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95th National Congress of the Italian Society for Experimental Biology

Trieste, Italy, 12-15 April 2023

ABSTRACT BOOK

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Trieste, Italy, 12-15 April 2023

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FROM OBSERVATIONS TO EXPERIMENTS AND BACK: THE NEW ALLIANCE

Ferdinando BOERO

Stazione Zoologica Anton Dohrn, Fondazione Dohrn, CNR-IAS, Napoli, Italy

Natural history is based on observations. The world is "looked at" and patterns are uncovered. The description of patterns is then conducive to the identification of the processes that generate them. Observation is usually "holistic" and comprises the description of the intricacies of the natural world. Experiments, instead, are usually "reductionistic" and are performed under controlled conditions: all variables are kept stable, and they are changed one at a time, in order to understand their bearing on the functioning of the investigated system. Darwin, a naturalist, performed both approaches. He "observed", but then he also made experiments, for instance on vegetation plots, the resistance of plant seeds to the immersion in salt water, the existence of seed banks, not to speak about the breeding experiments artificial selection is based on. Darwin's grand theory blends observations and experiments, with cross-fertilization of the two approaches. The advent of advanced technologies allowed for increasingly refined experiments and somehow relegated observational science to the margin of scientific investigation, especially in biology. The exploration of biodiversity, so as to answer the crucial question: how many species are there on Earth?, is pursued with genomic approaches based on advanced sequencing technologies, whereas the phenotypic appreciation of biodiversity (*i.e.* traditional taxonomy) is relegated to an inferior rank in terms of scientific respectability. The dismissal of taxonomy is the result of loss of respect for observational approaches. As a result, we perceive the world with the aid of machines and laboratories, and lose contact with nature. On the one hand, the last century saw prodigious advances in the experimental analysis of the facts of life, on the other hand, however, we almost stopped observing the natural world. Time is ripe, now, to join forces and reconcile the two approaches that, indeed, are not mutually exclusive and, instead, reinforce each other. Observations answer WHAT questions, experiments answer HOW questions; togheter, they answer WHY questions. Anton Dohrn founded the Zoological Station of Naples to demonstrate the validity of Darwin's ideas. He promoted both descriptive and experimental approaches. The monographs on the Fauna and Flora of the Gulf of Naples are an outstanding example of the exploration of marine biodiversity. Together with this descriptive approach, the Stazione promoted experimental biology on a wide range of organisms. In those early days, observations and experiments thrived with mutual benefit. The advent of model animals decoupled observation from experiments. Model animals share the exceptional trait of being easily reared in great quantities under laboratory conditions. This technical convenience has the inconvenience of basing our search for general principles on very special organisms. The exploration of biodiversity has still a lot to offer to experimental biology, from the discovery of GFP in *Aequorea victoria*, to the ontogeny reversal of the immortal jellyfish *Turritopsis dohrnii*. Both species are not easily reared in the laboratory and their exceptional properties have been discovered with observations first, and studied with experiments then.

TRIESTE AND SCIENCE: JOURNEYS, EXPLORATION AND DISCOVERY AT THE TIME OF FERDINAND MAXIMILIAN OF HABSBURG

Andreina CONTESSA[1]

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In an era of scientific discoveries and measurements, the botanical expedition to Brazil (1859-60) and the circumnavigation of the globe by the frigate Novara (1857-59), sent from Trieste under the auspices of Archduke Ferdinand Maximilian of Austria, marked an important step for the city's role in the history of science, study, and systematic research. Maximilian, in the same years he was building the Miramare Castle and defining the botanical collection of his large garden, promoted science by supporting scientific expeditions and a network of knowledge and contacts among scholars of the time. An eclectic collector, lover of art and botany, up-to-date on the scientific discoveries of his time, Maximilian was aware of the writings of Charles Darwin and was familiar with the work and thought of Alexander von Humboldt (1769-1859). The expedition around the globe sponsored by the Archduke on the frigate Novara had diplomatic, scientific, anthropological, commercial, and military character, with scientific and medical purposes. Its main objectives were the exploration and cartographic description of unknown areas of the globe, the cataloging and study of minerals, plant and animal species, the knowledge of indigenous populations, and the observation of physical and astronomical phenomena. The Novara returned to Trieste with thousands of notes, observations, and specimens, which were made available to scholars, formed the basis of numerous publications, and formed the collections of various museums, including the "Civic Museum Ferdinando Massimiliano" (now the Civic Museum of Natural History). Today, the Miramare Castle and its park, which have become a national historical museum open to a wide public, are in the heart of a citadel of science where numerous important international scientific institutions grow and develop. The museum maintains good relationships with these institutions and pursues fruitful collaborations with some of them in the spirit of that ancient vision that already in the nineteenth century sought to associate and promote science, art, and culture.

ELVEZIO GHIRARDELLI: THE REBIRTH OF MARINE BIOLOGY IN TRIESTE

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In Trieste, marine studies have a long history. Since the second half of the 18th century, the city has been the point of contact with the Mediterranean for zoologists and botanists of Central Europe. However, it was only in 1852 that the Museum of Natural History was inaugurated, and in 1875 the *Imperial Regia Stazione Didattica e di Osservazione Zoologica* (the Royal Zoological Observation and Teaching Station), a branch of the University of Wien and University of Graz. From the time of its establishment to 1900, many renowned names in the field of biology came and went in the Station's laboratories. The most illustrious remains Sigmund Freud. In 1915, with Italy's war declaration to Austria, the Station ceased all activities. Researches picked up again with the arrival, at the University of Trieste, of Professor Ghirardelli who founded the Institute of Zoology in 1962 and the Laboratory of Marine Biology in 1979.

FETAL DISPLACEMENT OF JAKOBSON ORGAN

Camilla TOGNONI[1], Francesca BUFFELLI[2], Valerio Gaetano VELLONE[1,2], Chiara CONCETTI[2], Martina AGRESTA[2], Ezio FULCHERI[1,2]

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Pheromones have a crucial role in social, reproductive, and foraging behaviours in several animal species. The vomeronasal organ (VNO), among other sensory systems, is designated for their perception. It is well represented in macrosmatic animals, while its function, apparently only vestigial, is unknown in man. It is identifiable in prenatal life until late phases. However, its functionality, apart from being a scaffold for LHRH cells towards the hypothalamus, is unknown. In this study we have tried to identify and describe the VNO histologically, morphologically, and functionally – through histochemistry and immunohistochemistry – in human fetuses between 13 and 21 GW, in order to describe its normal anatomy, and be able to univocally recognize it through easily attainable basal markers. Out of a total of 200 Diagnostic Findings from 2013 to 2022, 70 nasal pyramids were taken from both fresh and formalin-fixed subjects. Technically, the entire pyramid was removed due to incomplete ossification and welding of the maxillary and the corresponding splanchnocranium portion. The hemi-maxillary joints with 14 bones: three of the neurocranium and 11 of the splanchnocranium. The entire block was then formalin fixed and paraffin embedded in a single block so that the cut section could have a coronal plane orientation. Structures corresponding to the VNO description located at the base of the septum, in areas lacking Bowman's nasal glands and respiratory epithelium, were looked for on standard E.E. stained preparations. They can be recognised as tubular structures with pseudostratified columnar epithelium, only occasionally covered with cilia, and always separated from the respiratory epithelium. On a sample of 16 cases selected based on gestational age and optimal preservation conditions (without post-mortem autolytic changes), semi-serial sections were obtained and histochemical (AB-PAS, Masson's Trichrome, Azan Mallory) and immunohistochemical (CK19, P63, S100, Neurofilaments) staining was performed. While VNO structures could be topographically and morphologically observed, they could not be identified histochemically and immunohistochemically. With AB-PAS, S100 and NF it was not always possible to distinguish the VNO from other nasal glandular structures. Conversely, VNO was consistently positive for CK19, like the epithelia. Quite interestingly, neuronal marker-positive cells were always present in the area where VNO was located. The first result of this work is 1) drawing attention to this structure; 2) describing it topographically, histo-morphologically, and cytologically with histochemical and immunohistochemical characterization; 3) providing useful images to establish a diagnosis and to be used as benchmark to correlate VNO features to fetus development between 14 and 18 weeks. This is a crucial period when the involutional process of this organ is believed to have already begun and progressed. VNO has been demonstrated by us in all fetal ages of the selected period. This could open up some diagnostic routes in disembriogenetic disorders, such as in hyposmic hypogonadism (Kallman Syndrome).

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SPACE, A NEW FRONTIER FOR LIFE SCIENCES

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In recent years the Space sector has been approaching the world of research, universities and industry in a much more open way. This effect is evident at world level, also thanks to the so-called "New Space Economy" which wants to finally make the "Space" a resource at the service of the community life. The change has been notable at national level and local level, and involves all the research and industrial fields. This has been accompanied by a particularly active period in the sector in general for all the players, institutions such as the European and the Italian Space Agencies, the European Commission, as well as the industrial component. The University of Trieste has also been very active, with initiatives ranging from Engineering and Physics to Medicine, and now with a synergetic effort of all the cultural components of our University. Human spaceflight has indeed enabled the emergence of new research: physical and life sciences in space. By profiting of the unique conditions in space, scientists can examine many fundamental processes in physics, chemistry and biology that are masked by Earth's gravity. Experimental areas ranges from cell and developmental biology, biotechnology, integrated physiology, bone and muscle physiology and neurology. Future research into these fields promises significant contributions to the improvement of health on Earth and of astronauts during long-term missions. In the long-term, this research will pave the way for humans to travel beyond the Earth towards the exploration of the Space. Passion, dedication and curiosity. These are also typical characteristics of our Bruno Cester that in one of the last interviews in 2010 recalled the many nights spent observing the stars, measuring their light intensity across the abyss of space: "In the silence of the night, we were finally alone: me and the star. All night at the telescope, until dawn. It was a good job. But for me it was always a hobby, more than a job'.

NANOMEDICINES TARGETING THE CNS: A BRIDGE TO THE UNKNOWN

Sabrina PRICL

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The therapeutic challenges imposed by the brain and the nervous system, as well as the obstacles encountered by anything trying to target and interface with them, are largely due to their multifaceted architecture and physiology. The brain, in particular, is computationally and physiologically incredibly complex, with extremely limited anatomical access. Accordingly, designing new drug able to bypass the constraints imposed by the blood-brain barrier (BBB) requires – at least – an understanding of this biological firewall, a thorough understanding of the drug's physico-chemical properties, and how it engages the BBB to limit undesired side effects. Unfortunately, less than 5% of all newly synthesized chemical entities effectively pass through the BBB, and this renders the development of drug targeting disorders of the central nervous system (CNS) a long and winding process. Moreover, this discovery pathway is also bound to fail for a few allied reasons. Nanotechnology is an interdisciplinary field of science and engineering that focuses on technologies and methods for manipulating and controlling materials and devices at the molecular scale (1-100 nm) via physical, chemical, or combined methods. Within this ever-expanding area of research, the clinical application of nan-

otechnology to neurological pathologies has a real potential to contribute to novel practices for treating traumatic and degenerative disorders, as well as malignancies affecting the CNS that are extremely challenging to manage. Among the plethora of nanotechnology-related promising approaches allowing the CNS delivery of active compounds, nanovector-based drug delivery systems (NDDSs) are among those strategies currently showing encouraging outcomes for drug transport across the BBB while minimizing adverse results. The variegated world of NDDs is populated by a myriad of different platforms, including viral vectors, polymeric nanoparticles, liposomes and other self-assembling systems, nanobubbles and exosomes, just to name a few. In this talk I will try to offer a bird's eye view of these current strategies for the delivery of drugs to the brain parenchyma based on different NDDs, not only from the perspective of their successful and promising features but also from the viewpoint of the obstacles and challenges posed by the NDDs in their journey to the CNS.

I wish to acknowledge the enormous wealth of knowledge I have acquired in the field of NDDs by leading the COST Action CA17140 "Cancer Nanomedicine – from the bench to the bedside" and the supercomputing resources/financial support from ICSC – Centro Nazionale di Ricerca in High Performance Computing, Big Data and Quantum Computing (Spoke 7, WP4 (Pilot Applications) and 5 (Materials Foundry)) funded by European Union – NextGenerationEU.

