

Journal of Biological Research

Bollettino della Società Italiana di Biologia Sperimentale



**94th National Congress of the
Italian Society for Experimental Biology**

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ABSTRACT BOOK

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ANTHROPOLOGY: COMPARATIVE APPROACHES

THE CALVARIUM OF THE BLESSED MARIA LORENZA LONGO († 1539) FOUNDER OF THE “OSPEDALE DEGLI INCURABILI” IN NAPLES: PALEORADIOLOGICAL AND PALEOPATHOLOGICAL EVALUATION

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Maria Lorenza Requenses was born in 1463 to a noble family in Lleida (Spain). In 1506, she followed her husband Joan Llonc (Giovanni Longo) in Naples, where he died three years later. Here, she established the hospital of Santa Maria del Popolo degli Incurabili, and also founded the Order of the Capuchin Poor Clares. She died on 21 December 1539 and beatified 9 October 2021. The relic attributed to Blessed Maria Lorenza Longo is represented exclusively from a completely skeletonized calvarium lacking right temporal bone, part of the large sphenoidal wing, lacrimal bone and orbital lamina of ethmoid bone. A large fracture of the right parietal bone was recorded during the previous recognition and attributed to an accidental fall. The calvarium underwent autoptical inspection and digital radiology on different projections using a GMM Opera device. Computed tomography (CT) scanning was performed using a Toshiba Astelion 16-slice scanner with multiplanar reconstructions (MPR) and volumetric (3D) rendering. The analysis of the biological profile indicates a certainly mature individual, of a sex that tends to be female. No signs of vitamin deficiency were identified, on the other hand, a slight cribrosity on the glabella was identified, which however has a non-specific nature, which could be associated with an osteoporotic state, suffered by Blessed Longo. A moderate inflammatory process is active along the orbital frame of the zygomatic bone, with slight reactions of a periostitic nature. The endocranium is characterized by deep vascular impressions, with rather Pacchioni granulation, somewhere almost passing through the entire thickness. A small rounded osteoma was present on the endocranial surface of the frontal bone. The right frontal sinus bears the signs of a past inflammatory process, such as chronic sinusitis. An artificial, post-mortem hole was clearly visible at the top of the calvarium, probably made for suspending the relic by a small rope. From the morphological point of view, the tertiary syphilis hypothesized by some sources could not be confirmed, the alleged injury to the bregma, in the past interpreted as stigmata of syphilis, is instead to be attributed to a mechanical action of an anthropic nature, which over time has exposed the diploe.

ANCIENT BODIES, BIOLOGY AND CULTURE: CASE STUDIES FROM THE COLLECTION OF THE MUSEUM OF ANTHROPOLOGY AND ETHNOGRAPHY (UNIVERSITY OF TURIN)

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Advanced scientific techniques and cross-disciplinary collaborative approaches in the investigation of ancient human remains offer important insights into the lives of past populations and can provide us with information that written sources cannot always supply. Furthermore, the scientific analysis of human remains is now considered an integral part of care, conservation and storage practices. As a result, anthropological collections have acquired new roles in biological research as well as in museology and in public exhibitions.

The study presented is a part of ongoing research carried out on Egyptian human remains housed at the Museum of Anthropology and Ethnography of the University of Turin. Detailed information about biology, paleopathology, thanatology, tissue preservation and funerary rituals are being gathered using both traditional anthropological/paleopathological analysis and more advanced techniques, such as CT-scanning and non-invasive palaeoproteomics. Here we present the most significant case studies from recent investigations.

DIACHRONIC STUDY OF AN ITALIAN APENNINE VILLAGE THROUGH ANCIENT DNA, ANTHROPOLOGY AND PALEODEMOGRAPHY

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Remains of ancient people represent an invaluable opportunity to understand patterns of past population dynamics and familial relationships. In this study, the mummified remains of the ancient inhabitants (16th - 18th centuries) and the modern community of Roccapelago (Modena) were analyzed in order to examine population dynamics and relatedness over the centuries in this small mountain village. The collection of ancient samples (N=25) was guided by anthropological, biodemographical, archaeological and historical analyses. The analysis of the parish registers was of fundamental importance to identify founder surnames for the selection of present-day (N=14) samples. The aims of this study were to predict Y-chromosome paternal haplogroups and to assess genetic continuity between ancient mummies and the current population of Roccapelago. Moreover, this multidisciplinary study aimed at testing and combining archaeogenetic and forensic protocols, criteria and approaches, in order to maximize the yield of endogenous DNA, obtain more likely complete Y-STR profiles and minimize exogenous contamination. The 27 markers of the Yfiler Plus STRs kit were successfully amplified for at least 10 loci in 19 out of 25 ancient DNA samples and in all the 14 analyzed modern

individuals from Roccapelago. This study confirmed the value of the otic capsule of the petrous bone as a skeletal element in maximizing ancient DNA yield in order to increase the probability of obtaining complete Y-STR profiles and limit allelic dropout, but also in preventing contamination with exogenous human DNA. Overall, population relationships analyses supported a paternal genetic continuity of the analyzed population from the 16th century until today. The predicted Y-haplogroups revealed a homogeneous genetic composition between ancient and modern samples of Roccapelago, and despite reflecting lineages commonly observed in Italy, the searching against the Y-haplotype reference database (YHRD) resulted in non-matches between each ancient sample and any of the other Italian reference samples included in the database. In addition, estimation of the expected Time to the Most Recent Common Ancestor (TMRCA), in agreement with the network results, confirmed signals of genetic continuity between ancient and modern inhabitants of Roccapelago for 11 out of the 13 mummies considered for this analysis. These findings agree with historical data and the past geographical isolation of this small village that, thanks to this peculiar archaeological discovery, kept a precious witness of its past.

YAWN CONTAGION IN BONOBOB: OBSERVATIONAL VS. EXPERIMENTAL STUDIES

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Yawn contagion (YC) refers to the replication of a yawn shortly after the perception of the yawn emitted by another subject. This phenomenon has been investigated in some primate species, particularly great apes and humans, and has been linked to interindividual synchronization, coordination, and emotional contagion. Depending on the species considered, different individual factors (such as sex and age) and social factors (such as relationship quality) concur in explaining the presence and frequency of YC. In bonobos (*Pan paniscus*) yawn contagion has been found in several studies - either observational or experimental - but the results on the modulating factors shaping YC in this species vary depending on the study. Here, we investigated YC in two groups of bonobos living at Twycross Zoo (UK) by applying for the first time both observational and experimental methods to highlight similarities and differences in the results. To reach this purpose we recorded observational data (May-September 2021; 13 individuals) on yawn contagion and grooming (to test the effect of relationship quality) through the All Occurrences sampling method. Moreover, on the same individuals, we performed experiments by individually proposing videos showing either familiar or unfamiliar conspecifics' yawns (experimental condition) or mouth movements (control condition) via a Tablet (October 2021). Interestingly, the observational and experimental data led to opposite results concerning the effect of age and familiarity and we suggest that these differences could be largely explained by the methodology itself. Taken together, our results show the importance of investigating the same topic by applying different, though complementary, approaches and might help explaining the contrasting results previously found between in the studies on YC in bonobos.

ANALYSIS OF THE MANIFESTATION OF THE EAGLE SYNDROME AND THE CROWNED DENS SYNDROME IN *HOMO SAPIENS*: MORPHOMETRIC APPROACH THROUGH 3D TECHNOLOGY

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The acquisition and refinement of the upright posture have determined a series of remarkable anatomical modifications and functional adaptations in the cervical and basicranial region. In addition to substantial and progressive changes to the ergonomics position of the skull on the vertebral axis, such as the basal migration of the occipital foramen and the tendency to a progressive verticalization and lightening of the splanchnocranium, we detect a series of acquisitions and morpho-functional adaptations peculiar to *Homo* (which we call "obligate biped"). Among these adaptations are the shape and function of the axis, the second cervical vertebra, and those of the styloid process of the temporal. Both districts, effectively subject to intense stress during daily activities and therefore to strong cumulative stress, are characterized by a large degree of individual variability and pathological expression. Among pathological manifestations, we observe rare inflammatory rheumatoid arthropathies to neck and mouth articulations. In this case, the involvement of the temporomandibular and atlanto-occipital joint leads to degenerative phenomena of the ossification and/or calcification of the ligaments, in the form of anatomical anomalies typical of syndromes such as the Eagle syndrome (ES) and the Crowned Dens Syndrome (CDS). ES, also known as stylohyoid/styloid syndrome or styloid-carotid artery syndrome, is defined as a rare clinical condition, characterized by the elongation of the styloid process of the temporal bone, a slender pointed piece of bone on the inferior surface of the temporal bone, as a result of calcification or ossification of the stylohyoid ligament. Symptoms include pain in the ear, pain in the neck, that extends to the oral cavity and the jawbone, the feeling of a foreign matter in the pharynx, pain that is noticeable even in the case of tongue extension and neck turning. CDS is a rare occurrence of the pseudogout and pyrophosphate arthropathy, a calcium pyrophosphate dihydrate crystal deposition disease, with a peculiar case of ring-shaped calcification surrounding the dens or odontoid process of the axis. It is defined by strong occipital pain and neck stiffness. More acute cases are typically followed by fever and inflammation and can easily be misdiagnosed as *Polymyalgia rheumatica* o meningitis. Severe relapse chronic manifestations can be erroneously diagnosed as cervical pain or occipital neuralgia. Unlike the first works had focused on the description of morphological aspects, in recent literature studies concentrated on potential treatment and resolution of the syndromes, with the examination of clinical cases and related surgical treatments, putting aside any etiological aspects. Given this aporia, this work proposed a critical review of the literature data, with reference to the etiology and the biological and clinical significance of these two syndromes, with the use of a morpho-functional/3D analysis approach on osteological materials from different origins and chronology.

ANTHROPOMETRY CONTRIBUTION IN CLINICAL RESEARCH: BODY COMPOSITION ASSESSMENT THROUGH BIOELECTRICAL IMPEDANCE VECTOR ANALYSIS DURING PREGNANCY

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Dimensional characteristics and body composition are interesting in anthropometric research applied to different contexts concerning health and well-being. The knowledge of healthy human variability may be an opportunity for comparison and identification of anomalies, supporting the clinical research field. In particular, body composition assessment is showing an increasing utility in supporting routine medical examinations in various aspects of clinical practice, especially in relation to liquid gain and body mass changings: pregnancy and postpartum periods are exceptional moments in women life and such assessment can be very useful. Beyond gold-standard methods for body composition evaluation, Bioelectrical Impedance Vector Analysis (BIVA) offers a qualitative, fast, non-invasive and cheap approach to assess even little changes in concern to liquids, fat and fat free mass and is therefore suitable for monitoring pregnancy. Based on the few studies in this context (e.g. Lukaski *et al.*, 2007) we pointed out, in a previous longitudinal study (Moroni *et al.*, 2021) how during physiological pregnancy women gradually gained liquids (Total Body Water, TBW, assessed by Classic BIVA, Piccoli *et al.*, 1994) and fat mass% (FM%, assessed by Specific BIVA, Marini *et al.*, 2013; Buffa *et al.*, 2013) and how they tended to return to their first trimester body composition status in the postpartum period. It is to underline that throughout the gestation, the Extra-cellular/Intracellular (ECW/ICW) ratio remained constant, indicating no significant shift to one of these water compartments (Lukaski *et al.*, 2007; Moroni *et al.*, 2021). Based on the variables' physiological trend described in the abovementioned study, we compared the trend of pregnancies that at some point showed complications and their vector displacement differed. Due to the variability of the pathologies observed, we present some cases singularly: - a woman with periphery oedema (age: 28, primipara) at end of gestation showed a more pronounced shortening of the Classic BIVA vector, indicating an abnormal increment of TBW already at week 32, which was still not visible and so undetectable until the clinical observation at week 36. Moreover, the phase angle decreasing (from 5,8° at week 19 to 4,9° at week 36) indicated that liquid gain shifted to the ECW compartment and, although she did not manifest particular problems but the lower limbs swelling, she was anyway more exposed to a major risk for developing hypertension. FM%, unlike physiological pregnancies, decreased gradually; - a woman manifesting hypertension at the end of pregnancy (age: 28, third pregnancy) showed a very little liquid gain (no observable shortening of the Classic BIVA vector) in respect to what detected in healthy pregnancies (starting from week 25) and an important increase of FM%; - a woman showing a slight decrease in the rate of growth of the foetus (age: 24, primipara), showed a stable trend for Classic BIVA indicating low increment of liquids and a slight increase of FM% (Specific BIVA). As there is still a lot of research to do in order to create reference BIVA values for healthy pregnancy, such preliminary information in terms of anomalous liquid and fat mass gain/loss or stable variables, could be helpful for physicians, in order to better identify the components of weight gain and support the early diagnosis and treatment of possible disease which can occur during pregnancy.

3D-3D SUPERIMPOSITION OF PUBIC BONES: AN ADDITION TO THE PERSONAL IDENTIFICATION TOOLKIT

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When dealing with highly decomposed human remains, some of the primary identifiers may fall short due to the extent of postmortem decay and dispersion of body parts. In such conditions, the investigators must rely on alternative strategies to reach a positive personal identification. Superimposition of 3D models of anatomical structures from CT scans have brought about an innovative and efficient approach for the identification of unknown individuals, mainly focusing on the uniqueness of frontal and sphenoid sinuses. This short study applies the 3D-3D superimposition of pubic bones to the personal identification procedure, and aims at expanding the anthropological identification toolkit. Eighty abdominal CT scans were selected from a hospital database, including 40 males and 40 females. From each CT scan, pubic bones were semi-automatically segmented twice with ITK-SNAP, to simulate an antemortem (AM) and a postmortem (PM) model. For the segmentation, the right and left lateral limits were set at the medial margin of the iliopubic eminence and the inferior limit was at the middle point of the ischiopubic ramus. The two 3D models of pubic bones from the same and different individuals were then automatically superimposed according to the least point-to-point difference between the two surfaces through the VECTRA Analysis Module (VAM), recording the root mean square (RMS) point-to-point distance. For each sex, 40 matches and 40 mismatches were so created. Differences in RMS distance values between matches and mismatches were investigated through a paired Student's t-test ($p < 0.05$). Both in the male and female groups, RMS distance values were significantly lower in matches than in mismatches ($p < 0.0001$). In fact, males' RMS distance values ranged between 0.03 mm and 0.96 mm (mean 0.37 ± 0.26 mm) for the matches and between 1.06 mm and 5.26 mm (mean 2.30 ± 0.89 mm) for the mismatches. Female's RMS distances ranged between 0.01 mm and 0.97 mm (mean 0.28 ± 0.26 mm) for the matches and between 0.97 mm and 3.33 mm for the mismatches (mean 2.01 ± 0.54 mm). No overlaps between matches and mismatches were found in males; however, one superimposition of a mismatch in the female group produced a RMS distance values overlapping with the matches. This pilot study showed that 3D-3D registration of anatomical structures could provide a helpful addition to the identification procedures in the instance, for example, of unavailable ordinary anthropological indicators of identity, such as frontal sinuses. The quantitative outcome of the superimposition could then be used to strengthen a presumptive identifications or an exclusion, when appropriate AM CT images of this body portion are accessible.

THE AGAR PRE-EMBEDDING TECHNIQUE IN HISTOPALEOPATHOLOGY

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The agar technique represents a classic method in histology to obtain optimal orientation of small specimens and to avoid tissue loss during processing. Before automatic embedding, bacteriologic agar helped to keep specimens in the desired position until paraffin embedding. To date, when innovative embedding systems are not available, the agar technique is still advisable for mucosal and small skin biopsies, temporal artery specimens, ocular tissues, as well as in all cases of small, thin, or irregularly shaped tissues. As all types of solutions easily penetrate the solid agar, it can also be applied to fresh specimens, cell block preparation, and in transmission electron microscopy. We applied this method in mummified soft tissue samples to be submitted to histological examination. In the last years, we used to pre-embed almost all mummified specimens in agar prior to submitting them to rehydration, histology processing, and paraffin embedding. Aim of the present work is to describe the agar pre-embedding technique, emphasizing its unquestionable advantages in handling mummified soft tissue samples. A 5% agar solution was prepared and stored into test tubes at 4°C in a refrigerator. When needed, a test tube was placed into a water-filled beaker and microwaved for 30 seconds at 600 W. The mummified tissue specimen was placed in the desired position on a glass slide, while the fluid agar was dropped onto it with a pipette. The agar became solid in a few seconds, keeping the specimen in the desired position. Excess agar was trimmed in order to obtain well-defined borders of the block, placed into a plastic cassette, and put in the rehydrating solution. Once the rehydration process was completed, the specimen underwent histology processing and paraffin embedding in order to obtain microtome sections to be histochemically stained or immunostained. Solid agar prevented rapid tissue swelling, a major concern during ancient soft tissues rehydration. Sometimes, progressive bloating of bigger fragments produced cracks in the agar block, but they could be promptly refilled with additional fluid agar. The specimens were easy to recognize and handle during paraffin embedding and subsequent sectioning. Consistently thin and uniform sections were obtained from the paraffin blocks, improving the quality of the microscopic image. Agar pre-embedding did not hamper immunostaining. Histologic examination allowed to recognize tissue features as well as external/polluting materials and structures (fungal spores and hyphae, pollen grains, arthropods, and their dejections). The agar was microscopically visible as a slightly basophilic amorphous material circumscribing tissue sections and sometimes leaking into specimen crevices, but did not hamper the interpretation. In conclusion, agar pre-embedding technique represents a simple method that can greatly improve the quality of diagnostic information from mummified tissue samples.

BIOLOGY OF AQUATIC ECOSYSTEMS

MICROPLASTICS IN THREE COMMERCIALY IMPORTANT DECAPOD CRUSTACEANS FROM SOUTHERN TYRRHENIAN SEA

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Recently, the increasing discharge of microplastics (MP) into the oceans has been a major concern and this poses serious threats to the health of marine organisms. Though some studies already found microplastics in some organisms, further findings on commercially and ecological important species are essential. The giant red shrimp, *Aristaeomorpha foliacea*, is a deep-water benthopelagic species that mostly inhabits muddy bottom. It is a scavenger species and opportunistic carnivores that consumes a wide variety of prey. *Aristeus antennatus* (purple shrimp) is found across the Mediterranean and prefers muddy bottoms. It consumes other types of marine invertebrates, particularly crabs and molluscs. *Parapenaeus longirostris* commonly known as the pink shrimp is also found across the Mediterranean. This species lives in mud or muddy-sand substrates, and juveniles can be found up to 300 meters deep, but adult ones are often found deeper than 350 meters and preferably feeds on benthic organisms. These decapoda are important seafood in the Mediterranean including Italy and their exposure to microplastic contamination may pose a risk to marine food safety. It is therefore important to study the occurrence of microplastics in these species. A total of 50 samples of *A. foliacea*; 50 samples of *A. antennatus*, and 32 samples of *P. longirostris* were collected from Tyrrhenian Sea (GSA 10) in December 2020 at a depth range of 200 – 400m by trawling fishing (CAMP. BIOL. PROJECT). Results showed variation among species with *A. foliacea* having a higher concentration of microplastics (mean of 1.7 items/specimen) than *A. antennatus* (mean of 1.58 items/specimen) and *P. longirostris* (mean of 1.41 items/specimen). Fibers and fragments were the most prevalent kinds of microplastics isolated across the three species studied; fibers accounted for ca. 94% of the examined microplastic particles across all species, whereas fragments accounted for 6%. The plastic debris were also analyzed based on color and size which varied across individuals and species. As the results of this study confirm the presence of microplastics in the gastrointestinal tract of these species, it therefore raises concerns about marine species and ecosystems health.

AQUEOUS EXTRACTS FROM LEAVES AND RHIZOMES OF THE MARINE SEAGRASS *POSIDONIA OCEANICA* EXHIBIT ANTI-LIVER CANCER ABILITY IN VITRO

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The marine environment is a reservoir of bioactive compounds in terms of primary and secondary metabolites produced by aquatic species which exert a wide range of therapeutic effects in humans. Thus, they represent novel promising prevention and/or treatment agents and beneficial supplements for the formulation of functional food and food-packaging material. With the aim to identify novel substances endowed with anticancer action, aqueous extracts were prepared from the green and beached leaves and the rhizomes of the marine seagrass *Posidonia oceanica*. HepG2 hepatocarcinoma cells were used as an *in vitro* cell model to test the potential anti-liver cancer ability of the preparations by analysing their effects on cell viability and locomotory behaviour, cell cycle distribution, apoptosis and autophagy modulation, mitochondrial function and redox state. All the samples, apart from those obtained from *P. oceanica*'s beached leaves, were able to affect cell viability in a dose-response manner and the IC₅₀ at 24h was calculated and used as exposure concentration in subsequent assays. Cell cycle impairment, the increase of the percentage of cells in the subG₀ cycle phase and the accumulation of annexin-V⁺/PI⁺ cells at early times of exposure indicated the apoptosis-promoting effect of both leaf and rhizome extracts. In addition, the intracellular accumulation of acidic vesicular organelles, hallmarks of the autophagic process which is required by HepG2 cells to sustain their survival and active growth, decreased after both treatments although to a lesser degree in the presence of rhizome extracts. Interestingly, only the leaf extract was shown to induce mitochondrial dysfunction through the dissipation of the transmembrane potential and to maintain over 24h a steady down-regulation of intracellular ROS which are acknowledged redox-active signaling messengers necessary for cells' biological activities. As expected from the observed derangement of HepG2 cells' healthy state, the treatments were responsible for an early block of cell locomotory behaviour in wound healing assays. Collectively, the results so far obtained strongly suggest the potential anti-liver cancer ability of the preparations which appears to be stronger for green leaf than rhizome extracts and merits further investigation addressed to the isolation of the substance(s) responsible for the cytotoxic effect and to the opening of new interesting scenarios for its future biomedical and nutraceutical applications.

OCURRENCE OF *ZU CRISTATUS* (BONELLI, 1819) IN THE IONIAN SEA AT AN UNUSUAL DEPTH

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The biodiversity of Mediterranean fishes is continuously evolving in the last decades, due both to the effects of non-indigenous species invasions and the influences of the global climate change. Monitoring the occurrence and the distribution of fish populations is of fundamental importance, especially in a semi-closed basin as Mediterranean Sea. The peculiar morphology of this basin makes it divided into several biologically different areas, and this leads to enormous difficulties in carrying out research surveys that can cover it entirely. Moreover, due to their biological features, several fish species are difficult to detect during the commercial fishing activities and to be properly identified. Indeed, biologically interesting species often represents a fishing waste and therefore are not reported, leading to a huge

gap in data collection. Here we report the occurrence in the Ionian Sea of *Zu cristatus* (Bonelli, 1819), a mesopelagic species from order Lampriformes considered rare in the Mediterranean basin. The specimen was captured off the coast of Noto (Sicily, Italy) by deep-sea longline fisheries at a depth of 700m. The sample was transported to the University of Messina laboratories for morphological identification and in-depth analysis. Both biometrics and meristic data were collected including several lengths and widths of different fish body parts, weights, and numerical counts of vertebrae, gill rakers and its numerous fins. All measurements were performed by visual inspection using available identification keys. Tissue samples were collected for histological investigations and molecular characterization which confirmed the results of morphological identification as *Z. cristatus*. It is interesting to note that among the few records in the literature, most reports lower depth range for this species. In fact, it is quite known that juvenile specimens of *Z. cristatus* were frequently found in shallow waters, while little or nothing is yet known about the biology of the adult stage, which would seem to prefer much deeper environments. Considering the difficult to collect data on this rare fish, this contribution can help to improve the knowledge on the distribution of this species. Indeed, this report represent one of the first record of the scalloped ribbonfish in the Ionian Sea. Further studies on the geographical and bathymetrical distributions of this species are needed, to explore the adaptations that it encounters during its life cycle phases, moving to deep sea environments.

VALORIZATION OF FISHERY AND AQUACULTURE SIDE STREAMS FOR THE EXTRACTION AND VALORIZATION OF BIOACTIVE COMPOUNDS IN DIVERSE BIOREFINERIES

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The need to combine sustainable management of marine resources with incisive action to recover the intrinsic value of fishery by-catch and processing by-products, from fisheries and aquaculture, appears increasingly urgent, as highlighted by the SDGs in the 2030 Agenda. Fisheries by-catch and aquaculture processing by-products may represent unused or underutilized resources, still containing a large amount of components with high nutritional value. The use of these resources could be a link to seafood processing sector, generating development and economy from a resource that would otherwise be discarded. The use of wastes derived from farmed fish species processing as raw material to obtain bioactive compounds requires a preliminary study to verify the yield from processing and the effect of farm-related factors on body composition. In fact, the percentage of waste is extremely variable both in relation to the species, which influences the type of tissue discarded, and to the season, which influences fat deposition. The fish processing industry can produce a huge amount (ranging from over 25% to 70%) of by-products. Side streams from the processing of marine organisms, such as those from the tuna and anchovy supply chains, represent a resource from which to obtain fish oils rich in omega-3 polyunsaturated fatty acids (PUFAs), including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), well known for their beneficial effects on human and animal health. Farmed bluefin tuna by-products, that show a higher lipids and PUFA

content respect to the wild specimens caught in the same period, were considered for the production of ethyl esters enriched in omega 3 (PUFA_E) by the technique of short-path distillation (SPD). This procedure allowed to obtain up to almost 85% ethyl esters enriched in (PUFA_E), that could be addressed to nutraceuticals and pharmaceutical biorefineries.

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PERCEPTION OF FUNDAMENTAL FREQUENCY AND FORMANTS IN AFRICAN PENGUINS

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Animal vocalizations encode a wide range of important biological information about the age, sex, body size and social status of the emitter. In addition, vocalisations play a major role in signalling the identity of the emitter to a conspecific. The ecological pressure and the specific breeding ecology of penguins have shaped their vocalisations to maximise their propagation in noisy environments and to develop a complex mechanism of individual recognition. The application of the source-filter theory of vocal production and the use of modern technologies have allowed fine signal processing and in doing so, have advanced our knowledge of the identification, extraction and manipulation of specific acoustic parameters encoded in the calls. Recent studies have shown that acoustic cues to individual identity in the African penguin (*Spheniscus demersus*) are encoded in the fundamental frequency (sound source, produced by the syrinx through membranes vibration) and formants (resonant frequencies generated by the supra-syringeal vocal tract) of the vocalisations. However, this is only one side of the communicative process. Individuals may produce distinct vocalizations but not necessarily the receivers recognize the vocalising individual as unique. The investigation of the receiver's ability to recognize an individual as unique can reveal important aspects of communicative abilities and the impact of these abilities on the social structure of penguins. In this study, we used the Habituation-Dishabituation (HD) paradigm, one of the most powerful behavioural methods to investigate perceptual abilities in animals. This paradigm estimates the ability to discriminate whether two stimuli are perceived differently based on the behavioural responses that they elicit. When a stimulus is presented continuously, the subject's attention towards it declines (Habituation), however, when a new stimulus is presented (Dishabituation), and it is perceived as different from the previous one, the subject attention is re-engaged. In the proposed study, we used re-synthesised penguin contact calls recorded from non-familiar individuals and the HD paradigm to test the hypothesis that penguins perceive and respond to a shift ($\pm 20\%$) in the fundamental frequency (f_0) and formants frequencies (F_1 - F_4) spacing, corresponding to the natural variation of an adult individual. Preliminary evidence showed that African penguins can detect fine shifts in f_0 and formants. We demonstrate that contact calls which encode individuality are used for individual recognition in fission-fusion societies, such as the one where the African penguin lives. These findings are extremely important in our

understanding of the evolution of vocal communication in non-human animals.

A NEW FEMALE VIOLET BLANKET OCTOPUS (*TREMECTOPODUS VIOLACEUS*, DELLE CHIAJE, 1830) RECORD IN THE STRAIT OF MESSINA, SOUTHERN ITALY

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Reports of rare species are essential for increasing knowledge for biodiversity studies. The Tremoctopodidae is a small family of epipelagic to mesopelagic octopuses with a single genus, *Tremoctopus* and four recognized species (Diaz *et al.*, 2004). Specimens belonging to *Tremoctopus sp.* have been rarely found along the Italian coasts (Bello, 2008; Belluscio *et al.*, 2003; Capua, 2004; Mereu *et al.*, 2012). The reports of this genus in the Strait of Messina are very old and rare. In the present study an adult female of *Tremoctopus violaceus violaceus* (Delle Chiaje, 1830), was recorded from the Strait of Messina. Species of the family Tremoctopodidae are widely distributed in tropical oceans, but rare in coastal waters. It is considered present, but uncommon, in the Mediterranean Sea. A specimen of *T. violaceus* was found on the shore, in well preserved condition, during a sampling in Mortelle (38°16'28.11"N; 15°36'52.94"E) (Messina, southern Italy). The specimen was preserved on ice and sent to the Centre for Experimental Fish Pathology of Sicily (C.I.S.S.) for identification, measured fresh (HL, ML, TL), weighted (TW) and sex determination. The aim of this study is to report *Tremoctopus violaceus* in the Strait of Messina, expanding the knowledge in migration behaviour regarding this rare mollusc. This record of the uncommon *Tremoctopus violaceus* from the Strait of Messina, confirms the presence of this genus in the central Mediterranean.

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EFFECT EVALUATION OF AMINE-MODIFIED POLYSTYRENE MICROPLASTICS ON SPERM QUALITY OF *MITYLUS GALLOPROVINCIALIS*

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In the marine environment, microplastic (MPs) pollution represents an emerging problem on a global scale (Koelmans *et al.*, 2019). MPs are synthetic polymeric fragments with a diameter <5 mm, diffused in all seas both horizontally by currents and throughout the water column by gravity (Rochman, 2015; Ziccardi *et al.*, 2016). Thus, all aquatic organisms are exposed to MPs of different types and sizes, usually conjugated with various additives recognized as toxic, carcinogenic, and teratogenic (Chen *et al.*, 2018). Indeed, many studies have already shown toxic effects on reproductive system of different animal models, highlighting the ability of MPs to accumulate in gonads, stimulating inflammation and oxidative stress (Brandts *et al.*, 2018). In addition to the damages due to the ingestion of plastic waste and the bioaccumulation by adult marine biota, micro- and nanoplastics could also affect gametes that are released in water by organisms with external fertilization. Of all types of MPs, styrene polymers are the most frequently encountered in coastal surface and aquatic habitats worldwide. For this reason, the aim of the present study was to investigate the effects of amine-modified polystyrene particles (0.1 µm) on sperm parameters of *Mytilus galloprovincialis*. Following an acute exposure (30 min) to increasing concentrations of polystyrene (1 µg/ml, 10 µg/ml, 20 µg/ml), different parameters were evaluated: motility, viability and DNA fragmentation. The data obtained show that these parameters undergo an exponential worsening compared to the control group, at the increasing concentration tested ($p < 0.05$ and $p < 0.01$). The findings highlight the negative impact which plastic pollution could have on sperm quality and reproductive potential of organisms, altering the equilibrium of aquatic ecosystems.

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EFFECTS OF ACUSTIC STRESS ON BIOCHEMICAL AND MOBILITY PARAMETERS AND BEHAVIOUR IN THE CRAYFISH, *CHERAX DESTRUCTOR*

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This study examined the effects of acoustic stress on behaviour and biochemical parameters on freshwater crayfish, *Cherax destructor* (yabby). The experiment was conducted in a tank equipped with an audio and video recording system using ten groups (five control and five test) of three adult shrimps (30 yabbies in total). The animals in the test group were exposed to acoustic signals [a linear sweep ranging from 10 to 200 kHz

and lasting 1 s, with a sound pressure level between 138 and 157 dBrms (re 11PArms)] for 45 minutes. The following behavioural event and status were considered: velocity of movement, distance moved, angular velocity, tail flip, sounds emissions, encounters, fights and duration of the fights. Osmolarity, pH, protein concentration and enzyme activities (alkaline phosphatase, esterase and peroxidase) of hemolymph were measured. Animal exposed to acoustic stress showed higher motility and aggressivity with a significant changes in velocity of movement, angular velocity and distance of movement, sounds emissions and duration of the fights. Enzyme activities also show significant changes, with significantly lower values in stressed animals (alkaline phosphatase: TEST 0,014±0,014U/µg CONTROL 0,045±0,016U/µg; esterase: TEST 0,009±0,01U/µg CONTROL 0,037±0,016U/µg; peroxidase: TEST 5,1±3U/µg CONTROL 8,3±2,8U/µg). Our results suggest that acoustic stress can have both behavioural and physiological effects on the species.

