correction. The tests, carried out for a 10-day period, were conducted both in dark and illuminated conditions, performed by using a Phillips TLD 18 W/ 54 lamp. Then different food waste and agro-industrial by-products, such as: fruit and vegetable waste, collected from both large scale distribution and the agro-industrial sector, and pretreated whey were tested as cultivation media. These tests were conducted using the same conditions adopted in the first test. Extraction of astaxanthin was carried out using organic solvents together with ultrasound. Astaxanthin was determined by a UFLCXR liquid chromatograph combined with an LCMS-8040 (Shimadzu, Kyoto, Japan) equipped with a 100 x 2.1 mm, 1.7 µm C18 column. This study showed the possibility to produce astaxanthin from food waste and agro-industrial by-product as an alternative to expensive and non-eco-friendly synthetic products, thanks to microbial synthesis. Furthermore, the study confirms the important role of light in increasing the astaxanthin productivity from *X. dendrorhous*, even when agro-industrial by-products are used as growth media.

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METHYLMERCURY EFFECTS ON *Mytilus galloprovincialis* HAEMOCYTES ACTIVITY

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Bivalves, filter-feeding organisms, due to their wide distribution, are used by many countries in biomonitoring, particularly in the assessment of xenobiotics in the marine ecosystem. These sentinel organisms are able to accumulate trace metals and other substances in their tissues. Haemocytes are effectors of cellular immunity in bivalves and are capable of responding to stressors through activities such as phagocytosis or cytotoxicity. In order to identify cellular markers to investigate pollution, the effect of different concentrations of organic mercury (CH_3HgCl) on the morphology and responses of *Mytilus galloprovincialis* haemocytes was studied in this work. Sublethal concentrations of methylmercury, as evidenced by the Trypan blue exclusion test, were used to investigate its effect on morphology, the efficiency of phagocytosis towards yeast cells, the maintenance of the lysosomal membrane and the ability to release cytotoxic molecules. Alterations in haemocyte viability, morphological changes and alterations in the cytoskeleton were observed. The spreading ability, a cell morphometric parameter, was also used as an additional method. Exposure to CH_3HgCl influenced the percentage and index of phagocytosis. Finally, cytoskeletal and morphological modifications lead to a reduction in the ability to adhere to the substrate and incorporate the target. The cytotoxic activity of *M. galloprovincialis* haemocytes towards erythrocytes and the activity revealed from lysis plaque assay has not been modified by adequate concentrations of methylmercury in the medium. In addition, membrane permeability could be affected by methylmercury due to the reduced retention capacity of neutral red by the cells. This evidence confirms that the Mediterranean mussel *M. galloprovincialis* is a suitable model organism in the study of the state of health of the marine environment and in particular for investigation of pollution caused by xenobiotics.

EFFECTS OF MIXTURES OF EMERGING POLLUTANTS AND DRUGS ON MODULATION OF BIOMARKERS RELATED TO TOXICITY, OXIDATIVE STRESS, AND CANCER

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Over the years, there has been an increased interest in xenobiotic substances, including flame retardants, heavy

metals, and pharmaceuticals, because of their ability to accumulate in the environment and in organisms, resulting in a wide variety of effects, ranging from toxicity to health effects, to ecological unbalance. It is known that exposure to contaminants causes oxidative stress, resulting in overproduction of reactive oxygen species (ROS), which can affect signal transduction pathways that regulate the cell cycle, apoptosis, energy balance and cellular metabolism. The aim of the study was to evaluate the effect of sub-lethal doses of mixtures of pollutants and drugs *in vitro,* such as 2,2',4,4'-tetrabromodiphenyl ether (BDE-47), cadmium chloride ($CdCl_2$) and carbamazepine (CBZ) on the modulation of biomarkers related to toxicity, oxidative stress and cancer, in order to detect early exposure biosensors. The effects of exposure, were investigated at gene expression level, on the hepatoma cell line HepG2, through the evaluation of molecular markers related to the conjugation and detoxification of xenobiotics, DNA damage and apoptosis, to identify alterations to these pathways. Our results showed that contaminants tend to affect the enzymatic detoxification system, increasing oxidative stress levels, influencing the cell cycle, the DNA repair defense mechanisms, involved in resistance to oxidative stress. The observed alterations, at molecular level, could contribute to the definition of *early warning* markers useful for environmental monitoring and at the same time for identify the levels of toxicity and hazard of these stressors in the environment.

THE EFFECT OF TEMPERATURE CHANGES ON ARM REGENERATION IN THE BRITTLE STAR *Ophiactis virens* (ECHINODERMATA: OPHIUROIDEA)

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Climate change has a great impact on marine ecosystems and the relationship between abiotic variations and marine organisms is complex to investigate and predict. One of the most important climate change phenomena that can affect marine environments is ocean warming (OW): indeed, an increase in temperature has an influence on the growth and the development of different marine invertebrates. Regeneration, *i.e.* the replacement of body parts after damage, is a post-embryonic developmental process that can be highly affected by abiotic factor alterations. Regenerative capabilities are widely present in the animal kingdom and in this area of research echinoderms are considered as perfect animal models thanks to their great regenerative potential. In the present study, the poorly described Atlantic-Mediterranean brittle star *Ophiactis virens* (Echinodermata, Ophiuroidea) was selected as model to investigate the arm regenerative process in physiological conditions (14°C and 17°C) and in conditions of temperature increase (20°C) in order to estimate the effect of future ocean warming on this species survival and regeneration. The experimental brittle stars were collected in the Ligurian Sea. To identify the species, specimens were analysed under the stereomicroscope and the Scanning Electron Microscope (SEM). Moreover, a histological analysis of non-regenerating arms was performed. All experimental animals were subjected to traumatic amputation of an arm and regeneration was followed for selected endpoints in order to evaluate regeneration progression through time in different temperature conditions. From the emergence of evident regenerative buds (at about 7 days p.a.) regeneration rates were measured for each temperature. ANOVA and Tukeys *post-hoc* test were also performed to test the significance of the obtained data. The samples at 20°C show a statistically significant higher regeneration rate in the early phases, where more differentiated and longer regenerative buds emerged from the arm stumps, than samples at the other two tested temperatures. Indeed, at 14°C there is a lag phase prior the emergence of the regenerative bud. Despite the increased length and speed of the process, animals at 20°C present slower movement. In the advanced phase (from day 14 p.a. onwards) no significant difference arises in terms of morphology. Survivorship does not seem to be influenced by different temperatures. The results of this study raise the question if the increment in the rate of regeneration, correlated with the metabolic rate increment, could be a useful trait to minimize damage that will be derived from the stressing environments predictable in an ocean warming scenario.