SUDDEN CARDIAC DEATH: FROM GROSS ORGAN TO MOLECULAR PATHOLOGY

Gaetano THIENE

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Since the time of Morgagni and Lancisi, Sudden Cardiac Death (SCD) was found to complicate cardiovascular diseases, easily visible at naked eyes at autopsy, (coronary thrombosis, aortic dissection, pulmonary thromboembolism) or by enhanced optical resolution with microscopy view of histological slides (inflammatory, infiltrative, storage phenomena). However, there are cases of SCD in which the heart is normal, both at gross and histological examination ("mors sine materia"). These patients present ecg alterations of depolarization and repolarization of myocardial electrical activity (long and short QT, early precordial repolarization syndrome) or of electro- mechanical coupling (catecholaminergic ventricular tachycardia), due to Na+, K+ or Ca++ flows through cell membranes, known as channelopathies. They are hereditary dominant morbid entities. Rudolph Virchow in 1894, during the meeting in Rome entitled "*Morgagni and the anatomic concept*", stated "*…Any anatomic modification is material, but is any material modification anatomic? Why not molecular? Can a profound molecular modification occur in the setting of an apparently normal structure? These modifications belong more to physiology than to anatomy, they are functional-dynamic… the method of investigation will never be morphological*". He anticipated by a century what happened with the invention of Polimerase Chain Reaction by Kary Mullis, 1993 Nobel Prize. Molecular studies of SCD at autopsy include both detection of viral genomes in inflammatory cardiomyopathies and gene mutations in genetically determined heart diseases. For these purposes, 10 ml of EDTA blood and 5 g of heart and spleen tissues are either frozen and stored at –80°C or in RNA later at 4°C for up to 2 weeks. In conclusion the role of autopsy in SD is to establish: 1) whether the death is attributable to a cardiac disease or to other causes, including molecular investigations.; 2) whether the cardiac condition causing SCD may be inherited, requiring screening and counselling the next of kin; 3) ruling out toxic or illicit drug abuse and other unnatural deaths. Given that one-third of these cases are of genetic origin, it seems no longer justifiable to ignore genotyping in these victims of SD. Finding a disease-causing mutation will enable a rapid screening of all relatives, to identify those who carry the same mutation as the victim. The molecular approach should now become part of the routine postmortem study of SCD cases.

THE SCIENCE BEHIND SCIENCE COMMUNICATION: A DIVE INTO THE USE OF AI AND ML FOR CLEAR AND PRECISE COMMUNICATION OF SCIENCE

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It all started with newspapers. Then came radio and tv. Today, the great digital revolution has changed the way we deliver news to the people. everything is faster, content is easier to share, and audiences have increased exponentially but this always on, always connected world poses many challenges. misinformation is among the biggest issues of our times. Fake news can have profound consequences in real life and the enormous amount of information created and shared online every day is challenging even for experts. As technology advances, new trends in communication emerge. Examples are Machine Learning and Artificial Intelligence, which have a significant impact on the way we communicate. Deep Learning is a method of machine learning that uses artificial neural networks. As we learn, the connections between nodes become stronger or weaker. In the use of language, there are several uses: Image recognition, text writing, data processing, Language assistants, medical clarification, robotics. If we compare human use of language with that of artificial intelligence, what are the limitations that emerge? Artificial intelligence systems can be very accurate and efficient, but they still cannot match human language use, which is much more complex and nuanced. AI systems rely on algorithms and statistical models trained on large amounts of data so that they can understand and generate human-like language. However, they still have limitations in understanding the context, emotions, and other nuances of human language. In general, the brain processes information more slowly than a computer but, the human brain is still able to solve problems in a short time. It is superior to the computer especially when it comes to solving a completely new problem. Computer systems are still far from being efficient with the wealth of information that makes up the real world. There are several ways to control the way AI is used by researcher to communicate correctly and accurately: use of NLP (Natural Language Processing) techniques. Using NLP techniques, AI systems can be trained to understand and generate human language, which can help ensure that the language used is clear and accurate. AI systems must be monitored and reviewed by human experts to ensure that the language used is accurate and appropriate. It is important to remember that the development and use of AI systems is an ongoing process and that the language used by AI systems must be constantly reviewed and updated as necessary. For scientific results to be properly understood and replicated, it is critical that researchers use the correct terms to describe their methods and results. By using accurate terminology and effective communication techniques, experimental biologists can ensure that their research is communicated and understood by their colleagues and easily understood by a wide audience, including people with non-technical backgrounds. It is important to note that researchers must continually look for ways to improve their communication skills and stay up-to-date on the latest recommendations in their field, as well as keep abreast of the use of audio visual aids that support their research. .

ANTHROPOLOGY: COMPARATIVE APPROACHES

IDENTIFICATION OF SARCOPENIA IN A WORKPLACE HEALTH PROMOTION (WHP) PROJECT

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Sarcopenia is a pathology which involves the loss of muscular strength, mass and function from the age of about 50 years. A worsening of such condition is Sarcopenic Obesity, which adds the increase of adipose tissue and its infiltration in the muscle. Sarcopenia or the risk of developing the pathology is indeed a real problem to face, starting from the screening in the late adult phase, in order to precociously identify it. According to the European Working Group on Sarcopenia in Older People (EWGSOP2-Cruz-Jentoft *et al.*, 2019), such pathology can be identified using multiple and different diagnostic tests, from the first-point screening SARC-F questionnaire, handgrip strength (HGS) and functional tests (Short Physical Performance Battery, SPPB) to body composition assessment using different methods. Usually, sarcopenia is treated at clinical level and, as abovementioned, it occurs in the ageing period. The aim of this work was to use anthropometric, body composition (through Bioelectrical Impedance Analysis (BIA)) and functional data collected in a Workplace Health Promotion (WHP) project (involving mostly healthy employees) in order to precociously intercept values ascribable to sarcopenic condition. We evaluated HGS, lower limbs strength (Sit-to Stand test), Fat-Free Mass (FFM), Muscle Mass (MM) and Appendicular Skeletal Muscle Mass, (ASMM). Results highlighted 17% (females) and 10% (males) risk of sarcopenia (for at least 1 or more indicators) in a sample of 361 participants. Obtaining such important information could be a precious way of precociously identify sarcopenic condition in a general population screening, which aims to evaluations and interventions in the context of well-being.

INTERDISCIPLINARY STUDY OF A CASE OF ACROMEGALY FROM THE "LUIGI CATTANEO" ANATOMICAL WAX COLLECTION IN BOLOGNA

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In 1879 Cesare Taruffi, the first professor of Pathological Anatomy at the Bologna School of Medicine, reported the case of a man, named Luigi Marchetti (also called "Bottaro"), with evident prognathism and skeletal deformities. In his work he accurately described the skull and the skeleton, performed craniometric analysis and compared these data with measurements of other males from Paris and Bologna. The skull and the skeleton studied by Taruffi are currently hosted at the "Luigi Cattaneo" Anatomical Wax Collection, located at the Anatomical Institute of Bologna University and part of the University Museum Network (SMA). In addition to these skeletal remains, the collection hosts the wax bust of the man, the first known wax representation of acromegaly, and a dried preparation of a huge stomach. In 2021 the skull underwent a medical CT scan at the "Morgagni-Pierantoni" city hospital in Forlì, as part of a larger project to enhance and study the subject. The measurements in a virtual environment have returned a clear widening of the sella turcica. The sella length of 28mm and a depth of 13mm are well beyond the standard measurements (from the literature it emerges that the length measured from the tuberculum sellae to the dorsum sellae is 10.43+/- 1.48mm and that the depth measured from a line that goes from the base of the sella and perpendicularly intersects a straight line passing through the tuberculum sellae and the posterior clinoid process is 8.83+/-2.1 mm. There was also a significant deviation of the nasal septum to the right. Furthermore, progenism of the mandible with a type 3 occlusion relationship is evident. The thoracic spine showed wedge-shaped vertebral bodies, severe osteophytosis on the anterolateral aspect of vertebral bodies, increased anteroposterior diameter of the vertebral body, flattened cervical curve and dorsolumbar kyphosis (barrel chest), elongation and diversion of the ribs (due to overgrowth of the chondrocostal joints), supernumerary dorsal vertebra with rudimental ribs arising bilaterally from it. The data incontrovertibly suggests that we are dealing with a case of acromegaly from a GH secreting pituitary adenoma, which widened the sella turcica. Specific genetic analyses are underway aimed at verifying whether the % of endogenous DNA will be sufficient to conduct deeper sequencing; the characterization of the mitochondrial genome; the presence of any pathogenic microorganisms.

ENDOCRANIAL MICE NESTING IN THE BODY OF THE BLESSED ANTONIO DA FANO (DEAD 1435)

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Rodent nesting is not frequently described in bioanthropological literature, although it represents a common finding in mummified bodies. Aim of the present study is to describe a rodent's nest found during scientific investigation of the body belonged to a Franciscan friar. To the best of our knowledge, this is the first case of rodents nesting inside the mummified body of a Saint or a Blessed of the Catholic religion. The body of the Blessed Antonio da Fano (dead 1435) was found during restoration works in the church of Santa Maria Nuova in Fano, Marche region, central Italy. The partially skeletonized mummy underwent external inspection, digital radiology, and computed tomography scanning. Inner body cavities were inspected through endoscopy, followed by textile examinations and archeozoological investigations. The partially skeletonized mummy belonged to a 45-49 years old male. CT scanning and endoscopic examination of the cranial cavity revealed the presence of dense, amorphous material containing textile fragments and bony remnants of rodents. The latter included two hemi-mandibles, two skull fragments, and a smaller hemimandible morphologically referred to an adult and a subadult house mice (Mus musculus, Linnaeus 1758, subspecies domesticus, Schwartz & Schwartz 1943). It is quite possible that the textile fragments were brought into the cranial cavity by rodents. Rodent nesting is not frequently reported in bioanthropological literature, but it represents a rather common finding in mummified bodies. To the best of our

knowledge, this instance was reported in four mummified human remains and mentioned in a classical collection of medical anecdotes. Our present report represents the first description of rodent nesting occurring in the body of a Saint or a Blessed of the Catholic religion. Nesting and burrowing are spontaneous behaviors and daily activities in rodent species, increasing lifetime reproductive success, reducing heat loss, food consumption, and the risk of predation. Pests are unacceptable in buildings for many reasons such as legislation, contamination, depreciation, and reputation. As regards dried bodies, used to form microhabitats, rodents cause physical damage because of their need to gnaw constantly to keep their front teeth worn down and sharp, as well as chemical damage due to residue stains and surface modifications. Mice can be easily detected by their teeth marks, droppings, body hairs, and smears of grease on surfaces. Rodent nesting should be carefully checked and described, in order to better understand this occurrence and to plan effective countermeasures.

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BIOLOGY IN SPACE

AGGREGATION OF ALPHA-SYNUCLEIN IN PARKINSON'S DISEASE: EFFECTS OF MICROGRAVITY ON CELLULAR *IN VITRO* MODEL

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Presence of α-synuclein (α-syn) insoluble aggregates is a typical hallmark of different neurodegenerative disorders characterized by the presence of Lewy bodies, particularly Parkinson's disease (PD). Understanding the factors involved on the formation of such aggregates is crucial to identify potential therapies aimed to stop, or reverse, the progression of PD. In this study, we investigated the effect of microgravity on α-syn aggregation on neuroblastoma cell line SH-SY5Y, along with a mutant clone overexpressing an aggregation prone form of α-syn (3K-SNCA). Our main objective was to compare the aggregation of α-syn in microgravity-exposed, in order to identify processes able to prevent the aggregates formation or increase it. We exposed 3K-SNCA and SH-SY5Y cells to microgravity using a clinostat for different time points (0, 1 hour, 4 hours, 8 hours, 24 hours, and 48 hours). Exploiting western blot and confocal microscopy to quantify the levels of α-syn aggregates we showed that microgravity exposure resulted in a significant increase in the aggregation of α-syn. The aggregates number increased over time, with a peak at 48 hours. Our findings suggest that microgravity increases synuclein aggregation in cell lines in a time-dependent manner, which mechanism will be investigated further to uncover the pathways involved. These results provide new insights into the potential impact of microgravity as model for Parkinson's and aging related studies, offering a platform for rapid generation of otherwise complex cellular models.

ARCHITECTURE, NANOTECHNOLOGIES AND BUILDING MATERIALS: MAYBE TERMITES ARE READY FOR SPACE??