PLASTISPHERE: THE IMPACT OF PLASTICS FROM NEW PERSPECTIVE

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Plastic represents the most abundant class of litter in the Oceans [Canals *et al.* 2021; Pham *et al.* 2014; Worm *et al.* 2017] and its presence has been recognized globally [Bergmann *et al.* 2015; Cau *et al.* 2018; Chiba *et al.* 2018; Suaria *et al.* 2016]. Its impact on marine ecosystems is currently considered a hot topic for both scientific community and political sphere. Plastics, like any other artificial and hydrophobic surface, can be exploited and colonised quite rapidly by a wide range of organisms which, over time, can give rise to real biocenoses [Wright *et al.* 2020; Wright *et al.* 2021]. This aspect, for which the term 'plastisphere' has recently been coined, deserves attention as plastic debris on seabed tends to increase structural complexity of habitats, which paradoxically favours a local biodiversity increase [Song *et al.* 2021]. During sampling carried out within the MEDITS project, in October 2021, plastic substrates were collected by fishtrawl for approximately 3 km of coast in Calabria (start point: Latitude 39°12.576' N, Longitude 16°2.175' E; end point: Latitude 39°14.18' N, Longitude 16°2.126' E) at a depth range between 84 and 87 m. This area is included in GSA 10 (Central-Southern Tyrrhenian Sea). Using a stereomicroscope, several classes of organisms were identified at the lowest possible taxonomic level; these included 45% Anellida Polychaeta, 40% Bivalve Molluscs, 10% Cnidaria and 5% Ascidiacea. The results of present research can contribute to widening of currently limited knowledge about the role of plastic as a possible artificial substrate for benthic organisms. This aspect could be of particular interest in order to clarify and evaluate possible positive, negative or no effects generated by accumulation of plastic waste on seabed and in particular on biodiversity.

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OTOLITHS ANALYSES HIGHLIGHT DIFFERENCES AMONG THREE SPECIES OF MULLET (MUGILIDAE) FROM TRANSITIONAL WATER

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The importance of studying fish otolith morphology and morphometry is nowadays well recognized. These are one of the most useful anatomical structures to study many aspects of teleost life, due to their valuable time-keeping properties. 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, or saccular otoliths, are usually the largest and the most studied, having a high taxonomic value. In the present study, attention was addressed to the Mugilidae family that includes high-values fish species with a worldwide distribution and a high tolerance to variations in salinity. Three Mugilidae species were selected: golden grey mullet, *Chelon auratus* (Risso, 1810), thicklip grey mullet, *Chelon labrosus* (Risso, 1827) and boxlip mullet, *Oedalechilus labeo* (Cuvier, 1829). These teleosts share similar trophic position and habitats, inhabiting neritic environments. They usually form inshore schools and frequently enter brackish lagoons and estuaries. The diet of *C. auratus* is mainly composed by small zoobenthic organisms and detritus while the other two species alternate a vegetarian diet with the consumption of small invertebrates. Specimens were caught between March and June 2021, using traditional throwing nets called "rezzaglio", in Ganzirri Lagoon (38°15'57" N, 15°37'50" E), a brackish pond continuously in communication with the Strait of Messina through different channels. A total of 74 Mugilidae (31 *C. auratus*, 32 *C. labrosus* and 11 *O. labeo*) were sampled, identified using dichotomous keys, weighed and measured. Pairs of sagittae were manually removed from otic capsule, cleaned from tissue

and stored dry inside Eppendorf microtubes. Morphometry and microstructure of all saccular otoliths extracted 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 and other measurements 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 9 otoliths (3 for each species) were used for Scanning Electron Microscope analysis. The results showed relatively slight interspecific differences, probably related to the closeness at the taxonomic level, similar habitat and ecological niche shared by the three species of mullet. The *C. auratus* specimens showed a more rectangular sagittae, with margins of anterior region more regular than other specimens from different Mediterranean areas. Regarding *C. labrosus*, the rectangularity was higher, and circularity was lower than data reported by previous literature, and this was also the only species showing slight differences between right and left sagittae. The *O. labeo* specimens showed rectangular sagittae with an irregular anterior region, described for the first time for this species to our best knowledge. All these features detected could be related to transitional environment peculiarities, leading to changes in sagittae between different stocks of cryptic species like those belonging to the Mugilidae family.

ZFET AS A VIABLE TOOL FOR EXPERIMENTAL BIOLOGY

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Zebrafish (*Danio rerio*) is a prominent vertebrate in the field of experimental biology and is a useful animal model, nowadays even more used in experimental research. Zebrafish has an external fertilization and a rapid development *ex vivo*. The embryos are transparent, and this allows researchers to observe their anatomy and development. The zebrafish genome has been fully sequenced. Moreover, this fish assumes an ever-greater importance because its genome is highly conserved with that of humans (~70% orthologous), and it makes possible to recapitulate human disease states in zebrafish and to use it for translational studies. Zebrafish embryo toxicity test (ZFET) is one of the most promising alternative approaches to classical fish toxicity test. Given its excellent correlation with the acute fish toxicity test and the fact that non-feeding developmental stages of fish are not categorized as protected stages according to the new European Directive 2010/63/EU on the protection of animals used for scientific purposes, the ZFET is a good alternative for the acute fish toxicity test. The ZFETs here reported were carried out at the Centre for Experimental Fish Pathology (CISS) University of Messina, to evaluate acute toxicity effects of chemical and natural extract, drug discovery, and translation medicine. The Fish Embryo Acute Toxicity (FET) used to estimate the toxicity of various molecules on zebrafish embryos is carried out according to OECD guideline (OECD TG 236, 2013). Embryos are incubated at 26°C for 96 hours post fertilization (hpf). All the solutions and embryo media are renewed daily at 90% after recording any toxic or lethal effects on the embryos. The toxicity is estimate by several

endpoint: coagulation of the embryo, lack of somites, non-detachment of the tail, non-detection of the heartbeat and number of hatched embryos. All the observations are made at 24, 48, 72, 96 hpf under a stereoscope. Considering the high predictive capacity of the ZFET demonstrated by Belanger *et al.* (2013) in their retrospective analysis of acute toxicity, the ZFET can be defined a satisfactory assay to verify the toxicity of natural compound such as extracts of vegetable origin and synthetic substances like drugs, pollutants, and nanoparticles. ZFET was performed on environmental pollutants, to evaluate their potentially toxicity on the organisms, and on several bio-compounds, which can be destined for a variety of biotechnological application. It was applied to test the toxicity of crude extract from macroalgae, such as *Chaetomorpha aerea*, *Agardhiella subulata* and *Hypnea cornuta*; extracts of terrestrial plants such as *Rush coraria* and *Ocimum basilicum*. ZFET was used to test the toxicity of pollutant, such as polystyrene, and drugs, as N-Palmitoylethanolamide-oxazolinem, which confers a positive effect on Lipopolysaccharide-Induced Inflammation and on IBD (Inflammatory Bowel Disease). Furthermore, the toxicity of Graphene oxide nanoparticles was investigated. ZFETs carried out at the CISS laboratory are here reported to describe the possible application and versatility of this experimental model in different research field.

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EVALUATION OF POTENTIAL SIDE EFFECTS OF TiO₂-rGO NANOCOMPOSITES ON PARACENTROTUS LIVIDUS SPERMATOOZOA (LAMARCK, 1816)

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A lot of pollutants reach directly or indirectly the aquatic environments. Depending on the nature of such contaminants, they can negatively impact on aquatic ecosystems (Piwowarska *et al.*, 2021). In this context, water remediation can represent a valid solution to reduce the impacts of xenobiotics that reach the marine environment through wastewaters (Oliveira *et al.*, 2020). Among the most popular remediation method, nanomaterials cover an increasing importance and utilization in order to counter water pollution (Ahmed *et al.*, 2021). However, despite their great utility in do this, the effects of nanoparticles on marine systems are unknown and unpredictable (Timerbaev *et al.*, 2021). Therefore, it becomes necessary to test these nanomaterials to assess the presence of potential negative effects on aquatic life. Some studies have evaluated the toxicity of the most common nanoparticles such as ZnO, Ag and TiO₂ on spermatozoa, larvae and adults of the sea urchin *Paracentrotus lividus* (Lamarck, 1816), generally demonstrating toxic effects that dramatically affect the survival rate of embryos (Gambardella *et al.*, 2013; Manzo *et al.*, 2013, 2017). In particular, graphenic structures are the most promising materials that can

be efficiently combined with TiO₂ for enhancing the photocatalytic activity (Balsamo *et al.*, 2021). The aim of this study was to evaluate the effects of TiO₂-rGO nanocomposites on both viability and motility of spermatozoa of *P. lividus*. The spermatozoa were exposed at different times (30, 60, 90 minutes) and concentrations (10, 20, 40 µg/ml) of TiO₂-rGO nanocomposites. The results clearly showed a decrease in both viability and motility of *P. lividus* spermatozoa exposed to TiO₂-rGO nanoparticles. In particular, viability and motility resulted to be inversely related to both exposure time and concentration of TiO₂-rGO nanocomposites than control group. In conclusion, although nanomaterials represent a new generation of technologies involved in water purification, their release in aquatic ecosystems can potentially endanger aquatic life. Hence, the urgent need to further investigate and test the potential risks of this materials through both extensive laboratory and field studies.

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UTILIZATION OF HALOFILIC STRAINS AS PROBIOTIC IN AQUACULTURE AND EFFECTS ON FISH HEALTH: PRELIMINARY RESULTS

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Aquaculture is currently the fastest growing food production sector in the world, now accounting for about 50% of total aquatic production (aquaculture and fisheries). Currently it is facing three challenges on which its expansion depends: species diversification, improvement and optimization of nutrition, and disease control. In intensive farming, animals are subjected to stressful conditions that improve their immune systems, increasing their susceptibility to pathogens and promoting disease outbreaks. Traditionally, disease control and prevention strategies in aquaculture have relied on the use of vaccines, antibiotics and chemotherapeutics, however it is now widely accepted that prevention is more advisable than treatment and actually, the goals of nutrition optimization and disease control in aquaculture converge in the manipulation of diets with natural products. Immunostimulants, probiotics, prebiotics and other natural substances are among the most promising ingredients, useful to improve fish health and, consequently, the profitability of aquaculture. In this study, the effects of the integration of an halophilic probiotic strain in the artificial diets of seabream, *Sparus aurata*, was evaluated, after 30 days of treatment. The preliminary results indicate that the supplemented diet led to an improvement in growth

parameters and an enhancement of the immune response, suggesting positive effects on health, welfare and resistance to farming conditions. Further studies are in progress to evaluate other biochemical parameters related to fish health and quality of the product.

THE IMPACT OF WASTE WATER TREATMENT PLANTS ON ALPINE RIVERS AFFECTED BY WATER SCARCITY: STATE OF THE ART AND PRELIMINARY RESULTS

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Alpine rivers have always been considered the “water reservoir” of Europe, due to the quantity and quality of water stored in this area. Currently, Alpine rivers provide water for drinking, irrigation, artificial snowmaking, and hydroelectric power plants, as well as being a biodiversity reserve and a strategic resource for tourism. However, the ability of watercourses to offer such ecosystem services is increasingly threatened by climate change: the regime of our Alpine rivers is more and more altered, and the general reduction in flow rates occasionally causes the total disappearance of superficial water for several months each year. Consequently, the human demand on water, in particular for agriculture and for the production of electricity, is increasingly in conflict with the needs of river ecosystems. In this context, an often-overlooked topic is the ability of rivers to “self-purify” and to cope with pollution from diffuse and punctual sources, such as urban and industrial wastewater. The reduction of flows linked to climate change alters the effectiveness of these essential processes, and the consequences of that will be significant and serious, even though they are little known. During the more and more frequent periods of drought, emissions from point sources such as urban wastewater discharges are less diluted: this leads to an increase in the concentrations of pollutants and pathogenic microorganisms in watercourses. Therefore, the impacts determined on water bodies by the discharges of purified wastewater directly affects their ability to implement natural self-purification processes. Furthermore, in some cases, the collection and purification systems are inadequate to reduce the polluting load produced by urban or industrial settlements. In addition to this, it is important to consider the sewage discharges of non-purified civil and industrial wastewater and / or other inputs. Clearly, the presence of the aforementioned substances can affect the development of the biotic components of the river system by altering their balances and therefore modifying the expected communities (e.g. macroinvertebrates and diatoms communities). On the other hand, however, the ecosystem itself constitutes an important element for the reduction and the containment of these substances. Overall, therefore, what is recorded is a deterioration in the quality of natural waters, with an alteration of the biological balances that also regulate the life of ecosystems. The aim of this study is to analyse the impact caused by climate change (in particular by the reduction of flows) on the effectiveness of the water treatment processes of three different oligomesotrophic rivers of the Po basin, specifically Pellice, Stura and Malone rivers. In order to achieve this objective, it has been chosen a multidisciplinary approach, which contemplates ecological, chemical and microbiological aspects, and which provides scientific support to an innovative management model, more attentive to water enhancement.

CHERAX DESTRUCTOR (CLARK, 1836) AND CHERAX QUADRICARINATUS (VON MARTENS, 1868): SAFETY AND NUTRITIONAL QUALITY

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Two species of freshwater Parastacidae (*Cherax quadricarinatus* and *Cherax destructor*) are used for breeding in several countries and to date also in Italy in one aquaculture plant located near Catania (Sicily). Although they are Australian, they seem to have some of the peculiar properties that make it important, strategic and highly appreciated species for aquaculture facilities in Italy. In fact, they mature early, have multiple reproductive cycles in a year, females can lay over a thousand eggs in a single brood and seem to tolerate wide ranges of temperature (Haubrock *et al.*, 2021). Several authors have studied the *Cherax* genus from different points of view: diseases, moulting phases and the immune responses under stress conditions (Mac Loughlin *et al.*, 2016; Sacristán *et al.*, 2016; Foyssal *et al.*, 2020). To date, even if it is known the economic value of these species, the nutritional properties for human were not yet evaluated. The economic expenses behind the maintenance of an aquaculture facilities must certainly be justified by: i) maintenance of species that can guarantee a competitive product on the market; ii) their good nutritional levels as well as for the organoleptic characteristics. Our study, conducted as part of a project “POFEAMP 02/INA/17” (02/INA/17, PO-FEAMP 2014-2020”, represents for the first time a preliminary evaluation of some macronutrient parameters important to know the nutritional properties of these animals. For this reason, some parameters, as the level of total protein, cholesterol, triglyceride, glucose and lactate dehydrogenase in two *Cherax* species, *Cherax quadricarinatus* and *Cherax destructor* were evaluated in haemolymph and in muscle tissue. These results can be useful both for evaluating the good health of these animals kept in aquaculture facilities and for evaluating better their nutritional properties. In the future, these *Cherax* species could increase their consumption and allow their greater presence in dietary habits.

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CELLULAR STRESS

CHAPERONE SYSTEM IN DIAGNOSIS AND TREATMENT OF THE GLIOBLASTOMA MULTIFORME: IMMUNOHISTOCHEMICAL AND IMMUNOFLOUORESCENCE ANALYSIS

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Glioblastoma (GBM) is a devastating disease and the most common primary brain malignancy of adults for which there is no efficient treatment. Thus, there is a pressing need for new and effective ways to target the aggressive GBM cells and treat the GBM patients¹. Chaperone system (CS) members are known to play key roles in carcinogenesis². They are involved in vital mechanisms, such as cell proliferation, differentiation, invasiveness, neoangiogenesis, metastasis, and immune system interaction. The chief components of the CS are the molecular chaperones, part of which are named heat shock protein (Hsp), and some of these are biomarkers of carcinogenesis: their expression is correlated with the degree of differentiation and aggressiveness of the tumor, along with the vascular endothelial growth factor (VEGF). Consequently, we investigated the CS in GBM by immunohistochemical evaluation of the chaperones Hsp10, Hsp27, Hsp60, Hsp70, and Hsp90 in GBM tissue samples. In parallel, we also studied other molecules important to GBM biology, such as Flt1 (VEGFR-1), Flk1 (KDR, VEGFR-2), and Flt4 (VEGFR-3) in primary glioblastoma cell lines derived from patient biopsies. The aim of our study was to assess the presence and subcellular localization of the chaperones in biopsies of ten GBMs and in four primary cell lines derived from them by immunohistochemistry and immunofluorescence. We found elevated levels of Hsp10, Hsp27, hsp60, and Hsp90 immunopositivity in the cytosol of all GBM tissue samples as compared with the normal controls. Instead, we observed low levels of Hsp70 in the GBM tissues, similarly to the low levels of this chaperone in the stem line G166 used as a positive control. We also determined the levels of VEGFRs belonging to a superfamily of RTK (receptor tyrosine kinases) and found positive VEGFR-1 and VEGFR-2 reactions with cytosol localization in the cell lines and a weaker positive reaction for VEGFR-3 in the tumor biopsies. Our findings with the cell lines and the tumors, demonstrating increased levels of chaperones and factors connected to malignancy, make these molecules potential diagnostic-prognostic biomarkers and targets for anti-cancer compounds, for instance, negative chaperonotherapy.

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ER STRESS OF HEPATOCYTES AS POSSIBLE CONSEQUENCE OF FMO3 MISFOLDING

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Trimethylaminuria (TMAU) is a rare metabolic syndrome characterized by the trimethylamine (TMA) accumulation and consequent body excretion through biological fluids. Such situation determines an unpleasant rotten fish odor in affected patients. The primary form (TMAU1) arises from *FMO3* homozygous causative mutations which could impair the *FMO3* enzyme activity. Frequently, TMAU1 affected patients do not carry causative mutations in homozygous condition. Therefore, we hypothesized that compound heterozygosity and haplotype variants might also cause *FMO3* misfolding and impair the enzymatic kinetics of *FMO3*. *FMO3* misfolding might determine hepatocytes ER stress amplified by the TMAO levels reduction. In fact, it is known that TMAO binds the luminal domain of protein kinase R-like endoplasmic reticulum kinase (PERK), activating the unfolded protein response and consequently reducing endoplasmic reticulum stress. To confirm our hypothesis, we performed a mutational analysis of *FMO3* gene in 26 patients by Sanger sequencing. Then, docking and unbinding analyses involving misfolded *FMO3* and TMA were carried out, with the final aim of revealing how found variant combinations could influence the enzyme folding. Results revealed the presence of 17 variants distributed in 26 different haplotypes which might lead to possible impairments of *FMO3* activity, probably reducing the interaction time between the enzyme catalytic site and TMA or losing the wild-type binding site. Since little is still known about the role that the combination of multiple variants could exert on the enzyme activity, our in-silico analysis could represent a starting point to unveil new scenarios about the genetic form of TMAU.

LPS AND 17- β -ESTRADIOL AFFECT HUMAN MONOCYTES BEHAVIOUR IN A SEX-SPECIFIC MANNER

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Monocytes and macrophages are fundamental cells in the inflammatory process and in the immune response (Fujiwara and Kobayashi, 2005; Lotter and Altfeld, 2019; Campesi *et al.*, 2017; Campesi, *et al.*, 2013;). Interestingly, their activities strongly depend on sex (Campesi *et al.*, 2017; Franconi *et al.*, 2017; Ruggieri *et al.*, 2014), even if the precise mechanism of sex differences onset is not fully understood. It is known that oestrogens contribute to the sex differences of immune responses (Bhatia *et al.*, 2014), but only one study investigated the influ-

ence of sex on human monocytes migration (Ruggieri *et al.*, 2014), a fundamental process for inflammation and immune response. Therefore, we investigated the lipopolysaccharide (LPS) effects on tumor necrosis factor- α (TNF- α) release, autophagy, oestrogen receptors (ER) expression and chemotaxis in freshly isolated monocytes from healthy young men and women. In basal conditions, monocytes from male and female had similar TNF- α release, migration rate, and ERs expression (both ER α and ER β), while the autophagic index, the LC3II/I ratio, was significantly higher in male cells. After LPS treatment, qualitative and quantitative sex differences were observed. In particular, LPS reduced the LC3II/I ratio and increased TNF- α release only in male monocytes, while migration was significantly affected only in female cells. Moreover, LPS reduced the expression of ER α only in female cells, while ER β expression was reduced in both sexes. Finally, we observed an interesting relationship between LPS treatment and 17- β -oestradiol (E2) only in female cells. Our results add new and important information on the mechanisms underlying sex differences in human monocyte activity, underlining and expanding the concept that sex is a variable of fundamental importance in preclinical and clinical studies, being sex differences cell - and parameter - specific.

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ROLE OF MELATONIN IN MODULATING STRESS-INDUCED PREMATURE SENESCENCE IN HEMATOPOIETIC STEM CELLS

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Human hematopoietic stem/progenitor cells (HSPCs) are pluripotent cells, featured by self-renewing and able to differentiate into mature blood cells, maintaining the functional properties of hematopoietic system¹. Under oxidative stress, HSPCs become senescent, losing the ability to maintain tissue homeostasis. Several natural molecules are used to prevent premature senescence². Melatonin is a free radical scavenger molecule, bearing antioxidant and anti-senescence properties, able to induce cell cycle arrest, by p53 overexpression³. Myeloproliferative diseases are often associated with HSPCs aging and reduced human blood system function. Maintaining proper hematopoiesis is crucial for the proper management of leukemia and other

onco-hematologic disorders⁴. Within this context, in the present study we aimed at evaluating the ability of melatonin to modulate HSPC senescence and oxidative stress following H₂O₂ exposure, in HSPCs isolated from patients with onco-hematological disorders. We exposed HSPCs to melatonin, under different conditions, and then analyzed the expression of cell cycle-regulating genes and assessed cellular senescence by β -galactosidase and telomerase activity. We also evaluated the ability of melatonin to counteract nitric oxide production and enhance catalase activity. Our results show that melatonin exerts an anti-proliferative effect on cells, regulating their typical hyperproliferation and influencing cell size and shape. In addition, melatonin acts by modulating c-Myc gene transcription, as well as the expression of genes related to cell cycle arrest, respectively, improving the functional integrity of the hematopoietic system. Moreover we also demonstrate that melatonin is able to counteract HSPC senescence, enhancing their hematopoietic regenerative potential and thus paving the way for future successful clinical application.

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A 3D MODEL OF ORGANOTYPIC ORGANOTYPIC CULTURES OF NORMAL HUMAN SKIN HELPS TO GAIN INSIGHTS INTO THE HOMEOSTATIC ROLE OF INTERLEUKIN 22 IN HEALTH AND DISEASE

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More than twenty years after cloning, characterization, and identification of interleukin (IL-) 22, its biological role in healthy and unhealthy skin is not known completely. Although the increase of IL-22 levels is a common feature in two of the most common dermatological diseases, *i.e.* psoriasis and atopic dermatitis (AD), the role of IL-22 in skin homeostasis has to be elucidated yet. Experimental models mimicking as close as possible the physiological condition are needed to elucidate the direct effect exerted by this cytokine in the epidermal compartment. A simple, clean, but good, possibility is offered by the 3D model of organotypic cultures of normal human skin, which was well standardized in the last decade in our lab. Considering the absence of blood and lymphatic vessels, this experimental setting allows the study of the early response occurring in the epidermis after an exogenous stimulus, focusing on keratinocytes. In this study, bioptic samples are obtained from healthy young non smoking women (n=7) and cultured in a Transwell system at the air-liquid interface. After an overnight incubation in order to reduce the acute effects of surgery, the medium containing 10% fetal bovine serum supplemented with penicillin/streptomycin, amphotericin B, and glutamine and maintained at 37°C with 5% CO₂ overnight was enriched with IL-22 alone (100 ng/ml) or in combination with IL-17 (50 ng/ml) and TNF-alpha (100 ng/ml). No hydrocortisone is present to avoid an anti-inflammatory activ-

ity on epidermal keratinocytes. To evaluate cell proliferation, three hours before the end of the experiment, 5-bromo-2'-deoxyuridine (BrdU), a non-radioactive thymidine analogue selectively incorporated in DNA of S-phase cells, is added (400 $\mu\text{mol L}^{-1}$) to the culture medium. After 24, 48, and 72 hours of culture, samples are processed in parallel for histological examination by light and transmission electron microscopy. Indirect immunofluorescence analysis is performed on paraffin sections. BrdU incorporation is revealed using a monoclonal antibody and keratin 10 and 17 are considered as differentiation markers. We observed that IL-22 early impairs keratinocyte maturation as regarding keratin 10 expression, without affecting keratinocyte proliferation, highlighting its prevalent homeostatic role. On the other hand, the combination of the three cytokines induces a progressive and statistically significant decrease of keratinocyte proliferation at all considered time-points and no effects on cell differentiation are evident. The hypothesis herein advanced is that IL-22 profoundly affects keratinocyte terminal differentiation, whereas, in order to induce a proliferation impairment, a more complex psoriatic-like microenvironment should be present. As most of the data concerning IL-22 immunomodulating activity are obtained from mouse models, this work offers a new perspective on its clinical role. Further studies are needed in order to establish which clinical step better correlates with IL-22 increase in order to precisely tailor biological treatment.

MAP KINASES INVOLVEMENT IN STRESS SIGNALING DURING THE COLONIAL BLASTOGENETIC CYCLE OF *BOTRYLLUS SCHLOSSERI*

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Botryllus schlosseri is a colonial ascidian easily found in the Lagoon of Venice, which undergoes weekly generation changes called take-overs (TOs) [1]. A blastogenetic cycle is defined as the period between two successive TOs. During this phase, lasting 24-36 h, a diffuse apoptosis occurs in tissues of old zooids, which will be replaced by their primary buds representing the new generation [2]. An increase in oxygen consumption (respiratory burst) is observed, due to phagocytes removing apoptotic cells, which causes the production of reactive oxygen species (ROS) [3]. In order to protect the new zooid generation from ROS damages, these animals have evolved stress defense mechanisms, which imply the activation of anti-stress proteins through stress signaling transduction pathways, possibly driven by mitogen-activated protein kinases (MAPKs). MAPKs are a family of highly conserved serine-threonine protein kinases important for the regulation of cell growth and differentiation and apoptosis [4]. With this study, through the use of specific inhibitors for Erk, JNK and p38, the three main MAPK subfamilies, directly microinjected in *Botryllus* circulation, we want to evaluate the importance of MAPKs in the regulation of the blastogenetic cycle both from a morphological and a molecular point of view. Differences in transcription levels of stress-related genes, *i.e.* superoxide dismutase (*sod*), glutathione synthase (*gs*), glutathione peroxidases (*gpxs*), *tia-1* related nucleolysin (*tiar*) and tristetraprolin (*ttp*), have been evaluated by quantitative real time PCR (qRT-PCR). The last two genes are involved in the formation of stress granules, important cell foci involved in post-transcriptional control of stress-related genes [5].

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POTENTIALITY OF OXYGEN-OZONE THERAPY IN SKIN REGENERATION FOR TROPICAL DISEASES: PROTOCOL FOR THE TREATMENT OF BURULI ULCER

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If 2019 brings the signature of the medicine Nobel for "discovery of how cells sense oxygen", oxygen/ozone (O₂-O₃) therapy acquires a prestigious position. Indeed, if the low availability of O₂ leads the cell to rise growth factors (VEGF, *Vascular Endothelial Growth Factor*; PDGF, *Platelet-derived growth factor*; HIF, *Hypoxia Inducible Factors*) genes expression, some papers demonstrated that O₃ increases itself the levels of these factors, replacing the work of the cell. During regeneration processes, the mesenchymal stem cells release various paracrine factors that play key roles in mitogenesis, apoptosis, angiogenesis and scarring. Interestingly, several studies demonstrated the positive effect of O₃ on Epidermal Growth Factor (EGF), Transforming Growth Factor α , β , Insulin Growth Factor (IGF-1), Fibroblast Growth Factor, Brain Derived Neurotrophic Factor increasing their levels. O₃ treatment promotes the fibroblasts migration, increases the levels of collagen, α -smooth muscle actin (α SMA), TGF β and inhibits the inflammation in injured fibroblasts. Another important property of O₃ is its anti-bacterial/virus/fungi activity. In relation to skin diseases, it has been demonstrated that O₃ can have topic effects on the skin, producing an antioxidant response, blocking the pro-inflammatory pathway and promoting wound healing processes. Moreover, O₃ can have systemic effects on platelet activity increasing the production of VEGF, PDGF and TGF β . Particularly focusing on skin tropical diseases, we stipulated an official memorandum regarding the application of O₂/O₃ therapy for Buruli Ulcer (BU), one of the most widespread skin related neglected tropical diseases. As BU aetiology involves inflammatory-immune reactions that are equally implicated in the action mechanisms of O₃, it is reasonable to speculate that the molecules underlying these mechanisms could represent potential therapeutic targets for the cure of this devastating skin tropical disease by O₃ application, taking in consideration also its strong anti-bacterial activity. To this aim, we set-up a nursery protocol for the implementation of the O₂-O₃ therapy in a center around Abidjan, in Cote d'Ivoire. By this procedure, it is possible to reduce the healing times, antiseptics, disinfectants, antibiotics, painkillers anti-inflammatories administration, limit the medications cost, use one device to treat up to 100 patients per day, reduce the reconstructive plastic surgery, minimum maintenance of the appliance, easy to apply and no negligible side effects. We believe that this therapy will represent a new therapeutic option capable of being easily used by local healthcare providers in the poorly assisted areas.

IMPACT OF CIGARETTE SMOKE ON INFLAMMASOME-DEPENDENT RESPONSES IN HUMAN LUNG FIBROBLASTS

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Cigarette smoke exposure is a major risk factor for lung diseases. The innate immune system of the lung is complex and includes itinerant leucocytes such as monocytes, neutrophils and macrophages, as well as structural cells, such as epithelial cells and fibroblasts. The resident cellular components, when exposed to cigarette smoke, can trigger an inflammatory cascade resulting in the release of cytokines and chemokines promoting inflammation and remodelling. Inflammasomes consist of a family of cytosolic multi-protein pattern recognition receptors (PRR) that sense damage-associated and pathogen-associated molecular patterns (DAMPs/PAMPs). The most characterised is the NLRP3 inflammasome, whose activation requires the adaptor protein ASC and leads to proteolytic cleavage and activation of caspase-1. Caspase-1 promotes the maturation and secretion of Interleukin (IL)-18 and IL-1 β . Activated caspase-1 can also cleave and activate gasdermin D (GSDMD) leading to pore-formation and pyroptosis, a proinflammatory form of cell death. Several reports have shown that caspase-8 triggers also the cleavage of IL-1 β and GSDMD, displaying a pro-inflammatory role. The activation of both caspase-8 and caspase-1 has been associated with mitochondrial damage. The impact of cigarette smoke exposure on inflammasome-dependent responses in human lung fibroblasts is largely unknown. This study aims at investigating the impact of cigarette smoke extract (CSE) on inflammasome-dependent responses in human lung fibroblasts (MRC-5). MRC-5 cells were stimulated with 5% and 10% CSE for 24 hours. Presence of inflammasome components and associated proteins was evaluated by Western Blot (WB). The activity of caspase-1 and caspase-8 was investigated by an enzymatic assay (Caspase-Glo 1 and -8 luminescent assay); pyroptosis was evaluated by measuring the release of lactate dehydrogenase (LDH). Secretion of IL-1 β and IL-18 was evaluated by ELISA assay. Mitochondrial damage was explored by immunofluorescence analysis after staining the cells with MitoTracker (indicator of mitochondrial mass) and MitoSox red (indicator of mitochondrial ROS). WB results showed that ASC, GSDMD, caspase-1 and caspase-8 were constitutively expressed while NLRP3 and IL-1 β were undetected. The activity of caspase-1 and caspase-8 increased in response to CSE. Preliminary data using selective inhibitors of TLR4 (anti-hTLR4 neutralizing antibody) and RIPK1 (Necrostatin-1) suggested that activation of caspase-8 by CSE depended on TLR4-RIPK pathway. CSE did not induce LDH release indicating that no pyroptotic cell death occurred in response to CSE, despite caspase-1 activation. ELISA assay showed no release of IL-1 β and IL-18. Immunofluorescence analysis revealed increased mitochondrial superoxide and decreased mitochondrial mass in response to CSE. Overall, our data show that CSE triggers caspase-1 and caspase-8 activation in MRC-5 cells in the absence of pyroptosis and IL-1 β /IL-18 release. Preliminary data suggested that caspase-8 activation occurred via TLR4/RIPK1 pathway and was associated with mitochondrial damage. Further investigations could unveil mechanisms leading to caspase activation and downstream effects.