PRELIMINARY STUDIES ON THE EFFECT OF THIACLOPRID ON *Mytilus galloprovincialis*

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Neonicotinoids, an emerging class of insecticides, are neurotoxins that act as nicotinic acetylcholine receptor agonists. Among these thiacloprid is used mainly against aphids[1]. Due to its solubility and stability it potentially puts aquatic organisms at risk. Besides the long-term protection of agriculture, neonicotinoids can persists in the environment, causing possible adverse effects on marine organisms such as bivalve molluscs[2]. Thirty mussels of the genus *Mytilus galloprovincialis* for each experimental condition were exposed to two concentrations of thiacloprid (4.5 mg/L and 450 mg/L) for 20 days to evaluate possible physiological alterations of two fundamental tissues: hemolymph and the digestive gland. We rated: i) cell viability through neutral red retention time (NRRT) in the hemocytes and cells of the digestive gland; ii) regulation volume decrease (RVD) in the cells of the digestive gland. Preliminary data show excellent resistance of the two types of cells. in fact, after 10 days and 20 days, for both concentrations, cells remain viable and therefore, neither the components of the lysosome membranes were been modified nor the physiological components of the cell membranes responsible for maintaining of osmolarity were altered. These results suggest that thiacloprid, both, inenvironmental relevant concentration (4.5 mg/L) and in concentration100 times greater, could't caused any damage to the physiology of the model organism.

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DIET SHIFT AND FEEDING HABITS OF THE EUROPEAN HAKE (*Merluccius merluccius*, LINNAEUS, 1758) IN THE CENTRAL MEDITERRANEAN SEA

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The European Hake, *Merluccius merluccius* (Linnaeus, 1758), is a widely distributed species in the eastern Atlantic, from Norway to the Gulf of Guinea, Mediterranean Sea and along the southern coast of the Black Sea. The European hake is a commercial relevant fish species, and their stocks suffer overfishing. *M. merluccius* is a nektobenthic apical predator inhabits a depth range between 20 and 1000m in which the juvenile and small specimens usually live on muddy substrata; while largest individuals inhabit deeper than 200m. Deeping the knowledge about their diet and feeding habits, based on ontogenetic changes, helps in understand fish feeding ecology and dynamics that occur in the marine trophic web. *M. merluccius* represents an essential predator of the deeper shelf–upper slope Mediterranean communities. It is an opportunistic predator, and its feeding habits can change basing on the geographical distribution and abundance of the preys allowing the change of the diet composition of the European hake. Furthermore, *M. merluccius* tends to shift the diet composition in relation to ontogenetic development. In this study we examined the stomachs of 734 European hake collected in 2018 in southern and central Tyrrhenian Sea (GSA10). Total length (TL) of specimens ranged between 6cm and 73cm, and they were sorted in five size-classes: I<10cm, II 10.5-15cm, III 15.5-20cm, IV 20.5-32.5cm, V>32.5cm. Quantitative feeding indices for each class was evaluated. The stomach content analysis of the small hakes (Size class I) revealed that the most abundant preys were the Euphausiids (*Stylocheiron longicorne*) and Mysidacea, while crustaceans and fishes were predominantly in specimens of Size classes II, III and IV. Regarding the preys, the most abundant fishes was *Engraulis encrasicolus* followed by *Boops boops* and Myctophids. In the biggest hakes (Size class V) was recorded the presence of decapod crustaceans as *Plesionika martia, Aristaeomorpha foliacea, Pasiphaea sivado, Pseudosquillopsis cerisii* and *Pontocaris cataphracta* as well. Concerning the diet of specimens from Sicilian Tyrrhenian coast, our results showed the essential contribution of mesopelagic fishes community, with an high percentage of Myctophids and other bioluminescent preys in the stomach contents of European hake. This is a proof of the inverse energy flow from deeper mesopelagic to the shallower epipelagic communities.

SEPARATION OF LARGE VOLUME *Nannochloropsis* SP. CULTURES BY TUBULAR-BOWL CONTINUOUS CENTRIFUGE

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Marine microalgae, due to the great biodiversity and the ability to produce interesting primary and secondary metabolites such as pigments, antioxidants, polysaccharides, lipids and fatty acids, have high potential for pharmaceutical, nutraceutical, and cosmeceutical applications. Larger scale extraction of bioactive compounds produced by microalgae for pilot scale applications, *e.g.* for feed preparation or to produce functional food, requires significant quantities of microalgae. Technologies for microalgae cultivation and different types of photobioreactors allow to easily produce, even in laboratory, large volumes of high density microalgae culture. However, the laboratory scale techniques to separate microalgae from the culture medium are often expensive and time consuming, as they involve separation by centrifugation or filtration. Tubular-bowl continuous centrifugation technology allows processing large volumes of microalgae culture in a short time and at low cost, to separate microalgae without modification of cell structure. Microalgae are retained in the tubular bowl and can be recovered after the machine is stopped, clarified liquid is eliminated through an exit port, while centrifuge is running. To test the feasibility of separation by continuous centrifugation of microalgae from large culture, we used highly concentrated *Nannochloropsis* sp. cultures, containing 15.47±1.07 g/L of biomass, obtained in 200L photobioreactor. Microalgae cultures were processed using two different centrifuges: i) IEC Centra CL3 (Thermo Scientific Milford, MA), equipped with swing-out Rotor 243, 4x250 mL, for batch centrifugation; ii) CEPA® LE (CEPA Zentrifugenbau GmbH, Germany) tubular-bowl centrifuge, for continuous centrifugation. In this case, samples clarified liquid were collected, every ten minutes, to verify the efficiency of separation by microalgae cell counting. We were able to process 20L/hr by CEPA LE and 3L/hr by batch centrifugation. No alteration of cell structure was evident after cell past collection. This test showed that the CEPA centrifuge quickly and efficiently collected and clarified the microalgae *Nannochloropsis* sp. cultures, proving it to be a highly useful tool for large-volume microalgae cultures separation.