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Termites give the impression of building nests with a marked design skill. Numerous regular shapes and geometries of a cell or compartment can be recognized, multiple spaces destined for housing and a complex system that provides ventilation. Among individuals there is a high range of coordination and apparent cognitions available to termites. In addition, it leaves the choice of the construction material and, above all, of the consolidation substances of the structure particularly admired and amazed. In the world, as far as we know, there are 2200 species of termites (a small thing, compared to 3100 varieties of amphibians or 4100 of mammals). It is one of the smallest orders of insects and, at the same time, one of the most varied and ingenious. These invertebrates draw their sustenance from cellulose, the most widespread carbohydrate on our planet. But, although cellulose is nothing but a very long sequence of glucose molecules, in the presence of oxygen it is indigestible. The bond that unites the sugars of cellulose cannot therefore be broken by conventional enzymes, therefore it is the building material par excellence. Termites do not directly digest cellulose (any more than cows, giraffes and antelopes do) but leave other organisms to perform chemical digestive reactions; for this purpose there are two strategies: the most primitive termites (for example the *Reticulitermes lucifugus*, present in Europe and, in particular, in the sub-Danubian area) ingest the cellulose and transfer it to a fermentation chamber in which anaerobic bacteria and protozoa demolish it; the most advanced termites (for example *Macrotermes bellicosus*, present in the whole sub-Saharan area of Africa) present a different feeding strategy digesting "outside". In fact these termites deposit food in termite mounds where they bring fragments of fungi which, precisely because of their essence, break down cellulose in the presence of air, heat and humidity. The structure of the nest, therefore, is modified not only to give maximum protection from the queen, to eggs and nymphs but also to create some spaces dedicated to the fermentation of food. If, on the one hand, *Reticulitermes lucifugus* uses its own digestive secretion as a building cement, on the other hand *Macrotermes bellicosus* uses the result of mold fermentation not only as a food source but also as a substance for the construction of the termite mound. The molecular structure of this substance is being studied both to understand its "building" qualities (particularly surprising for the internal arches of the termite mound) and to verify its possible use as a nano-biomaterial in civil engineering. What has been observed and measured in the studies carried out on isoptera allows us to highlight that the termites are adaptable from desert to temperate climates, including strong temperature changes, as well as manage an advantageous water balance; also the balance between the gases inside a termite mound and in the vicinity of the ventilation holes is currently being studied.

BIOLOGY OF AQUATIC ENVIRONMENTS

QUALITY OF LARGEMOUTH BASS *(Micropterus salmoides),* CULTURED IN SICILIAN AQUAPONIC SYSTEM

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Largemouth bass *Micropterus salmoides*, is a freshwater species from North America, introduced in Italy at the end of the last century, being recorded at first in the Lake Trasimeno in the 1980s, and has since spread throughout the country, even to Sicily. Thanks to its rapid growth performance, short rearing cycle, strong adaptability, handling tolerance, flesh quality and absence of intramuscular bones, this species became target of the aquaculture sector in many countries. In particular, *M. salmoides* is one of the emerging species in aquaponics, a farming system that symbiotically combines aquatic animal culture (aquaculture) with plant culture (hydroponics). Aquaponics farms, in Sicily, have implemented this closed-loop farming system consisting of four elements: water, fish, bacteria, and plants, where the cycle begins with the farming of *M. salmoides* in fresh water. The aim of this study was to evaluate, for the first time, the quality and shelf life of the largemouth bass from an aquaponics farm in Sicily and to provide information about consumer perception (liking or disliking of the fish product), in order to define consumer acceptance and its commercial success. Obtained results showed that this species has a low lipid content and is an important source of omega-3, mainly DHA (22:6 ω-3 docosahexaenoic acid) and EPA (20:5 ω-3 eicosapentaenoic acid). Both sensory analysis and biochemical parameters resulted correlated with good acceptability of the product up to 12 days. These results highlighted that Sicilian aquaponics might contribute to add value to the seafood production sector by exploiting new species, enabling greater diversification of seafood products and helping to improve the sustainability and profitability of the sector.

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CHEMICAL CHARACTERIZATION AND BIOLOGICAL PROPERTIES OF MACROALGAE EXTRACTS

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Marine species are a rich source of bioactive molecules and among them, it is known that marine algae produce different secondary metabolites for which different biological activities such as: immunomodulatory (Raposo *et al.*, 2016), antioxidant (Fisch *et al.*, 2003) and antimicrobial (Pinteus *et al.*, 2015) were demostrated. The aim of this study was chemical characterize the extracts of three macroalgae species: *Carpodesmia crinite* (Duby, Orellana & Sansón, 2019), *Carpodesmia brachycarpa* (J. Agardh, Orellana & Sansón 2019, WoRMS, 2023), *Ericaria brachycarpa* (J. Agardh, Molinari & Guiry, 2020), *Asparagopsis taxiformis* (Delile) Trevisan 1845) to evaluate also their biological activities. The characterization of the secondary metabolites was performed by HPLC-MS and showed higher meroterpenoids levels. Then, the extracts were tested on the *Arbacia lixula* sea urchin and against the bacterial strains *Listeria monocytogenes* and *Staphylococcus aureus*. In the first case results showed a modulation in total and differential cell count demonstrating their involvement in immunity responses. In the second case important antimicrobial activities were observed against both bacterial strains tested. The results obtained, although preliminary, are certainly encouraging to understand better the biological potentiality of these metabolites.

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GELATINE FOR FOOD USE: FROM THE *Rhizostoma pulmo* MESOGLEA (EXTRACELLULAR MATRIX) A NEW COLLAGENE SOURCE

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Gelatine for food use is commonly produced from animal waste coming from the slaughter of cattle and pigs. In recent years this practice has highlighted several critical points, risks to human health such as infections with transmissible spongiform encephalopathy or other viral vectors (Z. Ahmed, "Marine drugs" 2021), tightening in the legislation on slaughterhouses that make it difficult to supply the raw material and the increase of those who do not consume products of animal origin. Given the importance of gelatine in both food and pharmaceuticals, the study of alternative sources has therefore been undertaken. Discarded plant sources, which were found to be inadequate, the research was directed towards animals that could constitute a source of gelatine of comparable quality with the one commonly used but without the risks and negativity of the traditional system. Such animals have been identified in the Cnidarians; in this study, in particular, we considered the sciphozoans of the species *Rhizostoma pulmo* in their medusoid form, the most suitable thanks to the composition of the extracellular matrix, consisting essentially from denatured collagen. The gelatine extracted from the *Rhizostoma* mesoglea has been analyzed; it has been seen that its composition is comparable, from the organoleptic point of view, with that of the commonly used animal gelatine. The analysis then took into account the possible presence of pathogenic microorganisms and these do not appear in the results, allowing us to affirm

that *Rhizostoma* gelatin is safe for human health and is free from microbiological risk. *Rhizostoma* jellyfish are of considerable size, an adult can reach 50-60 cm in diameter and has an average weight of about ten kilos; the species is widely diffused in the Mediterranean Sea, where the coastal "annual production" is estimated at around 3000 tons. The best method was therefore sought to obtain the extract by studying through the ashes and impurities, with plausible solutions from an economic point of view and in compliance with safety studies.The use of Cnidari Scifozoi and in particular *Rhizostoma* as a new source of animal gelatin well responds to the new critical issues affecting the traditional system currently in place, being *Rhizostoma* a safe source from food safety point of view and usable from a regulatory point of view. The research continues along two lines; the first to evaluate the best field of use for the gelatine from Cnidarians, whether pharmacological, food, cosmetic or other, the second focused on the method of supplying of the raw material, whether from direct fishing or possible farmin.

PRESENCE OF TRACE METALS AND RARE EARTH ELEMENTS (REEs) IN PHYTOPLANKTON COMMUNITIES FROM THE NORTHWESTERN MEDITERRANEAN SEA

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Phytoplankton plays a key role in marine food webs and in the neritic and pelagic ecosystems as, being at the base of it, can influence the availability of both essential and trace elements. Phytoplankton as primary producers is involved in the transfer of elements in the food web. Some essential elements, such as iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), and chromium (Cr), are of extreme importance for the biogeochemical cycles and for the metabolism of phytoplankton and other organisms. However, above a certain level essential and non-essential elements could cause damage to the marine ecosystem, and reaching higher levels of the food web could also cause toxicity risks for humans. REEs are represented on the periodic table by 15 elements ranging from lanthanum to lutetium, in addition to the two elements of group III B, scandium and yttrium. The study aims to investigate the presence of REEs and trace metals at different depths in phytoplankton populations from the Ligurian sea to understand if these components, which represent a truly important link in the food web, can be used as bioindicators to detect the presence of these elements in the marine ecosystem. The phytoplankton was collected seasonally during four expeditions between 2021-2022 at three stations with a mesh size of 20-micron (diameter 40 cm), frozen at -18 °C, and stored in dark glass bottles. Trace and rare earth elements were analyzed by inductively coupled plasma spectrometry (ICP-MS). Preliminary results show that, except for Fe, Zn, Mn, and V which appear to be more concentrated at 50 m, all the other trace elements and REEs had a higher concentration at 30-meter depth. The significative presence of these elements in the first meters of depth is related to biochemical processes such as the uptake of phytoplankton in surface waters, and the decomposition and demineralization of organic material in deep waters.

ASSESSMENT OF GONADIC MATURITY STAGE OF *Paracentrotus lividus* (LAMARCK, 1816) IN THE EASTERN COAST OF SICILY

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The common sea urchin, *Paracentrotus lividus* (Lamarck, 1816), is a species widely distributed in the Mediterranean Sea and in the northeastern Atlantic and covers a key role in coastal area ecosystems [1]. Furthermore, its gonads are edible and highly appreciated in many Mediterranean countries, including Italy. However, the fact that gonads are considered a sea food delicacy caused the collapse of several populations, depleted by legal and illegal fishing activities [2]. Data on the reproductive biology of the species based on large scale studies are essential as a prerequisite for efficient management strategies. However, available data are limited to a few limited areas [3,4,5]. Moreover, these studies reported a considerable reproductive variability in *P. lividus* at various spatial scales, and information is often contradictory [6,7]. Specific data and recent studies on the reproductive biology of *P. lividus* in the Ionian coast of Sicily are lacking. The aim of this study was to evaluate the maturity stages in the period May-June 2022 in three areas along the eastern coast of Sicily: in particular, S1 (Messina, Ganzirri Lake, Due Torri Channel) and S2 (Catania, Ognina) that are located near large commercial port and highly urbanized, and S3 (Syracuse, Natural Reserve of Vendicari) that represents a low impact area within a natural reserve. A total of 60 sea urchins were collected, 20 for each study area. The morphometric characteristics of each individual were measured, and histological preparations of their gonads with hematoxylin-eosin staining were performed to evaluate the stage of maturity through different index. A comparison was made between the most used maturity index: the Gonadosomatic Index (GI), the Gonad Maturity Index (GMI) and the innovative Pixelar Indices (PI_1 and PI_2) through image analysis performed with IMAGEJ software. The results obtained revealed a discrepancy between the gonads' maturity index used. Through the integration of the image analysis, it was possible to confirm that the sea urchins sampled in the three areas had different stages of maturity; and in particular sea urchins collected in S3 are in advanced stage of maturity (according to sampling period) compared to S1 and S2. Furthermore, in five specimens collected in S1 a pathology was histologically detected.

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COMBINATION OF CAFFEINE AND MICROPLASTICS ON SENTINEL ORGANISMS: DETRIMENTAL EFFECTS ON DIGESTIVE GLAND

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Currently, pharmaceutically active compounds (PhACs) represent the main class of micropollutants detected in aquatic environments. Among the various PhACs detected, caffeine is one of the most abundant substances, found obviously in drugs, but also in foods and personal care products. Therefore, there is a serious need to clarify the real environmental risk posed by this compound on non-target organisms, especially in combination with other substances, equally abundant in water: microplastics (MPs). In line with the above considerations, the present study aims to evaluate the sub-lethal effects induced by the combination of caffeine (CAF) and MPs. During the experiments, bivalve molluscs of *Mytilus galloprovincialis* (Lamarck, 1819) were divided into four groups: Control group, CAF Group (20.0 µg L^{-1}), MPs Group (1 mg L^{-1} - 35-50 µm), and one with their combination (Mix group), for 14 days. The tests performed were RVD and cell viability, to assess physiological changes following exposure, while multiple biochemical markers were considered to assess oxidative stress. The results from the RVD test showed that there is a significant alteration of the digestive gland volume regulation mechanism in the Mix group compared to the control group, as the return of the cell to its initial volume is inhibited. As for cell viability, while it remained above 90% in each treatment, it showed a significant reduction in the CAF group. In addition, the MPs group and the Mix group highlighted a decrease in Mn- SOD, catalase and GST activities, TBARS level, as well as the increase in the GSH/GSSG ratio by 1.4-1.5-fold, the metallothionein level and their Zn content, while the CAF group did not affect the oxidative stress indices. The distinguishing feature of the CAF group was the decrease in caspase-3 activity. In conclusion, from the results that emerged, the visible effects of the exposure to the individual substances were enhanced or attenuated in the group exposed to the combination of the two pollutants. That was probably because of their interaction. This study thus demonstrated how essential it is to evaluate pollutants in a wider context, considering not only the effects of the single contaminant but also its combination with others present in the same environment.