THE ROLE OF ACTIVITY-DEPENDENT NEUROPROTECTIVE PROTEIN (ADNP)-DERIVED PEPTIDE (NAP) IN THE CORNEAL EPITHELIUM: FOCUS ON ITS EFFECT AGAINST UV-B RADIATION-INDUCED ROS FORMATION

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The cornea is a transparent tissue with refractive and barrier functions comprising five main layers: the epithelium, the Bowman's membrane, the stroma, the Descemet's membrane, and the endothelium. The corneal epithelium, the outermost layer of the cornea, prevents access to harmful agents into the intraocular space by acting as a dynamic barrier. The corneal epithelium is subjected daily to different types of insults, including ultraviolet B (UV-B) radiations, representing one of the most common corneal injuries. Previously, we showed the protective role played by pituitary adenylate cyclase-activating polypeptide (PACAP) against UV-B radiation insults in human corneal endothelial cells. Since some PACAP effects are mediated by the stimulation of an intracellular factor, known as the activity-dependent protein (ADNP), our aim was to evaluate whether ADNP, a peptide constitutively expressed in the corneal epithelium, played a protective role against UV-B insult. Our results showed that the treatment of corneal epithelial cells exposed to UV-B radiations with NAP, the small peptide derived from ADNP, significantly reduced reactive oxygen species (ROS) levels. Moreover, NAP treatment decreased JNK pathway activation and consequently reduced apoptotic cell death. Our results highlighted the promising therapeutic benefits of NAP to counteract corneal UV-B damage.

PLACENTA-BRAIN AXIS: FOCUS ON ASTROCYTES

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The placenta is a transient organ that is pivotal for a successful pregnancy, it is involved in the maternal-fetal cross-talk and secretes many important factors during gestation. Recent studies show that the placenta plays an active role in the physiology of the central nervous system (CNS). This organ directs both fetal neurological development and structural/functional changes in the maternal brain during pregnancy. By releasing a wide range of molecules into the maternal and fetal circulation the placenta secretome regulates the physiology of several glial cells, such as astrocytes. The purpose of this study was to determine the pattern of prenatal insults associated with increased oxidative stress, that was moderated by placental secretome. We, first of all, cultured term placental explants to collect their secretome. Subsequently, we performed *in vitro* cultures of astrocytes activated toward an inflammatory phenotype and treated them with conditioned media to assess the protective and mitigating effects of the placental secretome. We specifically studied ROS formation and observed that these returned to physiological levels when astrocytes were treated with conditioned media from term placental explants. Our results indicated that molecules present in the placental secretome can mitigate the inflammatory behavior of astrocytes, giving the possibility to develop useful drugs to control/improve acute neurological injuries and/or chronic CNS diseases.

PROBIOTIC-MEDIATED NF- κ B REGULATION AND INDUCTION OF MOLECULAR CHAPERONES IN THE SMALL INTESTINE OF A MOUSE MODEL OF ETHANOL-INDUCED INFLAMMATORY DAMAGE

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In this study, we examined the protective effects of the probiotic *Lactobacillus fermentum* (L. fermentum) in the small intestine, using a mouse model of ethanol (EtOH)-induced inflammatory damage. Female 12-month-old mice (BALB/cAnNHsd) were orally fed with EtOH (96%) alone (n=5) or in combination with L. fermentum (10⁹ CFU) (n=5) (groups 12 EtOH, and 12 EtOH-P, respectively), every day for twelve weeks. Both groups were compared with mice (n=5) fed with the standard diet as per a previously calibrated experimental model (1). Immunomorphological, quantitative real-time PCR, and Western blotting analyses were used to evaluate the effects of L. fermentum on the NF- κ B signalling pathway, and on the levels of cytokines and molecular chaperones. The levels of phosphorylated NF- κ B were elevated in the 12 EtOH group but were lower in the 12 EtOH-P. L. fermentum induced downregulation of the tumor necrosis factor (TNF)- α production and NF- κ B activation via increased expression of the NF- κ B inhibitor- α (I κ B- α). The probiotic induced the expression of the chaperones Hsp60 and Hsp90, by inhibiting I κ B-kinase (IKK) and preventing NF- κ B activation. The data indicate that L. fermentum had anti-inflammatory and cytoprotective effects, pointing to its possible application to treat intestinal inflammatory disorders.

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SIRT5 AS CELLULAR MODULATOR IN NON-ALCOHOLIC FATTY LIVER DISEASE

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Sirtuin 5 (SIRT5) has emerged as a key intracellular regulator of mitochondrial post-translational modification (PTMs). GWA studies has been reported that the rs12216101 T>G non-coding SNP at the SIRT5 gene locus is associated with the number of carotid plaques, suggesting that the gene variation may modulate the susceptibility to cardio-metabolic disease related to obesity (1, 2). However, to date no study has been reported the association between the sirtuin SNPs and liver disease severity in patients with Non-Alcoholic Fatty Liver Disease (NAFLD). We evaluated the impact of the non-coding

SNP rs12216101 T>G of SIRT5 on liver disease severity, on hepatic SIRT5 expression and on transcriptomic signatures in patients with biopsy proven-NAFLD. In the present study, we observed that SIRT5 rs12216101T>G variant, analyzed in 2606 patients with biopsy-proven NAFLD consecutively recruited at three European centers, increases the risk of ballooning, NASH and F2-F4 fibrosis. The effect of the G variant on liver damage was larger in obese individuals, suggesting a possible interaction between metabolic risk factors and host background genetic, as already reported for PNPLA3 (3). Using transcriptomic approach in a sub-cohort of 112 Italian bariatric patients, we found that the variant rs12216101 G was associated with a more severe liver damage, leading to the up-regulation of genes involved in different metabolic pathways. Between them, oxidative phosphorylation, and fatty acid metabolism, as well as Myc pathway, involved in hepatic angiogenesis and fibrogenesis, were found up-regulated (4). These data confirm that the rs12216101 G variant affects SIRT5 activity, which, probably, influence the transcription of the regulatory SIRT5 isoform 4. In conclusion, this study reported that the rs12216101 T>G variant of SIRT5 is independently associated with liver disease severity in patients with NAFLD. Further studies are needed to clarify the mechanisms linking SIRT5 variation with development of steatohepatitis and fibrogenesis and the impact of these findings for disease risk stratification.

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PHYSIOLOGICAL RESPONSES INDUCED BY PFAS EXPOSURE IN FRESHWATER FISH OF THE VENETO REGION

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In recent decades, the interest towards per- and polyfluoroalkyl substances has grown exponentially around the world, due to the toxic effects induced by these chemical compounds in humans, as well as in other animals and plant organisms. However, the knowledge related to the antistress responses that organisms can express when exposed to these substances is still lacking and therefore requires further investigation. For this purpose, this study was launched on the possible physio-

logical responses that exposure to environmental concentrations of PFAS can induce on *Squalius cephalus* and *Padogobius bonelli*, two freshwater fish species widely spread in the Veneto Region, a geographical area directly involved in what is considered one of the most significant cases of PFAS pollution. Specimens of the two species were sampled in three rivers of the Vicenza area, characterized by three different levels of PFAS pollution. Several biomarkers of stress have been evaluated and the results obtained suggest an increase in the expression of mitochondrial antioxidant enzymes. Conversely, no change in total antioxidant capacity was observed, suggesting that the effect of oxidative stress detected in the mitochondria does not correspond to a more extensive cellular response. For both species, various morphological indices were calculated with the aim of determining both the general well-being of the organisms and identifying possible variations in the size of the liver, spleen and gonads. The obtained results are preliminary and certainly require further analyses and investigations, but they suggest an interesting protective mechanism against damage to the protein component based on lipid vacuolation in the liver.

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PEEL AND SEED EXTRACTS OF *MANGIFERA INDICA* REDUCE ADIPOGENESIS AND EXERT ANTIOXIDANT EFFECTS ON 3T3-L1 CELLS

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Several studies highlighted how many plants, due to the presence of bioactive compounds, exert beneficial effects on the prevention and treatment of obesity. Mango (*Mangifera indica* L.) is a plant belonging to the *Anacardiaceae* family which has been shown to exert anti-inflammatory, anti-oxidant and anti-tumoral effects [1,2]. Here, we demonstrated that extracts of peel and seed of mango, the main bio-waste products of mango processing, exert anti-adipogenic, anti-lipogenic and anti-oxidant effects in 3T3-L1 cells. In particular, Mango Peel (MPE) and Mango Seed (MSE) extracts significantly reduced lipid accumulation and triacylglycerol contents during 3T3-L1 adipocyte differentiation. The anti-adipogenic effect of MPE and MSE was the result of down-regulation of the key adipogenic and lipogenic transcription factors PPAR and SREBP-1c. In addition, both MPE and MSE significantly increased the phosphorylated and active form of AMPK with the consequent phosphorylation and inhibition of Acetyl-CoA-carboxylase (ACC), the main enzyme of fatty acid synthesis. AMPK and ACC phosphorylation induced by MPE and MSE was counteracted by the addition of compound C, a specific AMPK inhibitor, thus suggesting a key role of AMPK in mediating MPE and MSE anti-lipogenic effects. Notably, we demonstrated that MPE and MSE possess strong antioxidant properties, as suggested by their ability to counteract ROS increase produced during 3T3-L1 adipocytes differentiation. These effects seem to be mediated by MPE and MSE-activation of Nrf2 and its downstream targets MnSOD, HO-1 and Catalase. In conclusion, these data support bio-waste products of mango as a potential supplement to reduce the risk of obesity.

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OXIDATIVE STRESS IMPAIRS THE ACTIVITY OF KIR2.1 CHANNELS IN A MODEL OF AGING NEUROGLIA

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Epilepsy is a chronic disease of the brain and its prevalence increases with age. Strikingly, about 50% of all epilepsy cases diagnosed in elderly patients (>65 years) are idiopathic. Metabolic changes, including the production of reactive oxygen species, may contribute to epilepsy development. The neuronal glia plays a crucial role in epilepsy by controlling neuronal hyperexcitability. One of the key roles of glial cells is the spatial buffering of extracellular K⁺ ions that are released by excited neurons and transported through glial inwardly rectifying potassium (Kir) channels from extracellular regions of high K⁺ to those of low K⁺ to inhibit epileptogenesis. However, whether Kir channels can be the target of oxidative stress during aging is not known. Among experimental oxidative stress-related aging models, exposure to D-galactose (D-gal) is considered the most similar to natural aging. In the present study, we investigated the effect of D-gal-induced aging on Kir channel function in glioblastoma U87-MG cells. RT-qPCR was utilized to identify the isoform(s) of Kir channels expressed in glioblastoma U87-MG cells. In addition, cell viability and oxidative stress following exposure of cells for 24 hours to 30 or 100 mM D-gal have been assessed by the MTT colorimetric assay and estimation of thiobarbituric acid reactive substances (TBARS) levels - a marker of lipid peroxidation - as well as membrane total sulfhydryl (SH) groups, respectively. The membrane K⁺ conductance was measured by the patch-clamp technique in whole-cell configuration. As Kir channels are sensitive to Ba²⁺, all currents have been subtracted of the Ba²⁺-insensitive component. To obtain equal osmolality, equimolar amounts of mannitol were used as the control for D-gal. The oxidizing agent TBH70X (1 mM) was used as an alternative to D-gal for inducing oxidative stress. Screening of all 15 known isoforms of Kir channels by RT-qPCR revealed that the predominant transcript expressed in U87-MG cells corresponds to the Kir2.1 channel. Among other Kir channels known to be expressed in neuronal glia, Kir4.1 was 26-fold less expressed and other Kir2 members as well as Kir5.1 were virtually absent. D-gal (30 and 100 mM) or TBH70X had no obvious cytotoxicity, but 100 mM D-gal or TBH70X activated oxidative stress pathways, namely significantly enhanced TBARS levels and reduced the abundance of membrane SH groups. Interestingly, 100 mM D-Gal exposure was associated with a pronounced decrease of Ba²⁺-sensitive inwardly rectifying K⁺ currents, most likely mediated by Kir2.1 (p<0.05, n=5). Importantly, 30 mM D-gal and 30-100 mM mannitol failed to elicit oxidative stress and, accordingly, had no significant effect on the Ba²⁺-sensitive K⁺ current (n=5). Exposure of cells for 5 minutes or pre-incubation for 2 hours with 1 mM TBH70X significantly reduced the Ba²⁺-sensitive K⁺ currents compared to control, thus supporting the concept that the activity of Kir2.1 is highly sensitive to oxidative stress. These find-

ings reveal a novel Kir2.1 channel modulation that is likely to occur in oxidative stress. We suggest that inhibition of Kir2.1 in neuronal glia may alter the extracellular K⁺ buffering and contribute to oxidative stress-related neuronal hyperexcitability and epileptogenesis during aging.

β-CARYOPHYLLENE REVERTS FREE FATTY ACIDS-INDUCED CELLULAR STRESS IN HEPG2 HEPATOCYTES THROUGH CB2 AND PPAR-α RECEPTORS

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Nonalcoholic fatty liver disease (NAFLD) is one of the most common cause of liver disorder, defined by excessive accumulation of triglycerides in hepatocytes due to both increased ingestion of free fatty acids (FFAs) and de novo hepatic lipogenesis. The accumulation of lipids causes oxidative stress, anomalies in hepatocytes and inflammation, that may lead to the progression of NASH (nonalcoholic steatohepatitis) and liver cancer. The scientific interest for natural compounds as potential drugs has increased exponentially in the last years, along with the number of studies on nutraceuticals and herbal extracts, aimed to test their effects on many disorders, including obesity, NAFLD and also cancer. The sesquiterpene hydrocarbon (E)-β-caryophyllene (BCP), widely distributed in the plant kingdom [1], is one of the most investigated and promising natural compounds in chronic inflammation studies [2], with significant effects on reduction of lipid accumulation [3]. In our study we demonstrate its ability to revert FFA-induced steatosis and modify the lipid profile in HepG2 hepatocytes by *in vitro* biological assays and lipidomic analysis. To simulate the condition of steatosis, HepG2 cells were treated with palmitate and oleate (the most abundant fats in our diet), and lipid content was quantified by AdipoRed fluorescence staining. Our results demonstrate that the treatment with a 0.5 mM mixture of palmitate and oleate causes 80% increase in intracellular triglycerides, while the 24h co-treatment with 0.5 μM BCP, determines a significant reduction in triglyceride accumulation with respect to steatotic control cells, without altering the cell viability. Moreover, we show that the BCP-induced triglyceride reduction could be mediated by the cannabinoid receptor 2 (CB2) and peroxisome proliferator-activated receptor alpha (PPAR-α). It is known that *trans* fatty acids promote inflammation and endoplasmic reticulum stress, whereas *cis*-unsaturated fatty acids are protective [4]. To reveal the potential change in HepG2 cell lipid profile induced by BCP treatment, we used a lipidomic approach based on gas chromatography-mass spectrometry (GC-MS). Our GC-MS data show that co-treatment with BCP induces a reduction of palmitic and stearic acids (both saturated fatty acids), oleic acid (monounsaturated fatty acid), elaidic acid (*trans*-polyunsaturated fatty acid) and an increase in palmitoleic acid (monounsaturated fatty acid). Taken together these results reveal interesting and novel properties of BCP, suggesting potential applications in the reduction of *trans*-fatty acid accumulation and cellular damages caused by the accumulation of fats, typical condition of NAFLD.

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MANIPULATION OF HSP70 SIGNAL MODULATES THE PROCESS OF SH-SY5Y DIFFERENTIATION AND SURVIVAL TO OXIDATIVE STRESS-DEPENDENT CELL DAMAGE

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SH-SY5Y neuroblast-like cells are widely used in *in vitro* investigation in the field of neuroscience research. These cells can be differentiated into mature human neurons through a variety of different mechanisms including serum deprivation and the use of retinoic acid (RA). Advantages include the capacity for large-scale expansion, with relatively ease and low cost culture compared to primary neurons. Several studies have described important differences between undifferentiated and differentiated SH-SY5Y cells. Undifferentiated SH-SY5Y cells rapidly proliferate and appear to be non-polarized, with very few, short processes. They often grow in clumps and express markers indicative of immature neurons. When differentiated, these cells extend long, branched processes, decrease in proliferation, express markers of mature neurons, and in some cases polarize. Both undifferentiated and differentiated SH-SY5Y cells have been utilized for *in vitro* experiments requiring neuronal-like cells. In our studies we observed a different sensitivity of undifferentiated and differentiated cells to a powerful oxidizing agent. Indeed, treatment of undifferentiated cells with hydrogen peroxide induces a dose-dependent decrease in cell viability, which is not detectable in differentiated cells, even at high concentrations of hydrogen peroxide. Heat shock proteins (HSP) are key molecules involved in the correct folding of proteins and protection of cells from injury, including damage caused by oxidizing agents. Accordingly, we observed a higher expression of HSP70 protein in differentiated SH-SY5Y cells, as compared to undifferentiated ones. Interestingly, inhibition of HSP70 expression by KNK437 during the process of differentiation caused the failure of the differentiation process and a dramatic increase in cell death, suggesting that HSP70 expression is necessary for the overcoming of the cell stress associated to the process of differentiation. On the contrary, when HSP expression is induced by treatment with Oxotremorine, a selective muscarinic acetylcholine receptor agonist, differentiated SH-SY5Y cells becomes more resistant to oxidative stress-induced cell death. In conclusion, our data demonstrate that manipulation of HSP70 signal modulates the process of SH-SY5Y differentiation and survival to oxidative stress-dependent cell damage.

ERYTHROCYTE AGING IS ASSOCIATED WITH CHANGES IN BAND 3 PROTEIN FUNCTION DUE TO OXIDATIVE STRESS AND GLYCATION EVENTS

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Aging is described as a multi-factorial process developing through a complex net of interactions between biological and molecular mechanisms, and involves oxidative stress as well as glycation. The aim of the present work focuses on the role of Band 3 protein (B3p) activity, an anion exchanger essential to erythrocytes homeostasis, in a D-galactose (D-Gal)-induced

aging model. Anion exchange capability, measured by the rate constant of SO_4^{2-} uptake through B3p, TBARS levels -a marker of lipid peroxidation-, total sulfhydryl (SH) groups, methaemoglobin (MetHb) and glycated haemoglobin (Hb) levels, and GSH/GSSG ratio were determined following to exposure of human erythrocytes to different concentrations of D-Gal for 24 hours. Our results show that: i) the rate constant for SO_4^{2-} uptake is accelerated; ii) TBARS and glycated Hb levels are increased; iii) total -SH groups and GSH/GSSG ratio are decreased. Conversely, MetHb levels did not change compared to control conditions. In conclusion, these findings confirm that D-Gal exposure induces aging in human erythrocytes, altering B3p function, Hb and the antioxidant system. Further studies are needed to understand whether supplementation with antioxidant substances, namely quercetin, can restore the altered parameters.

INSIGHTS ON RECENT ADVANCEMENT IN CARTILAGE TISSUE ENGINEERING AND 3D JOINT BIOMIMETIC SYSTEM

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Osteoarthritis (OA) is a chronic degenerative disease of articular cartilage. This pathology interests people over forty years old: patients suffer from chronic joint pain, progressive loss of mobility and consequent loss in quality of life. So far, there is no therapy available that effectively stops the progression of the disease. The complex morphology of the articular cartilage and multifactorial aspect of the articular joint have recently prompted the development of advanced organ-on-chip engineering, perfusion bioreactors for 3D constructs, 3D cell culture models and cutting-edge 3D bioprinting technologies for cartilage engineering, trying to reproduce a complex cartilage architecture and joint environment in a microscale system to achieve the properties of the native joint tissues [D'Amora *et al.*, *J Tissue Eng Regen Med.*, doi: 10.1002/term.2457.]. The basic concept of cartilage engineering is to seed chondroprogenitor cells within a 3D polymeric scaffold [Calabrese *et al.*, *Front Physiol.*, doi: 10.3389/fphys.2017.00050]. The just settled scaffold might be then set on a BioReactor to reproduce cartilage tissue *in vitro*. Alternatively, a 3D biomaterial scaffold is used directly at a defect site, working as a carrier to deliver cells and/or therapeutic biomolecules to facilitate cartilage regeneration [Szychlinska *et al.*, *Materials*, doi: 10.3390/ma13102369.]. Critical to the success of these cartilage tissue engineering strategies are the physical and chemical properties of biomaterial scaffolds. The appropriate physicochemical properties that account for a physiological intracellular stress state deriving from the cell-biomaterial interaction, favor the recovery of a phenotypic cell expression. The 3D support must be also able to provide mechanical strength, and at the same time, regulate the biological activities of the seeded cells and their phenotype [Bucchieri *et al.*, *Exp Lung Res.*, doi: 10.1080/01902148.2017.1303098.]. In the past two decades, a great number of biomaterials have been introduced into cartilage tissue engineering. By including cutting-edge technologies, bioreactors, peptide synthesis and controlled release, novel scaffolds with "smart" materials and structures, capable of changing their physical or chemical properties in response to physiological needs to enhance cell growth and tissue regeneration, are currently developed and represent the future investigative trend in this area.

HUMAN HSP60 MUTATIONS p.GLU129LYS AND p.VAL287ILE ASSOCIATION WITH MITOCHONDRIAL DYSFUNCTION: NEW INSIGHTS FOR UNDERSTANDING SPASTIC PARAPLEGIAS

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The molecular chaperone Hsp60 plays a key role in maintaining mitochondrial proteome integrity. In humans, neurodegenerative conditions have been associated with missense mutations in the Hsp60-encoding gene *HSPD1*, which cause the amino acidic replacements p.Asp29Gly and p.Val98Ile [1]. These two conditions have been included in the group of genetic Neurochaperonopathies [2], but hundreds of other variants exist with the potential to negatively affect protein structure-function and to be pathogenic, as inferred from bioinformatics predictions and analyses of the mutant proteins three-dimensional structure [3]. Consequently, many other Chaperonopathies could occur that have not yet been diagnosed and about which no published reports are available. Most of these lesser-known variants of the *HSPD1* gene have been linked to different forms of Spastic Paraplegia (SP), for example p.Glu129Lys and p.Val287Ile. In this study, *in silico* analysis was carried out to assess the possible impact of these two missense mutations on protein structure-function and predict their pathological significance. The analysis was performed using the crystal structure of the tetradecameric Hsp60-Hsp10 football-shaped complex [4] as the template-reference model and considering: (a) the properties of individual amino acids; (b) the known functions of the amino acids in the human Hsp60 and/or in the highly similar bacterial ortholog GroEL; (c) the location of the mutant amino acids in the monomers and oligomers; and (d) structure-function relationships inferred from crystal structures. Glutamic Acid 129, located at the equatorial domain of the Hsp60 monomer, participates in an important inter-ring contact in the tetradecameric football-shaped complex and its replacement with a Lysine could abolish or weaken this contact and impair the formation and/or the stability of the macromolecular complex. Valine 287 is located at the apical domain of the Hsp60 monomer, near the residues directly involved in the interaction with the co-chaperonin Hsp10. Thus, its replacement with an Isoleucine, which has a different conformation, could impair the interaction with Hsp10, and compromise the formation and/or stability of the macromolecular complex. Experimentally, transfection of human dermal fibroblasts *in vitro* with plasmids carrying one or the other of the two *HSPD1* mutants affected cell viability, and mitochondrial morphology and activity. The data thus far encourage more research for elucidating the genetic and molecular underpinnings of these types of SP associated with Hsp60 chaperonopathies.

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COVID-19

SARS-COV-2 IN MARINE MAMMALS: SURVEY RESULTS ON THE POTENTIAL VIRAL SUSCEPTIBILITY IN CETACEANS STRANDED ALONG THE ITALIAN COASTLINE

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Due to marine mammal's demonstrated susceptibility to SARS CoV-2, based on homology level of the angiotensin-converting enzyme 2 (ACE2) receptor with the human one, along with the global spread of infection and the aquatic contamination, potential SARS CoV-2 transmission to marine mammals can be expected. Moreover, based on immune system and inflammatory responses to SARS-CoV-2 infection in humans, macrophages could also play an important role in antiviral defense mechanisms. In order to provide a more in-depth insight into SARS CoV-2 susceptibility in marine mammals, we evaluated the presence of SARS CoV-2 and the expression of ACE2 and CD68, as a pan-macrophage marker. A large number of lung tissue samples, belonging to cetaceans stranded along the Italian coastline during 2020-2021, was collected for SARS CoV-2 analysis by real-time PCR and Immunohistochemistry (IHC), along with ACE2 expression by IHC. In addition, ACE2 and CD68 were also investigated by Double-Labeling Immunofluorescence (IF) Confocal Microscopy. From samples analysed for the survey, no SARS CoV-2 positivity was found while ACE2 protein was detected in the lower respiratory tract but heterogeneous for age, sex, and specie, suggesting that ACE2 expression can vary between different lung regions and among individuals. Finally, IF analysis showed elevated colocalization of ACE2 and CD68 in macrophages only when an evident inflammatory condition is present, such as in human SARS CoV-2 infection. In conclusion, although no SARS CoV-2 spillover already occurred on cetaceans stranded along the Italian coastline and examined by our network within the investigated period, results on the expression of ACE2 allow us to hypothesize a possible susceptibility to SARS CoV-2 in marine mammals.

From a One Health perspective, it is therefore important performing a constant and systematic surveillance of SARS-CoV-2 infection on marine mammals by monitoring stranded specimens, in order to help and protect both human health and endangered marine mammal species.

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THE SIDE EFFECTS OF SARS-COV2 ON CHEMICAL COMPOSITION OF HUMAN SCALP HAIR

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The hair bulb is one of the biological memories of the human organism. Its structure stores is the result of the relationships between the internal environment and external events. The aim of the present research is to measure, through biomolecular analysis, the variation of the mineral and biological composition of the intracellular content of the hair bulb correlated by the presence of Coronaviruses linked to the SARS-CoV2 pandemic. To carry out the experiments aimed at the present work, biomolecular analysis of the hair of four groups of 10 individuals was performed. The groups were selected according to the following criteria: people who did not contract SARS-CoV2 and were not vaccinated, people who contracted SARS-CoV2 and were not vaccinated, people who did not contract SARS-CoV2 and they received at least the second dose of the vaccine and, finally, people who contracted SARS-CoV2 and received at least the second dose of the vaccine. The system that carried out the analysis is called BMT (Bio Molecular Test) which is offered on the market as a repeatable screening test and returns the values of 8 heavy metals, 23 minerals, 13 vitamins, 19 amino acids and 7 hormones. For the present research the values of 4 minerals and 4 amino acids were taken into consideration. Tests are carried out on the hair bulb with a polarized light technology. The chemical and biological content of the hair roots was determined using the BMT technique which, unlike the simple amount of trace elements, does not destroy the sample (so the analysis is repeatable) and, by operating on the bulb and not on the stem, goes further to the vision on minerals and heavy metals. From the results obtained it has been possible to find that the chemical and biological contents of the hair bulb vary as a result of the contraction of SARS-CoV2 while they remain indifferent to the administration of vaccines. The next steps of this research aim to analyze, over time intervals, the variations in the chemical and biological compositions of the hair bulbs associated to the variable linked to the contraction of SARS-CoV2. The intention is to take advantage of the current pandemic to establish a non-invasive method in order to have a continuous focus on the intracellular biochemical state aimed at elaborating corrective intervention strategies through the personalization of the temporary use of drugs and supplements.

GENDER ANALYSIS ON COVID-19 DATA IN PIEMONTE: THE VIRUS PREFERS MEN

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Several important sex and differences in clinical manifestation and response to treatments for many diseases are known since a long time, although they continue to be underestimated. Covid-19 pandemic has recently provided further evidence of the importance of gender-based approach. Many fields of study, such as medicine, law, psychology, sociology, as well as sciences applied to data analysis, highlighted the importance of this perspective in studying Covid-19 pandemic effects, and the actions to contain it. Sex factor is strongly present in this health crisis: Covid-19 mainly affects men, with a worse symptomatology and a general disease exacerbation. This is due to a different immune system, stronger and more responsive in women, in addition to the role of ACE2 (Angiotensin-converting enzyme 2), differently expressed among sexes. The gender factor also played an important role in the path of Covid-19 infection. Women are more inclined to maintain social distancing and continuous hand hygiene. Women smoke less. Aim of the work was to analyze data on Covid-19 testing in Piedmont region, northwest of Italy, from people admitted to Amedeo di Savoia hospital, regional referral center for infectious diseases. Data are referred to the whole of 2020, giving us a detailed image of the trend of the pandemic. We performed analysis on all testing records present in the regional platform: an high percentage of them was suitable for lack of informations or not evaluable for macroscopic errors in data entry by operators. Among suitable sample, we analyzed records negative for Covid-19 testing and positive, matching records for unique subjects, in order to evaluate recurrence of Covid-19 testing in the same person. On this number we performed disaggregation by age and sex. At the symposium we will also show analyses on the suitable sample concerning correlation of testing with hospital admission motivation and symptoms. Sex and gender approach should be recognized as part of the medical knowledge: a sex/gender-based approach to clinical practice also in the context of this pandemic seems to be mandatory for patients and for the sustainability of the National Health System.

WHY IS ABSOLUTE EXCESS MORTALITY IN ITALY IN 2021 SIMILAR TO 2020?

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This study focuses on the comparison with other European countries of the overall mortality in Italy in 2021. The data are provided by ISTAT, EUROMOMO and the open source data of Johns Hopkins University. After a brief note on the general limits of statistics produced in Italy for COVID-19, we reported the

mortality rates for all the pathologies (for males and females) in 2021 and 2020 in comparison with the average of the pre-Covid years (2015-2019). There is an *unexpected mortality* excess in 2021, not far away from the one in 2020, the year in which mortality probably increased due to COVID-19. We then proceed to compare the excess mortality from all causes in the age groups 65-74, 75-84, 85+, finding also in this case excess mortality in 2021 high or completely similar to 2020 (respectively +14.47%, + 5.97% and +10.25%). On a geographical map by province we have also displayed, with a color scale of increasing intensity, the 2020-2021 mortality rate. We found that in 2020 mortality was very concentrated only in some provinces of the North and much lower in those of Southern Italy. Unexpectedly, in 2021 mortality was distributed much more homogeneously among all provinces, but generally higher in each province. The comparison with other EU countries, is focused on the trend of general excess mortality in Europe, always for 2021 and 2020, with respect to the baseline (2015-2019). The data are provided by EUROMOMO and regards the overall excess mortality (males and females together) for the 23 European countries covered by EUROMOMO. Firstly, all age groups are considered, secondly the 0-14 and 15-44 age groups. The results indicate a very high cumulative excess of mortality from all causes in 2021 for the 0-14 age group compared to 2020 when it was even negative and more than double for the 15-44 age group in 2021 compared to 2020. We shall remind that the number of deaths in 2021 also includes an unknown number of deaths from adverse reactions to the vaccine, as found in autopsy tests (Sessa et al 2021). Finally, an attempt was made to verify the effect of lockdowns on the positivity rate and the mortality rate of COVID-19, by comparing the Johns Hopkins University data on closures with data on mortality per 100,000 inhabitants produced by Worldometers. For 39 European countries (according to a very broad definition of Europe, including Russia and other non-EU countries), is not found a statistically significant positive effect produced by lockdowns on mortality and positivity rate (Herby et al. 2022).

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SARS-COV2'S TROPISM IN NEURONS AND GLIA IMPAIRS NEURONAL NETWORKS ACTIVITY: AN IN VITRO STUDY ON RAT CORTICAL CELLS

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Since the start of COVID-19 outbreak, up to ~50% of convalescent individuals presented neuropsychiatric and neurological symptoms. A recent biochemical analysis of the plasma of

severe and moderate COVID-19 cases demonstrated an increase in biomarkers of CNS lesions (*i.e.* such as GFAP - glial fibrillary acid protein and Nfl - neurofilament light chain protein). This suggests the activation of astrocytes as well as the occurrence of neuronal damages, following viral infection. We examined the effects of SARS-Cov2 infection on neurons, studied in an *ex vivo* model of rat brain cortical networks. For the first time, we report that rat neurons and glial cells are susceptible to SARS-Cov2 infection, even if lacking hACE2 receptors. Infection in our primary neuronal cultures was confirmed by plaque assay and by real-time PCR. We observed nucleocapsid-positive cells as early as 3h post-infection. At 16 and 24h post-infection, glial cells showed a significantly increased rate of infection, when compared to neurons (*i.e.* 15-18% versus 5%). In addition, when we analyzed the electrophysiological phenotype of infected neuronal networks by microelectrode arrays (MEAs) recordings, we found altered electrical signaling and subsequent irreversible signal loss. In fact, at 24h post-infection both spontaneous and electrically induced generation of neuronal impulses and coordinated network activity was reduced to zero. Such a dramatic alteration however did not occur abruptly. At 3h post-infection, extracellular electrical stimuli still induced low-latency (*i.e.* <20 ms) action potentials, suggesting that antidromic (axonal) excitability was still preserved. However, the complete disappearance of "reverberating" electrophysiological events at larger latencies (*i.e.* 20-50 ms), strongly expressed before infection, suggests that a loss of synaptic function is occurring. We hypothesized that such a scenario results from inflammatory events including microglia activation. Interestingly, a similar – although weaker - downregulation of spontaneous and evoked electrophysiological events was observed in sister cultures exposed to a UV-inactivated SARS-Cov2 virus, in which replication was impaired but the spike protein and its membrane binding were preserved. This result suggests that membrane binding of SARS-Cov2 is sufficient to trigger inflammatory events. An additional cytokines analysis, using supernatant of infected neurons was performed, revealing a key role of TGF- β , TNF- α , IL-1 α , RANTES and MIP-1 β . Future studies aimed at preventing the neurological symptoms of long-covid syndrome could likely target the inhibition of these cytokines and benefit from our initial analysis of the impairments on the electrophysiological activity.