OCCURRENCE OF MICROFIBERS IN FARMED GILTHEAD SEABREAM AND COMMON CARP AT DIFFERENT LIFE STAGES

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Microplastic (MP) and microfiber (MF) pollution is a great concern worldwide due to their ubiquity in aquatic environment, becoming a potential threat to aquatic organisms. The accumulation of MPs in freshwater and marine environments can have strong ecological implications, mainly due to their

long persistence, toxicity and ability to adsorb other pollutants and pathogens. However, less attention has given to MFs pollution, although they are no less polluting. In fact, whereas the number of studies on MPs ingestion by wild fish is constantly increasing, the current knowledge on the occurrence of MPs and MFs in farmed fish species remains scarce. In the present study, we investigated the occurrence of MPs and MFs in the digestive tracts of gilthead sea bream (*Sparus aurata*) and common carp (*Cyprinus carpio*), at different life stages, reared with different technologies. Larvae, fry and adult individuals of gilthead sea bream and common carp, were collected from two fish farms, located in Italy and Croatia respectively. 700 gilthead sea bream larvae and 795 common carp larvae were examined. The gastrointestinal tract of 60 fry and 20 adult samples specimens (commercial size) of each species was analysed and undertaken to chemical digestion for microparticles extraction. Plastic and non-plastic microitems were found in both fish species investigated, with microfibers (90%) as dominant type. The presence of microparticles was not detected at larval stage. Fry and adult gilthead sea bream individuals showed microfiber abundances of 0.21 and 1.3 items/specimen, respectively. A lower concentration of microparticles occurred in fry (0.06 items/individual) and adult common carp (0.25 items/individual). Polymer composition of extracted microparticles showed significant differences between the fish species analysed in this study. Natural (20%), semi-synthetic (28%), and single or blended synthetic fibers (52%) were identified in gilthead sea bream. Linen, rayon, lyocell, cotton: polyester and polyester (12.5%) fibers were identified in common carp, while PTFE (37.5) was present as fragments. In conclusion, a considerably lower contamination level of synthetic polymers (average 0.11 items/individual) was detected in farmed fishes compared with data reported by other authors on feral fish. The low number of microplastics found could depend on the contamination degree of fish farms location, culture water and commercial feed used in the farms under study. To the best of our knowledge, this is the first study describing plastic and non-plastic microfiber contamination in farmed *S. aurata* and *C. carpio* at different life stages.

FREE COMMUNICATIONS

BEHAVIOURAL AND METABOLIC IMPLICATIONS IN TRIMETHYLAMINURIA: THE DUAL ROLE OF GUT MICROBIOTA

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Trimethylaminuria (TMAU), also known as "fish odor syndrome", is a metabolic disease characterized by a strong body odor similar to rotten fish one, due to an excessive excretion of trimethylamine (TMA) through sweat, saliva, urine, breath and vaginal secretions. Under physiological conditions, TMA is produced directly or indirectly from its dietary precursors (choline, carnitine, betaine) by the gut microbiota. Soon after, TMA is oxidized to an odorless tertiary amine, the TMAO, by the hepatic Flavin-containing monooxigenase 3 (FMO3) enzyme. To date, two forms of trimethylaminuria are known: the primary (TMAU1) and the secondary (TMAU2). While the TMAU1 phenotype is mainly determined by genetic mutations in *FMO3* gene, the secondary form is mostly determined by gut microbiome dysbiosis. Even if the metabolic and clinical manifestations of TMAU are generally considered benign, the strong body odor can interfere with many aspects of daily life, affecting interpersonal relationships and social life. This social impact is commonly considered the first cause of the psychiatric conditions exhibited by the most of patients, who frequently show behavioral disturbs like social exclusion, depression, anxiety, sleep-wake cycle and mood alterations, until to suicide attempt in most extreme cases. Nevertheless, it is still unknown if the psychiatric involvement is a cause, or conversely, a consequence of TMA altered metabolism. In recent years crucial associations between the gut microbiota-brain axis and neurological disorders have already been highlighted, but no connections are known with TMAU, yet. Thus, we performed the microbiota analysis of both psychiatric suffering secondary TMAU patients and TMAU psychiatric unaffected controls. Microbiota comparative analysis of TMAU cases *versus* controls highlighted very interesting differences, regarding both bacterial family heterogeneity and concentration. After retrieving detailed info on association between gut microbiota families and their own produced metabolites, it was found that such metabolites might be involved into TMAU psychiatric comorbidities acting directly as neurotransmitters (*e.g.* serotonin) or indirectly through their precursors. The most intriguingly aspect related to these metabolites is related to the ability to act also as TMA precursors, contributing to its accumulation. Thus, the "dual" nature of gut microbiota metabolites might represent the junction ring between TMAU and psychiatric disorders. Further experiments will confirm these preliminary results, permitting to develop new personalized therapy for each patient affected by TMAU.