2,2′,4,4′ TETRABROMODIPHENYL ETHER (BDE-47) EXPOSURE INDUCES OXIDATIVE STRESS BY MODULATING ANTIOXIDANT DEFENSES SYSTEM IN FISH CELL LINE

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There is a growing concern related to the potential effects that chemical contaminants may have both on marine organisms and human health, due to their transfer along the marine food web. Current evidences from marine organisms and fish cell line models suggest that the exposure to polybrominated diphenyl ethers (PBDEs) induces endocrine dysfunction, developmental toxicity, reproductive disorders, neurotoxicity and carcinogenicity. 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) is a highly persistent and easily bioaccumulated carcinogenic flame retardant, considered to be among the most abundant contaminants in the marine environment. Recently, the identification of molecular and biochemical biomarkers as an early warning indicator of stress, have received considerable attention, representing a sensitive tool to detect the toxic effects of pollutants, useful in environmental biomonitoring. One of the most important mechanisms induced from chemical toxicant contaminants is oxidative stress, which leads to the overproduction of reactive oxygen species (ROS), triggers several signal pathways in the organism and promotes cell transformation. In this study the effect of BDE-47 exposure was tested *in vitro* on *Sparus aurata* fibroblast (SAF-1) cell line, evaluating molecular markers related to cell cycle (p53), oxidative stress and antioxidant defenses system (nrf-2, sod and cat), in order to identify changes in ROS-mediated signaling. Our results showed that BDE-47 induced oxidative stress, as demonstrated by the inhibition of the event by pretreatment with antioxidant, affecting cell cycle biomarkers and the antioxidant defense system, represented by the scavenging enzymes (sod and cat). The obtained result highlight the role of antioxidant system in prevent the negative effects of environmental contaminants and its role in biomonitoring.

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MICROORGANISMS INHABITING POLAR LAKES WITH POTENTIAL APPLICATIONS IN THE BIOREMEDIATION OF COLD CONTAMINATED AREAS

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Arctic and Antarctic environments are generally considered pristine and unexplored, however, the presence of persistent pollutants, such as heavy metals (HMs) and petroleum compounds, has been recorded in these regions. Pollutants can be transported through natural processes, such as air, sea currents, volcanic activities, animals, or be released by anthropogenic activities [1]. Lake ecosystems, especially small lakes and ponds, which are widely distributed in Polar Regions, are very sensitive to environmental perturbations [2]. In fact, small variations in the dynamics and duration of their snow and ice covers, and the deposition of xenobiotic substances can have a noticeable effect on lake ecological variables. Microorganisms (prokaryotes and eukaryotes) inhabiting cold environments have been shown numerous adaptations also to the presence of pollutants. These microorganisms can be an important resource for the degradation of pollutants at low temperatures [3]. Our work was aimed at analyzing the microbial communities in Arctic and Antarctic lakes, with particular attention to the isolation of bacterial and fungal strains able to tolerate heavy metals (Iron, Fe; Copper, Cu; Mercury, Hg) and grow in presence of biphenyl as a unique carbon source. Samples were collected in five and seven coastal lakes at the Svalbard Islands and Antarctic Peninsula, respectively. More than 100 strains (including bacteria and fungi) showed the ability to tolerate high concentration of heavy metals. Particularly, 90 bacterial strains grew at concentrations of 1000 ppm for Fe and 3 for Cu at the same concentration. Eight bacterial strains showed the ability to tolerate Hg (up to 100 ppm). A total of 32 and 3 fungal strains were able to grow in Fe and Cu enriched media (1000 ppm), respectively, whereas

16 strains tolerated Hg (100 ppm). These isolates were further tested for tolerance to higher metal concentrations and for HM multi-tolerance, showing promising results. With respect to biphenyl analyses, we isolated 112 bacterial strains and 9 fungal strains able to use biphenyl as sole carbon source, from natural enrichment at the concentration of 0.1% w/v. All the results obtained highlight the importance of further studies in understanding the biogeochemistry of polar lakes, but in particular for the deep exploration of microorganisms inhabiting these environments for their possible use as biosensors or in the bioremediation of cold polluted areas.

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BLUE GROWTH AND GREEN DEAL INITIATIVES AT REGIONAL LEVEL IN SUPPORT OF THE AGENDA 2030 FOR THE SUSTAINABLE DEVELOPMENT GOALS (SDGs) 14

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The increasing pressure on marine/aquatic resources needs urgent actions aimed to optimize the sustainability of the marine food supply chains. The finding of alternative food/energetic resources, the reduction of food loss and waste, as well as their valorization, is crucial to follow the principle of the "circular economy" and of the green deal, contributing to the challenges of the AGENDA 2030 for the Sustainable Development Goals (SDGs). Marine biotechnologies, thanks to the trajectories of the blue growth, continue to give a strong contribution in implement and transfer procedures to realize sustainable marine biobased products, to cultivate organisms able to provide bioactive ingredients, in set up industrial green process. In this frame, the results of some projects, aimed to apply these principles at regional level, will be presented: extraction of omega-3 enriched oil from fish processed by-products, production of astaxanthin from shrimp by-products, algae cultivation in multitrophic aquaculture systems, are among some example of circular economy procedures, that were transferred at industrial level.

MERCURY AND MICROBIAL ACTIVITY IN SNOW INFLUENCE ARCTIC HYDROLOGICAL SYSTEMS

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Snow covers about 35% of the Earth's surface during the year and represents one of the most important climatic and ecological systems [1]. It is a physically, chemically, and biologically dynamic system that also strongly impacts the hydrological cycle, especially during the spring and summer periods when snow melting flows into streams, rivers, lakes, and seas [2]. Mercury (Hg) can be emitted both by anthropogenic processes and from land surfaces through natural processes. Once released, due to its long atmospheric residence time, Hg can undergo long-range transport and arrive in remote regions such as the Arctic, where it is subsequently deposited. Atmospheric Hg is principally deposited onto the cryosphere and finally comes to hydrological systems. The abundance of microorganisms on the snow varies significantly, ranging from 10^2 cells per milliliter of melted snow on the South Pole [3] up to 10^2–10^5 in high mountain and Arctic snow. Microorganisms on the snow in polar regions were also analyzed for their metabolic activity and for the production of molecules of interest [4]. Currently, there are few studies on the presence of Hg-resistant microorganisms in snow and on the possibility of using them in bioremediation. The research aimed at surveying Hg pollution level and the spread of Hg bacterial resistance in different environments (snow, river, and sea) and, in particular, to evaluate the contribution of Hg pollution made from snow in Arctic waters systems, to assess bacterial community activity in the snow, and to isolate biotechnological interesting bacterial strains in terms of bioremediation potentialities. Our preliminary results underline that the snow is an interesting and unique ecosystem, especially in terms of bacterial composition and activities. Very relevant results have been shown in intermediate snow deposition layers, where the concentrations of chlorophyll-a and enzymatic activities were the highest. The microbial activities detected in the snow were comparable to (and in some cases higher than) those retrieved in both sea and river water. The large snow falls and subsequent windblown remobilization, together with the ongoing formation of nearby sea ice makes the Hg depth profiles difficult to interpret. Finally, Hg-tolerant bacterial strains have been isolated. Obviously, many more analyses are still needed, with some of them that are currently in progress, especially for the evaluation and quantification of the presence of *mer* genes. However, it is possible to assert that the contribution of the snow to water systems in the Arctic [5], in terms of Hg concentration, bacterial abundance and composition is a very crucial point to understand Hg pollution dynamics in freshwater and seawater systems.

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BIOLOGICAL EFFECTS OF THE PRIMARY METABOLITE OF CHLOROACETANILIDE, PROPACHLOR ESA, ON THE MODEL ORGANISM *Mytilus galloprovincialis*

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The indiscriminate use of pesticides ends with concerns about their effects on the environment, and on non-target organisms. In this point of view, aquatic ecosystems are the most valuable for living organisms, and residual concentrations of pesticides and their metabolite products pose a threat to them. Chloroacetanilide metabolite propachlor ethanesulfonic acid (PROP-ESA) belongs to these pollutants and is founded in aquatic ecosystems worldwide. Due to its chemical-physical properties may be easily absorbed throughout the soil and water, resulting in an adverse impact on aquatic organisms and humans via the trophic chain. This study investigated the bioaccumulation of PROP-ESA in soft tissue and its effect on oxidative stress in the gills and digestive gland of the model organism *Mytilus galloprovincialis.* Mussels were exposed to PROP-ESA at 3.5 µg/L, the environmental concentration and its tenfold 35 µg/L for 10-d and 20-d. Bioaccumulation of PROP- ESA in soft mussels' tissue is presented with the bioconcentration factor (BCF). Oxidative stress measured in gills and digestive glands is shown by measuring superoxide dismutase (SOD) activity, levels of lipid peroxidation (LPO) and oxidatively modified protein (OMP). Results showed that PROP-ESA increases in tissue at higher concentrations and longer exposure times. The evaluation of oxidative stress biomarkers showed a different trend between gills and digestive gland. Protein carbonylation (OMP) increased in the gills at both concentrations only after the shortest exposure time, no changes were reported in the digestive gland. TBARS levels, a marker of LPO, increased in both tissues after 10-d of exposure but remained high only in the digestive gland after 20-d of exposure at the lower concentration. An increased activity of SOD enzyme was observed in gills after 20-d of exposure at both concentrations. In conclusion, this study shows that the *M. galloprovincialis* can absorb the pollutant into its tissue, but that over long periods of exposure an adaptation of the body to the stress conditions imposed by the pollutant may occur.

WASHED UP *Posidonia oceanica*: FROM WASTE TO SECONDARY RAW MATERIAL

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Posidonia oceanica (Linnaeus) Delile is a marine phanerogam very common in the Mediterranean Sea. *Posidonia* meadows represent important ecosystems both from biological and environmental point of view. The leaves are the habitat for many other organisms and perform an important role in reducing plastic pollution and mitigating wave motion. Due to their important ecological role, *Posidonia* meadows have been protected by European legislation (Habitat Directive 92/42 / EU). Most of the leaves, however, are not consumed by other organisms but become waste material and is washed up on the shore, especially in the autumn season, when the chorism of all deciduous plants takes place. On the beaches near consistent meadows, the dead leaves of Posidonia accumulate year by year to form deposits known as "banquettes" up to 2 meters high. In their journey between the meadow and the deposit, the dead leaves of *Posidonia* collect and retain sand and waste, including the so-called "special waste", ie the substances or objects that are classified as "dangerous" as they are composed of, for example, asbestos, rock wool, paint residues, objects of sanitary use and so on. The Convention for the Protection of the Mediterranean Sea from Pollution (in Italy transformed into Law no. of 21 January 1979. 30), following the amendment of the Conference of Plenipotentiaries of the Contracting Parties, held in Barcelona (Spain) in 1995, gave rise to a series of application protocols and has as its main purpose to protect the marine environment and the Mediterranean coast. It has been signed by the 21 countries bordering the Mediterranean and by the European Union. The Convention requires the Contracting Parties to take, individually or jointly, all necessary measures to protect and improve the marine environment and the coastline of the Mediterranean in order to contribute to its sustainable development. Starting from 2024 it will be fully operational in all States, therefore moratoriums and sanctions will be activated for countries that are not in compliance with the protocols. The main problem of the Public Bodies is the authorization to bathe, which from 2024 cannot be granted or will force the State to apply severe penalties, as the "banquettes" composed of dead leaves of *Posidonia* emerged for some time keep within them a fair amount of special waste. The proposal presented in this study aims to solve the problem through a system of cleaning the "banquettes". A third of the dead leaves "washed" by special waste will be returned to the beach in order to maintain the natural coastal anti-erosion purpose, and the remaining part will be used, always after appropriate cleaning treatment, as a secondary raw material for fiber for panels, compostable for biogas, fertilizers and so on.

MICROBIOLOGICAL AND BIOGEOCHEMICAL OBSERVATIONS IN THE GREENLAND SEA AT 75° N

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The Arctic and sub-Arctic ecosystems are environmentally sensitive regions, where the impact of global climate change is expected to make marked changes over the next decades and more rapidly than elsewhere. In fact, these regions have been warming more than twice as rapidly as the rest of the world for the past 50 years. The Arctic climate has undergone tremendous change, such as Arctic wetting, reduction of Arctic sea ice thickness and coverage, a decrease of snow cover extent and duration, thawing of permafrost and melting of Greenland ice Sheet. This positive feedback effect is among the main processes responsible for the "Arctic Amplification", which will likely strengthen in the coming years. In this context, in the framework of the Arctic Research Program (PRA) and as a contribute to the Synoptic Arctic Survey (SAS) observatory datasets, the Italian project CASSANDRA aims at quantifying the current state of the physical, chemical, biological and biogeochemical parameters of a historical subarctic transect at 75° N crossing a cyclonic vortex of the Greenland Sea. CASSANDRA oceanographic cruise was carried out from 29

August to 14 September 2021 onboard the N/R Laura Bassi. Preliminary results concerning the ecosystem functioning will be presented. Water samples were collected along the water column from six stations across the 75°N transect to evaluate the microbial abundance, biomass, activities and metabolism. Interesting results have been determined reflecting the contour characteristics of the large sampled marine area, the possible contribution from the continental shelf and the peculiar oceanographic conditions occurring across the sampled transect. Comparisons with previous bibliographic data in the same area and seasonal period will be made to verify any modifications of the microbial standing stock and its activity.
Funding: CASSANDRA (PRA; PI Maurizio Azzaro).