PREDICTED ROLE OF MOLECULAR MIMICRY IN COVID-19 PATHOGENESIS: NEW INSIGHTS FROM BIOINFORMATICS AND IMMUNOMORPHOLOGY

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COVID-19 is typically characterized by an acute respiratory syndrome but can also affect other organs simultaneously or separately. We performed an immunomorphological study

of lung samples from subjects who died of COVID-19 and a bioinformatics comparative analysis of SARS-Cov-2 and human proteins. Hematoxylin-eosin-stained sections showed a strikingly modified architecture of the lung parenchyma. Marked congestion, microthrombi of the small vessels, haemorrhages, and detachment of the alveolar lining with desquamation of pneumocytes within the alveolar space were evident. Immunomorphological evaluations were performed, using antibodies against cytokeratins (CKAE1AE3, and CK7) to detect pneumocytic hyperplasia and tendency to form aggregates. We also performed immunohistochemistry experiments to characterize the abnormal cells with large, irregular, and monstrous nuclei that were present in the lung parenchyma. The data showed that these cells are megakaryocytes (CD61 positive) as expected from the thrombotic pathology of COVID-19. Immunomorphological evaluation was performed, also, to verify the presence of two molecular chaperones, Hsp60 and Hsp90, which we hypothesize play a role in pathogenesis. We observed elevated levels of both chaperones in the COVID-19 lungs compared with control samples. Bioinformatics analysis showed that Hsp60 and Hsp90 share immunogenic epitopes with SARS-Cov-2 proteins. These shared epitopes could induce an autoimmune response by molecular mimicry and contribute to the establishment of Long Covid syndrome.

ENVIRONMENT AND HEALTH

NEW STANDARDISED PROCEDURE TO EXTRACT GLYPHOSATE AND AMINOMETHYLPHOSPHONIC ACID FROM ORGANIC AND INORGANIC MATRICES: TOWARD A PRACTICAL KIT FOR HPLC-UV DETECTION

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Glyphosate is used in agriculture worldwide and is the most popular herbicide because it is able, at least temporarily, to quickly eliminate all vegetation. It is widely used as herbicide in woody crops (such as vineyards, olive trees, hazelnut and almond) but also in horticultural and cereal crops, strawberries, sunflower, rice, soy, in industrial areas and railway sites. It has also been used for drying barley and wheat for the brewing industry. The herbicide is also sprayed on wheat spikes and used for legumes, potatoes and oilseeds. The substance is classified, according to Directive 67/548/CEE, irritating and dangerous for the environment (Xi, N) with the risk phrases R41 (risk of serious damage to eyes), R51/53 (toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment) and the formulations are classified as dangerous for humans and/or the aquatic environment. People, plants and animals can easily be exposed to this substance during applications. All natural terrestrial and aquatic habitats characterized by vascular plants that are located in the vicinity of the sprayed fields can be damaged and contaminated by this herbicide. Residues are frequently found in food and in the environment and is one of the most widespread substances in surface waters. In November 2017 a European Commission resolution has been approved and glyphosate-based herbicides are formally allowed for agriculture until 2022. Among its derivatives, the most stable and abundant is THE aminomethylphosphonic acid (AMPA). As of today, the safety of glyphosate in mammals is still under debate. Acute intoxications due to its ingestion are reported to strongly affect cardiovascular system, as well as to cause gastrointestinal and respiratory symptoms, hypotension and consciousness alteration. The long-term effects of a chronic exposure to glyphosate and AMPA are not clear. Some *in vitro* studies on different mammalian cell lines showed glyphosate to be genotoxic, cytotoxic and reprotoxic. Glyphosate toxicity is usually associated with oxidative stress and dysfunction in mitochondria dynamics and bioenergetics. In support of our basic research program on the biological effect of glyphosate and AMPA, we developed a fast, low-cost and reliable method to determine their concentration in biological samples. The chromatographic procedure has been validated based on several parameters including specificity, selectivity, matrix effect, accuracy, precision, calibration performance, limit of quantification, recovery and stability. Analytes extraction protocol has been tested on different specimens using SPE: anion exchange resin is used and elution is performed, after conditioning, with hydrochloric acid 50.0 mmol/L. For HPLC determination, the analytes are derived and injected in the HPLC with a C18 column and using mobile phase consisting of phosphate buffer 0.20 mol/L at pH 3.0 and acetonitrile (85:15). The eluate is monitored at 240 nm. All procedure steps are carried out at room temperature and analysis is performed in 8 min. In order to quantify substances levels each calibration curve will be

obtained using more calibration points. Calibration curves are created by internal standard quantification method. A quadratic regression is used for all curves. Our procedure aims to unify protocols to extract and determine glyphosate and AMPA in different biological matrices. The developed method will represent a practical resource for experimental, medical, quality control, alimentary and several other applications.

VIABILITY CELLS OF MYTILUS GALLOPROVINCIALIS EXPOSED TO ACETYSALICYLIC ACID

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In recent years one of the most discussed topics is environmental pollution. Among the contaminants, drugs play an important role. In fact, significative concentration in aquatic system was found; among them could be acid acetylsalicylic (ASA)¹. ASA is a non-steroidal anti-inflammatory drug, and it is the most used salicylate, but there isn't much information about the effects on mussels and other non-target organism¹. For this reason, the study was conducted on the marine invertebrate *Mytilus galloprovincialis*, chosen for bioaccumulation capacity and for its essential position in food chain and economy². The viability in haemolymph and digestive gland cells of *M. galloprovincialis*, was observed after chronic exposure to ASA, for 10 and 20 days to increasing concentrations: 0 µg/L (control), 10 µg/L (ASA 1), 100 µg/L (ASA 2). We tested cell viability of haemolymph and digestive gland cells by two assays: -T rypan Blue (TB) exclusion test; -lysosomal membrane stability by Neutral Red (NR). The data collected indicate a cell viability of more than 80% in both assays. In conclusion, after chronic exposure to ASA, membrane integrity was maintained. These results don't exclude the possibility of a bioaccumulation in tissues of mussels.

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ZEBRAFISH: OBESITY AND NATURAL COMPOUNDS

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Obesity is a pathological condition characterized by an excessive accumulation of body fat causing serious damage to health. It is a result of an alteration in energy balance in which energy intake exceeds energy expenditure. According to the World Health Organization (WHO) obesity represents a fast growing epidemic worldwide [1]. Obesity is a plurifactorial disease generated by both exogenous and endogenous

factors on which natural compounds have potential beneficial effects. In the last decade, many studies reported antioxidant, anti-inflammatory properties of natural compounds and their capacity to control adipogenic factors. Thus, their use as putative safe treatments and/or supplements for obesity and its comorbidities have been recommended. Zebrafish is emerging as an important model organism to study obesity and related metabolic disorders because it shares remarkable similarities in lipid metabolism and adipogenic pathway with mammals. This study evaluates the potential beneficial effects of melatonin and flavonoid-rich extracts from *Citrus sinensis* and *Vitis vinifera* on diet induced obesity zebrafish model (DIO) [2]. Histological analysis showed a subcutaneous and visceral adipocytes reduction, demonstrating a lipolytic action of the tested natural compounds. Moreover, the employed compounds modulated the orexigenic genes expression (leptin, POMC) and displayed an anorexic action (ghrelin, orexin, NPY). This study adds new insights into the anti-obesity properties of the tested natural compounds, suggesting a weight manager role through a lipolytic action linked to a restoration of metabolism.

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EARLY-LIFE EXPOSURE TO TRICLOCARBAN INDUCES OCULAR DEVELOPMENTAL TOXICITY IN THE ZEBRAFISH MODEL: HISTOLOGICAL, MOLECULAR AND BEHAVIORAL INVESTIGATIONS

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Triclocarban (TCC) is an antimicrobial compound widely used in personal care products. This chemical is recently classified as an endocrine-disrupting chemical due to its potential to affect the hormonal balance in exposed organisms, including humans. The detrimental consequences of hormonal disruption are mainly investigated through the evaluation of reproductive toxicity. However, a hormonal imbalance could be responsible also for neurotoxicological effects and ocular developmental toxicity. The study of TCC oculotoxic effects in the zebrafish model is a topic of high concern. The present study aimed to evaluate the developmental and long-term consequences of TCC exposure on the visual function of zebrafish. Two concentrations of TCC, including a sublethal concentration and an environmentally relevant concentration of human exposure, were tested in the zebrafish model. The expression level of two genes involved in visual function development, namely *mitfb* and *pax6a* was investigated through Real-Time PCR in 5 days post-fertilization zebrafish larvae. Behavioral tests were executed in zebrafish larvae to evaluate the general behavioral phe-

notype and the visual ability of exposed larvae. Moreover, the potential persistent effects of developmental exposure to TCC were evaluated through histological analyses and behavioral tests in zebrafish juveniles. The results of the present study confirmed the ocular developmental toxicity of TCC in the zebrafish model. The gene expression of *mitfb* and *pax6a* was downregulated in zebrafish larvae exposed to both concentrations of TCC, which showed also reduced activity and increased thigmotaxis (a measure of anxiety) compared to controls. The histological analyses revealed alterations in the thickness of retinal layers, without disorganization of the general structure. Moreover, zebrafish juveniles exposed during development to TCC showed impaired visual discrimination abilities and increased sociability. Overall, the results of the present research highlight the negative developmental and long-term consequences of TCC exposure on the visual function of the zebrafish model.

HYPOTHESIS OF HUMAN PATHOGENIC MICROBIOLOGICAL PRESENCES WITHIN THE CATABOLITES OF XYLOPHAGOUS INSECTS IN URBAN CONTEXT

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The presence of xylophagous insects in urban areas is a consolidated aspect that has recently been gaining a certain notoriety above all following reports of collapsing structures of buildings of architectural and cultural-historical interest. The relationship between xylophages and wooden structures inside buildings like beams, floors and fixtures, not only concerns the removal of the cellulose that makes up wood but also its replacement with "secondary" wood or with a pulp composed of cellulose digested and regurgitated by the insect, that the mere presence of insect catabolites. As for the replacement, its function is variable: mainly its purpose is to mark a territory making it exclusive as a foraging ground for an entire species or for a single family group; in the case of termites, the substitution also has the function of chemical marking of the path. As for the catabolites, these are typically composed of cellulose and a small percentage of substances deriving from its intake; as for woodworms, catabolites are notoriously present inside the tunnels dug by the larva and outside the artifact or structure transformed into a table by the woodworm. The burrows of the woodworm are often populated by "foreigners" such as the Scleroderma Hymenoptera, parasite of the Woodworm, which is a hematophagous and prefers humans for its foraging. The pandemic period we are experiencing, hopefully in its twilight phase, has stimulated us to study xylophagous residues in relation to the presence of viruses and bacteria that are pathogenic to humans. A large number of homes welcomed people in quarantine because they tested positive. Being positive for the swab corresponded to being a virus carrier. One of the things that have become clear since the start of the pandemic is that the SARS-Cov2 type virus is spread by means of aerosol in the air. The same air that xylophages breathe and that surrounds their catabolites, whether they are abandoned digestive residues or re-elaborations of their digestate for the purpose of marking the territory. The research, with regard to xylophages, was based on two main areas: the analysis of the traces of marking (termite walkways) and analysis of the stomach contents of the Scleroderma hymenoptera. The results obtained from the first

year of research have allowed us to understand that there is a permanence in life of microorganisms both within the replacement wood and in the catabolites present inside and outside the foraging areas. It was found that the humidity of the replacement wood and the catabolites is related to the duration of the half-life of the viruses present. It has also been noted that the type of wood (therefore the general composition of the rosura and the catabolites) can influence, all other things being equal, the half-life time of the viruses present. From the point of view of the transmissibility of the viruses, given the stomach contents of the collected *Scleroderma* specimens, it was possible to verify that the microorganisms survive for a certain period and can be transmitted to humans.

THE USE OF NATIVE SPECIES FOR URBAN FORESTRY TO PREVENT ALLERGIES

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The persistence of populations migratory flows from the rural to the urban context certainly alter the habits in the connection with nature and, generally, decrease the quality of life and the well-being of inhabitants of cities. The pandemic highlighted how crucial is for citizens the connection with nature and the key role of vegetation in parks and gardens defined as a real cultural heritage to be protected and enhanced. It is not important design and develop new green areas only but also to re-evaluate and re-appropriate existing ones. Urban reforestation projects must not be just green restoration actions, but real systematic and structured programs, which consider the whole territory and its complexity. Each "green" action must be integrated inside the territory and connected with the context. The planting of thousands of new trees in the several development plans requires basic and applied research to have adequate material to effectively perform the required functions such as air purification, climate mitigation, water regulation, environmental rehabilitation, etc. (Anguelovski *et al.* 2018) The identification of native species that can be successfully used for urban landscaping and reforestation in the Mediterranean environment is crucial. Native species have the advantage of already being adapted to the environment in which they will be used, while the exotic species currently used have been selected based on aesthetic characteristics and their resistance, with low consideration of the risks on human health and ecosystem's biodiversity. One of the most underestimated issues is the allergenicity of species used for urban reforestation. Allergies are the result of a hypersensitive response of the immune system to foreign agents, called allergens, which can be represented by very different substances. (Bro ek *et al.*, 2017) In particular, pollen allergies affect the respiratory system and are characterized by seasonality and recurrence during the year, determined by the cycle of plants that produce and release into the environment the different types of pollen, large quantities of which enter the respiratory tract (Lake *et al.*, 2017). Normally this event does not have consequences, whereas in allergic people the release of pollens and their "migration" causes allergic rhinitis, commonly called hay fever, and in more serious cases it can cause real asthma attacks. (Cariñanos, *et al.* 2016.) The best fight against allergy is to try to avoid contact with the allergenic substance. For pollen this is very complicated because it means not staying outdoors during the migra-

tion period, closing windows and using air filters and air conditioning systems. In order to avoid the worsening of this problem during urban reforestation programs, it is recommended the introduction of species with low pollen emission and low or no allergenicity.

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METABOLIC EFFECTS OF POLYSTYRENE MICROPLASTICS IN MARINE MUSSELS AFTER SHORT-TERM EXPOSURE

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Over the last 50 years, plastic has saturated our world due to the high demand across all sectors because of its favourable properties. Despite the societal benefits, plastic is today a global concern owing to its persistence and bioavailability as microplastics (MPs) to aquatic biota. MPs have been found in all compartments of the environment, and numerous life forms are known to take up the anthropogenic particles. Marine filter feeders are particularly susceptible to ingest suspended MPs since their size overlaps with the size of planktonic organisms and sediments, making them bioavailable and thus facilitating their entry into the food chain. In the last years, mussels were used as test organisms in many laboratory studies to assess the ecotoxicological effects induced by MPs, but the majority of these studies covered more than one week of exposure. With the aim to fulfil this research gap and elucidate mechanistic insights into the early toxicity effects of MPs on aquatic invertebrates, this study was designed to conduct a short-term (up to 72 h) exposure to 3 mm red polystyrene MPs (50 particles/mL) in marine mussels *Mytilus galloprovincialis*, selected as model organism because being filter-feeders and thus able to ingest MPs, besides for their commercial relevance. The application of an innovative protonic Nuclear Magnetic Resonance (¹H NMR)-based metabolomics, associated to chemometrics, enabled a comprehensive exploration at fixed exposure time-points (T24, T48, T72) of the metabolic effects of MPs accumulated in mussel digestive glands, chosen as the major site for contaminants storage and detoxification processes. Specifically, a Principal Component Analysis (PCA) clearly separated ¹H NMR metabolic fingerprints of MP-treated mussels from control, and a clear grouping was observed according to experimental time-points. Numerous metabolites, including amino acids, osmolytes, metabolites involved in energy metabolism, and antioxidants, participating in various metabolic pathways significantly changed over time in MP-exposed mussel digestive glands related to control, reflecting also the fluctuations in MPs accumulation and pointing out the occurrence of disorders in amino acid metabolism, osmotic equilibrium, antioxidant defense system and energy metabolism. Overall, findings from this work elucidate time-dependent metabolic disor-

ders induced by polystyrene MPs in mussels and therefore the early mechanisms of toxicity of MPs in marine filter-feeder invertebrates, which are indicative of the health risk for biota associated to MPs exposure.

TIME-DEPENDENT BIOLOGICAL IMPACT OF ENVIRONMENTALLY REALISTIC DOSES OF CAFFEINE VS. CAFFEINE-SALICYLIC ACID MIXTURES ON MYTILUS GALLOPROVINCIALIS

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Nowadays, wastewaters are frequently impacted by a significant input of pharmaceutical active compounds (PhACs) from urban and hospital activities. In several instances, the performance of wastewater treatment plants (WWTPs) are ineffective to counteract the PhACs contamination of aquatic environments, particularly in coastal areas close to high anthropogenic impacted sites. In details, by a chemical investigation within the area of Mahdia (Afsa *et al.*, 2020), among the various PhACs, caffeine (CAF) and salicylic acid (SA) were detected in relevant concentrations at different sampling points (hospital, WWTP, coastal area), highlighting their high degree of persistency in various water compartments. Thus, the assessment of the impact on biota of such permanently present pharmaceutical residues, namely CAF and SA, as single components or as a mixture of compounds is crucial for the correct estimation of the environmental risk caused by insufficiently efficient management of municipal and hospital wastewater. After a period of acclimation, specimens of mussel *Mytilus galloprovincialis* were exposed, under controlled lab condition, to five realistic concentrations of the single drug CAF (C1:0.005 µg/L; C2:0.05 µg/L; C3: 0.5 µg/L; C4: 5 µg/L; C5:10 µg/L), and five environmental concentrations of a CAF+SA mixture (C1: 0.005 µg/L+0.05 µg/L; C2: 0.05 µg/L+0.5 µg/L; C3: 0.5 µg/L+5 µg/L; C4: 5 µg/L+50 µg/L; C5: 10 µg/L+100 µg/L) during a 12-day exposure. The effects of the two experimental conditions were investigated in mussel gills, sampled at different time points (T0; T3: 3 days; T5: 5 days; T12: 12 days) in order to evaluate the temporal modulation of the biological responses. Histological (haematoxylin & eosin staining) and biochemical analysis (spectrophotometric method) were employed to assess alteration in morphology, in antioxidant system (superoxide dismutase SOD, catalase CAT, glutathione S-transferase GST, lipid peroxidation LPO), and in nervous system (acetylcholinesterase AChE). Changes in gill morphology and in antioxidant and neurotransmission systems were observed during the two experimental conditions. In details, the influence of the CAF+SA mixture compared to the single CAF exposure seemed to have a different time-related impact. Indeed, in the CAF+SA mixture, the biological activities of AChE and GST arose more intensely and at earlier stages than CAF exposure. Also, contrarily to what found after CAF exposure, mussels exposed to the combined CAF+SA exposure showed a more regular oxidative system (SOD, CAT and LPO) comparable to control, therefore suggesting that the presence of SA is able to reduce the harmful effects induced by CAF, particularly at T12. In light of the data reported, the assessment of the biological effects of PhACs as single or mixed compounds enables to enquire in a more realistic perspective the impact of environmental contamination due

to pharmaceutical residues, and to emphasise the urgency of defining new guidelines for the improvement of sewage treatment plant activities.

ENVIRONMENTAL CONTAMINANTS IN ALGERIAN AROMATIC HERBS

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Aromatic herbs play an important role like anticancer, antimicrobial, antioxidant, anti-inflammatory. They are used in cosmetic, medical sciences, culinary sciences (in this field they improve the flavour and contribute to the reduction of salt added, with health benefits). It is also seen some aromatic herbs, by their antiviral properties, could be used as prophylactic/preventive drugs or compounds against COVID-19. However, aromatic herbs may contain residues of chemical contaminants due to phytosanitary treatments or absorbed by the soil or the environment. It is well known that contaminants can pose a serious risk to human health. In this study, 43 samples of aromatic herbs (7 samples of *Mentha L.*, 9 samples of *Verbena officinalis L.*, 9 samples of *Foeniculum vulgare Mill.*, 11 samples of *Laurus nobilis L.* and 7 samples of *Origanum L.*) purchased in Algeria, big exporter in UE market, are analysed to evaluate the residues of 119 environmental contaminants. Polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAH), organochlorine pesticides (OCPs), organophosphorus pesticides (OPPs), pyrethroid insecticides (PYRs), fungicides (Fs), herbicides (Hs), synergists, carbamates, acaricides, and insect growth regulators (IGRs) were extracted with QuEChERS method and simultaneously analysed by GC-MS/MS. The analyses showed that the levels of environmental contaminants in herb samples are less to the maximum limits established by the European Regulatory Commission. From the results of this study, it can be assumed that human consumption of herbs does not pose a health risk.

AFLATOXIN B1 TOXICITY IN ZEBRAFISH LARVA (DANIO RERIO): PROTECTIVE ROLE OF HERICIUM ERINACEUS

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Aflatoxin B1 (AFB1), a secondary metabolite produced by fungi of the genus *Aspergillus*, has been found among various

foods as well as in fish feed. However, the effects of AFB1 on fish development and its associated toxic mechanism are still unclear. In the present study, we confirmed the morphological alterations in zebrafish embryos and larvae after exposure to different AFB1 doses as well as the oxidative stress pathway that is involved. Furthermore, we evaluated the potentially protective effect of *Hericium erinaceus* extract, one of the most characterized fungal extracts, with a focus on the nervous system. Treating the embryos 6 h post fertilization (hpf) with AFB1 at 50 and 100 ng/mL significantly increased oxidative stress and induced malformations in six-day postfertilization (dpf) zebrafish larvae. The evaluation of lethal and developmental endpoints such as hatching, edema, malformations, abnormal heart rate, and survival rate were evaluated after 96 h of exposure. *Hericium* inhibited the morphological alterations of the larvae as well as the increase in oxidative stress and lipid peroxidation. In conclusion: our study suggests that a natural extract such as *Hericium* may play a partial role in promoting antioxidant defense systems and may contrast lipid peroxidation in fish development by counteracting the AFB1 toxicity mechanism.

MULTI-STRAIN PROBIOTIC FORMULATION IMPROVE INTESTINAL BARRIER FUNCTION BY MODULATION OF TIGHT JUNCTION PROTEINS

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In healthy individuals, tight junction proteins maintain intestinal barrier integrity and avoid the entry of endotoxins, pathogens and pro-inflammatory molecules in systemic circulation. Dysbiosis and increased intestinal permeability were observed in several diseases including intestinal bowel disease (IBD), irritable bowel syndrome, diabetes, acute pancreatitis, non-alcoholic steatohepatitis and neurodegenerative disorders [1, 2]. Antibiotic use, stress, dietary changes and aging represent the most common causes that lead alteration of intestinal microflora and dysbiosis. Many studies highlight the role of probiotics in preventing intestinal barrier dysfunction [3, 4]. The aim of this study is to investigate the effects of a probiotic formulation of *L. rhamnosus* LR 32, *B. lactis* BL 04 and *B. longum* BB 536 (Serobioma, Bromatech s.r.l, Milan, Italy) toward tight junction proteins (*i.e.*, Zonula Occludens, E-Cadherin, Occludin, Claudins) expression and intestinal epithelial barrier integrity. Moreover, we evaluated the capability of this formulation in preventing the inflammation-associated damage induced by lipopolysaccharide (LPS) of the intestinal epithelial barrier. We set up an *in vitro* model of intestinal barrier using 21-days Caco_2 cell monolayer. mRNA expression levels of tight junction genes were analysed using RT-qPCR. Moreover, changes in protein amount were evaluated with Western Blotting. Finally, the effect of Serobioma on intestinal epithelial barrier function was evaluated using Trans-Epithelial Electrical Resistance (TEER) measurement. Preliminary results suggest that Serobioma modulates the expression of tight junction proteins and prevents inflammatory damage in Caco_2 cells challenged with LPS. Our results provide new insights into mechanisms with which probiotics prevents intestinal epithelial barrier damage and contributes in maintaining gut health.

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BIOACCUMULATION AND IDENTIFICATION OF BIOMARKERS ON BULL EXPOSED TO ENDOCRINE DISRUPTORS

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The interest of the scientific community regarding the potential effects on human health and the environment deriving from the exposure of substances that may interfere on the endocrine system, is significantly increased in the last decade (Rim, 2017). Endocrine disrupting chemicals (ECDs) are substances which are capable of simulating the activity of hormones, and therefore responsible for dangerous functional imbalances. In particular, the ability of these molecules to weaken the activity of male hormones (androgens) and to strengthen that of female estrogens results in a reduction in the number and motility of spermatozoa (Diamanti-Kandarakis *et al.*, 2009). All of these chemicals can be dispersed into the environment or through air pollution or into water and soil (Salvaggio *et al.*, 2019; Gonsioroski *et al.*, 2020). The aims of this research is to evaluate the expression of exposure biomarkers on bull (*Bos taurus*) correlated to bioaccumulation of plastic additives. Samples, from controlled and certified farms, were taken from local slaughterhouses and transported to the laboratory. A quantitative and qualitative analysis of contaminants in target tissues (liver and gonads) has shown a higher mean value especially for 2-ethylhexyl phthalate (DEHP) (19.49 ng/g in testis, 14.83 ng/g in ovaries, 13.85 ng/g liver male and 40.97 in liver female) and mono-n-butyl ester phthalate (MNP) (12.73 ng/g in testis, 11.69 ng/g in ovaries, 8.58 ng/g liver male and 11.15 in liver female). Moreover, a immunohistochemical analysis has demonstrated a positivity for Heat Shock Proteins 70, Cytochrome P450 1A and Vitellogenin biomarkers in all samples, indicating a response towards environmental stress.

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ANALYSIS OF SPATIAL AND TEMPORAL VARIATIONS OF ALL-CAUSE MORTALITY BY TOWN URBAN UNIT: UPDATE ON A MORE DETAILED SPATIAL DEFINITION OF THE EXPERIENCE IN GENOA

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The Municipal Epidemiological Report makes it possible to carry out a reliable, and economic monitoring of total mortality in all the districts of an entire municipality, such as Genoa. The main objective of this study was to analyse the space-time epidemiological differences by sex during the 2009–2020 period in the total mortality recorded among residents in each of the 71 urban units of the Genoa municipality, net of the age effect. The analysis was based on official statistical data relating to total mortality and on the resident population (on average about 591,000 inhabitants during the period considered, starting from 609,000 inhabitants in 2009 to 572,000 in 2020). An estimate of the expected deaths was made to calculate the sex-specific age-standardised mortality ratio (SMR). The temporal trends and age-standardized death rates (SDRs) with respect to those of the European population specific to sex and calendar year were identified for each urban unit. Over the entire observation period, the total SMR ranged from 157.7 (Begato unit) to 77.0 (Puggia unit); for females, the values ranged between 172.7 (Begato) and 78.5 (Puggia); for males, the values ranged between 142.9 (Ca' Nuova) and 74.7 (Puggia). Between 2019 and 2020, Genoa recorded an increase in SDR of 24.5%, more pronounced in males (+26.7%) than in females (+22.4). This epidemiological methodology is replicable and allows to quickly identify spatial, temporal, sex, and age differences in the general mortality within a municipality.

THE ANTIMICROBIAL POTENTIAL OF AN HEXANE OIL FRACTION AND POLYPHENOLS-RICH EXTRACTS FROM *PISTACIA VERA* L

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The excessive use of antibiotics, both in the health care setting and in the agricultural industry, has been associated to the spread of resistant microorganisms. Antimicrobial resistance became a global threat for public health and for the economy [1]. To date, more global effort is focused on novel therapeutics and natural drugs, to use alone or in combination with conventional antibiotics. Plant oils have been known for several years as source of pharmaceutical agents and promote a wide spectrum of beneficial effects on human health. Plant oils and extracts have been shown to have antimicrobial properties as well as active effects when used in combination

with existing antibiotics. In this study, *Pistacia vera* L. was used. Pistachio nuts are a good source of nutrients and phytochemicals, which are responsible of their health benefits [2]. *Pistacia vera* polyphenols have been shown to have antimicrobial and antiviral potential [3–4], as well as a modulatory effect on the gut microbiota. In particular, RP-HPLC-DAD analysis revealed the presence of thirteen compounds, belonging to flavonoids and phenolic acids in the hexane oil fraction and the polyphenols-rich extracts. The main identified compounds were gallic acid, catechin, cyanidin-3-O-galactoside and isoquercetin in the range of 0.9–2.7 mg/100g. Protocatechuic acids, caffeic acid, epicatechin, luteolin, chlorogenic acid and quercetin-3-O-rutinoside were detected in significant amounts (0.1–0.8 mg/100g), while the other compounds (eriodictyol-7-O-glucoside, daidzein and eriodictyol) were only present in trace. *Pistacia vera* hexane oil fractions, obtained from both raw and roasted pistachios, have been tested against a range of Gram-positive and Gram-negative bacteria and the yeast *Candida albicans*, showing MIC values ranging between 0.25–2.0 mg/ml. In addition, the antibacterial activity of pistachio polyphenols-rich extracts has been investigated against a range of *Listeria monocytogenes* food isolates, which represent a significant cause of food-borne illness, with MIC values ranging between 0.25–2.0 mg/ml. These results have highlighted that phytochemicals obtained with different extraction methods from both raw and roasted pistachios, alone or in association with known antimicrobial agents, could represent a starting point for the development of novel formulations and treatment also in the cases of drug resistance.

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CNIDARIAN JELLYFISH AS A NEW FOOD SOURCE. A TOOL TO CHANGE A PROBLEM TOWARDS A RESOURCE?

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Cnidarian jellyfish are ancient inhabitants of marine waters worldwide. These organisms have a great importance because of their role in ecosystems and in the food web of the marine environment, as well as for their relationships with other organisms, such as predation, commensalism, symbiosis, etc. Jellyfish are also a subject of concern for their toxicological properties which put them among the most dangerous marine organisms, some species being able also to kill humans and anyhow to cause serious pathological consequences. As a matter of fact, a lot of envenomations were reported and treated in emergency first aid services worldwide, and an abundant literature is available about the serious damage suffered by bathers and sea workers, notably in

some coastal areas, such as in Australia and in many tropical zones, where the most dangerous jellyfish live and exert their toxic/venomous activity which may induce dermonecrosis, as well as respiratory and cardiological complications. These aspects greatly affect some recreational and working activities of high economic concern. But also in the Mediterranean area several toxicity phenomena consequent to jellyfish stinging, some of them required the resort to first aid care in emergency services, and some lethal cases were reported. In spite of this, during the last decades cnidarian jellyfish have been viewed more and more as a source of interesting extracts and compounds having beneficial properties from the pharmacological and nutraceutical point of view. Really, new research projects have emphasized the great potential for the direct use of jellyfish as food and in the food industry, due to their high content of bioactive peptides and proteins, mainly collagen, and to the occurrence of other interesting bioactive compounds useful for their antioxidant activity. Really, jellyfish are for long utilized in Asian - mainly Chinese, Vietnamese and Japanese - cultures, where they are already on the menu. But for all above reasons, at present jellyfish are starting to be considered as a source of food for human consumption also in western Countries. From the nutritional point of view, it should be considered that jellyfish are rich in protein and collagen, fat-free and furnish a quite low caloric input. Furthermore, from the point of view of the conservation of marine resources, the utilization of jellyfish seems to have a scarce impact, because the removal of jellyfish stocks from the sea doesn't stop new one specimens being born, due to the high spawning from polyps attached to the sea bottom. This aspect profoundly differs the utilization of jellyfish from the environmental dangerousness consequent to the permanent damaging of stocks of other edible organisms, such as fish; therefore, this aspect makes the utilization of jellyfish an environmental sustainable food source. Finally, considering that jellyfish are viewed as a dangerous problem in many coastal areas around the world, due to their toxic properties, affecting also many human activities, such as fishing, tourism and many other recreational aspects having strong economic consequences, the utilization and fishing of jellyfish could contribute to change a possible problem to a useful resource.