EVALUATION OF GALECTIN-3 IN THE CROSS-TALK BETWEEN MUSCLE AND BONE

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Galectin-3 (Gal-3) is a pleiotropic lectin that has been recognized over the past two decades as being involved in many physiological and pathological processes. Although the galectin field has been a lot studied remain incompletely understood, in particular the molecular mechanisms of their actions in intra and extracellular space and the relationship between these activities, as well as an associated regulatory loop. Since it is well described that Gal-3 is widely distributed both in the developing and mature bone and that its expression is under control of the master regulator of bone growth RUNX2, it could suggest that Gal-3 may be a key player in all stages of bone biology. Many types of bone pathologies are strikingly related to severe damage inputs affecting the bone-associated tissues. In particular, it is well recognized that bone and muscle have recently been identified as endocrine organs, that secrete cytokines and chemokines, through which they interact to promote bone formation, repair and maintenance in the bone-muscle cross talk. For that, the general aim of our study is to define the role of Gal-3 on bone tissue and its possible involvement in skeletal muscle crosstalk. First, we found an increase of Gal- 3 expression during myogenic differentiation and that it was secreted in the culture medium. Through a coculture experiments between myogenic cells and osteoblast cells we documented that recombinant Gal-3 inhibited osteoblast differentiation and since it may be argued that it may have a different conformation state in comparison to endogenous Gal-3, we next tested whether the silencing of Gal-3 secreted by muscle cells affect osteoblast differentiation at the same manner. In order to investigate the effects induced by extracellular Gal-3 on bone cells, focusing on signaling events unleashed by the recombinant protein, by a mass spectrometric approach, we analyzed the entire proteome obtained by differentiated osteoblasts differently affected by the presence of recombinant Gal-3. Moreover, through an *ECM* Cell *Adhesion* Array *kit* we monitored the impact of Gal3 on the extracellular matrix complex, evaluating specific cell surface Integrins and Adhesion molecules upon Gal-3 silencing, inhibition and overexpression.

EFFECTS OF SIMULATED MICROGRAVITY ON HUMAN PANCREATIC ADENOCARCINOMA (PDAC) MULTICELLULAR SPHEROIDS

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Gravity is a well-known physical force that represents a fascinating area of interest in biology and medicine, considering relevant physiological changes in organisms during space exploration. Spaceflight opportunities are rare and expensive and access to space experiments is limited, but studies on the effect of gravity variation can be carried out using devices able to simulate microgravity, like Random Positioning Machine (RPM, Airbus, Defence & Space, ADS, Dutch Space, Leiden, NL) used in ours experiments. The aim of this project was to evaluate the effect of simulated weightlessness on human pancreatic adenocarcinoma cell line, PaCa-44 (Neuroscience, Biomedicine and Movement department, University of Verona). Pancreatic adenocarcinoma remains a major unsolved health problem nowadays, with conventional cancer treatments that have little impact on disease course. Pancreatic cancer co-opts multiple cellular and extracellular mechanisms to create a complex cancer organ with an unusual proclivity for metastasis and resistance to therapy. Exploiting simulated microgravity (SM), we designed a reproducible and easy method for scaffold-free PaCa-44 spheroids formation, that better reproduce the physical communications and signaling pathways observed in firsts stages of solid tumors. Morphological analyses were performed at different time-points to evaluate cell's aggregation and spheroid's structure under SM. LC-MS/MS proteomics analyses were performed on spheroids obtained by SM and on ground control samples. Results showed variations in protein's expression related to the process of epithelial mesenchymal transition (EMT) coupled with an increased glycolysis pathway related to Warburg effect, typical in cancer cells, as confirmed by quantitative RT-PCR assay. Moreover, both proteomic and PCR results showed an increase in expression levels of genes and proteins related to stemness pathway in spheroids samples compared to ground control non-spheroid cells. All these data suggest that PaCa44 cells exposed to gravitational unloading changed their growth behavior and started growing in a three dimensional (3D) manner in RPM. Moreover, obtained data showed that simulated microgravity plays a role in activation of molecular pathways implicated in stemness and in overexpression of genes involved both in stemness and in EMT under SMG condition, supporting the assumption that microgravity induces cancer cell transformations towards the acquisition of cancer stem cells-like features.

COMBINING TRANSLATION READTHROUGH INDUCING DRUGS AND NONSENSE MEDIATED DECAY PATWHAY INHIBITION TO THE CFTR RESCUE IN CYSTIC FIBROSIS CELL MODEL SYSTEM

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Nonsense mutations affect 10% of patients with cystic fibrosis and produce a premature termination codon in *CFTR* (Cystic Fibrosis Transmembrane Conductance Regulator) mRNA causing early termination of translation and leading to lack of CFTR function. A potential therapy for nonsense mutations provides the use of small molecules able to overcome the premature stop codon (PTC) by a readthrough mechanism that lead to synthesis a complete CFTR protein. Despite the good results obtained from this approach, TRIDs efficiency is considerably reduced by the poor amount of target transcript, that is the mRNA containing the PTC. The readthrough, indeed, does not occur on the totality of target transcripts because of their degradation due to the nonsense mediated decay pathway (NMD). This pathway provides the degradation of mRNA harboring premature stop codon to prevent the production of altered polypeptides. In contrast, the activity of this pathway interferes with the effectiveness of the readthrough drugs, limiting the mRNA concentration of the target protein. Thus, a promising strategy for nonsense mutation treatment is a com-

bined use of readthrough agents and factors that attenuate the nonsense mRNA decay. By silencing the UPF1 mRNA/protein, the activity of the NMD pathway was reduced, in FRT cells CFTR ^{W1282X}. Alternatively, caffeine was used as specific inhibitor of the UPF1 activity, to increase the efficiency of readthrough molecules (NV848 and NV914) in FRT cells CFTR^{W1282X} cells. In both cases, the combined treatment: NV848 or NV914/caffeine and NV848 or NV914/UPF1siRNA caused an increase of CFTR^{W1282X} mRNA level followed by the rescue of the CFTR expression and functionality. However, unexpectedly, despite the higher CFTR^{W1282X} mRNA level in caffeine treated samples, both expression and functionality CFTR rescue resulted slightly lower than the recovery achieved by UPF1 silencing. Our results indicate that modulation of NMD pathway, although still to be optimized, could be a promising approach in order to increase TRIDs effects in presence of stop mutations.