MICROBIAL COMMUNITIES ASSOCIATED WITH THE FRESHWATER SPONGE *Spongilla lacustris* FROM THE ARCTIC PASVIK RIVER, NORWAY

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Freshwater sponges are affiliated to the order Spongillina within the class Demospongiae that tolerate extreme physical and chemical conditions, like desiccation and cold temperatures. Freshwater sponges play important ecological roles in aquatic ecosystems often supported by microbial symbionts with important functions in nutrient dynamics, and by unicellular algal symbionts. Despite sponge microbiomes play pivotal roles in their habitats, the characterization of bacterial populations associated with freshwater sponges is scant in comparison with those of marine sponges, especially in Arctic areas. The diversity and abundance of the microbial communities associated with *S. lacustris*, living in tight contact with soft substrates in slow-flowing areas of the Pasvik River (Northern Fennoscandia) were investigated. First data were also provided on the microbial enzymatic activity rates, prokaryotic abundance, biomass and heterotrophic bacterial viable counts. Persistent pollutants (*i.e.*, polychlorinated biphenyls, heavy metals, polycyclic aromatic hydrocarbons, and organochlorine pesticides) were determined in sponge mesophyll tissues. Extraction of total DNA and sequencing of the 16S rRNA genes was performed on sponge, water and sediment samples, to investigate sponge transient and core microbiome and the taxonomic sharing level. Results provided insights into the phylogenetic bacterial diversity of this sponge species, confirming Proteobacteria as the dominant associated phylum. It also revealed the co-dominance (with Alpha- and Gammaproteobacteria) of Acidobacteria and Chloroflexi. Our results suggested that *S. lacustris* harbours its own distinct bacterial community which differ from those occurring in waterbody and the sediment, supporting the common belief that sponges selectively acquire the microorganisms from the surrounding environment. Enzyme activity rates confirmed that leucine aminopeptidase was the most abundant enzyme, followed by alkaline phosphatase and beta-glucosidase, comparable to those recorded in sediments for leucine aminopeptidase and beta-glucosidase. In our study cell morphologies were characterised by a numerical prevalence of rods and cocci. Different widespread classes of contaminants were searched and retrieved in *S. lacustris* mesophyll. To our knowledge, the present study is the first addressing the diversity and composition of the bacterial communities associated with *S. lacustris*. Our results extend the so far restricted knowledge of bacterial community in freshwater sponges of the north.
Funding: International Network for Terrestrial Research and Monitoring in the Arctic (INTERACT), within the project SpongePOP (grant agreement n. 262693).

NEW INSIGHTS INTO THE DISTRIBUTION AND THE DIVERSITY OF THE MEDICINAL LEECHES IN ITALY (ANNELIDA, HIRUDINEA)

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Currently, seven leech species belonging to the genus *Hirudo* are known in the Palearctic realm. The most charismatic species is the European medicinal leech, *Hirudo medicinalis* Linnaeus, 1758, historically known for its use in treating human diseases. For decades, the Italian populations of medicinal leeches have been ascribed to *H. medicinalis*. Conversely, from the beginning of the XXI century onwards, based on morphological and molecular evidence, the Italian *Hirudo* populations were ascribed to the Mediterranean medicinal leech *Hirudo verbana* Carena 1820. However, the scarce data currently available for the genus *Hirudo* in Italy as well as its scattered distribution in the country prevented from obtaining an adequate knowledge of its taxonomic status and current distribution. For this reason, we investigated the diversity of the genus *Hirudo* in Italy comparing morphological and molecular diversity patterns based on the barcode region of the mtDNA gene cytochrome oxidase subunit I. Our results proved the existence of two well-characterized evolutionary lineages of *Hirudo verbana* in Italy: an eastern lineage, including the Italian populations located east of the Apennine ridge, and a western one, including the populations located west of the Apennines and in Sicily. Furthermore, we confirmed the presence of the long-forgotten "dragon leech" *Hirudo troctina* Johnson, 1816 in Sardinia, adding the species in the Italian fauna. No recent data or observations are available for medicinal leeches on the Alps, so that the identity of these populations is still to be assessed. Since all these taxa are nowadays included in the Annex V of the Habitats Directive (Council Directive 92/43/EEC) *sub Hirudo medicinalis*, with a conservation status classified as Unfavourable-Inadequate (U1) in the 2018 Italian reporting, monitoring and management actions should be implemented in order to protect their populations.

A UNIQUE BARCODE GAP TO RULE THEM ALL: A RELIABLE METHOD FOR TARDIGRADE SPECIES DELIMITATION?

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Taxonomic identification was traditionally based on morphological characters, but a significant revolution has occurred over two decades ago with the introduction of DNA barcoding, a molecular tool that still plays a vital role for species delimitation across all organisms. DNA barcoding uses a standardized DNA region (principally *cox1* for metazoa) as a tag for rapid and accurate species identification. Despite its high potential and taxonomic utility, one of the main difficulties of DNA barcoding is to define

a clear-cut method to determine species boundaries. One of the most effective and less computationally demanding methods is the identification of the barcode gap, defined as the limit between smaller intraspecific distances and larger interspecific distances. In the first studies on water bears, the proposed barcode gap threshold value was set at around 3% p-distance. Due to the adoption of the integrative taxonomic approach by many tardigrade research groups, there is presently a substantial amount of *cox1* sequences available to determine the feasibility of identifying a universal barcode in the class Eutardigrada. The Assemble Species by Automatic Partitioning (ASAP) method was used to analyze all available *cox1* sequences of both eutardigrade orders of Apochela and Parachela. The ASAP analysis revealed that the threshold value of the barcode gap varied considerably depending on the taxa examined. For instance, threshold values for the genus *Macrobiotus* and the corresponding Macrobiotoidea family differ significantly, with respective values of 4.5% and 13.9%. Thus, establishing a clear barcode gap for tardigrades is challenging at present. Furthermore, using a single method for species delimitation may result in a potentially distorted assessment of species boundaries. These findings suggest that incorporating different tools based on various methods and frameworks could facilitate a more comprehensive biodiversity assessment.

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CELLULAR STRESS

AUTOPHAGY AND APOPTOSIS MODULATION BY AQUEOUS EXTRACTS FROM LEAVES AND RHIZOMES OF *Posidonia oceanica* ON HEPG2 HEPATOCARCINOMA CELLS

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Bioactive compounds produced by aquatic species exhibit a wide range of therapeutic effects in humans, representing encouraging prevention and/or treatment agents and beneficial supplements for the development of functional food and food-packaging material.[1-2] Within this context, aqueous extracts, obtained from green leaves (GLE) and rhizomes (RE) of the seagrass *P. oceanica*, were tested on HepG2 hepatocarcinoma (HC) cells to study cell viability/proliferation, cell cycle, apoptosis and autophagy modulation.[3,4] Both GLE and RE affected cell viability in a dose-response manner and the IC_{50} at 24h was calculated and used in the subsequent assays. Analyses of cell cycle and Annexin-V FITC binding indicated the apoptosis-promoting effect of both extracts, as also proven by the detection of the activation of caspase-1, -2 and -6 after exposure to both extracts and the additional and prominent activation of caspase-3 in the presence of the sole GLE. The intracellular accumulation of acidic vesicular organelles, hallmarks of autophagy, decreased after both treatments, more drastically after exposure to RE. Viability inhibition was not reverted by co-treatment of RE with the autophagy-stimulator rapamycin, suggesting a more extensive cell damage. The intracellular accumulation of the protein markers LC3, Beclin-1, p62/SQSTM1 and hsp60, related to the autophagic process and cytoprotection, at 4, 14 and 24 h of exposure was further studied through Western blot. Protein levels were downregulated in treated cells *vs.* controls with the exception of LC3 II/I ratio at 4 h and p62/SQST1 at all experimental times. The expression levels of *BCL2*, *BAX*, *BAD*, *FOS*, *JUN* and *DAPK* genes, involved in the apoptotic and autophagic processes, were analyzed through Real time-PCR. The results showed the downregulation of *DAPK*, *BAX* and *FOS* at 4 and 14 h of exposure, the upregulation of *JUN* at both times and the variation of *BCL2*/*BAX* and *BCL2*/*BAD* expression ratios in treated cells *vs.* controls. The results obtained suggest the prominent involvement of apoptotic promotion and autophagy downregulation in the anti-HC ability of both extracts from *P.oceanica*, and prompt further investigation aimed to identify the substance(s) responsible for the cytotoxic effect, thus opening the way to promising biomedical and nutraceutical applications.

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THE POST-TRANSCRIPTIONAL RNA EDITING LANDSCAPE OF STRESSED RETINAL EPITHELIAL CELLS: AN EPITRANSCRIPTOMICS APPROACH

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To date it is known that oxidative stress plays a fundamental role in retinal degenerative diseases, especially in inherited retinal dystrophies, but the molecular genetic causative mechanisms are not yet fully understood. In the present work, we carried out a comprehensive profiling of RPE cells treated with the oxidant agent N-retinylidene-N-retinyl ethanolamine (A2E). We considered a follow-up of four time points (1 h, 2 h, 3 h and 6 h) after exposure and compared them to untreated controls (time zero). We detected a total of about 63,000 between annotated and de novo RNA editing sites throughout all time-related samples. Approximately 19% of these RNA editing sites were found within 3′ UTR, including sites common to all time points that were predicted to change the binding capacity of 359 miRNAs towards 9654 target genes. After GO and KEGG enrichment analyses, different clusters of RNA editing sites associated with ECM and vascularization alterations, possibly related to RPE cell apoptosis, were identified. In addition, gene expression differences in deaminase family ADAR, APOBEC and ADAT members, already known to be involved in canonical and tRNA editing events, were detected. Collectively, the transcriptomics approach used in this work showed dynamic RNA editome profiles in RPE cells for the first time, elucidating new molecular mechanisms of retinal degeneration.

THE CHAPERONE SYSTEM IN SALIVARY GLAND DEVELOPMENT

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The chaperone system (CS) canonical function is to maintain protein homeostasis. Proper folding of nascent peptides is crucial in developing tissue. The chief components of the CS are

the molecular chaperones, which play key roles in development as indicated by their presence in embryonic tissue as early as at two-cell stage. However, scarce information on the CS in developing tissues is available, especially at advanced stages of embryogenesis and its role is not fully understood. In our previous study, we reported the presence of molecular chaperones in the ducts and acini of human adult salivary glands. Here, we extend our work and report the distribution pattern of the molecular chaperones Hsp10, Hsp27, Hsp60, and Hsp90 in adult mice submandibular glands (SMG) and in human and mice embryos at late stages of development. We also report tissue levels and expression of these molecular chaperones determined by means of immunohistochemistry, Western blot, and RT-PCR. The four molecular chaperones were present in salivary gland tissue with Hsp90 levels being the highest at both stages, embryonic and adult. The data indicate that the CS is active throughout the ontogeny of salivary glands, with time-course changes in levels and distribution that reflect a dynamic functional involvement specific for each developmental step. The implications of these findings for understanding the pathology of salivary glands with roots in abnormal development are worth exploring, and the data presented here should serve as a basis for such studies and, consequently, boost the invention of novel diagnostic and treatment tools.