NEURODEVELOPMENTAL TOXICITY OF SODIUM VALPROATE IN THE ZEBRAFISH MODEL: MORPHOMETRIC PROFILE AND BEHAVIORAL CHARACTERIZATION OF NEUROTOXICITY ENDPOINTS

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Valproic acid (VPA) and its sodium salt, sodium valproate (SV), are well-known anti-epileptic drugs used therapeutically for migraine, bipolar disorder, and anxiety. Despite remarkable advantages in improving the patients' quality of life, the presence of pregnancy and teratogenic risks represents one of its major health concerns. VPA maternal exposure during the first trimester of pregnancy has teratogenic effects and it is responsible for an increased risk of developing congenital spina bifida and craniofacial malformation during the early

phase of development. The teratogenic effects of VPA are mediated principally by its ability to inhibit histone deacetylase (HDAC), which leads to the increase of histone acetylation levels, thus inducing chromatin remodeling and gene transcription regulation. The VPA neurotoxicity phenotype is quite different among species (e.g., spina bifida in humans and exencephaly in mice), and it is related to neural tube defects induced by VPA-HDAC inhibition. In fish, which have exclusively secondary neurulation, the investigation of the classic neural tube defects is particularly complex to achieve, since an open neural tube would be lethal in the non-sterile environment of aquatic ecosystems. Moreover, in the field of teratology, the high concordance of the zebrafish model with human toxicity has often been noticed. Given the fundamental differences in neurulation processes between mammals and fish, additional and surrogate end-points are extremely useful to investigate the neurodevelopmental effects of teratogens chemicals, such as VPA, on zebrafish early-life stages. Among these end-points, craniofacial deformations, ocular size, and behavioral alterations are good indicators of changes potentially associated with malformation of the brain that can be related to developmental neurotoxicity. The present study aims to evaluate the lethal and sublethal alterations induced by SV in the zebrafish model, focusing on the quantitative characterization of the three developmental neurotoxicity end-points. The eye size, in terms of interocular distance, eye length, and eye width, was affected by SV exposure. Moreover, the craniofacial deformations were studied using Alcian-blue staining and morphometric analysis of craniofacial structures, including Meckel's-palatoquadrate (M-PQ) angle, the distance between left and right PQ structures (PQ-PQ distance), PQ length, ceratohyal (CH) angle, M angle, the distance between left and right M structures (M-M distance), the distance between M and CH cartilage (M-CH distance) and PQ-CH angle were altered in zebrafish larvae exposed to SV. The behavioral alterations developed by zebrafish larvae exposed to SV were characterized using automated measurements of distance moved, thigmotaxis, visual startle response, and photic entrainment of circadian rhythms.

SPONDYLUS GAEDEROPUS CELL LINES AS AN INNOVATIVE TOOL FOR ENVIRONMENTAL TOXICITY ASSAYS

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The bivalve mollusk *Spondylus gaederopus* (Linnaeus 1758) is a species indigenous to the Mediterranean Sea, popularly known as "spiny oyster" because of its irregular-shaped hollow spines on the shells. Morphologically, these bivalves can reach a maximum length of 15 cm in proper conditions and can live up to 18 years. Populations are characterized by a low density, random distribution, with specimens isolated or in aggregations of no more than 9 individuals. *S. gaederopus* lives in the infralittoral plane, mainly in shallow waters (<50m) in pristine areas with a specific hydro dynamism, firmly anchored through the valves to rocky grounds¹. During our study, the suffering of the specimens was already highlighted when they were taken from their natural habitat. For this reason, it was decided to maintain the animals for a week in artificial settings reflecting the original conditions, to minimize the stress. The study done stems from the need to fill the gap of information regarding the morpho-functional aspects of the

hemocytes of this poorly known bivalve. Therefore, this work aimed to characterize the circulating cells in the hemolymph: *i.e.*, the hemocytes². Hemocytes play a key role in the immune response of *Spondylus gaederopus*, so, in addition to cell characterization, a phagocytosis assay was added, using the yeast *Saccharomyces cerevisiae* (1×10^7 yeast mL⁻¹) as a foreign body. The ratio of hemocytes to yeast was maintained at 1:10 throughout the experiment. From the tests performed, multiple cell lines with and without phagocytic capacity were observed. In conclusion, this study is the first in the use of *S. gaederopus* to assess environmental toxicity, as an early step in building background knowledge on a wider set of promising biomarker organisms.

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SODIUM LAURYL SULFATE EXPOSURE ON ZEBRAFISH: LOCOMOTOR AND SOCIAL ALTERATIONS

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Detergents are now defined as emerging pollutants, primarily because of their ubiquity in the environment, and their toxic effects even at low concentrations. Sodium lauryl sulfate (SLS) is an anionic surfactant and is a common ingredient in household cleaning and personal care products. Due to the heavy use of all these products, SLS is commonly found in wastewater and sewage systems, becoming a highly concentrated pollutant and a toxic substance to aquatic species. Therefore, the aim was to evaluate the toxicity of this detergent in zebrafish (*Danio rerio*). For acute exposure (30 hours), two different concentrations of the pollutant were chosen: 0.5 µg/L and 1 µg/L plus control. In contrast, for chronic exposure (14 days), four different concentrations were evaluated: 0.25 µg/L - 0.50 µg/L - 1 µg/L - 1.5 µg/L. For both exposures, the individuals were randomly selected and divided into aquariums. During the experiment, these were monitored using video-tracking software to assess their locomotor parameters, such as distance moved, active movement, and acceleration. For the variable "active movement" during acute exposure, significant differences (*p < 0.05 ANOVA) were observed between the control group and the group with 1.5 µg/L SLS concentrations. The increasing values may be indicative of substance-induced stress in animals. During chronic exposure, however, the group most affected by SLS seems instead to have been the one exposed to the lowest concentration (0.25 µg/L). This is evidence that at lower concentrations but with a long persistence time in the environment, SLS appears to be more toxic. Heat maps were also produced to highlight how much time individuals spent in contact with "social stimuli". In both treated groups, individuals altered their sociability. Indeed, they tended to isolate themselves or move away from other animals placed as stimuli.

SPARC PROTEIN: INDUCER OF REMODELING ALSO IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD)?

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The SPARC (secreted protein acidic and rich in cysteine) proteins family is a highly conserved group of matricellular molecules. SPARC family proteins are characterized by an N-terminal acidic domain, a cysteine-rich follistatin-like (FS) domain, and an extracellular α -helical (EC) calcium-binding domain with an EF-hand motif. One of the most studied members of this family of proteins is SPARC. It is more frequently encountered as a secreted glycoprotein but is also expressed on the cell surface and in the intracellular compartment. Expression of SPARC is elevated during embryonic development and decreases in normal adult tissues. Therefore, this molecule is multifunctional as it is involved in extracellular matrix remodeling and turnover processes. In particular, SPARC regulates cell adhesion, proliferation, and migration. It mediates interactions between cells and their surrounding extracellular matrix (ECM), regulating, in addition, ECM-associated growth factors and signaling: TGF- β , for instance. Its expression in non-small cell lung cancer (NSCLC) is well known. It has been identified as a key mediator of cell invasion and thus may play an impactful role in the metastatic process. The expression of SPARC, in contrast, in chronic airway diseases such as COPD (chronic obstructive pulmonary disease) is poorly understood. Therefore, in this work, we aimed to investigate the expression of SPARC protein in subjects with COPD, in comparison with two control groups, divided between smokers and non-smokers. COPD is a heterogeneous inflammatory disorder of the respiratory tract characterized by airflow limitation, typically progressive and irreversible. SPARC may contribute to changes in airway structure and function through several mechanisms. Changes in ECM setting, angiogenesis, and epithelial-mesenchymal transition (EMT), for instance. Altered ECM protein deposition is a key feature of airway wall remodeling in COPD, so any change in SPARC expression or activity will likely affect ECM changes in these conditions. The study was conducted through the application of immunohistochemical techniques combined with an enzymatic detection system (DAB). The results obtained highlight the expression of SPARC protein in the apical cellular compartment that characterizes the bronchial epithelium. In detail, a mild expression of SPARC protein was observed in the "smoker control" tissues. This expression decreases to minor in "non-smoking control" tissues and, finally, appears slight or almost absent in COPD patients' tissues. These preliminary results are in keeping with the possible inhibition of SPARC following the altering of the epithelial-mesenchyme communication pattern. This modification is more pronounced in COPD than in potentially "healthy" tissues. In addition, these results suggest a connection between this protein expression and the level of TGF- β , which is known to be more marked in "smoker tissues" rather than in "non-smokers". This study lays the foundations for

advancing future research to investigate and evaluate SPARC activity in chronic airway diseases, poorly known so far.

MICROGRAVITY EFFECT ON CYANOBACTERIA UNDER EXTRA-TERRESTRIAL GROWING CONDITIONS TO SUPPORT MANNED SPACE MISSIONS

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The growing interest of several space agencies to undertake manned missions on asteroids, Moon and Mars over the next 40 years is well known. In view of this, supplementary resources cannot be continuously provided from Earth due to the relative unaffordable cost of the mission and must be produced by exploiting available *in situ* resources. However, food production cannot avoid the exploitation of microorganisms and bioengineering techniques. In this context, a recent field of research, known by the acronym bio-ISRU (*In Situ Resource Utilization*), is being developed to investigate the possibility of producing food in-situ through bioengineering techniques using regolith and the Martian atmosphere. The use of cyanobacteria and microalgae leads to the additional positive effect of producing photosynthetic oxygen which is crucial for the crew and can supplement the quantities produced through physico-chemical ISRU processes. In this context, the possibility of cultivating *Spirulina Platensis* to produce food for crew members on Mars was studied. The experiments were carried out with a device capable of simulating microgravity and an atmosphere very similar to the Martian one in terms of chemical composition. Preliminary results show that the considered strain was capable to growth better under Martian simulated conditions using the in-situ produced growth medium than under Earth ones using optimal growth medium.

OXYSTEROLS PROFILE IN ZEBRAFISH EMBRYOS EXPOSED TO TRICLOCARBAN AND PROPYLPARABEN. A PRELIMINARY STUDY

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Oxysterols have long been considered as simple by-products of cholesterol metabolism, but they are now fully designed as bioactive lipids that exert their multiple effects through their binding to several receptors, representing endogenous mediators potentially involved in several metabolic diseases. There is also a growing concern that metabolic disorders may be linked with exposure to endocrine-disrupting chemicals (EDCs). To date, there are no studies aimed to link EDCs exposure to oxysterols perturbation-neither *in vivo* nor *in vitro* studies. The pres-

ent research aimed to evaluate the differences in oxysterols levels following exposure to propylparaben (PP) and triclocarban (TCC) in the zebrafish model using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Zebrafish embryos were exposed, immediately after fertilization, to two different concentrations of PP and TCC up to 24 h post-fertilization (hpf). 22-Hydroxycholesterol (22-OH), 25-hydroxycholesterol (25-OH), 24-hydroxycholesterol (24-OH), 27-hydroxycholesterol (27-OH), 20d-hydroxycholesterol (20-OH), 7 β -hydroxycholesterol (7b-OH), 7 β -hydroxycholesterol-d7 (7b-OH-d7), and 7 α -hydroxycholesterol (7 α -OH) were detected, in the zebrafish embryos after exposure to these substances, at 8 hpf and 24 hpf. Following exposure to PP and TCC, there were no significant changes in total and individual oxysterols compared with the control group; however, some interesting differences were noticed: 24-OH was detected only in treated zebrafish embryos, as well as the concentrations of 27-OH, which followed a different distribution, with an increase in TCC treated embryos and a reduction in zebrafish embryos exposed to PP at 24 hpf. The results of the present study prompt the hypothesis that EDCs can modulate the oxysterol profile in the zebrafish model and that these variations could be potentially involved in the toxicity mechanism of these emerging contaminants.

PINCTADA IMBRICATA: AN EFFICIENT "ALIEN" AS A BIOMARKER

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The "Lessepsian" migration, although circumscribed to the Mediterranean Sea, represents the most significant biogeographical event worldwide. The pearl oyster *Pinctada imbricata* (Röding, 1798), was one of the first species to reach the Mediterranean throughout the Suez Canal, and since that moment had the opportunity to spread up to the western basin. To use the species as an experimental model in environmental studies, we analyzed the amount of haemolymph cells, characterized the haemocytes, and investigated their phagocytosis using the yeast *Saccharomyces cerevisiae*. Morpho-functional aspects of the haemocytes of *P. imbricata* have already been studied in some organisms from tropical Australian coasts (Kutchel *et al.*, 2010), but no information is available in the literature about the haemocytes from this species in temperate waters. *P. imbricata* is able to adapt itself to high ranges of climatic, hydrological, and ecological conditions, which allows it to successfully settle in habitats very different from the native area. Moreover, it is tolerant of habitats characterized by polluted water. The species, reported from marine and transitional waters in the Strait of Messina (Central Mediterranean) by Giacobbe *et al.* (2010), is particularly abundant in the natural reserve "Laguna di Capo Peloro". From such environment, several specimens have been collected to assay our analyses on haemocytes, according to Matozzo *et al.* (2016). In this respect, preliminary positive results indicate *P. imbricata* as a promising biomarker in natural and anthropogenic stressed environments.

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ACETYSALIC ACID: PHYSIOLOGICAL CHANGES IN NON-TARGET ORGANISM *MYTILUS GALLOPROVINCIALIS*

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Drugs are considered emerging contaminants and they are often found in water in various concentrations. Among the most common are anti-inflammatory drugs. Acetylsalicylic acid (ASA) is one of the most widely produced and consumed non-steroidal anti-inflammatory drugs, in the order of several kilotons per year (Piedade *et al.*, 2020). As these substances can remain in the environment for long periods, they can also interact with non-target organisms. Particularly exposed could be filter-feeding organisms such as *Mytilus galloprovincialis*, which has physiological and cellular mechanisms that can be used as markers to assess possible interaction with pollutants (Stara *et al.*, 2021). *Mytilus galloprovincialis* for each experimental condition were exposed to two concentrations of ASA (10 mg/L and 100 mg/L) for 20 days to evaluate possible physiological alterations in regulation volume decrease (RVD) in the cells of the digestive gland and histological condition of gills and digestive gland. The concentrations chosen are in the same range as those found under ambient conditions. The data show that cells exposed to higher concentrations over a long period of time lose the ability to regulate their volume. While already after 10 days tissue damage is evident. These results suggest that ASA interacts at both concentrations, causing alterations in non-target organism. This could affect the animal itself, but also the whole ecosystem.

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THE CONTRIBUTION OF MOLECULAR TRACEABILITY TO UNCOVERING COMMERCIAL FRAUDS AND PROTECTING THE HEALTH OF CONSUMERS

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The growing worldwide diffusion of seafood products destined for human consumption requires ever more accurate health and quality controls to also fight the food frauds. A common commercial fraud is based on replacing species of value with species of lower commercial value for the evident economic gain. This illegal practice is very frequent in seafood commercialized products where the morphological identification of the species is impracticable because of food processing. The DNA analysis is the best approach to authenticate the species in seafood products and to unveil the frauds based on species substitution. In this study, the COIBar-RFLP, a molecular strategy coupling Cytochrome Oxidase I (COI) DNA barcoding with the consolidated methodology of Restriction Fragment Length Polymorphism Analysis (RFLP), was applied to search for pattern of restriction enzyme digestion, useful to fast and easily discriminate five different fish species (juveniles of *Engraulis encrasicolus* and *Aphia minuta* sold in Italy respectively as "bianchetto" and "rossetto"; icefish *Neosalanx tangkahkeii*; European perch, *Perca fluviatilis* and the Nile Perch, *Lates niloticus*). A total of 25 fresh and frozen samples were processed for DNA barcoding and analyzed against a barcode library of COI sequences retrieved from GenBank to validate some samples to be used for COIBar-RFLP analysis. Cases of misdescription were detected: 3 samples labeled as "bianchetto" were substituted by *N. tangkahkeii* (2 samples) and *A. minuta* (1 sample); 3 samples labeled as "persico reale" (*P. fluviatilis*) were substituted by *L. niloticus*. The aforementioned species were simultaneously discriminated through specific digestion profiles obtained with the restriction enzyme *MspI*. Two issues emerge in the case of the "bianchetto": the first concerns the attention paid by the European regulations which adopted a common fisheries policy to promote the conservation and the sustainable use of the fishery resources and to avoid the excessive catches of undersized individuals. For these reasons, certain areas where juveniles congregate are protected and fishing for juveniles has been prohibited as it is the case of the juveniles of *E. encrasicolus*. The second concerns the economic fraud giving that the "bianchetto" is much more expensive than icefish. The substitution of "bianchetto" by *Aphia minuta* is probably unintentional due that the two products are sold with the same price on the market. As regards the substitution of *P. fluviatilis* by *L. niloticus*, in addition to the commercial fraud (the "persico reale" is very expensive with respect to the Nile perch) there is also a serious food safety problem. Indeed, a case of patient with immediate-type allergy to *L. niloticus*, caused by cross-reactive fish allergens other than parvalbumin, has been reported. It is evident that, the fraud based on species substitution entails, in addition to the economic damage, serious threats to the health of consumers and the COIBar-RFLP resulted an effective tool to authenticate fish in seafood products by responding to the emerging interest in molecular identification technologies that reduce processing time and costs bypassing the need for DNA sequencing.

PESTICIDE EXPOSURE AND PREMATURE IDIOPATHIC THELARCHE IN GIRLS: THE PEACH PROJECT

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Several pesticides are recognized as endocrine disruptors (EDs) since they can interfere with the dysregulation of sexual, thyroid and neuro-endocrine hormones contributing to earlier pubertal onset. Exposure to pesticides can be considered an important factor associated with precocious puberty and premature thelarche in girls. Children are particularly vulnerable to the effects of EDs due to their developmental stage, peculiar lifestyle and dietary habits. The main objective of the PEACH project is to evaluate the association between exposure to pesticides and idiopathic premature thelarche in girls, through the measurement of pesticides in urine and the dietary intake, by analysing locally produced foods. Girls living in an agricultural area of Marche region (Centre of Italy) and with idiopathic premature thelarche (2-7 years old), matched to healthy subjects (controls), were enrolled (N=60+60). They are asked to fill in the food frequency questionnaire (FFQ) and to deliver urine samples. Furthermore, sampling of locally produced foods was performed. Food and urine were analysed by LC or GC-MS/MS to detect the pesticide levels. The FFQ was organized in three parts: the first part included personal information on their area of residence, the second part the eaten food, as well as their place of purchase or local production (farm or private garden), the third part concerned the food diary. The activities are still ongoing, but the preliminary results obtained in the urines (N=43 premature thelarche and N=13 controls) showed pesticide levels below the quantification limit of the method. Otherwise, several pesticides were detected in fruits and vegetables (N= 11 samples consumed by cases and N=11 by controls) sampled in the local farms. Small fruits and berries, in particular grape and strawberry, and stone fruits (apricots, peaches, cherries, and plums) reported the highest number of pesticides including carbamates, pyridinylethylbenzamide, benzamide, phenylpyrrole and triazole fungicides and also insecticides as neonicotinoids and carbonylhydrazide. The pome fruit and cucurbits (melon and watermelon) reported only the presence of neonicotinoids. Leafy vegetables reported the presence of a systemic fungicide, the metalaxyl. All vegetables and fruit from private gardens are still under analysis as well as the other commodity categories (meats, eggs, oil, honey). At the end of the project, data integration will provide an estimate of the risk of potential effects of pesticides on premature thelarche, linked to dietary intake.

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ANTIMICROBIAL AND ANTIBIOFILM ACTIVITY OF A PEPTIDE IDENTIFIED IN THE SEAGRASS *POSIDONIA OCEANICA*

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The present study was carried out to evaluate the antimicrobial and antibiofilm activity of a peptide identified in a polypeptide-enriched extract from green leaves of the Mediterranean seagrass *Posidonia oceanica* (L. Delile) (Posidoniaceae). The sequence NVVELNAPGDK was charac-

terized through RP-HPLC/nESI-MS/MS and the synthetic peptide was obtained by GenScript Biotech (Netherlands). According to the database BLAST, the sequence showed 75% identity with a carboxypeptidase and endopeptidase of a bacterium, *Candidatus schekmanbacteria*. In order to predict the potential of this molecule as antimicrobial peptide (AMP) and similarities with already described AMPs, the "APD3: Antimicrobial Peptide Calculator and Predictor" tool of the Antimicrobial Peptide Database (APD) has been used. The synthetic peptide has a length of 11 amino acids and is characterized by a negative net charge -1 and a hydrophobic ratio of 36%. The sequence has three hydrophobic residues on the surface and a good Boman index (1.69 kcal/mol). According to the prediction of the database APD3, this synthetic peptide may have good chances to form alpha helices and interact with bacterial membrane. In addition, the sequence also shows 50% amino acid similarity with acidocin LCHV, a bacteriocin from *Lactobacillus acidophilus* whose anti-Gram positive, anti-Gram negative and antifungal properties have already studied in literature. The antimicrobial properties have been evaluated against four relevant bacterial pathogens (*Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 15442, *Escherichia coli* 25922, *Enterococcus faecalis* ATCC 29212) and a yeast (*Candida albicans* ATCC 10231). Synthetic peptide was tested in concentrations ranging from 800 mg/ml to 6.2 mg/ml. The results, expressed in terms of Minimum Inhibitory Concentration (MIC), showed no antimicrobial activity of the synthetic peptide towards planktonic tested strains. The antibiofilm activity of the synthetic peptide has been analyzed against *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 15442, *E. coli* ATCC 25922 and *E. faecalis* ATCC 29212. The assays were performed using sub-MIC concentrations of 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.2 mg/ml. The results showed that the inhibition of biofilm formation was strongly evident in *E. coli* ATCC 25922 at IC₅₀=49.8 mg/ml and low in *S. aureus* ATCC 25923. In contrast, the synthetic peptide did not inhibit the formation of biofilm in *P. aeruginosa* ATCC 15442 and in *E. faecalis* ATCC 29212. With the aim to increase the antimicrobial and antibiofilm properties of the natural peptide, the initial sequence has been modified, according to the database APD3, by replacing some hydrophilic and negative amino acids with hydrophobic and positive amino acids, obtaining the derivative WVRLNAPGKK. In conclusion, bioinformatic analysis has revealed that natural peptide identified in green leaves of *P. oceanica*, may represent a new scaffold for the design of novel antimicrobial and antibiofilm peptides, making them promising candidates for the treatment of drug-resistant pathogens.

MICROPLASTICS AS A SOURCE OF ENDOCRINE DISRUPTING CHEMICALS: EVALUATION OF THE POSSIBLE EFFECTS ON *IN VITRO* AND *IN VIVO* MODELS

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Environmental pollution caused by plastic wastes is a well-known global issue. Although environmental damages are

clearly visible, the impact of macro- and microplastic particles (MPs) on living organisms is not yet well understood. For a long time, MPs have been considered non-toxic inert particles, however it has been recently found that they can distribute systemically within an organism and induce various damages in tissues, including cytotoxicity, oxidative stress, and chronic inflammation. At present, there is no evidence of a direct action of MPs as Endocrine Disrupting Chemicals (EDCs) on organisms, there is instead growing evidence on the potential of MPs to be vehicles of a wide range of compounds such as hydrophobic organic contaminants (HOCs) including EDCs, and Persistent organic Pollutants (POPs). Some indirect effects of MPs may therefore be linked to these chemicals leached or adsorbed on these particles. In order to demonstrate this hypothesis, environmental MPs were collected in the Adriatic Sea in the harbor of San Benedetto del Tronto (AP) and at different distances from the coastline. Organic extracts were obtained and analyzed, showing that MPs can be vehicles of environmental contaminants like various polycyclic aromatic hydrocarbons (PAHs), organophosphorus (OPs), organochlorine pesticides (OCs), and polychlorinated biphenyls (PCBs) congeners. Interestingly, we observed that MP extracts act *in vitro* as EDCs, in particular as metabolic disruptors, inducing both adipogenesis and lipid uptake/storage in 3T3-L1 preadipocyte cells. To better define the contribution of each compound to the overall pro-obesogenic effect of MP-associated endocrine disruptors, we began to test single substances alone or associated with virgin microplastics. Our preliminary results obtained in 3T3-L1 preadipocytes show that the well-known metabolic disruptors Pyraclostrobin, Tributyltin chloride and Bisphenol A (BPA) induce relevant pro-obesogenic effects. Interestingly, pro-obesogenic effects of BPA were partially reverted by simultaneous exposure of 3T3-L1 cells to (E)- β -caryophyllene (BCP), suggesting beneficial properties of nutraceuticals on reducing metabolic disruption driven by EDCs. In order to better understand the impact of MPs and associated contaminants on living organisms, *in vivo* studies were conducted using the zebrafish model. *Danio rerio* embryos were exposed to fluorescence-tagged polyethylene and polystyrene MPs and the rate of MP internalization in different organs and cell types was determined. Preliminary results show an effect of both MPs alone and combined with BPA on embryo survival, hatching rate and morphological abnormalities outcomes. Also, by means of qRT-PCR, the expression of marker genes of development was found modified in treated *versus* control zebrafish embryos. Our results prompt to deepen investigations on how microplastics and environmental pollutants may globally affect the health of human and wildlife organisms.

VALORIZATION OF WASTE RASPBERRY SEED POWDER: PHYTOCHEMICAL PROFILE, ANTIOXIDANT PROPERTIES AND NUTRACEUTICAL APPLICATIONS

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Social, economic, and demographic changes of the last fifty years have made functional nutrition one of the main directions for a healthy lifestyle. Despite the popularity of foods naturally rich in functional substances, consumers also look with growing

interest and confidence at foods industrially enriched with bioactive ingredients. Plants represent the main source of bioactive molecules, but several factors limit the use of plant preparations as functional ingredients. Stability, bioaccessibility and bioavailability of bioactive plant components in the fortified food matrix must be considered as well as the possible negative influence of functional ingredients on sensory and technological properties of fortified food (1). In this regard, bread is particularly suitable for fortification, also because it is widely consumed in large quantities worldwide and it has a very limited functional value (2). Recently, in the search for useful strategies to minimize food waste impact, plant food waste has been considered as a source of ingredients to improve healthy properties of foods (3). Red raspberry is the fruit of *Rubus idaeus*. Due to its profitability, its production has significantly increased in recent years in Italy. Although the fruit is highly appreciated for fresh consumption, much of the fruit production is destined to industrial processing. Seeds are the main waste from this processing and strategies for their valorization have been explored. Cold pressing of seeds to obtain a very appreciated cosmetic oil produces an insoluble residue (Waste Raspberry Seed Powder, WRSP) that has not yet found application and represents an agro-industrial waste. The aim of this work was exploring the phytochemical composition and antioxidant properties of WRSP so to evaluate a potential nutraceutical application. Folin-Ciocalteu and DMAC assay indicated that WRSP is a very rich source of antioxidant polyphenols, including proanthocyanidins. In addition, HPLC analysis identified presence of high amounts of flavon-3-ols. Redox active properties of WRSP components, as measured by in solution assays, prevent lipid peroxidation in HepG2 cells with very high efficacy. Our results also suggest that WRSP can be an effective functional ingredient. In particular, we found that the replacement of a small percentage of wheat flour with WRSP significantly increases the polyphenolic content and the antioxidant activity of the dough and consequently of the final product. Our results also suggest that the antioxidant compounds in WRSP are stable at high temperatures and then suitable for the functionalization of other bakery products. In conclusion, the obtained results indicate that WRSP, an agri-food waste derived from the industrial processing of red raspberries, possesses a very high nutraceutical potential being an extraordinary source of antioxidant molecules that can effectively improve functional food quality.

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PHYTOCHEMICAL PROFILE AND ANTIOXIDANT PROPERTIES OF FRUITS OF *EUGENIA INVOLUCRATA* DC

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Bioactive compounds are extranutritional constituents able to exert effects on organisms, tissues or cells. Epidemiological studies demonstrate how high intake of bioactive compound-rich foods has been linked to reduced risk of developing sever-

al diseases, including cancer, cardiovascular disorders and chronic inflammation. Fruits and vegetables are the richest sources of biologically active molecules, whose biological activity has been frequently related to the protection of cells from oxidative stress phenomena (10.3390/nu12061748). In the last few years, many studies have been carried out to determine functional properties of exotic fruits, to date increasingly diffused and cultivated in regions other than those of origin. In particular, Sicily and Calabria, thanks to their peculiar climatic conditions, are areas suitable for the cultivation of tropical and subtropical fruits (10.1016/j.foodchem.2022.132137). Moreover, recent studies demonstrated that nutritional, nutraceutical and sensorial traits of exotic fruits grown in Sicily were comparable respect to fruits cultivated in tropical territories (10.1016/j.foodchem.2018.10.109; 10.3390/foods10010035). *Eugenia involucrata* DC is a native tree species from the southern Brazil, belonging to the Myrtaceae family and distributed in tropical and subtropical regions. Although *E. involucrata* is mainly used as an ornamental plant, it produces edible small red-purple fruits commonly known as cerejeira, cerella, or Cereza de Rio Grande, with a sweet cherry taste. Studies concerning *E. involucrata* fruits grown in the Mediterranean environment are not present in the scientific literature. Consequently, this study evaluated the phytochemical profile and antioxidative properties of *E. involucrata* fruits grown in Sicily. The phytochemical analysis, highlighted the presence of several bioactive compounds. In particular, HPLC-Orbitrad determined the presence of 25 phenolic derivatives, among them, anthocyanidins, flavones and flavonols; and different triterpenoids. Concerning antioxidant properties, *E. involucrata* fruits showed prevention of lipid peroxidation in a cell-based model. Moreover, the analyses suggested that the antioxidant protection involves both redox-active properties, as evaluated in in solution assays, and gene expression modulation of antioxidant enzymes. In particular, the fruit extract was able to increase *CAT*, *GPx* and *POX* expression. Collectively, the results indicate this tropical specie as a potential valuable raw material for the nutraceutical, cosmetic and pharmaceutical industry.

FEEDING HABITS OF THE BLACKMOUTH CATSHARK (*GALEUS MELASTOMUS*) FROM CENTRAL MEDITERRANEAN SEA, WITH EMPHASIS ON PLASTIC INGESTION

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The blackmouth catshark *Galeus melastomus* (Rafinesque, 1810) is one of the most abundant Scyliorhinidae in the Mediterranean Sea. Scavenger and opportunistic feeder, this species lives between 50 and 1400m depth from Norwegian sea to Senegal and in all the Mediterranean Sea. Although the vast distribution, little is known about the feeding habits, especially in the Tyrrhenian Sea. Specimens analysed in this study were obtained from October 2020 to August 2021. They came from five populations of the Tyrrhenian and the Ionian Sea at a mean depth between 40 and 700m. The main purpose of this work was to investigate the diet of the blackmouth catshark analysing the stomach contents. A total of 302 samples and 253 stomachs were collected and analysed. All

the stomach contents were sub-divided in macro-categories and identified with the help of some taxonomic guide at the lower taxa possible. Crustacean like *Parapeneus longirostris* and *Pasiphea sivado*; Cephalopods like *Heteroteuthis dispar*, *Todarodes sagittatus* and *Oynchoteuthis banksii* and Osteichthyes, above all specimens of Mychiophidae, were found. Plastic debris was found too among stomach contents. They were classified according to their colour (black, blue, green, grey, orange, red, yellow and white) and shape (fibres, fragments, film and sphere) in accordance with Eriksen *et al.*, 2014 and Valente *et al.*, 2019. Osteichthyes represented the most abundant item found in the stomachs, except for the population from Tuscany, where Crustaceans and Molluscs were dominant. Also for plastic debris, we observed significant differences between the population from Tuscany, where fibers were dominant, and the other populations, where fragments were more abundant. These differences could be explained with the different depths at which the blackmouth catshark populations live: the population of Tuscany, in fact, is the only one of depth, considering that it was sampled at -600 m.