BIOCHEMICAL RAMAN SPECTRAL SIGNATURE OF PLASMA LIPOPROTEINS

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Lipoproteins (LPs) are complex macromolecules, consisting of a hydrophobic lipid core, rich in triglycerides and cholesterol esters, surrounded by an envelope formed by amphipathic molecules, such as phospholipids, non-esterified cholesterol and apolipoproteins. Plasma lipoproteins are classified into five main classes: chylomicrons, VLDL (very low-density lipoprotein), IDL (intermediate-density lipoprotein), LDL (low-density lipoprotein) and HDL (high-density lipoprotein), based on their density and play a key role in the transport lipids throughout the circulation. Lipoprotein fractionation, as well as the quantification of the amount of cholesterol and triglycerides carried by LPs, has become one of the main clinical approaches to assess the increased risk of cardiovascular complications in individuals. However, conventional lipidomics approaches used to characterize LPs are notoriously *laborious* and extremely time-consuming. In this context, Raman spectroscopy (RS) represents an innovative, optical technique that allows to analyze biological samples without any sample preparation. Moreover, this technique allows to obtain simultaneous information about both the biomolecules composing lipoproteins and the relative amount. Therefore, the aim of the present work is to demonstrate the suitability of RS to rapidly get access to an impressive amount of valuable information about the lipids and proteins of the main classes of LPs present in blood – such as VLDL, LDL and HDL – thus providing new tools that could be effectively used in clinic to perform LPs analysis. We investigated spectra differences between major lipoproteins classes isolated from plasma of six fasting healthy donors, by ultracentrifugation in discontinuous KBr density gradient. All spectra were acquired and analyzed in two different regions, the low frequency region (between 400 and 1800 cm^{-1}) and the high frequency region (between 2600 and 3200 cm^{-1}). The obtained spectra showed peaks related to the different biomolecules that compose the lipoproteins: cholesterol, triglycerides, membrane lipids, unsaturated fatty acid, carotenoids, proteins, and their intensity well reflects their relative composition. Moreover, the typical structure of VLDL, LDL and HDL were evaluated and confirmed by transmission electron microscopy. Our preliminary results, obtained on a limited number of subjects, suggesting that Raman spectroscopy could be a viable and reproducible approach to provide a biochemical signature of the main classes of LPs. Since the composition of LPs is known to be altered in many pathological conditions, we are conducting a new study to demonstrate the possible application of RS in diagnostics, in order to identify the changes in lipoprotein composition.

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DEVELOPMENT OF A SCREENING STRATEGY FOR THE IDENTIFICATION OF NLRP3 SELECTIVE INHIBITORY COMPOUNDS

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NOD-like receptor pyrin domain-containing 3 (NLRP3) is the central component of the signalling complex "inflammasome" that promotes caspase-1 activation leading to processing and release of IL-1β and IL-18. NLRP3 activation is involved in different chronic age-related inflammatory diseases, therefore selective inhibition of NLRP3 is a promising strategy for the treatment of these diseases. The aim of this project is the discovery of novel NLRP3 selective inhibitors by using a semi-automated screening approach. To this purpose a primary screening assay must be developed, miniaturized and validated. Also, the development of an orthogonal assay is required for the validation of the primary actives (compounds that give positive results in the primary assay). Then, the real screening campaign can start. Herein we present the results of the development and validation of the primary and the orthogonal assays that will be used for the screening campaign to find selective NLRP3 inhibitors. A robust phenotypic primary assay was developed using human THP-1 macrophages stimulated with lipopolysaccharide (LPS) followed by nigericin, to induce selective activation of the NLRP3 inflammasome. At the end of the experiments the release of IL-1β was measured using Homogeneous Time-Resolved Fluorescence (HTRF) technology. This primary assays was first tested and validated in 6-well plates. The selective NLRP3 inhibitor MCC950 was used as positive control and three additional reference compounds, 3,4-methylenedioxy-β-nitrostyrene (MNS), parthenolide and glyburide, known to modulate NLRP3 activity, were included in the assay. Herein we report half maximal inhibitory concentration (IC_{50}) and IC_{max} for MCC950 and IC_{50} curves for the three reference compounds. We also report the results of assay miniaturization in 384-well plates and validation of the protocol using an automated liquid handling system following a stringent statistical evaluation of results reproducibility and variability. Finally, we present the development of the orthogonal assay that allows to measure NLRP3 activation using an independent approach compared to primary assay in order to discriminate false positives. The proposed orthogonal assay measures the

release of lactate dehydrogenase (LDH) as readout of inflammasome activation. Our results confirm that there is a linear correlation between the release of LDH and IL-1β release. Overall, based on the above results showing that the assay has been validated the next step of this project will be the screening of different libraries of compounds for the discovery of selective NLRP3 inhibitors. These compounds will be in turn tested in *ex-vivo* experimental models of chronic inflammatory lung diseases.