CHEMICAL CHARACTERIZATION AND CYTOTOXIC AND ANTIOXIDANT ACTIVITY EVALUATION OF THE ETHANOL EXTRACT FROM THE BULBS OF *Pancratium maritimum* COLLECTED IN SICILY

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BACKGROUND AND AIM: Plant extracts have found application in folk medicine as remedies against several disorders, due to their components with a large spectrum of biological activities. Among these, Amaryllidaceae plants have been employed for centuries as herbal remedies for inflammatory, circulatory, and neurological conditions due to their high alkaloid content[1], as lycorine-type or galanthamine-type ones. The Mediterranean species *Pancratium maritimum* L., belonging to this family, is well known as an important source of biomolecules and it has been suggested as a source of antioxidant compounds. Overproduction of proinflammatory mediators and Reactive Oxygen Species (ROS) are crucial pathophysiological mechanisms involved in different chronic diseases, such as the inflammatory bowel disease (IBD). Thus, the aims of this study were: 1) to characterize the composition of the ethanolic extract of bulbs from *P. maritimum* L., growing in sandy coastal area of Sicily; 2) to evaluate its eventual cytotoxic effect and 3) to estimate its antioxidant proprieties in lipopolysaccharide (LPS)-treated differentiated Caco-2 cells, as an *in vitro* model of inflamed gut epithelium. METHODS: Bulbs of *P. maritimum* L. were collected at Salinelle beach, Lascari (PA) in Sicily. One hundred grams of the dried sample were soaked in 99% ethanol for 48 h at room temperature. The content of biomolecules was determined by mono- and bi-dimensional Nuclear Magnetic Resonance (NMR) spectroscopy analysis, as well as by Liquid Chromatography Tandem Mass Spectrometry (LC-DAD-MSn). The cytotoxic effect on differentiated Caco-2 cells after 24h of treatment was tested by Trypan blue assay. The antioxidant activity on Caco-2 cells pre-stimulated with lipopolysaccharide (LPS) for 2h and then exposed to increasing concentrations of *P. maritimum* L. extract for 24h was assayed by flow cytometry after probing with 2′,7′-Dichlorodihydrofluorescein diacetate (H_2DCFDA). RESULTS: The phytochemical screening of the extract revealed the presence of alkaloids as major components, such as lycorine and oduline. Differentiated Caco-2 cells were exposed to increasing concentration of the extract (50-200 µg/mL) for 24h and no cytotoxic effect was observed, showing a decrease of cell viability down to 87% compared to controls only at the highest concentration tested. The treatment with LPS caused oxidative stress in Caco-2 cells, resulting in the significant up-regulation of intracellular ROS compared to controls. The exposure to *P. maritimum* L. extracts reversed in a dose-dependent manner the LPS-induced increase of ROS levels down to 44% at the highest concentration tested. CONCUSIONS: In conclusion, our findings suggest that Sicilian *P. maritimum* L. is a good source of Amaryllidaceae alkaloids and it can supply antioxidant compounds, as potent radical scavengers, conversely exerting no cytotoxic activity.

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EFFECTS OF "GOLDEN TOMATO" ON OXIDATIVE STRESS AND HEPATIC DYSFUNCTION IN A DIET-INDUCED RAT MODEL OF METABOLIC SYNDROME

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Oxidative stress is a phenomenon caused by an imbalance between production and accumulation of reactive oxygen species (ROS) in cells and tissues and the ability of a biological system to detoxify these reactive products. Previous research revealed that specific systemic biomarkers of redox homeostasis are robustly predictive of the development of metabolic dysfunctions, strengthening the impact of oxidative-based alterations in MetS. Tomato fruits defined as "Golden" refer to a food product harvested at an incomplete ripening stage with respect to red tomato at full maturation, representing an excellent source of bioactive compounds. Aim of this work is to highlight the ameliorative effects of Golden Tomato (GT) on the oxidative stress and on hepatic dysfunction in an *in vivo* rat model of Metabolic Syndrome (MetS). Male Wistar rats were fed with a high-fat diet (HFD) for 8 weeks. This MetS model is able to alter metabolic parameters and redox balance similarly to what occurs in humans. Firstly, we prove that GT possesses a different phytonutrient composition and better antioxidant and radical scavenger properties than the red tomato (RT). The present data revealed that one month of GT oral supplementation at daily dosage of 200mg/kg body weight combined with the HFD diet (HFD-GT), managed to reduce the body weight gain in HFD rats. It also improved glucose and lipid metabolism by reducing LDL and increasing HDL. Furthermore, both GT and RT notably modulate systemic antioxidant defences in the *in vivo* HFD model. Relevantly, not only does RT or GT supplementation

reduce systemic oxidative stress but also significantly counteract the HFD-induced, hepatic production of reactive oxygen and nitrogen species (RONS) and of the reactive aldehyde species (MDA). Consistently with the reduction of hepatic RONS levels, treatment with either RT or GT also markedly reduced the HFD-induced increase of hepatic lipid peroxidation. The protective effect of GT on hepatic lipotoxicity induced by the HFD diet is also demonstrated by histological analysis showing regression of hepatic steatosis. The current experimental evidence demonstrates, for the first time in an *in vivo* model of MetS, the ability also for Golden Tomato to reduce the HFD-dependent, systemic, and hepatic oxidative stress and lipid peroxidation.

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EMBRYOGENESIS IN AN INVERTEBRATE CHORDATE: A STRESS GRANULES-RELATED GENE TALE

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Stress granules (SGs) are dynamic non-membranous foci formed in the cytoplasm of eukaryotic cells exposed to a stress condition, especially through the aggregation of mRNA-binding proteins. Inside SGs, mRNAs for anti-stress proteins are temporally silenced, allowing the regulation of various cellular processes, especially those involved in cell defence. The protection from stress is a critical issue during embryogenesis, important for survival of the organisms and the perpetuation of the species. The aim of this research is to investigate the transcriptional regulation of the genes for TIA1 related nucleolysin (TIAR), tristetraproline (TTP) and GTPase activating binding protein (G3BP), important molecular markers of SGs, during early stages of development of the solitary ascidian Ciona robusta. Electroporation experiments on embryos were carried out with constructs for reporter gene (LacZ) expression, containing the promoter region of tiar, ttp or g3bp. The gene reporter assays allowed us to study level, time and cellular specificity of the transcription of the studied genes, which reflects the action of the regulatory sequences, especially occurring in mesenchyme of larval stages, under both normal physiologic conditions and in response to metal-induced stress.

SKIN CARE AND CELLULAR STRESS

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The skin helps to regulate the temperature, performs a secretory function, protects against external toxic chemicals and harmful microorganisms as well as against the sun's ultraviolet rays. Skin aging has become an important indicator of health and it is affected by multiple intrinsic and extrinsic stressful factors; however, cutaneous aging physiopathological processes have not been fully clarified. The transient receptor potential vanilloid1 (TRPV1) is a nonselective cation channel linked to heat, cell stress, pH and pain and it is expressed also at cutaneous level. The TRPV1 signalling pathway in skin have not been elucidated clearly. In this study, our research group investigated the putative cutaneous TRPV1 signalling pathway related to aging and lifestyle habits/vices. We observed in face skin biopsies from human donor specimens that cutaneous responses to aging-related stressful stimuli may be mediated by TRPV1 signalling pathway, displacing the notion that epidermal keratinocytes function strictly in a barrier role for the body. The TRPV1 levels of expression differed among the face skin sites, indicating both that this signal pathway may differ according to the anatomical site and that TRPV1 may be a promising target to prevent/limit skin aging. Furthermore, the present study confirmed that various lifestyle habits/vices may alter skincare in elderly.

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CITRAL-ENRICHED FRACTION OF LEMON ESSENTIAL OIL MITIGATES THE LPS-INDUCED EFFECTS IN HUMAN HEPATOCYTES

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In recent years, the biological role of Citrus fruits is becoming crucial for human health. One of the most interesting compounds derived from Citrus fruits is essential oil, natural and aromatic liquids with multiple biological activities. Made up of volatile components, they are extracted from different parts of the plant, such as peels, bark, flowers, buds, seeds, twigs and roots [1]. In detail, Citrus essential oil consists of a mixture of different classes of components, such as monoterpenes, aldehydes, alcohols, esters, sesquiterpenes and coumarins [2]. On the other hand, Lipopolysaccharide (LPS), a component of the Gram-negative bacterial cell membrane, is well known to be implicated in liver injury. Via Toll-like receptor 4 (TLR4), LPS stimulates the parenchymal and non-parenchymal liver cells to produce oxidative and inflammatory mediators, as well as to induce the activation of epithelial to mesenchymal transition (EMT) driving liver fibrosis [3]. This study aims to demonstrate the beneficial role of Citral-enriched fraction of lemon essential (Cfr-LEO) in human healthy hepatocytes. In particular, Cfr-LEO was studied as an anti-inflammatory and an antioxidant product and its role in the reduction of EMT was investigated. To evaluate the protective effects of this natural compound, THLE-2 cells were pre-treated with Cfr-LEO and subsequently with LPS. The non-cytotoxicity of the used doses of LPS and Cfr-LEO was validated using MTT assay. Subsequently, we observed that the pre-treatment with Cfr-LEO downregulated the expression of IL-6 and TNFα compared to the LPS alone, effects probably associated to the inhibition of the NF-kB pathway. Indeed, in cells pre-treated with Crf-LEO we found a significant reduction of the NF-kB phosphorylation levels and of its nuclear translocation. Moreover, the ability of Cfr-LEO to decrease ROS level production, to downregulate the expression of Nrf2 and P53 (known mediators of oxidative response) as well as to contrast the ability of LPS to induce the expression of the EMT markers (vimentin and N-cadherin) was also observed. Our future studies will be

focused on evaluating the beneficial effects of selected components of Cfr-LEO to understand what the "actor" could be responsible for the health effects observed. Therefore, having evaluated its non-toxicity and beneficial properties also in a human healthy hepatocytes cellular model, we confirm that Cfr-LEO, can certainly be applied in the agri-food industry not only for its organoleptic properties but it can also represent a preventive tool for improving human health, exerting a protective effect against hepatotoxic stimuli and suggesting its possible beneficial effect for a complex organ frequently exposed to damage such as the liver.

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THE MANAGEMENT OF ANTI-AGING FOOD SUPPLEMENTS THROUGH THE ANALYSIS OF THE HAIR BULB WITH THE BMT SYSTEM

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Managing the intake of food supplements in the diet has become significant especially from an anti-aging point of view. Even if the supplement is taken following a specialist indication, on the market it is available in a standardized form, both in quantity and quality. So the user receive a standard amount of substances regardless of the correct need. The BMT system analyzes the hair bulb through cytological and histological investigation in polarized light microscopy. The hairs, once collected "*in vivo*", are mounted and fixed on a slide, to be observed under the microscope and preserved. The observation is carried out on an optical microscope in polarized light with a black background and recorded via CCD in digital format. The image is stored on the PC and a copy of it is processed to extrapolate the visible frequencies. The algorithms for identifying the individual components of the hair bulb are applied to the processed digital image. Further steps allow to quantify each component. The spreadsheet thus obtained is compared with the spreadsheet with the standards and the differences between the two tables are highlighted. The differences are evaluated directly, identifying the problems connected to the elements, and in a "panel" manner, in order to understand the presence and correspondence between the different radicals. The overall results are compared with a spreadsheet containing the components of the food supplements in relation to the standard analysis data and the absorption and variability indices in the unit of time. A predictive table is therefore drawn up to indicate, by type and composition of food supplement, the amount of supplementary substances to be taken and the time intervals for a correct intake. In this way we obtain a forecast of the rearrangement of elements and radicals and a time interval necessary to bring the situation to an optimal state. The spreadsheets obtained are integrated by the control with the data and the method of the BMT system applied periodically. These allow to calibrate the quality and quantity of supplements so that any discrepancy between expected data and measured data is corrected. The BMT system is non-invasive, it is repeatable, and the samples are storable. This research was initially carried out to demonstrate that the BMT system is able to identify the presence, quality and quantity of 37 elements essential for human life, through the study of the hair bulb and its conservation over time for comparison. It has been established that the BMT system, applied to the hair bulb, allows to understand which food supplements may be necessary to reach and maintain a good lifestyle and the doses in which they must be taken.

MESENCHYMAL STEM CELL BEHAVIOR UNDER MICROGRAVITY

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Mesenchymal stem cells from Wharton's jelly (WJ-MSCs) are multipotent stem cells, able to acquire a specific phenotype under stimulation, to restore tissue damage. Particularly interesting is the behavior of stem cells in stressing conditions. In particular, in the present work we evaluated WJ-MSCs features under microgravity. Simulation was achieved with a random placement machine (clinostat) that mimics weightlessness. Cells were exposed to simulated microgravity for periods of 6, 12, 24 and 48 hours. We then analyzed the molecular program of stemness (Oct-4; SOX2; Nanog) and stress response SIRT1 and HSP70. From our results, we can infer that the simulated microgravity environment is able to influence the behavior of WJ-MSCs by modulating the expression of stress- and stemness-related genes. Our results suggest a survival-oriented cellular adaptation, occurring mainly during the first hours of simulated microgravity, followed by a decrease in stemness and proliferation capability, probably related to the onset of a molecular program of senescence.

BRAIN-GUT-MICROBIOME AXIS: ROLE OF THE GUT MICROBIOTA AND CIRCULATING EXOSOMES IN TRYPTOPHAN METABOLISM

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In recent years, accumulating evidence has disclosed that gut microbial communities have beneficial effects on various aspects of host physiology, including brain functioning, due to their antioxidant activities [1-3]. Among the metabolites that play an important role in gut health, tryptophan (Trp) is an essential aromatic amino acid that in humans is acquired exclusively from the diet. Trp metabolism is a multi-path and complex process occurring in the host and its intestinal symbiotic microbiota and is a precursor to numerous microbial and host metabolites, including serotonin [4,5]. Consequently, it is likely that alter-

ations in the composition of gut microbiota would remotely affect the mental state of the host. In this study, we examined the protective effects of some probiotic strains in protecting human colon cancer cell line HT-29 cells from stress. In particular, we examined data on microbial regulation of Trp metabolism, highlighting the contribution of homeostasis of the microbiota-gut-brain axis to stress-related central nervous system disorders. In order to do this, HT-29 cells were treated with a mix of probiotics (*Lactobacillus and Bifidobacterium*) alone or in combination with H_2O_2 for 24h. Furthermore, since the well-known role of extracellular vesicles (EVs) in the regulation of intercellular signaling by transferring proteins and other metabolites to local or distant organs, including the brain, here we set out to also explore whether the metabolites discussed above are found in the EVs load in the plasma of subjects with gut dysbiosis treated with the probiotic mix.