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NEURODEGENERATION AND NEUROINFLAMMATION

DEVELOPMENT OF A HOME-BASED TRAINING PROGRAM FOR PATIENTS WITH PARKINSON DISEASE: NEUROBIOLOGICAL AND MOTOR SKILLS EFFECT

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Neurodegenerative diseases are inherited diseases of the central nervous system, which cause progressive damage to specific populations of neurons and lead to a deterioration in the quality of life (1,2). Parkinson disease (PD) is a progressive neurodegenerative disease and is the second most common after AD, and is characterized by postural instability, tremor and rigidity. Moreover, physical activity can reduce risk of other geriatric diseases such as diabetes, hypertension, osteoporosis and cardiovascular disease, which may also contribute to PD pathogenesis (3). We enrolled 12 subjects (age: 62.74±4.94; height: 175,5cm±7,41 cm; weight: 75,5±17,95 kg) affected by PD. An home-based training program was developed for 12 weeks, 3 times a week, an hour and a half for each session. At the beginning (T₀), at the end of the study (T₁) physical parameters (strength, balance and eye-hand reactivity) and hematochemicals parameters (glycemia, insulin, bone metabolism markers and neurotrophins) were tested. Statistical analyzes were performed using R version 3.4.3 and IBM SPSS Statistics 22. Changes in anthropometric, physical parameters, and hematochemical measurements between T₀,T₁ were evaluated using *t* Student test for paired data. The results obtained have confirmed that this protocol can influence over all bone metabolism markers; in fact parathormone decrease T₀ 62,250 to T₁ 35,675 ng/ml, *p* value <0,05, whilst vitamin D increase from T₀ 16,500 to T₁ 23,875 ug/ml, *p* value <0,05. These are just a preliminary results that need to be deep investigated to better understand the effect of physical activity in PD.

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THE AMYLOID AGGREGATION STUDY ON BOARD THE INTERNATIONAL SPACE STATION

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Alzheimer disease (AD) is the most diffuse neurodegenerative disease among elderly people. As far as neuropathology is concerned, neurofibrillary tangles and amyloid plaques are the main hallmark of the disease. The primary component of amyloid plaques is Amyloid β peptide (A β) constituted of 37-43 amino acids. "Amyloid Aggregation" is an Italian Space Agency (ASI) simple test tube aiming to assess if and how amyloid fibrils aggregation are affected by microgravity, identifying a possible professional risk in astronauts spending long periods in space. The experiment was performed on the International Space Station (ISS) during the "BEYOND" mission sponsored by European Space Agency (ESA) in the frame of a specific agreement between ESA and ASI, which allows Italian utilization of the ISS resources available to ESA. Amyloid β peptides of different size and propensity to aggregate were encapsulated in the cap of special jars, also containing the reaction fluid in a separate lower compartment. Once on the ISS, the amyloid β peptides were mixed with the reaction fluid and kept for various interval of times (IT) at ambient temperature (ISS-Columbus module temperature) to allow for amyloid β peptide aggregation. At the end of each interval, samples were frozen to stop amyloid aggregation. Frozen samples were returned to Earth and analysed to determine the degree and timing of peptide aggregation. The experiment was repeated on Earth in an identical experimental protocol recapitulating the same ISS conditions except for the absence of weight. We have already completed all the parts regarding the preparation of peptide aggregates in orbit and on Earth. Four major techniques for amyloid analysis (Western Blotting, Mass Spectrometry, Atomic Force Microscopy, Dynamic Light Scattering) characterization have been optimized which are expected to provide insight into the structure and kinetic of aggregate formation. Even if sample analysis has been started only a few months ago, interesting preliminary results are summing up comparing the ISS samples with those processed on earth. The possibility to perform experiments on board of the ISS represents a unique opportunity to study if and how microgravity influences amyloid fibril formation. Hopefully, results from this project will help to design more stringent scientific studies for a better understanding of the possible risk of developing protein aggregation diseases in astronauts and eventually for implementing and monitoring prevention systems to protect human health during long-lasting space missions.

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EFFECTS OF PERINATAL EXPOSURE TO BISPHENOL A OR S IN EAE MODEL OF MULTIPLE SCLEROSIS

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Epidemiological studies support the idea that multiple sclerosis (MS) is a multifactorial disease, overlapping genetic, epigenetic, and environmental factors. A better definition of environmental risks is critical to understand both etiology and the sex-related different of MS. Exposure to Endocrine Disrupting Compounds (EDCs) fully represents one of these risks. EDCs are natural or synthetic exogenous substances (or mixtures) that alter the functions of the endocrine system. Among synthetic EDCs, exposure to bisphenol A (BPA) has been implicated in the etiology of MS, but to date controversial data has emerged. Furthermore, nothing is known about bisphenol S (BPS), one of the most widely used substitutes for BPA. As exposure to bisphenols will not disappear soon, it is necessary to clarify their role also in this pathological condition defining their role in disease onset and course in both sexes. In this study we examined, in both sexes, the effects of perinatal exposure to BPA and BPS in one of the most widely used mouse models of MS, experimental autoimmune encephalomyelitis (EAE). Exposure to bisphenols seemed to be particularly deleterious in males. In fact, both BPA- and BPS-treated males showed an anticipation of the disease onset and an increased motoneuron loss in the spinal cord. Overall, BPA-treated males also displayed an exacerbation of EAE course and an increase in inflammation markers in the spinal cord. Analyzing the consequences of bisphenols exposure on EAE will help to better understand the role of both xenoestrogens and endogenous estrogens on the sexually dimorphic characteristics of MS.

TARGETING OF ALTERED MITOCHONDRIAL GENES AS A THERAPEUTIC STRATEGY FOR SPINAL MUSCULAR ATROPHY

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Spinal Muscular Atrophy (SMA) is a motor neuron (MN) disease affecting children and young adults. It is caused by a low expression of survival motor neuron (SMN) protein, due to mutation/deletion of the *SMN1* gene. The current therapies (SMN-dependent) available, despite their efficacy, show several limitations including high costs, still unknown long-term effects and some side effects; moreover, their efficacy is strictly linked to the onset of the treatment (late-treated patients show a lower rescue) and they disregard SMN-independent targets. Therefore, the identification of new targets and of new therapeutic strategies for SMA are necessary. In the

recent years, mitochondrial alterations have been described in several neurodegenerative diseases, including Parkinson's, Alzheimer's and Huntington's diseases and Amyotrophic Lateral Sclerosis: similar abnormalities have been described in SMA too, both in MNs and peripheral tissues. Therefore, mitochondria are becoming a new field of investigation for the identification of novel targets aiming at the maintenance of their integrity. Here, by exploiting a bioinformatic approach, we identified mitochondrial genes whose expression could be normalized in order to regulate mitochondrial activity and functions. We first focused on *COX7A1* that resulted to be strongly anticorrelated with clusters of genes involved in similar disease phenotypes, since *SMN1* belonged to them. Then, we moved to the analysis of strongly SMN-anticorrelated genes in at least three different coexpression networks in mice. We considered only the genes that were listed to the Gene Ontology (GO) keyword "mitochondrion" and we further selected those expressed in the most affected tissues in SMA (CNS, muscles and heart) obtaining a list of seven genes (*GCSH*, *BAG1*, *GOLPH3*, *DNAJC5*, *SLC25A36*, *GLRX2* and *UQCRC2*). To better evaluate their upregulation in SMA conditions, by exploiting SMNdelta7 mice (an intermediate mouse model of SMA), we sacrificed WT and SMA animals in an early symptomatic stage of the pathology (postnatal day 5, P5), after a period of behavioral testing (P2-P5). We performed rt-PCR on spinal cord, brain, quadriceps, gastrocnemius and heart samples that revealed in particular a marked upregulation of *GCSH* in SMA spinal cord and brain in comparison to WT samples. Since these outcomes support the idea of an existing relationship between SMN and mitochondria, we can hypothesize that approaches aiming to modify mitochondrial gene expression could provide a new strategy to counteract mitochondrial alterations in SMA pathology.

A CONVENIENT METHOD FOR EXTRACTION AND ANALYSIS WITH HIGH-PRESSURE LIQUID CHROMATOGRAPHY OF CATECHOLAMINE NEUROTRANSMITTERS IN PLASMA AND BRAIN SAMPLES OF MICE

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The extraction and analysis of catecholamine neurotransmitters in biological fluids is of great importance in assessing nervous system function and related diseases, but its precise measurement is still a challenge. Many protocols have been described for neurotransmitters measurement by a variety of instruments, including high-pressure liquid chromatography (HPLC). However, there are shortcomings, such as complicated operation or hard-to-detect multiple targets, that can no longer be avoided. Presently, the dominant analysis technique is still HPLC, due to its high sensitivity and good selectivity. Here, a detailed protocol is described for the pretreatment and detection of catecholamines with high pressure liquid chromatography coupled with ultraviolet detection (HPLC-UV) in plasma and brain samples of mice. The calibration curve of catecholamines was established in the concentration range of 0.01 – 5.00 ug/mL. 100 µl of plasma or brain samples were extracted by protein precipitation using 300 ul of freeze solution of formic acid 0.5%v/v in acetonitrile. Each sample was vortexed for at least 15 s and then stocked in freezer at -20°C for 15' and later centrifuged at 4,000 rpm for 10 min. The 250ul of supernatant

was transferred to an injection vial. Chromatographic separation was performed at 35°C. Catecholamines plasma concentrations were reported as ug/mL, instead brain amount were converted in ng/mg of tissue weight. The established protocol was applied to assess the differences of plasma and brain levels of catecholamines between genetically different mice. Applications of our methods are very broad as wide is the field of neurodegenerative diseases.

A BIODEGRADABLE PBS-BASED SCAFFOLD IN PROMOTING REGENERATION OF THE PERIPHERAL NERVOUS SYSTEM IN A RAT SCIATIC NERVE TRANSECTION MODEL

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Several nerve guide conduits fabricated from biodegradable polymers are currently in use or under investigation for management of traumatic peripheral nerve injuries, when the nerve gap cannot be surgically approached by a direct end-to-end epineurial repair method (1,2). Poly(1,4-butylene succinate) (PBS) is a water insoluble biopolymer synthesized by the polycondensation of succinic acid and 1,4-butandiol (3). Given its chemical structure, PBS shows a proven biocompatibility and biodegradability, and a good versatility when employed as material for various biomedical applications and in tissue engineering, in the form of films as well as microfiber and nanofiber flexible tubular scaffolds produced by electrospinning technique (3). In this study, we evaluated the biocompatibility and effectiveness of an electrospun microfibillar PBS-based 3D scaffold to bridge a 10-mm nerve gap and improve and guiding peripheral nerve functional regeneration in a rat model of sciatic nerve transection. In Group 1 (G1; Control; n=5) we used conventional epineurial microsurgical sutures. In Group 2 (G2; Microfiber wrap; n=5) a PBS-based scaffold was implanted following neurotmesis at the severed nerve stumps without epineurial repair. The outcomes were evaluated by *in-vivo* magnetic resonance imaging (MRI), electrophysiological assessment and histological analysis after a post-surgery long-term period of 12 months. MR images and gross observation of the G2 repair site showed that the PBS wrap and the transected nerve was totally reconnected and the scaffold was completely reabsorbed, with absence of inflammation process. Histological analysis of the nerve confirmed the absence of infiltration by inflammatory cells, Wallerian degeneration and perineural fibrosis at the repair site in the G2. Histomorphometric evaluation exhibited that the total fiber number was found to be significantly higher in the G2 vs. G1 (9867±735.63 vs. 7890±124.13). As regard the electrophysiological findings, no statistical significance ($p > 0.05$) in the mean MUNE (number of estimated motor units) was observed between the operated (right, R) and healthy contralateral (left, L) limbs, for gastrocnemius (GA) and tibialis anterior (TA) muscles, both in G1 (GA-R vs. GA-L, 19.85 vs.

56.85; TA-R vs. TA-L, 13.34 vs. 77.12) and in G2 (GA-R vs. GA-L, 23.12 vs. 42.89; TA-R vs. TA-L, 19.47 vs. 31.83).

Overall, the results obtained demonstrate that the use of the bioabsorbable PBS scaffold is a more effective method of fixing the injured two portions of the sciatic nerve, to preserve nerve continuity and promote faster and better axonal regeneration and remyelination following repair.

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GLIOBLASTOMA MULTIFORME: IDENTIFICATION OF A NEW PHARMACOLOGICAL TARGET INTERFERING WITH UNCONTROLLED NEOANGIOGENESIS

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Glioblastoma (GBM) is the most common brain cancer with poor prognosis. This solid tumor is characterized by a stroma containing cells with different phenotype, blood vessels and infiltrating cells conferring to the mass epigenetic and genetic heterogeneity directly responsible of cancer progression. The uncontrolled cell proliferation creates hypoxic areas in the cancer mass overexpressing hypoxia-inducible factors (HIFs). HIF-1 α triggers vascular endothelial growth factor (VEGF) responsible of neoangiogenesis process. Recently, we have demonstrated that pituitary adenyl cyclase-activating peptide (PACAP) exerts anti-invasive in GBM by binding to its related receptor, PAC1R. In central nervous system, some of peptide effects are mediated through activity-dependent neuroprotective protein (ADNP) activation. The functional role of ADNP in human cancer is still poorly characterized and no evidence exists regarding its involvement in GBM. In the present study, we have investigated for the first time the ADNP involvement in GBM malignancy. Here, we have demonstrated that ADNP is overexpressed in hypoxic areas of human GBM displaying increased levels of HIF-1 α . To investigate whether ADNP interferes with hypoxia-mediated aberrant angiogenesis, we exposed human glioblastoma cells (U87MG) to a hypoxic mimetic agent, deferoxamine (DFX), and treated them with an eight amino acid peptide synthesized from ADNP, known as NAP. Results have shown that NAP downregulates HIF-1 α expression as well as its nuclear localization in cells exposed to DFX. NAP treatment also reduces VEGF expression and release in culture medium of cells exposed to hypoxic insult. Furthermore, data have demonstrated that it also interferes with new vessels formation by reducing tube-like structures formed by H5V microvascular endothelial cells cultured in conditioned medium derived from U87MG grown under hypoxia and treated with NAP. Finally, we have demonstrated that NAP treatment decreases cells migration along the wounded area as compared to DFX-treated cells. Although further investigations are needed, the present study suggested that PACAP effect in GBM could be due to ADNP induction.

RHEUMATOID ARTHRITIS INDUCED BY INTRA-ARTICULAR INJECTION OF COMPLETE 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 immune system attack on the joint lining. This disease results in an increased release of inflammatory mediators peripherally and centrally. Some of the major peptides involved are glutamate, Substance P (SP) and Calcitonin gene-related peptide (CGRP) which sensitize sensory neurons and cause further hyperexcitability, unadorned pain, the severe symptoms of RA and the persistent neurogenic inflammation. Furthermore, several studies have highlighted the protective role of HSPs (27, 60, 70 and 90) in different animal models of pain and inflammation. The aim of the present study is to examine the time course of HSPs expression in the spinal cord specifically sensory neurons and glial cells in the dorsal horn, and its role besides the receptors responsible for this alteration following induction of joint inflammation with intra-articular injection of Complete Freund's adjuvant (CFA) and to examine if increased cellular proliferation in the spinal cord of arthritic rats correlate with nociceptive behaviour and increased HSPs expression. Male Sprague-Dawley rats were assessed for sensory and motor behavioural changes prior to and at 7, 14, and 21 days post induction of inflammation. In addition, spinal cord tissues, serum, and synovial membranes were collected at the same time points. The level of different HSPs expression in the spinal cord (centrally) and in the synovial membrane (peripherally) was assessed using Western blot analysis. Immunofluorescence experiments were also done to reveal the expression site of these proteins in the spinal cord. NMDA and non-NMDA glutamate receptors antagonists will be used to determine the role of these receptors in HSP release. All inflamed rats developed sensory hypersensitivity and motor incoordination. The severity of joint inflammation was assessed by measuring the knee joint circumference. Alteration in the expression level of different HSPs was found in the spinal cord tissues at different time points: a significant increase of the proteins Hsp90 and Hsp70 in inflamed rats compared to control rats was detected after 14 and 21 days, respectively; a significant decrease of the Hsp60 in inflamed rats was observed at day 21; no significant differences were observed for Hsp27. Increased cellular proliferation was detected in different regions of the spinal cord of inflamed rats and peaked at day 14. HSPs (27, 60, 70 and 90) can be found in both neurons and glial cells in the dorsal and ventral horns of the spinal cord. Our preliminary data provide evidence for dual role either HSPs involve in the development of pain and inflammation associated with RA or an anti-inflammatory role contributing to the alleviation of progression of this disease, however depending on whether it is upregulated or downregulated in relation to the time points of variation. Furthermore, in correlation with the cellular events and behavioural changes taking place.

THE ROLE OF LOCUS COERULEUS DEGENERATION IN ALZHEIMER'S DISEASE AND ITS ASSOCIATION WITH NEUROINFLAMMATION

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Locus Coeruleus (LC) is the main noradrenergic (NA) nucleus of the brain, providing the NA innervation for the whole cortical mantle and many subcortical structures. *Post-mortem* studies have shown an early and dramatical degeneration of LC in Alzheimer's Disease (AD). Such an involvement may not only represent a mere histopathological feature of the disease but could also play a key role in its pathogenesis. Many preclinical studies have clearly shown that LC-NA takes part in the regulation of neuroinflammatory pathways, modulating microglial cells activity and neurovascular unit functioning. In AD animal models, LC lesioning caused neuroinflammation worsening, alongside with abnormal protein accumulation and increased neuronal death. If the LC degeneration bore the same effects in humans affected by AD, this may partly explain the pathogenetic process of the disease. In the present study, we assessed LC integrity *in vivo* in humans and explored its association with neuroinflammation. We submitted 160 subjects (53 cognitively intact healthy controls, 73 Mild Cognitive Impaired individuals and 34 Alzheimer's Disease Dementia patients – HC, MCI and ADD respectively) to a 3Tesla Brain MRI scan with LC-sensitive sequence. Through a template-based post-hoc analysis, we measured LC volume for both the hemispheres (left LC and right LC) for each subject. In a subgroup of subjects, blood cytokines (IL1, IL6 and its receptor – IL6R - IL10, IL12, TNF- α and TGF- β 1) were also assessed at the time of the MRI. All subjects underwent to a 2.5-year follow-up to evaluate disease progression and to monitor the conversion of MCI individuals to the dementia stage (MCI converters). We found that the volume of the left LC was significantly reduced in ADD patients when compared to HC ($p=0.036$), while no differences were observed considering the right LC. Moreover, the volume of the rostral part of the left LC was also significantly reduced in MCI individuals which had converted to dementia at the end of the follow-up, when compared to both HC ($p=0.015$) and stable MCI subjects ($p=0.030$). The volume of left LC was also associated to IL6 signaling; in particular, it was inversely correlated with IL6 blood levels in HC ($p=0.026$) and with IL6R levels in ADD ($p=0.005$). No significant associations were observed within the MCI group or in the whole sample.

Our results are in line with preclinical literature. For the first time, we observed *in vivo* in humans that the LC volume is reduced in MCI individuals converting to dementia. This may represent an *in vivo* marker of disease progression, supporting a possible role of LC impairment in AD pathogenesis. Such a contribution to AD-related neurodegeneration may be explained at least in part, in light of the inverse association between LC volume and the pro-inflammatory IL6 signaling, which highlights a possible link between the disruption of the NA system and the aberrant neuroinflammatory activity occurring in AD.

ENDOCANNABINOID AND TRPV1 CHANNELS: BIOELECTRIC INTERPLAY IN HIPPOCAMPAL NEURONS

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Endocannabinoids (eCBs) have been associated to neuronal excitability and neuroprotection acting on the cannabinoid receptor type 1 (CB1r) and Transient Receptor Potential Vanilloid type 1 channels (TRPV1). CB1r and TRPV1 seem to be involved in the transduction of stimuli at synaptic level, however the molecular mechanism implicated still remains unidentified. We aimed to investigate the role of the interplay among CB1r and TRPV1 in the rat hippocampal neurotransmission using whole-cell patch clamp technique to assess excitatory bioelectrical activity in CA1. We pharmacologically modulated the CB1r/ TRPV1 pathway with anandamide (AEA), CB1r and TRPV1 agonist, capsaicin (CAP), TRPV1 agonist and capsazepine (CPZ), TRPV1 antagonist. Our data reveal that the administration of drugs significantly modifies synaptic activity by influencing action potentials parameters and mini excitatory post-synaptic currents (mEPSC). Especially, CPZ increases mEPSC amplitude whereas CAP reduces it. As for the implication of presynaptic terminal, our results suggest that CPZ decreases mEPSC frequency and CAP increases it, also modifying in accordance the cumulative probability of inter-event intervals. AEA co-administration with CPZ reverts its effects, while Cap activity is potentiated in co-administration with AEA. These preliminary outcomes suggest the hypothesis that CB1r/TRPV1 pathway could modulate excitatory neurotransmission improving synaptic efficiency at various levels. Taking into account our results, TRPV1 appears to be a potential target in the cannabinoid neuroprotective modulation of hippocampal bioelectrical activity, with a possible impact on neurodegenerative processes.

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DIFFERENTIATION POTENTIAL OF HUMAN ADIPOSE-DERIVED MESENCHYMAL STEM CELLS

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Adipose-derived mesenchymal stem cells (ASCs) have been extensively investigated in the last decades for their potential therapeutic application in a number of human diseases, especially when other approaches fail. In fact, for their multipotent differentiation ability, they are able to give rise not only to cells of mesodermal origin, but also to neural or epidermal elements. In previous works, it was possible to induce a neural differentiation of ASCs using of conditioned media derived by glial cultures such as Schwann cells or Olfactory ensheathing cells (Lo Furno *et al.*, 2018). Results obtained by

immunofluorescence and flow cytometry showed a marked increased expression of neural marker (Nestin, Synapsin I, Microtubule Associated Protein 2, Glial Fibrillary Acidic Protein). In addition, a neural pattern of Connexin expression was also induced. Taking into account the coexistence of both neuronal and glial markers, it was concluded that neural-like differentiated ASCs might still be at early stages of differentiation, as it was reported for neural progenitor cells. Presumably, at later stages of differentiation, on the basis of micro environmental cues, the expression of some markers would be enhanced, while some others would be downregulated. Overall, neural-like differentiated ASCs may represent a valuable tool for the treatment of neurodegenerative diseases. More recently, a pericyte-like differentiation was obtained in ASCs cultured in a medium specifically designed for pericytes (Mannino *et al.*, 2020). This is particularly interesting for the treatment of some ocular diseases, such as diabetic retinopathy, characterized by a breakdown of the blood-retina barrier, subsequent to a progressive and irreversible pericyte loss. It was shown that, even better than native pericytes, pretreated ASCs were able to improve the expression of junction proteins (VE-Cadherin, Zonula Occludens and Occludin 1) between retinal endothelial cells. An improvement of blood-retina barrier was also indicated by the increased trans endothelial electrical resistance. Moreover, three dimensional co-cultures in Matrigel demonstrated their tendency to localize in the typical pericyte position, around endothelial-derived tubular formations. Interestingly, when cultured in high glucose conditions, pericyte-like differentiated ASCs showed high viability, proliferation rate and migration ability (Mannino *et al.*, 2021). They also induced increased mRNA levels of anti-inflammatory cytokines (IL 10), as well as decreased levels of pro-inflammatory cytokines (TNF- α), angiogenic factors (VEGF, angiopoietin 2) and ROS production.

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MOXIFLOXACIN RESCUES SPINAL MUSCULAR ATROPHY PHENOTYPES IN BOTH ANIMAL MODEL AND PATIENT-DERIVED CELLS

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Spinal Muscular Atrophy (SMA) is a neurodegenerative disease, affecting children and young adults and determining motor neuron (MN) impairment, skeletal muscle atrophy and premature death. It is caused by the mutation/deletion of the Survival Motor Neuron 1 (SMN1) gene. Even if spared in case of SMA, its human-specific copy (SMN2 gene) fails in rescuing SMA phenotype, since it produces a low amount of

functional protein due to an alternative splicing. Improving therapeutic strategies aimed at the increase of SMN2 function is still a hot topic in the SMA field. Recently, thanks to a *Drosophila*-based screening of FDA-approved drugs (Konieczny and Artero, FASEB J., 2020), the antibiotic Moxifloxacin demonstrated to exert positive effects on SMN2 exon 7 splicing and efficiently increase SMN protein level on several SMA models (a *Drosophila*-based reporter system and SMA patients-fibroblasts). To further validate the Moxifloxacin efficacy, here we investigated its effects in SMN7 mice (a murine model of moderate SMA) and in SMA type I patients-derived cells. We demonstrated that daily subcutaneous injections of Moxifloxacin in moderate SMA mice increased the SMN levels in both the spinal cord and skeletal muscles ($\geq 34\%$ in the spinal cord, $\geq 91\%$ in the quadriceps), leading to improved motor skills and extended lifespan. In addition, the analysis on lumbar spinal cord sections of treated mice showed that Moxifloxacin treatment had significant effects in i) delaying MN degeneration and reducing the apoptotic marker cleaved caspase 3 levels ($\leq 46\%$), ii) modulating neuroinflammation (by reducing astrogliosis: $\leq 48\%$), and iii) modifying the microglia ramification/activation. Finally, skeletal muscles analysis showed that Moxifloxacin treatment significantly enhanced muscle trophism (in terms of fiber size, perimeter and Feret's diameter) and improved neuromuscular junction (NMJ) phenotype (maturation, innervation) in treated mice compared to controls. We further demonstrated that Moxifloxacin rescued the SMA-related molecular and phenotypical defects in muscle cells and MNs derived from SMA type I patients-induced pluripotent stem cells (iPSCs) by improving the SMN2 splicing, the survival rate and the SMN levels ($\geq 30\%$) compared to controls. Moreover, Moxifloxacin improved the *in vitro* MN ability to establish functional NMJs with SMA myotubes and promoted their growth. To conclude, we suggest that Moxifloxacin, originally used as an antibiotic, can be potentially repositioned for the SMA treatment.

ACTIVATION OF THE HEPCIDIN-FERROPORTIN1 PATHWAY IN THE BRAIN AND ASTROCYTIC-NEURONAL CROSSTALK TO COUNTERACT IRON DYSHOMEOSTASIS DURING AGING

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During aging, iron accumulates in brain regions which are particularly vulnerable to neurodegeneration: the cerebral cortex and the hippocampus. However, knowledge on the mechanisms of iron regulation and the age trend of iron concentrations in the brain, together with its cellular distribution within neuronal tissue, remains scarce. We studied brain iron metabolism in C57BL/6 wild-type mice and we observed an age-dependent accumulation of iron in the brain that triggers neuroinflammatory and antioxidative stress response, especially in the cerebral cortex and hippocampus. Indeed, in old mice we found alteration of the Blood Brain Barrier (BBB) integrity, and increased neuroinflammation and oxidative stress measured by SAA1 and Nrf2 gene expression and reactive GFAP and IBA-1 positive astrocytes and microglia. Interestingly, we demonstrated for the first time that there is the activation of the Hepcidin/Ferroportin1 pathway in the brain

during aging. Hepcidin, a peptide produced mainly by hepatocytes, is the major regulator of iron content and availability. In iron overload conditions, Hepcidin interacts with the iron exporter Ferroportin1, causing its degradation and iron retention within the cell. Moreover, we observed NCOA4-dependent ferritinophagy of ferritin heavy-chain isoforms determining the increase of light-chain enriched ferritin heteropolymers that are more efficient as iron chelators. By immunocytochemistry, we observed that both in the cerebral cortex and hippocampus Ferroportin1 colocalizes with astrocytes, while the iron storage protein ferritin light-chain with neurons. This differential distribution suggests that astrocytes mediate iron shuttling and neurons are unable to metabolize it. Altogether, these data highlight the involvement of the Hepcidin/Ferroportin1 axis and NCOA4 during mice aging as a response to a higher iron influx to the brain and represent the starting point to clarify the basis of iron handling in the brain on which one could act to avoid brain iron overload, a typical pathological feature of aging and several neurodegenerative disorders, such as Alzheimer's disease. We are grateful to our colleague Pr. Sonia Levi from the University of Vita Salute, Milan (Italy) who provided us with the antibodies used to detect Ferritin.

ANTI-INFLAMMATORY POTENCY OF EXTRACELLULAR VESICLES DERIVED FROM HUMAN WHARTON'S JELLY STEM CELLS IN NEUROINFLAMMATION OF SCI

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Neuroinflammation is triggered upon inflammasome complex assembly which induces the active form of the pro-inflammatory cytokines, interleukin-1beta (IL-1 β) and interleukin-18 (IL-18) after spinal cord injury (SCI) (1, 2). Extracellular vesicles derived from mesenchymal stem cells (MSC-EVs) represent effective immunomodulatory characteristics (3, 4). We studied the inflammasome complex activity through applying intrathecal injection of human Wharton's jelly mesenchymal stem cells derived extracellular vesicles (hWJ-MSC-EVs) in a compressive SCI rat model. Inflammatory factors, IL-1 β , IL-18 and TNF- α , were evaluated at the mRNA and protein level. Also, caspase1 was examined to assume the inflammasome complex formation. Moreover, histopathological alternations and locomotor function of hind limb were analyzed. We observed that all investigated factors were significantly reduced and normal neurons are more preserved after EVs treatment in the injured spinal cord tissue. In addition, motor activity was relatively improved in hind limb. Our finding may suggest that MSC-EVs could be promising anti-inflammatory agents to modulate the neuroinflammation in neuropathic context.

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THE STUDY OF MITOCHONDRIAL MORPHO-FUNCTIONAL DYSFUNCTIONS IN THE PATHOGENESIS OF SPINAL MUSCULAR ATROPHY REVEALS MITOCHONDRIAL ACONITASE AS A POSSIBLE BIOMARKER OF THE DISEASE

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Spinal Muscular Atrophy (SMA) is a paediatric and juvenile onset neurodegenerative disease due to the autosomal recessive mutation in the Survival Motor Neuron (SMN) 1 gene causing the decrease of SMN protein levels and the selective and early death of spinal cord (SC) lower alpha motor neurons (MNs). Despite the genetic cause of SMA is known, many aspects of its pathogenesis are still poorly understood. In the last years, mitochondrial dysfunctions have been found in murine models already during the pre-symptomatic stages of the disease and their alterations are considered a risk factor for SMA. Mitochondria are highly dynamic organelles able to maintain cellular homeostasis. However, being extremely stress sensitive, insults that accumulate within the cell directly affect their functionality, consequently promoting disease development and progression. Interestingly, due to the ubiquitous expression of SMN protein, the sites of cellular damage in SMA are not only MNs but also peripheral tissues such as skeletal muscle and fibroblasts that share a strong dependence on energy and are indeed particularly enriched in mitochondria. In this study, we aimed to deepen the knowledge of the role of mitochondrial dysfunctions in SMA both at central (murine SC) and at peripheral (murine and human fibroblasts) level. From a screening with 2D gel and MALDI-TOF mass spectrometry on pure mitochondria isolated from SC, we noticed an altered expression and post-translational modifications in the mitochondrial Aconitase (mAcn) enzyme, accompanied by a strong reduction in its functionality. Moreover, by western blotting analysis, we identified alterations in mitochondrial dynamism and respiration without any change in mitochondrial content. In order to verify if mAcn alterations were present also at peripheral level, we cultured fibroblasts derived both from SMA Δ 7 embryos and from human SMA patients. Enzymatic and imaging (MitoTracker staining and multidimensional analysis by MiNA toolset from ImageJ) techniques confirmed both the reduction of mAcn functionality and the mitochondrial morpho-function-

al defects observed in the SC, together with a significant reduction of mitophagic processes. In conclusion, we suggest mAcn as a potential biomarker of the disease. Moreover, in a future perspective, we would like to test mAcn functionality also in human blood samples in view of obtaining a non-invasive way to monitor the status of the disease and/or the effectiveness of a treatment.