ANALYSIS OF EXPOSURE TO MULTIPLE AND EXTREME STRESS CONDITIONS ON A THREE-DIMENSIONAL HUMAN RESPIRATORY MUCOSA MODEL

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The lung structure is characterized by a network of air and capillary spaces. The balance between the processes of ventilation and perfusion guarantees the optimum gas exchange on earth. On the other hand, if human organism is exposed to a microgravity environment, the balance appears to be compromised, causing both ventilation and irregular perfusion. Space Biology studies used standard cell culture models to describe possible effects of spaceflight on morphology, differentiation, and physiology in respiratory cells. At present, studies on models of respiratory mucosa reproduced *ex vivo* and exposed to conditions of microgravity have not yet been conducted. In this study, the primary attention was placed on the transport phase (from the earth to the international space station) of respiratory mucosae reproduced. During this phase, the samples could be exposed to lower temperatures (<37°) or not suitably supplied with a fresh culture medium. The possible occurrence of phenotypic alterations or the development of an inflammatory condition were analyzed through different techniques (Asage ELISA, LUMINEX Multiplex Magnetic, TEER measurements, Western Blot). To simulate the impossibility of refreshing the culture medium, a medium stored at 37°C was provided to the cultures. Possible extreme environmental conditions have been reproduced: the samples have been exposed to a temperature of 20°C compared to the canonical 37°C. A further testing was carried out to reduce the percentage of carbon dioxide necessary for the cultures. The following parameters were analyzed: HSP 70 and HSP 90 expression as stress markers, release values of different proinflammatory cytokines (IL-2, IL-4, IL-5, IL-10, IL-12, IL-13, IL-16, IL-33, CCL19/MIP3β), direct morphological observation to evaluate the insurgence of any tissue damage, and trans-epithelial resistance measurements. The results showed that *ex vivo* respiratory mucosa cultures have a considerable adaptive capacity, and except for small exceptions, no variations in the expression of the markers analyzed were detected compared to standard culture conditions. In conclusion, this study showed that this three-dimensional culture model of respiratory mucosa is not influenced by the variations imposed on it by the experimenters, and the experiments carried out improved the development of the engineering automatized device by European space agency engineers.

MICROGRAVITY EFFECTS ON THE RESPIRATORY SYSTEM: OPTIMIZATION OF AN *EX-VIVO* RESPIRATORY MUCOSA CULTURE MODEL FOR THEIR EVALUATION

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Space Medicine studies showed that gravity force has a profound influence on the function of the respiratory system. In this regard, several pre- and post-flight longitudinal studies have shown that the respiratory mucosa of astronauts, if subjected to long-term exposure to microgravity conditions, undergoes the same pathological changes comparable in the course of chronic respiratory diseases, such as asthma and chronic obstructive pulmonary disease (COPD). To this end, the respiratory mucosa 3D culture model that our working group developed in these last years will be implemented aboard the International Space Station (ISS). This study is part of an ESA-funded project "International Space Life Sciences Research Announcement 2014" in collaboration with NASA. The project aims to better understand the effects of microgravity on biological processes and cell differentiation and to prevent any alterations, to support human life in space. In the ground phase of the project, several experiments have been carried out in the Bi.N.D. laboratories aimed at studying preliminarily the growth and differentiation of the 3D culture model subjected to the different stressful conditions foreseen by the project: i) Culture model isolation from the external environment and deprivation of the possibility of gas exchange; ii) Culture model adaptation on breadboards of the automated device that will be used on the ISS; iii) Samples long-term exposure at 4°C. During the various experiments to identify the onset of tissue damage, the cultures were monitored by a phase-contrast microscope. Transepithelial resistance values were detected during all phases of the experiments, to identify early the onset of damage to the respiratory mucous membranes. The pattern of released cytokines was analysed to evaluate the stress conditions, measuring their apical and basal release levels. Preliminary tests have been conducted to evaluate the adaptation of the 3D model to the breadboard of the automated device, designed by the Norwegian company CIRiS. The results obtained so far demonstrate that the model can withstand the multiple stressful conditions analyzed, and it guarantees both a faithful reproduction of the respiratory mucosa and the properties necessary to face experiments in conditions of microgravity.

IN VITRO EVALUATION OF ANTIFUNGAL AND ANTIBIOFILM ACTIVITY OF CHOLINIUM BASED IONIC LIQUIDS AGAINST *Candida SPP.*

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Several species of the genus *Candida* can cause opportunistic infections, including superficial infections, persistent infections and life threatening systemic infections through biofilm formation on tissues or implants. The increased resistance of *Candida* spp. to available antifungal drugs points to

the urgent need to find novel therapeutics with both antifungal and antibiofilm activities and with minimal cytotoxicity and side effects to the host. With this in mind, our study aimed to evaluate the *in vitro* antifungal and antibiofilm activity of newly designed cholinium based ionic liquids (ILs) N-(2-hydroxyethyl)-N,N-dimethyl-1-heptanaminium bromide (2), N-(2-hydroxyethyl)-N,N-dimethyl-1-dodecanaminium bromide (3), N,N'-tetramethyl-N,N'-bis(2-hydroxyethyl)-1,6-hexanediaminium dibromide (4), N,N'-tetramethyl-N,N'-bis(2-hydroxyethyl)-1,8-octanediaminium diiodide (5), N-(2-hydroxyethyl)-N,N-dimethyl-1-heptanaminium bromide dodecylbenzenesulfonate (2a), N-(2-hydroxyethyl)-N,N-dimethyl-1-dodecanaminium dodecylbenzenesulfonate (3a), N,N'-tetramethyl-N,N'-bis(2-hydroxyethyl)-1,6-hexanediaminium bisdodecylbenzenesulphonate (4a) and N,N'-tetramethyl-N,N'-bis(2-hydroxyethyl)-1,8-octanediaminium bisdodecylbenzenesulphonate (5a) against two strains of *Candida albicans* (ATCC 10231 and CBS 562), one strain of *Candida parapsilosis* (ATCC 22019), two strains di *Candida glabrata* (CBS 12544 and CBS 860) and three strains of *Candida tropicalis* (CBS 13075, CBS 13076 and CBS 13077). The antifungal activity of each compound was assessed using the agar disc diffusion test, and broth microdilution method was carried out to establish the minimum inhibitory concentration (MIC). The minimum fungicidal concentration (MFC) and the antibiofilm activity, using the crystal violet assay, were determined for ILs which showed higher antifungal efficacy. The results showed that some of the ILs tested are able to inhibit fungal growth. Interestingly, they exhibit this property even at very low concentration, thus reducing a possible citotoxicity. The compounds 2, 3 and 3a displayed antifungal activity against all *Candida* spp. strains. Compound 3 registered the lower concentration of MIC (1,17 μmol/ml for *C. albicans* CBS 562 and ≤ 0,15 μmol/ml for the other strains) and both good fungicide and antibiofilm properties. These preliminary data may suggest a potential application of these compounds for drug or disinfectant formulations.