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MORPHOLOGICAL ALTERATIONS AND OXIDATIVE/GLYCATIVE/LIPOXIDATIVE STRESS IN THE TUBAL AMPULLAE OF PCOS MICE: EFFECTS OF CARNITINES

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Polycystic ovary syndrome (PCOS) is a multifactorial disease commonly affecting reproductive-age women. After having assessed the metabolic features and morpho-functional effects of this syndrome on mouse ovaries (Di Emidio *et al*., 2020a; Di Emidio *et al*., 2020b) and uteri (Palmerini *et al*., 2023), we here investigated morphological and molecular alterations in the PCOS tubal ampullae, to also assess the antioxidant properties of L-carnitines over the oxidative, glycative and lipoxidative stress determined by this syndrome. To these aims PCOS was induced in adult CD1 mice by subcutaneous administration of dehydroepiandrosterone (DHEA, 6 mg/100 g body weight) for 20 days; two groups of mice concomitantly received orally 1) L-carnitine (LC) and acetyl-L-carnitine (ALC) or 2) LC, ALC and propionyl-L-carnitine (PLC). Control animals were untreated. At the end of the treatments, tubal ampullae were collected and subjected to histology and immunohistochemistry to evaluate morphology, collagen deposition, steroidogenesis and oxidative, glycative and lipoxidative stresses. Respect to controls, the luminal epithelium of PCOS ampullae showed hyperplasia, hypertrophy and hyperfibrosis, as evidenced by hematoxylin-eosin, Azan-Mallory and Col1 staining. Glycative stress was confirmed by MG-AGE accumulation. The decreased TOMM20 expression accompanied by an increased HNE staining was indicative of mitochondrial, oxidative and lipoxidative damage. Finally, altered steroidogenesis was demonstrated by 17 β-HSD4. The two L-carnitine formulations reduced, even if at a different extent, the observed PCOS tissue alterations and stress damages. These results provided new data on the PCOS-induced damage of Fallopian tubes, highlighting that the protective effect of L-carnitine may be mediated by a reduction of oxidative/glycative/lipoxidative stress.

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ROLE OF GAL-10 IN NASAL EPITHELIUM REMODELLING IN AN *EX VIVO* THREE-DIMENSIONAL MODEL OF RESPIRATORY MUCOSA

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Galectin 10 (Gal-10) is a glycoprotein recognized as a biomarker of eosinophilic type 2 inflammation; Although spontaneous protein crystallization is a rare event *in vivo*, Charcot-Leyden Crystals (CLCs) consisting of Gal-10 protein are frequently observed in eosinophilic diseases, such as asthma. This study aims to strengthen the role of Gal-10 not only as an eosinophilic inflammation marker but also as a predictive marker and a possible therapeutic target. The first step of this study was to evaluate the correlation of Gal-10 with other inflammatory markers in the nasal wash of patients with SAR. The results showed a correlation between Gal-10 and IL-5 and between Gal-10 and Muc-5AC. The second step of this study was to evaluate the role of Gal-10 and IL-5 in nasal epithelium remodelling by stimulating a three-dimensional culture model of respiratory mucosa with pre-characterized nasal washes. The *ex vivo* human mucosa was replicated using the respiratory outgrowth model. The 3D respiratory cell outgrowths were obtained from patients undergoing turbinectomy. The tissue was cut into fragments, and each piece was placed within a transwell covered with BME (Basal Membrane Extract). The culture medium used was a 1:1 mixture of DMEM/BEGM. The stimulation of cell cultures started one week after growth. Subsequently, cell cultures were divided into two groups, treated and controlled. The first group was stimulated for two weeks with nasal washes showing a positive correlation between Gal 10 and IL-5. After two weeks of stimulations, the morphological analysis was performed by fixing the samples in 4% PFA, embedding them in paraffin, and staining them with a hematoxylin-eosin procedure. The preliminary results showed a significant presence of nuclei cilia cells in samples stimulated with nasal washes, compared with the control group. Our next goal will be to confirm these results by performing immunohistochemical staining to study epithelial-mesenchymal transition markers.

LEVELS OF MOLECULAR CHAPERONES ON TUMOR AND NORMAL SAMPLES OF TRUE VOCAL CORDS

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Laryngeal squamous cell carcinoma represents 4.5% of all malignancies and is the most common type of head and neck cancer in male subjects. Tumor biology, clinical behavior, and prognosis differ based on their location: glottis, supraglottis, or subglottis region. Between 85% and 95% of laryngeal cancer is squamous cell carcinoma. In the United States, approximately 13,150 cases of laryngeal cancer were diagnosed in 2018, resulting in roughly 3710 deaths. The male-to-female incidence ratio is 3.9:1 for laryngeal cancer. Tobacco and alcohol use are the two primary risk factors for cancer of the larynx. The risk is proportional to the intensity and duration of tobacco or alcohol consumption, and the risk decreases slowly after cessation. It takes 15 years to return to baseline risk levels. Consequently, the clinical treatments vary according to location and the stage of the disease. Organ preservation strategies are based on several operative techniques such as radiotherapy, transoral laser microsurgery (TLM), and open horizontal partial laryngectomy to definitive surgery (total laryngectomy). Surgical treatment, whether carried out for diagnostic or therapeutic purposes, provides essential information about the biology of the tumor and the patient's course of treatment. The correct histological classification of the neoplasm and accurate knowledge of the tumour's biology is relevant. Several studies have shown that heat shock proteins (HSPs) can be associated as prognostic markers in specific carcinomas. The objective posed in this experimental study is to evaluate the localization and tissue levels of Heat Shock Protein 10, 27, 60, and 90 at three different pathophysiological moments of the vocal cord carcinogenic process: healthy mucosa, mucosa with moderate-grade dysplasia, and squamous cell carcinoma, by immunohistochemistry techniques. Through the preliminary data obtained by immunohistochemical experiments, we can attribute to Hsps a possible role in the process of vocal cord oncogenesis. These molecular chaperones are found to be increased and displaced within the tumor cell compared with their physiological location in healthy tissue.

HEPATIC ALTERATIONS IN THE MOUSE MODEL OF AUTISM SPECTRUM DISORDERS: A POSSIBLE IMPROVEMENT WITH MELATONIN

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The role of the liver in autism spectrum disorders (ASD), a neurodevelopmental disease characterized by impairments in social interactions and repetitive or stereotyped behaviors, has been poorly investigated. In ASD, it has been shown a dysregulation of gut-brain crosstalk, a communication system that can influence metabolic homeostasis, as well as brain development, mood, and cognitive functions [1]. Additionally, redox imbalance and oxidative stress seems to play a critical role in ASD pathophysiology [2]. The liver is a central vital organ that helps to regulate vital events and to detect and destroy pathogens that enter the organism throughout the intestines. Based on the above, this study aims to explore the pathophysiology of ASD in liver open the way for new therapeutic strategies. We used as autistic model BTBR T+Itpr3tf/J mice (or BTBR mice) treated and not treated by melatonin, an indolamine that has many important functions [3]. The control group shows a normal liver morphology; the hepatocytes display central nucleus with a regular shape and cytoplasm with a diffuse localization of glycogen in the cytoplasm of the same cells. On the contrary, even if the hepatic cytoarchitecture is preserved, hepatocytes of BTBR mice show many intracellular vacuoles and very low presence of glycogen droplets. In BTBR mice treated with melatonin the number of vacuoles appears to be reduced. We analyzed oxidative stress by immunohistochemical evaluation of oxidative and pro-inflammatory markers, such as HO-1 and IL-1β, and observed variation in the expression of these markers among the different groups of mice studied. Evidence from morphological and immunohistochemical analysis confirmed liver involvement in autism spectrum disorders and showed a possible beneficial role of melatonin in the ameliorating autism-induced liver alterations in mice.

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EFFECTS OF A PSORIATIC INFLAMMATORY MICROENVIRONMENT ON KERATINOCYTE MORPHOLOGY IN 3D AND 2D BIOLOGICAL EXPERIMENTAL MODELS OF NORMAL HUMAN SKIN

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The interplay between proinflammatory circulating cytokines and keratinocytes is a crucial event in the development and progression of psoriatic lesions. However, the early phases of the pathogenesis of psoriasis are still to be elucidated, in particular regarding the epidermal barrier. A pivotal role is played by tight junctions (TJs), *i.e.* claudin-mediated intercellular attachment structures, localized in the upper granular layer. A barrier impairment associated to an alteration in TJ proteins is described in psoriasis as a response to a proinflammatory microenvironment. We investigated by immunofluorescence analysis the modulation of the expression of claudin 1 (CLDN-1), a transmembrane integral TJ protein, and of Zonula Occludens 1 (ZO-1), a scaffold plaque protein, after the incubation with MIX, a combination of

interleukin (IL)-17, IL-22, IL-23, tumor necrosis factor (TNF)-alpha, for 24 (T24) and 48 (T48) hours. We considered as experimental models the standardized 3D organotypic cultures of normal human skin (n=7) and *in vitro* cultures of primary normal human keratinocytes (n=3) in basal or differentiating cell growth conditions. On skin bioptic samples, ultrastructural analysis by transmission electron microscopy (TEM) was performed. In control skin samples, CLDN-1 immunopositivity increased from the basal layer upwards, but its expression was early reduced in the basal and suprabasal layers starting from T24 in MIX-incubated group. At this time point, ZO-1 expression in control samples increased gradually, starting from the basal layers towards the epidermal surface and the incubation with MIX induced its immunopositivity in the basal and suprabasal layers. At T24, CLDN-1 expression was unaffected by MIX in undifferentiated and also calcium- differentiated keratinocytes. Unexpectedly, undifferentiated cells relocated ZO-1 at cell-cell contact points after the incubation with MIX, and in calcium-differentiated keratinocytes, ZO-1 synthesis was stimulated, too. By TEM, after MIX incubation, the overall architecture of the epidermal compartment was maintained, but apoptosis and enlargement of intercellular spaces were evident. The present results strongly suggest that the i) broadening of ZO-1 expression and ii) the downregulation of CLDN-1, typical features of psoriasis, can be induced as early as 24 hours in both models, suggesting that they represent a valid experimental approach. To complete this study, the effect of this microenvironment on keratinocyte proliferation and differentiation will be investigated, obtaining further insights into the early processes leading to the formation/progression of psoriatic plaques.

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ANTI-INFLAMMATORY ACTIVITY OF *Diospyros digyna* JACQ. FRUIT EXTRSCTS IN AN *IN VITRO* MODEL OF INTESTINAL INFLAMMATION

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Diospyros digyna Jacq. is a tropical fruit tree native to Mexico. Although it is almost completely unknown in Europe, recently, its cultivation has been successfully tested in the Mediterranean area, including Sicily. Its fruit, due to a peculiar soft and dark chocolate pulp, is called black persimmon or chocolate pudding fruit and some studies suggested that it possess an interesting nutritional and nutraceutical value (1). We previously demonstrated that black persimmon is a rich source of bioactive compounds, peculiarly distributed in the different parts of the fruit, showing notable radical scavenging and metal-reducing activity and ability to prevent oxidative damage in cells (2). Nowadays, the role of oxidative stress in human diseases is well demonstrated. In particular, several experimental data indicate that cellular oxidative damage is involved in etiology and progression of several chronic diseases, including cancer and inflammatory diseases (3). In addition, epidemiological studies show that dietary intake of antioxidants can provide a significant protection against oxidative stress-related diseases. Here we evaluated the anti-inflammatory potential of different parts of *Diospyros digyna* fruit obtained from plants grown in Sicily. For our experiment we used an *in vitro* model of intestinal inflammation consisting of differentiated Caco-2 cell monolayers subjected to proinflammatory activity of IL-1b. qRT-PCR analysis demonstrated that cell exposure to extracts from pulp, seeds, and peel of black persimmon prevents, in a concentration-dependent manner, the IL-1b-induced up-regulation of the genes encoding the main proinflammatory mediators, including soluble ones (IL-8, IL-6, IL-12, and TNF-α) and proinflammatory enzymes (iNOS, and COX-2). On the other hand, results from western blot and ELISA analysis, showed that the observed transcriptional effects result in a reduced level of the corresponding proinflammatory proteins. Interestingly, black persimmon extracts produce opposite effects on the expression of the gene encoding the anti-inflammatory cytokine, IL-10. Finally, our results showed that the observed anti-inflammatory activity of the black sapote extracts is associated with positive effects on the expression of genes encoding the main antioxidant enzymes (CuZnSOD, MnSOD, and GPx) suggesting that redox-sensitive signalling pathways could be involved in the anti-inflammatory activity of fruit components. Collectively, the obtained data showed for the first time that *Diospyros digyna* fruit components, at very low concentration, are active in attenuating the inflammatory response of intestinal epithelial cells suggesting local effects at the gut lumen useful for the physiology of the gastrointestinal tract.