NEUROTROPHIN SPECIFIC TRK RECEPTORS IN THE INNER EAR AND LATERAL SYSTEM OF NOTHOBRANCHIUS GUENTHERI

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Neurotrophins (NTs) are growth factors regulating neuronal proliferation, synaptic plasticity, development, survival, growth, and differentiation. Neurotrophins' biological functions are mediated by signal-transducing systems that are initiated by their interactions with the Trk and the p75 neurotrophin receptors (p75NTR). Neurotrophins and their receptors are evolutionarily conserved and have been found, among other vertebrates, in fish. Some neurotrophins and their receptors have been localized in the fish nervous system and sensory organs, for instance, the inner ear and the lateral line system whose functional unit is called neuromast. These two systems share structural and functional parallels such as similar hair cells for mechano-electric transduction that are very similar to those present in the inner ear of higher vertebrates. However, evidence of the occurrence of neurotrophins and their receptors in an annual fish, *Nothobranchius guentheri* is still scarce. Fish of the genus *Nothobranchius* have a short life expectancy, so they became a suitable model for aging studies. Therefore, our study aimed to investigate the localization of neurotrophin-specific Trk receptors in the inner ear and the lateral line system of *N. guentheri*. Thus, adult specimens of *N. guentheri* were processed for histological and immunohistochemical analysis. Antibodies against Trk receptors and S100 protein were employed. Sensory cells of the inner ear as well as the hair cells of LLS displayed an intense immunoreaction for TrkA and TrkC. Additionally, a specific immunoreaction for all Trk receptors (TrkA, B, and C) analyzed has been found in the acoustic ganglia neurons too. Thus, for the first time, our results demonstrate that neurotrophins and their specific receptors could play a pivotal role in the biology of the sensory cells of the inner ear and LLS of *N. guentheri*. Further studies are needed to better understand their possible role in the hair cells regeneration process in normal and aged conditions.

MODULATING PERIPERSONAL SPACE IN A VIRTUAL REALITY ENVIRONMENT

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Unilateral neglect is a neuropsychological syndrome in which patients fail to perceive or respond to stimuli in the con-

tralateral hemifield. Pseudoneglect is a phenomenon in which neurologically intact subjects tend to overestimate left space: literature data show that in real life pseudoneglect occurs exclusively in Peripersonal Space (PPS, also called reaching distance), while it does not occur in Extrapersonal Space (EPS, also called walking distance), unless tool utilization is involved (*i.e.* a stick). In this work we explored the visual-spatial responses of subjects immersed in an Augmented Reality platform (AR) experience and evaluated if there is a correspondence between the AR experience and the Virtual Reality platform (VR) experience; more specifically if the human brain treated PPS and EPS in a virtual reality (VR) and augmented reality (AR) context in the same way as it treats them in real life. We used a VRBox with a smartphone inside it as a headset and the software Unity3D to generate the VR/AR environments. In VR, subjects are immersed in an environment totally created in CGI, while in AR condition subjects see the real world but through VRBox cameras. 27 neurologically healthy right handed (*i.e.* Edinburgh Handedness Index >80) volunteers between the ages of 18 and 35 were enrolled and randomly divided into two groups. The first group ($n=16$) underwent to a line bisection judgement task, in four conditions for VR: NEAR (60cm, peripersonal space), FAR (120cm, extrapersonal space), TOOL (120cm with a stick), LONG-ARM (120cm with virtual arms over 120cm long). The second group (11 subjects) underwent the same task, with the same method, but only in three conditions: NEAR, FAR and TOOL. In VR, attentional bias to left occurred in FAR condition, while it came back to right in NEAR and TOOL condition: data showed relative differences comparable to those obtained in literature but the absolute values were inverted (NEAR vs. TOOL *n.s.*, NEAR vs. FAR $p=0.1$, TOOL vs. FAR $p=.01$). Furthermore, LONG-ARM condition showed no significant differences in all other conditions, placing itself between FAR and TOOL conditions (vs. NEAR *n.s.*, vs. TOOL *n.s.*, vs. FAR *n.s.*). In AR, results showed relative differences comparable to those obtained in real life. The results in VR allow us to hypothesize a paradoxical inversion of the physiological pseudoneglect and also confirm that even in VR the tool allows remapping EPS as PPS. Furthermore, the fact that NEAR, FAR and TOOL conditions AR showed relative differences comparable to those obtained in real life imply that the proximity of the VRBox device itself does not affect the inversion of the pseudoneglect. The explanation of this phenomenon is therefore unknown. We can hypothesize that the virtual environment is not recognized as analogous to real life by the brain and that this causes a sensory incongruity that is resolved by the brain, according to a Bayesian model, preferring the higher precision stimuli. In conclusions: more research is needed, also to verify the potential usefulness of VR in the rehabilitation field for patients affected by neglect syndrome.

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2D AND 3D IN VITRO EXPERIMENTAL MODELS TO STUDY SPINAL MUSCULAR ATROPHY AND PRELIMINARY PERFORM DRUG SCREENING

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Spinal muscular atrophy (SMA) is a genetic neurodegenerative disease affecting children and young adults, caused by a mutation/deletion of the survival motor neuron 1 (SMN1) gene. This determines the SMN protein lack, leading to motor neuron (MN) impairment, skeletal muscle atrophy and premature death of patients. Nowadays, three drugs FDA-approved are available to treat SMA acting. Even if all treatments efficiently increase SMN protein level, they show some non-negligible limitations, making essential the identification of alternative/synergistic therapeutic strategies. To easily perform a preliminary screening of alternative promising treatments, we propose here two SMA *in vitro* models, which well recapitulate the main pathogenetic features of SMA. In both cases, the samples were obtained from delta7 SMA mice (one of the most used SMA model) and compared with WT samples. For the cortical neuron cultures, P0–P1 newborns were sacrificed and their cortex dissected and dissociated by enzymatic and mechanical protocols. The neurons were grown in Neurobasal Medium supplemented with B27, L-glutamine and penicillin–streptomycin: at 7 days *in vitro*, different assays were performed to assess cell viability and morphology. SMA cortical neurons showed a reduced viability and significant morphological alterations, compared to WT, including decreased soma area and length/branching of neuronal processes. Instead, organotypic spinal cords cultures were obtained from P8 mice and prepared according to the membrane interface method. Morphometric analyses revealed significant differences in the MN soma area and axonal length between SMA e WT MNs. Furthermore, to validate the model, we tested the effects of human Mesenchymal Stem Cells or murine C2C12 cells conditioned media, added to the conventional medium for 7 days to the organotypic culture: we observed that the treatments positively influenced the MN soma size and the axonal length respectively, without modulating neuroinflammation. Thus, we can confirm the proposed 2D and 3D culture models are excellent tools to in depth study the pathogenetic mechanisms of SMA and preliminarily screen molecules and drugs.

CAN HUMAN SPATIAL ORIENTATION BE MODULATED BY MEANS OF GENERAL PURPOSE NON-INVASIVE BRAIN STIMULATION?

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Galvanic Vestibular Stimulation is a form of tDCS capable of eliciting a vestibular response through direct nerve stimulation and involvement of posterior labyrinth hairy cells. The main purpose of this preliminary study is to develop an affordable and relatively unexpensive experimental set-up for GVS modulation that can be used in specialized centers during flight simulations and spatial illusion simulations, to reduce the space disorientation in pilots. 9 healthy subjects (22-45 years) underwent

posturographic study during the GVS, using BrainSTIM™ stimulator through a continuous bipolar current of 1 mA using 20 cm² electrodes positioned over the mastoid process with the anode on the left. Wii Balance Board™ was used to obtain data on the COP (Center Of Pressure), deriving them from the four force sensors in the platform on the X and Y axis, and communicate these data to the computer by Bluetooth protocol to BrainBloX software. Subjects were asked to stand on the platform with their eyes closed, the head looking straight, the upper limbs along the body and the lower limbs spread about 30°. 3 tests lasting 40 seconds each were performed: the first with the objective of recording the baseline oscillation without stimulation; the last two, on the other hand, were aimed at assessing the inclination of the subject during the GVS. An inter-test rest period of approximately 1 minute was observed. A rmANOVA was performed with the variable COP X as an intrasubject factor and two conditions (pre tDCS vs. post tDCS). The analysis of the COP position showed a significant ($p < 0.05$) variation of approximately 0.45 cm (95% CI 0.44-0.46), in line with international studies performed on the healthy population. Remarkably, subjects experienced no harmful effects. The experimental protocol developed seems able to effectively modulate vestibular system and could constitute a future application in flight simulation, with the purpose of training or preventing spatial disorientation in aeronautical pilots by means of commercially available non-invasive brain stimulators. Thus, this protocol could provide an excellent tool for studying Human Factor in aerospace. In conclusion, further studies are needed to verify the usefulness of GVS also in the rehabilitation field for patients suffering from vestibular diseases

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LONG-TERM TRANSPLANTATION OF STRIATAL PROGENITORS AND ENRICHED ENVIRONMENT PROMOTE CIRCUITS RECONSTRUCTION AND MOTOR RECOVERY IN A RAT MODEL OF HUNTINGTON'S DISEASE

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Huntington's Disease (HD) is a neurodegenerative disorder

characterized by the prominent loss of medium spiny neurons (MSNs) in the striatum. To replace the affected cells, we grafted human striatal progenitors derived from H9 embryonic stem cells into adult immunodeficient rats in which the striatum was lesioned by unilateral injection of quinolinic acid (QA), as a preclinical model of HD. We assessed the survival, maturation and integration of the graft as well as its impact on lesion-dependent motor alterations up to 6 months post-graft. Moreover, by exposing a cohort of QA-lesioned animals to environmental enrichment (EE), we tested whether this protocol could improve graft maturation, integration and motor recovery. We found that the transplanted progenitors survived in the host up to 6 months post-graft, and underwent maturation as striatal MSNs positive for Ctip2, Darp32, Enkephalin, or interneurons expressing CB, CR, TH. Also, grafts displayed only residual proliferation. Interestingly, we observed that human striatal grafts were able to self-organise and recapitulated key striatal developmental features. Grafts domains with distinct differentiation potencies were populated by either hMSNs or human interneurons and hMSNs subsets appeared to start expressing striosome markers. Viral vector-based tracing experiments revealed that grafted cells were also integrated into the host circuits, receiving connection from both cells of the graft and of the host (in striatal and extrastriatal areas), and extending neurites at long distance (subthalamus and substantia nigra). Of note, EE increased both cell differentiation into MSN phenotype and graft connectivity, compared to standard housing condition. Moreover, behavioural analyses showed that the graft improved motor performances affected by QA; the exposure of grafted rats to EE further improved task execution. Taken together our data support the therapeutic potential of human MSN progenitor grafts for the replacement of degenerated striatal neurons and suggest that generalised training protocols (such as those provided by EE) can effectively stimulate the maturation and integration of transplanted human progenitors.

AMPLIFICATION OF THROMBIN SIGNALLING IN CEREBRAL CAVERNOUS MALFORMATIONS

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Cerebral cavernous malformation (CCM) is a pathological condition consisting of benign lesions affecting brain microvessels that appear enlarged and tangled, following cell junction impairment. This results in increased bleeding and seizure risk that are the main symptoms of the patients. In many cases, CCM arises sporadically and its molecular bases are still poorly understood. However, it can also be inherited following germline mutations in the *KRIT1*, *CCM2* and *PDCD10* genes. According to our previous expression data obtained by endothelial cells isolated from human CCM specimens, we investigated the role of thrombin signalling in pathogenesis of sporadic CCM. In brain, thrombin can bind peculiar receptors named protease-activated receptors (PARs) activating non-conventional transduction signals that are independent from coagulative cascade. PARs comprise 4 members (PAR1-4) and, in particular, PAR-1 activation, leads to matrix metalloproteinases

activation, resulting in increased blood-brain barrier permeability. These receptors are encoded by *F2R*, *F2RL1*, *F2RL2*, *F2RL3* genes, respectively. In order to clarify if this signalling can be involved in CCM pathogenesis, by quantitative real time-PCR we first evaluated expression of PAR encoding genes in CCM-derived endothelial cells and compared it with values observed in human Cerebral Microvascular Endothelial Cells (hCMECs), used as negative control cultures. Moreover, we wanted to investigate about CCM gene expression in hCMECs, following thrombin exposure, at 5 different time points. Results showed that CCM-derived endothelial cells over-express PAR receptors and, in particular, *F2R* and *F2RL2*, encoding for PAR1 and PAR3, respectively. Moreover, thrombin treatment affects CCM gene expression in a time-dependent manner causing a drastic reduction of their expression. These findings suggest that amplification of thrombin signalling in brain microvascular endothelial cells can be involved in CCM onset and progression.

ANTIOXIDANT AND ANTI-INFLAMMATORY ROLE OF GRAPEFRUIT INTEGROPECTIN

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Rich in *Citrus* flavonoids and terpenes and retaining the highly bioactive rhamnogalacturonan RG-I region, IntegroPectin is a new family of pectins extracted from citrus processing waste via hydrodynamic cavitation in water only. Tested on neuronal cells, microglial cells and on peripheral blood mononuclear cells, grapefruit IntegroPectin is effective in protecting different cell types from death after exposure to oxidizing agents, reducing the amount of intracellular reactive oxygen species (ROS). Preliminary results also suggest that IntegroPectin may modulate inflammatory phenomena. These data, alongside recent results concerning the *in vitro* anti-proliferative activity of this new pectin, suggest a potential therapeutic role of grapefruit IntegroPectin. Though preliminary, these results support experimentation on preclinical models of complex pathologies marked by extensive phenomena of oxidative stress and inflammation such as neurodegenerative diseases.

NEURODEVELOPMENT AND NEURO-GLIA INTERACTIONS

SPATIO-TEMPORAL DYNAMICS OF GLIAL CELLS, MACROPHAGES, AND EXTRACELLULAR MATRIX FOR GLIOBLASTOMA CHALLENGE

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Glioblastoma (GBM) is a lethal tumor and accounts for more than 60% of all brain cancers. GBM progression is promoted by a complex interaction with glial cells, that work as the brain's innate immune system. Microglia, astrocytes, and resident macrophages undergo (epi-)genetic, molecular, and morphological changes to acquire a tumor-supporting phenotype, that could be related to the phase of the disease. Therefore, the identification of time-dependent molecular targets may help to understand the pathways through which GBM affects glial cells. GL261 glioma cells were injected in the right striatum of immuno-competent C57Bl6J mice and animals were sacrificed after 7, 14, and 21 days (7D, 14D, 21D). The tumor development was assessed through 3D reconstruction of tomographic imaging and brains were processed by immunohistochemistry, immunofluorescence, and western blotting. In the early stage, the proliferating tumor (ki67+) appeared with a spotted distribution and triggered astrocytes (GFAP+, glial fibrillary acidic protein) reaction. Microglia and macrophages (Iba1+, ionized calcium-binding adaptor molecule 1) were scarcely represented at the site of the tumor injection, and the chemokine-CCL2-dependent recruitment of inflammatory monocytes appeared to be reduced. The tumor bulk became established at 14D and was surrounded by a dense scar of reactive astrocytes, paralleled by an increase of the phagocytic cells in the peritumoral area (CD68+; Iba1+; GFAP+), which may remove tissue debris and contribute to the extracellular matrix (ECM) remodeling. Accordingly, the ECM modifier metalloproteinase 9 (MMP9), and tenascin C (TnC) protein levels peaked at this stage, allowing CD133+ glioma stem cells to migrate out of the primary bulk, which appears inflamed, necrotic, and infiltrated by microglia/macrophages at 21D, as indicated by the rise in the level of CCL2 and Iba1. However, the protein expression of the specific microglial activation marker TMEM (transmembrane protein 119) was downregulated, suggesting differential regulation for tumor-associated-resident microglia and peripheral macrophages. The present study emphasizes the role of functional changes in the microenvironment during the GBM progression, fostering the studies on novel multi-targeted, time-dependent therapies in an experimental model similar to the human disease.

UNDERSTANDING THE FUNCTION OF *AHDC1* USING *IN VITRO* MODELS

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Xia-Gibbs syndrome (XIGIS; OMIM #615829) is a very rare neurodevelopmental disorder caused by *de novo* heterozygous truncating mutations in the AT-hook DNA-Binding Motif-Containing 1 (*AHDC1*) gene (OMIM# 615790)¹⁻³. XIGIS is a phenotypically heterogeneous disorder in which patients usually present poor muscle tone and severe developmental delays with symptoms of autism spectrum disorders^{2,3}. The genetic basis of XIGIS were discovered in 2014 by Fan Xia and Richard Gibbs and more than 270 cases have been reported so far. Human *AHDC1* gene is a protein-coding gene located on chromosome 1p36, it contains 5 noncoding 5-prime exons, a single 4.9-kb coding exon and a noncoding 3-prime exon¹. The single coding exon encodes for a protein of 1,603 amino acids, containing two AT-hook DNA binding motifs. All XIGIS-associated mutations described to date are located in the single coding exon and likely lead to the translation of truncated forms of *AHDC1* protein¹⁻³ which could be involved in defective neural development, causing the neurological features of Xia-Gibbs syndrome. However, the functions of *AHDC1* are unknown and indeed the gene belongs to the group of T-dark genes. Thus, our research focuses on the characterization of *AHDC1* functions. Since the presence of AT-hook DNA binding motifs suggests that *AHDC1* might bind DNA, we started investigating its possible role in gene expression regulation by identifying genes responding to its perturbation in *in vitro* cellular models (SH-SY5Y and U-87MG cells, of neuronal and glial origin, respectively). By performing RNA-seq analysis upon *AHDC1* silencing, we found that the biological pathways related to intellectual disabilities, neural differentiation and nervous system functioning and development are enriched among differentially expressed genes in *AHDC1* knocked-down cells. In addition, studying *AHDC1* protein, we confirmed the nuclear localization of the endogenous *AHDC1* by immunofluorescence. Finally, by coupling overexpression of *AHDC1* with mass spectrometry analysis, we are investigating its structure and post-translational modifications as well as its interactors. Our preliminary results support the hypothesis that *AHDC1* could be crucial in regulating development and differentiation in the nervous system and therefore could help in the identification of the mechanism at the basis of pathogenesis for XIGIS which is still poorly understood.

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A NEW ANIMAL MODEL OF INTERNET GAMING DISORDER: SEXUAL DIFFERENCES IN BEHAVIOR AND BRAIN ACTIVITY

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In 2013 the American Psychiatric Association included Internet Gaming Disorder (IGD) as a mental disorder in the Diagnostic and Statistical Manual of Mental Disorders (DSM-5). IGD mainly occurs among adolescents, who after developing addiction, show psychopathological traits such as social anxiety, depressive disorder, attention deficit.

However, the different studies conducted so far show several limitations, such as exposure period, duration, and gender. Trying to address the lack of experimental model for such disorder, in the present work we proposed an IGD rat model to investigate some peculiar tracts of the disorder, such as sexual dimorphism, and to better understand the alleged role of hormones or the brain areas involved in IGD. In fact, previous studies in adolescents showed sexual differences in both play behavior and activation of related brain areas, such as mesocorticolimbic reward system (Nucleus Accumbens, NAc; Orbitofrontal Cortex, OFC; Prefrontal Cortex, PRL; Ventral Tegmental Area, VTA), the visual processing and cognitive control areas (Inferior Parietal Lobule and Middle Occipital Gyrus), the Thalamus and the Insula. We developed, for the first time, using a new apparatus provided with a touchscreen platform, an IGD rat model that resembles the fundamental features of the disorder (e.g., addiction, hyperactivity) and also sexually dimorphic activation of related brain areas. After five weeks of training, male and female Wistar Kyoto (WKY) rats were assessed for: a) their attachment to the game, as time spent in front of the screen, under different conditions (alone, together with a new object, and in a social and sexual context), b) their compulsiveness during gaming (duration and number of touches on apparatus), and c) the maintenance of these conditions after a period of game pause and a reward interruption. According to multicriteria described in the literature, it was possible to identify IGD-rats in 16/18 males and in 21/21 females, which obtained scores between 66 and 99%. IGD-rats showed a significant increase in frequency and duration of play, and time spent in front of the screen compared to both controls and rats which have been trained but did not develop addiction. Moreover, IGD-females showed greater interaction and duration of play, which was maintained even in the presence of sexual or social stimuli, compared to IGD-males. Last, with immunohistochemical techniques, we analyzed the c-fos (a neuronal activity marker) immunoreactivity to investigate different neural areas correlated to addiction disorder: cortex (PRL, M1, Orbital cortex), Amygdala, NAc, and paraventricular nucleus of the thalamus (PVT). Quantitative analysis of c-fos immunoreactivity showed a significant increase in all areas of the cortex and the amygdala of IGD-rats compared to controls. Sexually dimorphic immunoreactivity in PVT and NAc was reduced in IGD-females and increased in IGD-males compared to control rats. In conclusion, we developed a first animal model of IGD in rats, with great translational potential. In fact, it presents characteristics also found in IGD patients: the development of addiction-like behavior, the sex difference in susceptibility, and the changes in brain activity. The use of our animal model of IGD will allow us to further investigate the neurological basis of the disorder taking into account also the sex differences.

TRANSCRIPTIONAL AND EPIGENOMIC PROFILING OF THE VISUAL CORTEX REVEALS MYELIN DISORGANIZATION IN CDKL5-DEFICIENCY DISORDER PATIENTS

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CDKL5 deficiency disorder (CDD) is a rare and severe X-linked genetic disease, characterized by a broad spectrum of clinical manifestations, including developmental impairment, treatment-resistant epilepsy, autistic-like traits and sensory abnormalities (*i.e.*: cortical visual impairments). Currently, very little is known on the neurobiological alterations underlying the disease in humans, a main reason why patients affected by CDD, mostly females, are without any resolutive therapy. CDD is driven by mutations of the Cyclin-dependent kinase-like 5 (CDKL5) gene, which encodes a serine/threonine kinase essential for circuit organization and function. CDKL5 is highly expressed in the forebrain, both in glutamatergic and GABAergic neurons, and can shuttle between nucleus and the cytoplasm. While the phospho-targets and roles of CDKL5 in the somato-dendritic processes have been recently understood, the nuclear functions of CDKL5 remain poorly investigated. Several lines of evidence suggest that CDKL5 may participate in the regulation of gene expression by interacting and regulating the activity of epigenetic factors, *i.e.*: MeCP2, DNMT1 and HDAC4. Since impaired epigenetic and transcriptional regulation has been proposed as being one of the leading causes in neurodevelopmental diseases (*e.g.*: Rett syndrome), it is thus crucial to understand the impact of CDKL5 mutations in the nuclear compartment. In this study, we assessed whether CDKL5 may be involved in the regulation of both epigenetic and transcriptional processes to provide novel insights on CDD pathophysiology. To this aim, we employed a multidisciplinary approach on post-mortem BA17 cortical samples from two CDD patients and two neurotypical, age- and sex-matched, controls. By using immunofluorescence and reduced representation bisulfite sequencing (RRBS) techniques, we found that CDD brains displayed profound epigenetic landscape abnormalities impacting both histones' modifications and DNA methylation pattern. Moreover, RNA-seq and bioinformatics analysis revealed that these epigenetic alterations were paralleled with changes in expression levels of genes involved in the formation and/or maintenance of myelin. Additionally, integrative analysis of CDD-associated transcriptional profile and dysregulated genes of human postmortem brains from Rett syndrome and autism spectrum disorder patients, revealed a significant overlap between the datasets, suggesting that these clinically-related neurodevelopmental disorders may arise from shared dysregulated biological processes. Finally, we show that myeloarchitecture in CDD patients is abnormal, a defect that is also expressed by a mouse line modelling CDD. Our study, even though generated on a limited number of samples, discloses for the first time that the process of myelination is a mechanism involved in CDD pathophysiology.

MORPHOLOGICAL CHARACTERIZATION OF ADULT-BORN HIPPOCAMPAL NEURONS IN A MOUSE MODEL OF THE NEURODEVELOPMENTAL DISORDER BBSOAS

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Intellectual disability (ID) is a neurodevelopmental pathological condition characterised by limitations in intellectual functioning and adaptive behaviour, which affects worldwide 1.56 million people, 2 to 3% of the general population. Although the causes are highly heterogeneous, genetic factors take a large part in the etiology of ID. The Bosch-Boonstra-Schaaf optic atrophy-intellectual syndrome (BBSOAS; OMIN#615722), is a rare disorder caused by mutations in the NR2F1 gene, characterized by mild to moderate ID associated to global developmental delay, optic nerve atrophy, hypotonia, seizure and autism spectrum disorder (ASD) traits. The transcriptional regulator Nr2f1, also known as COUP-TFI, acts as a key player in multiple cellular processes, from orchestrating the development of neocortical and hippocampal circuitries to the control of adult neural stem cell fate choice in the adult mouse hippocampal dentate gyrus (DG). Interestingly, alterations of proliferation, maturation, and functional integration of adult-born granule neurons in the hippocampal circuit have been reported in animal models of ID and recent findings suggest that a deficit in hippocampal plasticity may contribute to BBSOAS. Here, to investigate the possible effects of Nr2f1 haploinsufficiency on the hippocampal circuit we took advantage of a recently validated BBSOAS mouse model (*i.e.*, constitutive Nr2f1 heterozygous mice) and focussed on the adult hippocampal DG. First, quantification of doublecortin (DCX)-positive immature neurons revealed no difference compared to control mice, suggesting that constitutive Nr2f1 haploinsufficiency does not alter the number of new DG granule cells generated in adult mice. Next, we carried out an in-depth characterization by confocal imaging and 3D morphometric reconstruction of the adult-born DCX+ immature neurons. Interestingly, data collected so far strongly suggests that Nr2f1 haploinsufficiency influences dendrites architecture and proper development of adult-born neurons within the adult mouse DG, leading to the appearance of atypical and peculiar neuronal morphologies, which are usually associated with pathological conditions and aberrant hippocampal circuitry rearrangements. Moreover, our ongoing analysis focusing on the expression of immediate early genes - used as a proxy for neuronal activation (*e.g.*, Npas4) - started to depict an altered recruitment of neuronal ensemble of neurons in the DG of Nr2f1 heterozygous mice. Future analyses will be aimed at elucidating the underlying mechanisms for a better understanding of the cell-intrinsic versus cell-extrinsic components of the observed defects.

GFAP DELETION INDUCES MOLECULAR CHANGES IN THE SPINAL CORD NOT PARALLELED BY BEHAVIORAL ALTERATIONS

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Peripheral nerve injury has been used as a model to induce maladaptive changes in the central nervous system (CNS). The primers of this cascade-like events are glial cells (namely astrocytes and microglia) that rapidly undergo morpho-functional modifications, leading to a reactive state (reactive gliosis). Although reactive gliosis is well documented, the role of intermediate filaments (IFs) in maladaptive plasticity processes is yet to be established. Glial acid fibrillary protein (GFAP) is the hallmark of the reactive astrocytes and is significantly upregulated in reactive astrogliosis. However, how GFAP is linked to astrocyte's pathophysiology is far to be demonstrated. To verify the specific role of GFAP in spinal maladaptive plasticity, we used a GFAP-KO mice model of sciatic spared nerve injury (SNI) and compared it to wild type (WT) animals. Animals were studied with behavioral tests (von Frey and plantar test) and ex-vivo with immunohistochemistry and WB of the spinal cord lumbar tract for astrocytic (vimentin) and microglial (Iba1) markers. Glial and neuronal markers of the glutamate/GABA system (GLAST, GLT1, vGLUT, vGAT, GAD) were also analyzed. Our results demonstrated that normal phenotype, neuropathic behavior and reactive gliosis following SNI are GFAP-independent processes. However, GFAP influences spinal cord homeostasis and morpho-molecular characteristics such as microglial density, neurotransmitters metabolism and transport.

EARLY POSTNATAL TREATMENT WITH ESTROGEN RECEPTOR ANTAGONISTS: SEXUALLY DIMORPHIC ORGANIZATIONAL EFFECTS

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Many hypothalamic systems, controlling metabolism and reproduction, are programmed and stabilized during critical periods of development by many factors, including gonadal steroids [1]. In particular, estradiol (E₂) appears to have an important role on organization of these circuits [2-4]. E₂ acts through three different receptors: ER α , ER β and GPR30. To understand the role of these receptors on organizational effect of E₂, we treated male and female CD1 mice from post-natal day (PND) 5 to PND12 with subcutaneous injections of vehicle (corn oil), E₂ and E₂ associated with selective antagonist of estrogen receptors (MPP; PHTPP; G15) alone or together (mix). We analyzed, during the development, different physiological parameters related to food intake (body weight, food eaten, daily feed efficiency, gonadal and brown fat), reproduction (gonads, puberty onset, estrus cycle) and behavior (Y-maze, sexual behavior). Furthermore, in the adult, we have immunohistochemically highlighted the expression of some hypothalamic neuronal circuits closely associated with food-intake and metabolism, but also with the reproductive sphere: Pro-opiomelanocortin (POMC), Neuropeptide Y (NPY), Orexin and Kisspeptin systems. In general, E₂ induced effects mostly in females both on sexual and feeding behaviors. The treatments with G15 alone or in combination (mix) altered all the

considered parameters in both sexes. On the contrary MPP and PHTPP showed sexually dimorphic effects. MPP modified, in males, feeding parameters, but not those related to reproduction, whereas PHTPP modified parameters related to reproduction, but not those related to feeding. In females the situation was exactly the reverse. In conclusion, our data demonstrate that E₂ has a strong organizational role on different neuroendocrine systems, acting primarily on GPR30 and, in a sexually different way, on ER α and ER β .

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DEVELOPMENT OF A CHITOSA-BASED MEDICAL DEVICE FOR IMPROVING FUNCTIONAL RECOVERY AFTER RADICAL PROSTATECTOMY

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Prostate cancer is the most frequent cancer in males, the current most popular treatment of localized prostate cancer in patients with a life-expectancy >10 years is radical prostatectomy (RP). Unfortunately, in patients who undergo RP, frequently iatrogenic damage to the periprostatic neurovascular bundles (NVBs) occurs, leading to erectile dysfunction and impairment in quality of life. Chitosan is a derivative of chitin obtained from the exoskeleton of crustaceans and its useful properties in intra-operative field such as hypoallergenicity, biocompatibility, bioavailability and lack of toxicity has demonstrated to enhanced somatic nerve regeneration with effects compared to those elicited by nerve autografts. Recently, two patents about the clinical use of chitosan membranes for protection of periprostatic nerve plexus have been issued, while, from *In vitro* and *Ex vivo* experiments, the pro-regenerative effect of flat chitosan membrane on autonomic explant ganglia reported was very high and prostate cancer cells cultured in the presence of chitosan, showed a significant reduced proliferation rate. In order to improve the regenerative performance achieved by the flat membrane, the present project focuses on nanostructured chitosan membranes with two different topographies, a grating arrangement and a zig-zag pattern. At this purpose primary neuronal cultures and glial cell were cultured on the different membrane types in order to evaluate the ability of the membrane to sustain cell survival, adhesion and migration and, for the neuronal models, the neurite outgrowth. At the same time, preliminary *in vivo* experiments were performed to test the ability of different chitosan membranes to improve regeneration of cavernous nerve on adult male rats: 3 mm of cavernous nerve was bilaterally transected and repaired with chitosan membranes (flat, grating, zig-zag). At 30 and 60 days from the surgical procedure, samples were harvested and morphological analysis were carried out in order to identify the presence of nerve fibers. Samples were processed for immunofluorescence analy-

sis allowing to detect nerve fibers and numerous nuclei. These *In vivo* preliminary results provide the first experimental evidence supporting the ability of the chitosan membrane to allow autonomic axonal regeneration demonstrating the safety of the device for clinical use and supporting its application as an effective adjunct strategy to reach the functional recovery of the periprostatic plexus.

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MOLECULAR AND HISTOPATHOLOGICAL FEATURES OF A GENETIC NEUROCHAPERONOPATHY ASSOCIATED WITH A MUTATION IN THE CHAPERONIN SUBUNIT CCT5

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The chaperone system (CS) has canonical and non-canonical functions and the former pertain to the maintenance of protein homeostasis. The chief components of the CS are the molecular chaperones that are typically cytoprotective but, if abnormal, they can cause diseases, the chaperonopathies, which can be genetic or acquired (1). The neurochaperonopathies (NCP) affect primarily the nervous and muscular systems (2), for example, those associated with mutations in the CCT5 gene (3, 4). The chaperone CCT is a chaperonin of Group II that to function in protein homeostasis forms a team of 8 subunits and networks with teams of other chaperones. It is critically important for the cell since at least of 15% of the cytosolic proteins, such as actin and tubulin are its obligate or preferential substrates. Two mutations, His147Arg and Leu224Val, in the CCT5 subunit are known to be linked with NCP (4). In this study, we examined the muscle molecular and histopathological abnormalities associated with the Leu224Val mutation. We found muscular atrophy and muscle-cell apoptosis, and disruption and abnormal distribution of various muscle proteins with intra- and extra-cellular aggregates. Desmin was fragmented and disorganized and the chaperones normally located at the Z-disk were diminished. Our findings present for the first time a detailed picture of the muscular abnormalities associated with an NCP and provide clues regarding an extended spectrum of substrates for CCT, beyond what is currently known.