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FISH ANALYSIS ON FFPE FOR DETECTION OF SINGLE AND DOUBLE TRISOMIES IN SPONTANEOUS ABORTION

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In Pathology Laboratory, FISH analysis (Fluorescent In Situ Hybridization) still maintains an important role in diagnosis of chromosomal aneuploidies when preparations are not suitable for cytogenetic analysis. Several miscarriages end without ovular chamber expulsion and prolonged retention occurs: in this cases autolytic processes may alter embryo morphology features and chorionic villi assessment (Fulcheri E, J. Biol. Res. 2011). Hence it is impossible to set up cell cultures and it becomes necessary to obtain formalin-fixed paraffin-embedded (FFPE) tissue preparations. Furthermore, some samples from other Hospitals are centralized and undergo the same treatment for the material conservation for further analyses. FISH allows analysis of these preparations flanking and completing histomorphological assessment (specific histochemical and immunohistochemical protocols). To our knowledge, chromosome anomalies are a major cause of early spontaneous abortion. In our Laboratory, from January 2016 to January 2021, 481 cases of first trimester spontaneous abortions have been studied by FISH for detection of X, Y, 9, 13, 15, 16, 18, 21 and 22 chromosome aneuploidies. The median maternal age was 36 years (range: 15-52 years). We found 171 chromosomal anomalies out of 411 tested samples (41,6%): 60 polyploidies, 25 X monosomies, one autosomal monosomy, 81 trisomies that are 47% of our case report and intriguingly, four double trisomies (2,3%). Double trisomy is a rare condition (0,2%-2,8%) (Reddy KS, Hum Genet 1997) even more when chromosomes involved are autosomes; it's associated with advanced maternal age. In our experience we detected one case of double trisomy X and 18 (48,XXX,+18), three cases of double autosomal trisomy: 48,XX,+21,+22; 48,XY,+21,+22; 48,XX,+16,+21. Median maternal age was 42 years (range: 38-45 years). In addition, in 2020 we have completed the study of a further double trisomy (48,XX,+18,+21) in a fetus (maternal age: 45 years old) presenting anomalies of both chromosomes involved, whose diagnosis was performed at 12 weeks 4 days gestational age by cytogenetic analysis and followed by histomorphological assessment (Fiorio P, J Ultrasound Med. 2020). This complete study is made possible by the synergy of different specialist departments in a second level Institute. Finally, FISH analysis on FFPE allows the correct identification of chorionic villi and embryo tissue and despite of few number of chromosomes investigated, our probe panel allows to uncover over 83% of all abnormalities identified by karyotyping. (Russo R, Prenat Diagn. 2016). Diagnostic histomorphological examination and FISH analysis in spontaneous abortion may provide the diagnosis of the cause of miscarriage, in order to planning future pregnancies and providing psychological support.

MORPHOLOGICAL STUDY ON THE OVARY OF A DEHYDROEPIANDROSTERONE-INDUCED MODEL OF POLYCISTIC OVARIAN SYNDROME IN MOUSE

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Advanced glycation end-products (AGEs) are involved in the pathogenesis and consequences of polycystic ovary syndrome (PCOS), a complex metabolic disorder associated with female infertility. The most potent precursor of AGEs is methylglyoxal (MG), a low-molecular-weight dicarbonyl compound derived from metabolic processes, involving the Sirtuin 1 (SIRT1) network in the female gonads. To assess the role of MG-dependent glycative stress on the PCOS phenotype, we performed a morphological study. Four-week-old CD1 mice were administered or not (controls) with dehydroepiandrosterone (DHEA) (6 mg/100 g body weight) for 20 consecutive days. Part of the ovaries was subjected to 1) H&E staining for ovarian follicle classification and counting; 2) Heidenhain's AZAN Trichrome Staining; 3) Immunofluorescence Analysis for Von Willebrand Factor (vWF), alpha-smooth muscle actin (-SMA), BODIPY, 17 beta-hydroxysteroid dehydrogenase type 4 (17β-HSD4), translocase of outer mitochondrial membrane 20 (TOMM20), and 4) Immunohistochemical Analysis for MG-AGE and 4-HNE. Histologic examination of DHEA

ovaries showed alterations in the collagen deposition, as seen by a looser appearance of the stroma and a fibrotic aspect of the ovarian cortex, respect to controls. The follicular wall of secondary and antral follicles evidenced a concentric and network-like collagen distribution, more intense in DHEA than in the control group. Also, the vascularization increased in DHEA ovaries, with both vWF and -SMA more expressed. BODIPY and 4-HNE immunostaining after DHEA administration were stronger than in controls, evidencing an increased lipid droplet accumulation and lipid peroxidation. Intense staining in the ovarian surface epithelium for 17β-HSD4 was also observed. Moreover, confocal analysis of TOMM20 ovarian staining showed a reduced expression of the mitochondrial transporter in DHEA ovaries. MG-AGE staining increased in DHEA mice's ovaries with an intensive immunoreactivity in luteal cells, vessels and stromal cells. These results represent an important contribution to the characterization of the DHEA mouse model's morphological markers and contribute to the elucidation of new mechanisms underlying PCOS development or progression at the ovarian level.

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RESCUE OF LRBA GENE EXPRESSION IN PRIMARY HUMAN FIBROBLASTS CHARACTERISED BY NONSENSE MUTATION c. 5047 (C>T)

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Primary immunodeficiencies (PIDs) are rare genetic diseases characterized by susceptibility to infections, increased risk of autoimmunity, hypogammaglobulinemia, and lymphoproliferative syndromes[1]. PIDs are associated to genetic alterations in about 400 known genes, among which, mutations of the LRBA gene[2]. LRBA gene encodes a widely expressed multidomain protein with highly conserved BEACH domain, involved in regulation of endosomal trafficking, particularly endocytosis of ligand-activated receptors[3]. It was reported that stop mutations affect this gene leading to the loss of the protein expression[4]. Recently, we identified three Translational Readthrough Inducing Drug (TRID), that showed high readthrough activity in cystic fibrosis nonsense model systems, in this study we tested one of our TRIDs in a LRBA model system[5]. To evaluate the readthrough activity of the TRID molecule in the rescue of the LRBA expression in human primary fibroblasts characterized by an homozygous stop mutation, we treated LRBAstop cells with compound 1 after 24, 48 and 72 hours of treatment. The expression of the LRBA gene was analyzed by Real time RT-PCR and Western blot analyses. LRBA expression resulted increased after 72-hours of chronic treatment with our TRID molecule and the cells showed to growth normally. Our results confirmed that compound 1 is able to promote the readthrough of premature stop codon, besides in CF model systems, also in a different genetic context such as in LRBA expression.

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MERTK SNPs AND CLINICAL FEATURES OF LIVER DISEASE

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MERTK is a member of TAM receptor tyrosine kinases, mainly expressed in M2 macrophages. GWA studies reported that thers4374383 (A/G) SNP of MERTK is associated with the risk of developing liver fibrosis in patients with hepatitis C virus (HCV) chronic infection (1). We have evaluated the association between the SNP of MERTK and the clinical features of patients with HCV and HBV infection and in patients with Nonalcoholic Fatty Liver Disease (NAFLD) (2). The polymorphic state of MERTK seems play a key role not only in the control of apoptosis, in the HSCs epithelial-mesenchymal transition and in the immune response, but also in the mechanisms involved in fibrosis progression and carcinogenesis. Our results suggest that the polymorphic status of rs4374383 represent a useful tool for a more stringent monitoring to detect early the development of cancer in patients with HCV and HBV and genotype AA/AG or progression to cirrhosis and its complications in patients NAFLD and genotype GG/GA. The rs4374383 SNP of MERTK gene falls in an intronic sequence, but it is in high linkage disequilibrium with a dubbed enhancer SNP (deSNP) rs6726639 A/C. The deSNP is located within the binding sites of Transcriptional Repressor Factor (TRF) which are able to bind stronger the A allele (3,4). A bioinformatic approach Tsitescan was used to predict TFsspecifically targeting MERTK deSNPs. Finally, differentially binding TFs were considered for subsequent analyses and the target genes were downloaded from ENCODE (5) and TRRUST (6). Array approach inhuman tissue samples show that the deSNP polymorphism is involved in the regulation of key pathways of liver disease progression.

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IN VITRO SENSITIVITY TO RADIOTHERAPY OF CANCER STEM CELLS PREDICTS THE EFFICACY OF TREATMENT *IN VIVO*

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Radiotherapy represents a first-line treatment for many inoperable lung tumors. New technologies offer novel opportunities for the treatment of lung cancer with the administration of higher doses in smaller volumes. Since both therapeutic and toxic treatment effects are dose-dependent, the identification of a specific lower effective dose protocol which minimizes toxicity maintaining efficacy for each individual patient. Cancer stem cells sustain tumor growth, promote metastatic dissemination and may give rise to secondary resistance as a consequence of their intrinsic resistance. The identification of effective protocols targeting these cells may improve disease-free survival of treated patients. In this work we evaluated the existence of individual profiles of sensitivity to radiotherapy in patient-derived CSCs using *in vitro* and an *in vivo* model. Both CSC and CSC derived tumor bearing mice were treated with radiotherapy at different doses and dose rates. CSCs response to different radiation doses greatly varied among patients. In vitro radiation sensitivity of CSCs corresponded to the therapeutic outcome in the corresponding mouse tumor model. The dose administration rate did not affect the response. These findings suggest that *in vitro* evaluation of CSC may support the clinical decision predicting the response in patients.

IMMUNO-ONCOLOGICAL TREATMENT OF NON-SMALL-CELL LUNG CANCER IN ADVANCED STAGE WITH NIVOLUMAB

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In recent years, significant scientific progress has been made in the therapy of non-small cell lung cancer (NSCLC), which has made possible a better knowledge of this pathology and above all the realization of new personalized therapies. The main therapeutic revolution in advanced NSCLC is immuno-oncology, a new therapeutic strategy that aims to awaken the immune system to fight cancer cells. Our work helped us evaluate the therapeutic efficacy of monotherapy with Nivolumab in the treatment of patients with advanced stage IIIB/IV non-small-cell lung cancer beyond the second line. We can conclude that in the treatment of non-small-cell lung cancer, the use of Nivolumab improves the prognosis and quality of life of the patients, without causing serious side effects compared to other treatments. We hope that in the future the combination of predictive biomarker research combined with the improvement of Immunoncology protocols will led to ever greater overall survival data.

DETECTION, ANALYSIS AND PROCESSING OF ACUTE ALCOHOL INTOXICATIONS COLLECTED AT EMERGENCY DEPARTMENT OF THE "GARIBALDI CENTRO" HOSPITAL IN CATANIA

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The study we conducted was highlighted the main clinical aspects of voluntary or accidental acute alcohol intoxication. These intoxication were identified over the five-year period between 2015 and 2019, and we evaluated its pharmacological management by statistical analysis of clinical cases detected at the Emergency Department of "Garibaldi Centro" Hospital in Catania. The extrapolated data showed that acute alcohol intoxications are the main reason of access to the Emergency Department (65%) to which drugs (17%), addicted substances (8%), and other substances (caustics, food, gases and vapours, detergents and soaps, benzene and pesticides, 10%). It has been shown that the most commonly used treatment in intervention therapy of acute alcohol intoxications is Metadoxil (methadoxine), a metabolic accelerator that prevents alcohol admixation by facilitating metabolism and increasing urinary elimination of ethanol and its toxic metabolite, acetaldehyde. The prevention study of clinical cases detected shows that the age group most exposed to acute alcohol toxicose is the age range 18-24 years with a greater prevalence of male sex. It is therefore clear that prevention and information campaigns need to be increased by means of different professional channels and professions such as pharmacist.

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