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CCT CHAPERONIN MUTANTS ASSOCIATED TO SENSORY AND MOTOR NEUROPATHIES: ABNORMAL MOLECULAR SHAPE RESULTS IN DYSFUNCTION

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The health of the human body depends on the correct state of its organizational levels, *i.e.*, atoms, molecules, cells, tissues, organs, and systems. Each of them has specific structural features (form) designed to perform one, or more, roles (functions). If structure changes, functions may be altered. Different point mutations in the same gene can cause distinct amino acid substitutions, resulting in distinctive protein forms (shapes) with diversely altered functions. This situation is of utmost interest when members of the chaperone system (CS) are affected because they are essential for protein homeostasis in the entire body. A member of the CS is the CCT chaperonin, which is present in all human cytotypes and participates in the folding of about 15% of cytosolic proteins (1). Mutations in the gene encoding the CCT subunit 5 (CCT5) are associated with disorders of the nervous and neuromuscular systems now classified as neurochaperonopathies (NCP) (2). In the present work, we analysed *in silico* the structure of CCT5 wild-type and mutants Leu224Val and His147Arg, the former associated with a motor NCP and the latter with a distal sensory NCP (3,4). CCT5 functions are ATP-dependent, therefore, we examined it by molecular dynamics simulations under three conditions: nucleotide-free, ADP-bound, and ATP-bound. The results demonstrated that the His147Arg mutation, occurring in the equatorial domain of the CCT5 molecule, also affects the apical domain. A similar remote effect on the apical domain may be caused by the Leu224Val mutation, which occurs in the intermediate domain, three amino acids before the start of the apical domain. This is, however, very near the apical domain and our finding may instead indicate

that this domain starts at least three amino acids before of what is now considered its initial position in the amino acid sequence. In any case, these results emphasize the importance of the apical domain conformation in determining CCT5 functionality. The findings also provide the basis for investigating the structure-function deviations of the CCT chaperoning team underpinning the molecular anomalies and histological lesions observed in patients with a CCT5 NCP.

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HUMAN IPSCS-DERIVED OLIGODENDROCYTES AND ASTROCYTES: A MODEL FOR AUTOSOMAL DOMINANT LEUKODYSTROPHY

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Autosomal Dominant Leukodystrophy (ADLD) is a rare genetic disease, characterized by autonomic dysfunction and movement disorder, and associated with white matter loss in the central nervous system (CNS). The genetic cause is the presence of three copies, instead of the two normally present, of the gene that contains the instructions to produce the lamin B1 (LMNB1) protein, which belongs to a group of structural proteins forming the nuclear membrane of the cell. Although it is well known that LMNB1 regulates nuclear mechanics and integrity, interacts with chromatin, and regulates gene expression, pathogenic mechanisms in ADLD have only initially been explored. Moreover, a therapy to treat this disease is not available at the moment. Disease-relevant human models are therefore crucial to study disease pathogenesis and to further screen for effective therapies. Based on evidence showing glial pathology in ADLD patients, we set out to generate human glia cells (oligodendrocytes and astrocytes) from human-induced pluripotent stem cells (hiPSC) derived from fibroblasts of either ADLD patients or healthy donors (CTRL). We established a differentiation protocol based on three

stages: the commitment to neural progenitors, the production of gliospheres and a further maturation step into authentic astrocytes and, in lower percentage, oligodendrocytes. Preliminary observations indicate a lower gliogenic potential in the hiPSC ADLD lineages, as revealed by gliosphere production. In both ADLD hiPSC and astrocytes, an increased expression of LMNB1 was confirmed by real time PCR and protein expression analyses, together with morphological alterations affecting the nuclear shape. Functional analyses (e.g. Calcium Imaging) are ongoing to investigate possible alterations correlated to LMNB1 overexpression. Thus, the developed model appears as a promising "disease-in-a-dish" platform to further reveal so far unknown dysfunctions of the diseased cells and, prospectively, aid the development of effective therapeutic strategies for this rare genetic disease.

MIGRATING SCHWANN CELLS USE BLOOD VESSELS LIKE A PATHWAY TO COLONIZE NERVE CONDUITS

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The intrinsic regenerative ability of the peripheral nervous system is not sufficient to guarantee a functional recovery after a damage with the loss of nervous substance. Indeed, the use of autograft or conduit to repair the aforementioned severe nerve injury is required to bridge the gap and avoid axon dispersion. Although autograft is the gold standard technique, the conduits are widely used to repair gaps because of their comparable effectiveness. A deeper comprehension of the cellular interactions inside a hollow tube could improve the design of nerve conduits in order to be able to repair even longer gaps. Until now, the migration of Schwann cells on endothelial cells has only been studied on the nerve bridge model, a spontaneous formation of nervous tissue to connect nerve stumps 2-3 mm apart. In this study, we investigate whether endothelial cells play a guide role for migrating Schwann cells also within the nerve conduits used in the surgical practice to repair larger nerve gaps. Median nerves of adult female rats were injured and repaired with 10 mm chitosan conduits. The regenerated nerves, collected at different time points (7-, 14-, 21- and 28-days after the repair), were cut into slices 50 µm thick to be analyzed by confocal imaging. Our results show endothelial cells to form a dense capillary network, while only polarized vessels are used by clusters of Schwann cells to migrate from the two nerve stumps within the conduit. In conclusion, angiogenesis plays a key role to restore the functional architecture of the nerve by providing a directional pathway for the migration of newly formed Schwann cells within conduit. The use of methods to boost vascularization might be an interesting strategy to support and enhance nerve regeneration when angiogenesis is impaired, as in long gap.

ONCOLOGY AND MICRO AND NANOVESICLES IN BIOMEDICINE

QUANTITATIVE PATTERNS OF THE CHAPERONES HSP10 AND HSP90 IN TUMORS OF THE HUMAN MAJOR SALIVARY GLANDS: DIFFERENCES WITH NORMAL TISSUE AND PATHOGENIC IMPLICATIONS

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The chaperone system (CS) is involved in the cellular physiology and pathology, including tumorigenesis of tissue and organs. Current understanding of tumorigenesis is incomplete without having a complete background on the CS involvement. It is increasingly clear that the CS is involved 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 major salivary glands, on which scarce literature can be found. Clinicians, in particular histopathologist lack the proper training to properly classify and identify the different tumors of salivary glands due to the rarity of cases and the heterogeneity as well as the various types of salivary gland tumors. Additionally, lack of standardized protocols of treatments for patient care calls for urgent need to individualize etiopathogenic factors that can be targeted for therapy or on which physicians can base their work. Here, we present results pertaining to the chaperones Hsp10 and Hsp90 in the parotid (PG) and submandibular (SMG) glands. Quantitative evaluations of both chaperones by immunohistochemistry (IHC) and immunofluorescence-confocal microscopy (IF-CM) showed marked differences between different tumors and between tumoral and normal tissue for Hsp90 but not for Hsp10. These new findings in comparison with our previous results on Hsp27 and Hsp60 demonstrate that Hsp90 distinguishes itself from the other chaperones studied because it shows quantitative patterns distinctive of each salivary gland pathology. Consequently, determination of tissue levels of Hsp90 by IHC and/or IF-CM offers a potentially useful means for differential diagnosis of diverse salivary glands pathologies. In addition, the data open new avenues for investigating the role of Hsp90 in carcinogenic mechanisms in the salivary glands. Finally, our data offer a new methodology for untrained histopathologist to be able to identify and differentiate between benign and malignant tumors of salivary glands while relying on chaperone-based IF or IHC which may reduce ambiguity and doubts when routine examination is performed.

MALIGNANT PLEURAL MESOTHELIOMA – DISCOVERY OF NEW POTENTIAL THERAPEUTIC TARGETS AND PERSONALIZED THERAPY BASED ON SELECTIVE DELIVERY OF DRUG-LOADED EXTRACELLULAR VESICLES

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First line treatments in the handling of MPM are not resolute in the most of cases, driving the patient in an ineluctable relapse of the disease after few years from the surgery and/or chemotherapy (1,2). In this context, emerges a realistic possibility of the utilization of a “Trojan horse” self-produced by the tumor itself as a paracrine communication mechanism. In fact, we underline that none of the current therapies or the ongoing clinical trials were deemed efficient at prolonging the overall survival of the affected population and consequently a complete remission of the affected patient. The use of appropriately engineered extracellular vesicles (EVs) has been investigated to exploit a consolidated paracrine communication network in most solid tumors to selective delivery of chemotherapeutic compounds directly to the target site. The engineered EVs used derive from the tumor itself. The main goal of the project consists of the setup of an efficient method of extraction, purification, and engineering of the MPM's EVs. In this work, EVs have been isolated by differential ultracentrifugation and ultrafiltration steps and then, have been characterized (1, 3-5). To monitor the drug and the effective achievement of the drug loading into the extracellular vesicles, fluorescent cisplatin variant, a compound that we synthesized, was used. Two variants of the same compound were made using the amino to amide mechanism: a variant with a fluorescent tag (FLUOTAG) and a canonical one, completely comparable in structure to the variant in commercial use. The two variants were created to investigate possible cytotoxicity that the size of the FLUOTAG could have produced in the *in vitro* model. Moreover, after a molecular characterization, preliminary tests were assessed to find the best way to create stable and fully loaded cisplatin EVs. Preliminary tests show significant EVs production by the tumor model itself, a good extraction yield and promising results that suggest a good prospective applicability in precision medicine.

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EXTRACELLULAR MOLECULAR CHAPERONES IN CANCER THERANOSTICS: FROM BENCH TO BEDSIDE

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Cancer diagnosis comprises a variety of tests, including microscopic examination of tumor biopsies. In recent times, liquid biopsy has been introduced and shown promise for early diagnosis and patient follow up (1). In parallel, the identification of extracellular vesicles (EVs) shed by tumor cells has provided a new source of useful biomarkers and a mechanism for delivering anti-cancer drugs to the chosen target (2). EVs transport molecules that reflect the contents and metabolic status of the cell of origin, therefore collecting them from peripheral blood, liquid biopsy, is easy, minimally invasive, and can be done periodically. Another advance in oncology has been the realization that members of the chaperone system (CS) are involved in pro- and anti-cancer mechanisms (3). The chief components of the CS are the molecular chaperones, some of which go through quantitative changes during tumor development and in response to anti-cancer treatment. These characteristics make them convenient biomarkers for diagnosis, prognostication, and patient follow up. Chaperone types and levels can be assessed in fluids and tissues by various methods. Lately, they have been found along with corresponding microRNAs in EVs shed by tumors (4). For this reason, we have directed our research to standardize methods for preparing EVs from liquid biopsies to examine their contents under various conditions during the disease evolution, focusing on chaperones and pertinent microRNAs. Measurement of these biomarkers carried by EVs is providing key data on tumor spread and resistance to anti-cancer drugs. The EVs content reveals the nature and degree of malignancy of the tumor that shed them. Another important aspect of EVs is that they constitute a means by which tumors deliver molecules at distant sites to prepare them for the arrival of metastases. By examining EVs one can obtain information about the molecules that will prepare the microenvironment for the successful implantation of metastatic cells and, thus, learn how to block/inactivate them.

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INTERPLAY BETWEEN ONCOGENIC BRAF AND P53 IN MELANOMA CELLS AND MOLECULAR TARGETING BY HISTONE DEACETYLASE INHIBITORS

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Histone deacetylase inhibitors (HDACI) are widely considered very promising antitumor agents since they induce histone acetylation and the consequent expression of different target genes including tumor suppressor and pro-apoptotic ones. Here, we focus on the effects of HDACI in two melanoma cell lines characterised by BRAF V600E oncogenic mutation but with different p53 status. SK-Mel 28 cells express an oncogenic mutated p53 while A375 express wild type p53. Our results indicate that two pan-HDAC Inhibitors, SAHA and ITF2357, dose dependently reduce the viability of both SK-Mel 28 and A375 melanoma cells and induce histone acetylation. Interestingly, both inhibitors markedly decrease oncogenic BRAF protein level in the two cell lines. We also found that oncogenic BRAF protein has a nuclear localization in both melanoma cell lines and that HDACI also markedly decrease BRAF at the nuclear level. Immunoprecipitation revealed that oncogenic BRAF interacts with oncogenic p53 in SK-MEL28 cells and that HDACI reduce this interaction. In this cell line, HDACI induced proteasome-mediated degradation of oncogenic p53 while in A375 wild type p53 increased and was most likely involved in HDACI-induced apoptosis. In conclusion, our data indicate that HDACI exert a potent antitumor effect in melanoma cells and target oncogenic BRAF and p53, thus supporting a strong potential of these agents in melanoma targeted therapy.

EXTRACELLULAR VESICLES RELEASED BY PROSTATE CANCER CELLS ENDOW ADIPOCYTES WITH TUMOR-PROMOTING PROPERTIES

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It is known that an association exists between obesity and risk of prostate cancer (PCa). A crosstalk between adipocytes and PCa has been demonstrated; however, the study of this dialog has been limited to soluble factors, although emerging evidence points to a key role of extracellular vesicles (EVs) in the control of tumor progression. We have previously demonstrated that adipocyte-derived EVs can stimulate PCa growth, metastasis and chemoresistance, by triggering an Akt-HIF1 α -mediated Warburg effect in tumor cells. Herein, we found that PCa cell-released EVs can promote both lipolysis and adipokine (interleukin 6, MCP-1 and TNF α) production in adipocytes, accompanied by Akt, ERK1/2 and p38 activation. Interestingly, conditioned media from EV-treated adipocytes further stimulated PCa cell proliferation, migration/invasion and docetaxel resistance. Overall, these data indicate that an EV-mediated crosstalk exists between PCa and adipocytes, endowing the latter with pro-tumor properties. Further studies will be performed to confirm this evidence *in vivo* and to identify the EV molecular cargo responsible for the modulation of the interactions between adipose tissue and PCa.

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FROM PHYSICAL EXERCISE TO MOLECULAR PATHWAYS: INSIGHT TO TREAT CANCER-RELATED CACHEXIA

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Cancer-related cachexia is a multiorgan syndrome characterized by an involuntary loss of muscle mass and changes in adipose tissue. Recent advances highlighted an abnormal tissue cross-talk, driven by inflammatory cytokines signaling, which cause a structural and metabolic shift in skeletal muscle. On the other hand, it is well known the health effects of physical exercise in restoring the whole body homeostasis through the modulation of anabolic and catabolic stimuli. In line with this background, the present study was aimed to compare the effects of different training protocols on lifespan, tumor growth, cachexia onset, and skeletal muscle homeostasis. One hundred and fifty 3-month-old male mice (BALB/c AnNHsd), subcutaneous inoculated with a fresh fragment of C26 colon carcinoma were divided into six 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), training progressive-inoculated-training high intensity (TR_P/I/TR_H), training (SED/TR), and control (SED/SED). Immunofluorescence and quantification analysis were performed on tumor, gastrocnemius, plantaris, and soleus muscles slices to detect Hsp60, Isolectin, and Pgc1 α proteins expression as a marker of oxidative stress, vascularization, and mitochondrial biogenesis, respectively. The results showed a significant increase in lifespan's average of the TR_P/I/TR_H group compared to the other groups and a significant difference between the TR_P/I/TR_H and the SED/I/TR_P in cachexia onset. The immunofluorescence analysis showed increased immunoreactivity of Hsp60 in soleus muscle compared with plantaris and gastrocnemius muscles, and the Hsp60 signal was stronger in the trained groups than in sedentary counterparts. Immunoreactivity of Isolectin was significantly increased in white gastrocnemius and soleus muscles from SED/I/TR_P and TR_P/I/TR_H groups compared to the not-bearing tumor groups. In plantaris and red gastrocnemius muscles from TR_P/I/TR_H group was assessed a higher level of Isolectin compared to the SED/SED group, while the amount of this protein was significantly increased in the SED/I/TR_P group compared to both SED/SED and SED/TR groups in the same muscles. Immunofluorescence analysis for Pgc1 α showed higher protein levels in soleus, plantaris, and red gastrocnemius muscles from SED/I/TR_P and TR_P/I/TR_H groups than SED/SED group. All these findings suggest a promising role of physical exercise in improving life quality in chronic diseases such as cancer-related cachexia. Moreover, knowledge about the molecular pathways underlying the health effects of physical exercise could represent a crucial issue to develop a new therapeutic target.

THE WORD-OF-MOUTH MESSAGE OF MICROGLIA EXTRACELLULAR VESICLES

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Extracellular vesicles (EVs) represent a heterogeneous group of cell-derived membranous structures released by all cell types, including brain cells. There are three types of EVs, classified on the basis of their origin and size, exosomes, microvesicles, and apoptotic bodies which originate from the endosomal system, shed from the plasma membrane, or produced by cells undergoing apoptosis respectively. EVs are present in biological fluids, involved both in multiple physiological and pathological processes, and are now considered as an additional mechanism for intercellular communication, allowing cells to exchange proteins, lipids and genetic material. Microglia, the resident immune cell in the brain, is involved in immune surveillance and inflammatory responses. When the immune homeostasis is disturbed, microglia can be phenotypically transformed into two types of state: the classically activated M1 (pro-inflammatory) and alternatively activated M2 (anti-inflammatory) phenotype. Microglia release EVs both in normal conditions and after addition of proinflammatory stimuli. Upon activation, microglia also undergo evident morphologic changes, from resting ramified shape into activated amoeboid morphology. These changes are concomitant with up-regulation of several transcription factors and release of soluble factors, such as proinflammatory cytokines. In our study we have analyzed the effects of the EVs obtained from microglia with and without stimuli, on microglia activation. For this purpose, Lipopolysaccharides (LPS) is a potent pro-inflammatory stimulus, in this study we have used LPS to activate BV2 microglia cells and induce EVs release; the EVs obtained with LPS stimulation, called LPS-EVs, were then utilized to stimulate BV2 resting cells to investigate their ability to induce microglia polarization towards pro-inflammatory state. The change of BV2 cells morphology, proliferation and migration of the microglia after LPS-EVs stimulation were also investigated, in addition to the expression and the release of pro-inflammatory cytokines. The encouraging results of this study have demonstrated that LPS-EVs are able to induce microglia activation in the same way as the LPS alone, instead the EVs obtained from control cells are unable to induce microglia polarization towards a pro-inflammatory state. These observations underline the important role of EVs in cell communication and demonstrate that EVs produced in an inflammatory environment may contribute to exacerbate the inflammatory responses, as observed in activated microglia that, in turn, may influence all the brain cells.

THE RNA PROFILE OF LEMON-DERIVED EXTRACELLULAR VESICLES: THE SECRET OF THE CROSS-KINGDOM COMMUNICATION?

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Cross-kingdom interaction allows communication between living organisms. Plant-derived extracellular vesicles (PDEVs)

are pillar of cross-kingdom communication, rising interest in the scientific community for their biological properties and possible application. We isolated PDEVs from the juice of Citrus limon L. (LEVs); vesicles were characterized at dimensional, morphological and biochemical levels. We have recently demonstrated that LEVs have a protective role on the inflammatory and oxidant processes, in murine and primary human macrophages, as well as in human skin fibroblasts. Here, in order to correlate the observed biological effects with LEV-RNA profile, we carried out a Next-generation sequencing analysis. We identified 825 differentially expressed genes between LEV and juice, with 67 up-regulated ($\log_2FC > 1$) and 758 down-regulated ($\log_2FC < -1$) in LEVs. Enrichment analysis of KEGG terms revealed two significantly enriched pathways (FDR < 0.05) in up-regulated (EV) genes: Protein processing in endoplasmic reticulum (map04141) and Ribosome (map03010). In addition, we identified 29 long-non coding RNAs (lncRNAs) more abundant in LEVs compared to the juice. Computational analysis showed that these lncRNAs may sponge several human microRNAs whose targets are involved in the signal transduction and cell communication processes. In conclusion, here we reported for the first time the existence of lncRNAs in plant-derived extracellular vesicles; ongoing studies are aimed at correlating the observed biological effects with lnc-RNAs identified in LEVs.

VITAMIN D BINDING PROTEIN: A NEW PLAYER IN THE ONSET OF CANCER CACHEXIA

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Vitamin D binding protein (VDBP) is a multifunctional serum glycoprotein belonging to the albumin gene family and synthesized by hepatocytes, whose main function is the transport of vitamin D in the bloodstream. Proteomic analysis of samples from patients affected by pathologies susceptible to cachexia, including several types of tumors, shows an upregulation of VDBP. Coherently with the upregulation of VDBP observed in cancer patients, we observed an upsurge of VDBP also in two commonly used models of cancer cachexia, C26 colon carcinoma and Lewis Lung Carcinoma (LLC) tumor-bearing mice. We therefore hypothesized that VDBP may contribute to cancer cachexia-associated muscle loss. *In vitro*, treatment of C2C12 myotubes with increasing doses of VDBP induced atrophy, seen as myotube diameter reduction, without the involvement of the ubiquitin-proteasome system and likely through exacerbation of autophagy. Furthermore, VDBP treatment caused a severe mitochondrial dysfunction and impaired the formation and stability of acetylcholine receptor (AChR) clusters, an *in vitro* model of neuromuscular junctions (NMJ). The proof that VDBP induced atrophy acting directly on C2C12 myotubes and not by putatively sequestering trophic factors in the culture media was provided by the fact that VDBP-induced atrophy was prevented by silencing of megalin, the endocytic receptor that mediates VDBP entry in cells or by the coadministration of cilastatin, a recently identified blocker of megalin. To assess if VDBP had a causative role in muscle homeostasis also *in vivo*, we experimentally induced the expression of VDBP in VDBP knock-out (KO) mice by adeno-associated virus (AAV)-mediated gene expression. A relatively small amount of circulating VDBP was sufficient to reduce muscle performances and mass. Moreover, coherently with the *in vitro* results, VDBP induced morphological abnor-

malities in NMJ, seen as a reduction of AChR clusters and endplates surface area, and increased fragmentation. To assess if VDBP was involved in cancer cachexia-associated muscle wasting, we induced cancer cachexia in VDBP KO mice by inoculation of LLC cells. Tumor-bearing VDBP KO mice preserved their body weight, their performances, and the muscle loss was minimal, compared to cachectic WT mice. Moreover, while mitochondria of cachectic WT muscles were visibly damaged, mitochondria of cancer-bearing VDBP KO mice were preserved. Finally, preliminary data suggest that cachexia in WT mice is accompanied by NMJ dismantling, while NMJ architecture was mainly maintained in tumor-bearing VDBP KO mice. Although the primary function of VDBP is to transport vitamin D metabolites, we showed that VDBP acts as a hormone *per se*, having a direct pro-atrophic activity on skeletal muscle.

TRANSLATIONAL APPROACHES TO EXPERIMENTAL BIOLOGY

GENETIC DETERMINANTS OF SENSORY IMPAIRMENT IN ITALIAN SEMI-SUPERCENTENARIANS

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Age Related Sensory Decline (ARSD) is the slow and gradual deterioration of function of single or multiple senses simultaneously during the life span. Sensory impairment, especially the olfactory deficiency, has been shown to be associated with mortality [1,2,3]. Centenarians are widely considered an ideal model of healthy aging owing to their abilities to avoid or largely postpone major age-related diseases. The aim of this research is to investigate if centenarians are protected from sensory impairment by their genetic background. We here first describe sensory impairment in centenarians, centenarian's offspring and a group of controls recruited in Milano, Bologna and Calabria regions at phenotypic level (N=202). Visual problems (nearsightedness, farsightedness) and hearing issues were evaluated. Then we identified the most significant genetic signals associated to sensory impairment for the Italian populations and then we investigated them in a whole genome sequencing dataset of Italian semi-supercentenarians and controls (N=117) [4].

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BIOACTIVE TRITERPENES OF PROTIUM HEPTAPHYLLUM GUM RESIN EXTRACT DISPLAY CHOLESTEROL-LOWERING POTENTIAL

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Hypercholesterolemia is a form of hyperlipidemia that is characterized by the presence of high levels of circulating low-density lipoprotein cholesterol (LDL-C). It is the major cause of arteriosclerosis [1], which may lead to serious cardiovascular diseases (CVDs) such as hypertension, cerebrovascular disease, peripheral arterial disease, coronary disease, deep vein thrombosis, and pulmonary embolism [2]. CVDs are the leading cause of death globally and were responsible for 31% of all global deaths in 2016, as estimated by WHO (World Health Organization) [3]. According to the different hypercholesterolemia risk levels, a number of therapeutic strategies are actually available [1]. As a first step, changes in lifestyle and diet behaviors are strongly recommended [1,4]. In particular, the consumption of foods with high carbohydrates, saturated fats, and high cholesterol levels should be avoided, while increasing the intake of foods rich in fiber, potassium, unsaturated fats, and saponins [5,6]. Statins represent the most common therapeutic approach, but they may be insufficient due to the onset of resistance mechanisms and side effects. Consequently, patients with mild hypercholesterolemia prefer the use of food supplements since these are perceived to be safer. Here, we investigate the phytochemical profile and cholesterol-lowering potential of *Protium heptaphyllum* gum resin extract (PHE). Chemical characterization via HPLC-APCI-HRMS² and GC-FID/MS identified 13 compounds mainly belonging to ursane, oleanane, and tirucallane groups. Studies on human hepatocytes have revealed how PHE is able to reduce cholesterol production and regulate the expression of proteins involved in its metabolism. (*HMGCR*, *PSCSK9*, *LDLR*, *FXR*, *IDOL*, and *PPAR*). Moreover, measuring the inhibitory activity of PHE against *HMGCR*, moderate inhibition was recorded. Finally, molecular docking studies identified acidic tetra- and pentacyclic triterpenoids as the main compounds responsible for this action. In conclusion, our study demonstrates how PHE may be a useful alternative to contrast hypercholesterolemia, highlighting its potential as a sustainable multitarget natural extract for the nutraceutical industry that is rapidly gaining acceptance as a source of health-promoting compounds. For this reason, *Protium heptaphyllum* may become an interesting raw material for the nutraceutical industry in addition to gaining acceptance as a source of health-promoting compounds. Finally, the obtained results may boost the production demand of this resin, improving the income of local rain-

forest communities. This, together with the application of innovative and sustainable models in the production chain, based on the use of vegetable nontimber forest products, are the base concept of the circular economy.

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OVERCOMING SIZE IN MICROSCOPIC ANIMALS: NEW APPROACH TO STUDY TARDIGRADES WITH CONFOCAL LASER MICROSCOPY

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Tardigrades (aka waterbears) are eight-legged micrometazoan (0.5-1 mm in length) present all over the world. They belong to the clade Panarthropoda and share with its sister clades the presence of chitin and autofluorescent molecules in the teguments and in some cuticular organs. In tardigrades these cuticular structures *i.e.*, cuticle, claws, and parts of feeding apparatus, are the morphological characters useful for their identification and classification. Moreover, the structural, morphofunctional, and chemical investigations on those structures are crucial for evolutionary and phylogenetic studies in a frame of integrative taxonomy. The paucity of retrieved specimens, size of animals and organs, and the presence of a chitinous cuticle (that often hinders fixatives perfusion) are common impediments to used specimens for high-resolution techniques, *e.g.*, electron microscopies. A possible overcoming of these problems arises from the innovative use of Confocal Laser Scanning Microscope which combines the fluorescence microscopy from a laser point-like source, multilayers stack acquisition allowing high-resolution imaging and reconstruction of 3D objects, and chemical investigation without peculiar preparatory protocols. The aim of this presentation is to highlight the capability of Confocal Laser Scanning Microscopy as a tool for fine morphology and systematic study of micrometazoans both from fresh and old museal materials in a non-destructive fashion. The results from a number of case studies on which the analyses of tardigrades serve as model for the possible investigation on other micrometazoans will be shown. Firstly, we solved systematic issues on the genera *Ramazottius* and *Cryoconicus* highlighting how the 3D reconstructions of permanently mounted specimens are significant for comparative studies both at low and high taxonomic levels. Then, to confirm the presence of chitin in different taxa we improved an easy way to identify the structural molecules distribution on whole alive animal using the combination of specific vital dyes and this vanguardist technique. Besides, during these analyses we revealed structures otherwise invisible or hardly detectable with light microscopy that could become characters relevant for taxonomy. Lastly, we developed an easy-to-replicate protocol that maintain the integrity of entire animals to perform immune-histochemical stainings. Such technique will be displayed together with the results useful to contribute to the disentanglement of the evolution of Panarthropoda on the muscular and nervous systems of tardigrades.

DIGITAL TWIN RESEARCH IN BIOHEALTH SECTOR: TOWARDS THE CONCEPT OF DISTRIBUTED AND SHARED FACILITY

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The current state of applied research in the “Digital Twin” field, which began on various fronts since health systems required an “intelligent” digitalization of medical / surgical, technological, management and administrative procedures, is in a phase in which they begin to see the first practical results, that is the adoption of the digital medical record and the process of dematerialization of reports and prescriptions. The latest step forward was the development of digital medical documents to be distributed to individuals (green-pass). These facts show that the so-called “patient archive” has been consolidated and that this is dynamically connected to other archives. The core of this project is the organization of the enormous amount of data available and the development, on one hand, a customized archive system for the physical patient and, on the other, a method that makes the non-data warehouse usable. Only as a replacement for papers but, above all, as an aid for personalized medicine and precision surgery. These branches, from the application instrumental point of view, represent the clinical avant-garde: more and more instruments related to diagnosis and surgery are developed and produced as extremely precise aids for doctors and surgeons of all specializations. The final goal to be achieved in a period between 3 and 4 years, is to offer the market a digital system which, can dynamically manages information relating to patients understanding as individuals and as a whole, furthermore it offers a common platform for diagnostic and surgical tools available from a single clinic to the general market in health sector. The result will allow the healthcare system to manage each patient individually in an innovative way, represented in a triple dynamic aspect: real, Vitruvian and virtual. The “real patient” will consist of all the data deriving from anamnesis, diagnosis, analysis, etc... and will correspond to the subject in the current state and will represent the complete set of the health situation; the “Vitruvian patient” (abbreviation to indicate the patient in a forecasted state following treatment and maintenance) will act as a projection of protocols applied to the physical patient and reported to the “real patient”, its main function will be to manage, follow-up and foresee the reorganization according to the objectives to be achieved within the set times; the “virtual patient” will be the platform on which to apply the medical, health and surgical protocols in order to agree them in advance with the teams of operators and with the individual physical patient, making them aware of the benefits, times and methods relating to each protocol. The system will be developed in such a way as to be considered as a general facility and not as a simple aid to a single instrument or to a specific category of patients, even if the prototype phase can be limited to specific sectors.

CHARACTERIZATION OF A NOVEL *IN VITRO* MODEL OF SARCOPENIA

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Sarcopenia is the age-related progressive loss of skeletal muscle mass and functionality, which finally leads to poor physical performance and frailty. This pathophysiological process results from the interaction of many mechanisms, such as the establishment of an imbalance between protein synthesis and breakdown in favor of the latter, and the impairment of the regenerative activity of the muscle fibers by muscle stem cells, called satellite cells (SC). Although many factors can cause this impairment, interestingly, in sarcopenic patients, it has been found an increased number of senescent SC, which cannot differentiate into myocytes anymore, and, finally, to restore muscle fiber integrity and functionality. To date, there are different models of sarcopenia *in vivo*, which, involving the use of aged or genetically modified mice, are time- and resource-consuming. However, there are not any *in vitro* models of sarcopenia able to mimic such a complex phenomenon. Here, we propose a novel *in vitro* model of sarcopenia, which could allow to better characterize the mechanisms underlying this condition and to investigate potential therapies. To this aim, we reproduced an *in vitro* system of skeletal muscle from the accelerated aged phenotype obtained in mice by transplanting senescent preadipocytes (Xu *et al.*, 2018). In detail, we induced a sarcopenic phenotype in C2C12 myotubes through incubation with the conditioned medium of doxorubicin-induced senescent 3T3-L1 preadipocytes (SCM). Treatment of C2C12 myotubes with SCM causes atrophy seen as both reduction of their diameter and induction of the ubiquitin-proteasome system, one of the main mechanisms leading to muscle wasting. In addition, treatment with SCM significantly reduced both differentiation and fusion ability of C2C12 myoblasts, in agreement with the reduced myogenic capability of sarcopenic SCs. We developed a new *in vitro* tool that could accelerate and simplify the research in the field of sarcopenia, both allowing a deeper understanding of the mechanisms and paving the way for the discovery of targets of possible therapies. In fact, we believe that our model will be relevant in screening and characterizing the potential effects that molecules or compounds could have on the aged or aging skeletal muscle *in vitro*, reducing the time, costs, and the number of animals required in the study of this pathophysiological condition.

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