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COMPLEX MAGNETIC FIELDS AS STORAGE OF INFORMATION AND PROCESSING OF BIOACTIVE RESPONSES

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From the scientific literature it is known that electromagnetic signals of a certain type significantly increase the tolerance to hypoxia in post-infarction patients, have strong angiogenic properties and strongly reduce the cardiovascular risk factor. To prove this feature, *in vivo*, *in vitro* and cohort tests were carried out with the aim of monitoring performance and cardiopulmonary function at the same time. The first trial set involved a total of 300 mice with the aim of monitoring performance and cardiopulmonary function at the same time. The Rotarod Performance Test is a bin test that uses a rotating cylinder to test the animals' ability to balance and move their legs in a coordi-

nated manner. The test provides objective measures regarding balance, coordination, physical condition, motor pattern and motor coordination. The benefit of this test is that it creates a continuous, measurable variable that can be used to quantify the effects of different conditions. The test is computerized, so it is not based on the subjective judgment of an operator. The Rotarod was performed inside a metabolic chamber which allowed the measurement of the percentage of maximum oxygen consumption (V02MAX), the maximal heart rate (HRMAX) and to calculate the aerobic threshold (maximal aerobic speed, MAV) for each type of workout. The second trial set was conducted on 60 male athletes (competitive triathletes) with a mean age of 27.63±1.54 years, a mean weight of 77.23±1.90 kg and a BMI (Body Mass Index) of 23.73±0.91. The athletes were subjected, after 3′ of heating, to 6 total trials (3 with placebo and 3 with the device), seven days apart from each other, of 10, 20, 30, 40 and 50′ of running at 9km/h with an incremental difference in altitude of 0.5% every 10′, using a Technogym® Myrun treadmill (Technogym® SpA Via Calcinaro 2861 47521 Cesena, FC). Heart rate expressed in beats per minute was monitored using a Polar M460 heart rate monitor (Polar Italy), while lactate levels, expressed in mmol/L, were measured using a LACTATE PRO 2 device (Arkray ™ Global Business.inc, Kyoto, Japan). Lactate and HR were measured in the minute of elevation gain. During the test period, no athlete used the T·NES device, which was only used on test days. The results of this study aligned with expectations based on the pilot observations. Only Group A mice showed a marked increase in maximal oxygen uptake (VO2MAX) and aerobic threshold, which was higher in the SST protocol. Overall, the device added a 30-35% increase in overall performance.

IMPACT OF MICROPLASTICS ON CELLULAR STRESS RESPONSES IN EARLY DEVELOPMENTAL STAGES IN ZEBRAFISH LARVAE

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In recent years, the bioaccumulation of discarded plastics within aquatic habitat, has raised an increasing scientific and social concern. It has been widely demonstrated that the microplastics (MPs) derived from degrading plastic debris in the environment, have a highly toxic effect on the biota. An emerging model species to study the accumulation and toxicity of MPs in the freshwater habitats is represented by the zebrafish *Danio rerio*. Zebrafish has been widely used as a successful bioindicator in the field of environmental toxicology because of several forceful features, *i.e.* its small size, short life cycle, ease of breeding and maintenance, high fecundity and genetic similarities with humans. Moreover, the transparency of zebrafish embryos and larvae provides an additional advantage in studying the localization of fluorescent-labeled contaminants. In the last years, it has been reported that the exposure to MPs in zebrafish larvae caused several behavioral and morphological alterations along with the induction of high toxicity at different tissue levels. However, the impact of the MPs exposure on the cellular stress responses in early developmental stages, still remain unclear. Here, zebrafish embryos have been exposed to fluorescent 1 µm Polystyrene MPs (PMPs) at 500 µg/L and 1 g/L, up to 120 h post fertilization. The distribution and bioaccumulation of PMPs in the embryos have been assessed at different time points by a fluorescence microscope and the expression of cellular stress markers has been assessed by immunofluorescence and gene expression analysis. These analysis are still in progress and, in our opinion, the expected results will be useful to elucidate the impact of PMPs on the embryogenesis of freshwater fish species.

HSP90 QUANTITATIVE DECREASE INDUCED BY A COMBINATION OF GANETESPIB AND SAHA IN TWO BREAST CANCER CELL LINES

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Breast cancer (BC), the most diagnosed female malignancy (about 16% of all cancers). The molecular chaperone Hsp90 assists in the folding of nascent polypeptides, and other aspects of protein homeostasis with clients in normal and tumor cells, including BC cells. Several known Hsp90 clients are involved in signal transduction, including estrogen and progesterone receptors, tyrosine kinase, and epidermal growth factor, all relevant to BC biology. Also, Hsp90 overexpression is thought to participate in the mechanism through which BC cells become resistant to various stressors. Consequently, Hsp90 is considered a convenient therapeutic target in various cancers, including BC[1]. Ganetespib is a second-generation small-molecule inhibitor of Hsp90 that affects proteins involved in BC pathogenesis[2], and SAHA (vorinostat) is a histone deacetylase inhibitor with anti-proliferative activity in human cancer cell lines, including BC lines[3]. SAHA has been used in cancer therapy with some success, but the clinical trial results were not fully satisfactory. There is however the possibility that combining SAHA with other anti-cancer treatments may bring about more satisfactory results. Thus, we are testing the combination Ganetespib with SAHA and comparing results with those obtained with each separately, using the MDA-MB-231 and MCF-7 cell lines and applying the [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) test, Western blotting, immunohistochemistry, and the scratch-wound assay. Results thus far show a dose-dependent decrease of Hsp90 in both cell lines after treatment with the combination Ganetespib-SAHA. Further tests will evaluate properties of the cell lines related to Hsp90 functions and pertinent to BC carcinogenesis.

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CURCUMIN AND ANDROGRAPHOLIDE CO-ADMINISTRATION SAFETLY PREVENT STEATOSIS INDUCTION AND ROS PRODUCTION IN HEPG2 CELL LINE

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Non-alcoholic fatty liver disease (NAFLD) is an emerging chronic liver disease worldwide, defined by excessive lipids accumulation in hepatocytes due to impair balance between oxidation of free fatty acids (FFAs) and hepatic *de novo* lipogenesis. The hepatic FFAs deposition leads to chronic inflammation, characterized by endoplasmic reticulum stress, mitochondrial dysfunction, lipid peroxidation, oxidative stress and cell death, leading to non-alcoholic steatohepatitis (NASH) progression and fibrosis development. Researchers are focusing more on herbal medicines as potential drugs due to presence of natural bioactive compounds, such as phenolic acids and flavonoids, famous for their antioxidant property, useful in the prevention of NAFLD. Curcumin and Andrographolide are known to improve hepatic functions, being able to reverse oxidative stress and release of pro-inflammatory cytokines. The aim of our study was to evaluate the effects of the phytochemicals, Curcumin and Andrographolide, against steatosis and ROS production. Thus, we tested Curcumin and Andrographolide separately and in combination to determine their effect on triglyceride accumulation and ROS production, identifying the differential expression of genes involved in fatty liver and oxidative stress development. *In vitro* steatosis was induced in HepG2 cells and the protective effect of Curcumin, Andrographolide and their combination was observed evaluating cell viability, lipid and triglyceride content, ROS levels, and microarray differential gene expression. Curcumin, Andrographolide, and their association were effective in reducing steatosis, triglyceride content, and ROS stress. The hepatoprotective effect shown by Curcumin–Andrographolide co-treatment, seems to be imputable to the observed upregulation of genes related to cell survival, such as V-AKT murine thymoma viral oncogene homolog 1 (AKT1), mitogen-activated protein kinase 8 (MAPK8), phosphoinositide-3-kinase, catalytic, alpha polypeptide (PIK3CA), and Fas, TNF receptor superfamily, member 6. The association showed an interesting activity, increasing the anti-inflammatory interleukin 10 (IL 10) cytokine expression. The present study showed that the association of Curcumin and Andrographolide could be used as a therapeutic approach to counter high lipid content and ROS levels in steatosis liver, avoiding the possible hepatotoxic effect of Curcumin. Furthermore, this study improved our understanding of the anti-steatosis and hepatoprotective properties of a Curcumin and Andrographolide combination.

NUTRACEUTICAL CHARACTERIZATION AND EVALUATION OF "GOLDEN" TOMATO JUICE INTAKE ON REDOX HOMEOSTASIS: A SINGLE-ARM PILOT STUDY ON PLASMA FROM HEALTHY DONORS

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"Golden" tomato (GT) represents a mixture of tomato fruits at different ripening stages, with respect to red tomato at full maturation. Tomato (Solanum lycopersicum L.), which is frequently included in the Mediterranean diet, is one of the most cultivated and consumed vegetable in the world. It is also widely used in many processed products such as puree, sauces and juices. Tomato and tomato-based foods represent a large source of bioactive compounds, including antioxidant molecules that play beneficial roles in inhibiting reactive oxygen species (ROS) by scavenging free radicals [1]. Notably, compared to fresh tomatoes, tomato juice has an elevated antioxidant capacity as result of an increased biodisponibility of some phytonutrients that could be due to tomato processing [2]. Interestingly, it has already been reported that red tomato juice intake suppresses excess ROS in stress conditions, like in individuals performing extensive physical activity or in breast cancer patients after radiotherapy protocol [3,4]. However, the impact of GT juice consumption on the redox plasma balance of healthy subjects not exposed to specific stress factors is still to be unveiled. Thus, our aim has been to characterize for the first time the GT juice used in the present study in terms of composition of phytonutrients and antioxidant properties. Secondly, we pointed to examine the effect of its consumption on plasma from healthy donors in a single-arm study. In particular, through HPLC analysis we have found that the GT juice, similarly to fresh product, shows a different composition of phytonutrients compared to red tomato. Furthermore, we have estimated the antioxidant activity using the Folin-Ciocalteu (FC) Assay to evaluate the total polyphenols, the Crocin Bleaching Assay (BCA) for the radical scavanger capacity as well as the Ferric Reducing Antioxidant Power (FRAP) Assay for the reducing capacity. Once established the considerable antioxidant capabilities, we have recruited fourteen healthy donors from the University of Palermo, and we have asked them to drink 200 ml of unsalted tomato juice every day for ten days, without modifying their eating behaviour. Before (T0) and after (T1) supplementation period, their blood samples have been collected and we have assessed specific plasma biomarkers of redox homeostasis, *i.e.* hydroperoxidation, lipid peroxidation, thiols balance and antioxidant barriers. Based on our results, the GT juice consumption in healthy subjects does not appear to have modified the levels of hydroperoxidyl free radicals but seems to have reduced circulating lipid peroxides. Moreover, after supplementation we have detected an enhancement in thiols groups and non-enzymatic antioxidants which have the ability to adsorb radical species. These findings highlight that GT juice affects the plasma redox balance shifting towards an increase in endogenous antioxidant barriers. Importantly, this shift has been encountered in subjects in physiological conditions. Notwithstanding, our data should be considered as preliminary and future studies are necessary to increase the small number of healthy donors and add a placebo control group.

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ENVIRONMENT AND HEALTH

Sarcoptes scabiei: CELLULAR ASPECTS OF HOST-PARASITE RELATIONSHIP IN SCABIES INFESTATION

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Sarcoptes scabies is the arthropod species, belonging to the class of arachnids, that leads to the establishment of scabies in humans. Scabies develops in all countries, especially in cold and dry seasons. Development of *Sarcoptes scabiei* occurs principally in crowded domestic environments, in health care facilities, and in places with poor hygienic conditions. The body length of the mite is less than 1 mm in both sexes and is visible to the naked eye as a small black dot. *Sarcopotes scabiei* burrows under the skin surface about 0.3 mm in diameter and 5-10 mm in length. The male and female mites, move across the skin surface in search of a new partner and new burrowing sites. Mating lasts about 4 days and then the female lays eggs (about 2 or 3 per day) until she dies. Once incubation occurs, the larvae begin to move across the skin surface until they reach the adult stage post maturation. With burrow establishment, the adult mite initially causes mild physical reactions, only as it establishes itself, it causes damage at the level of keratinocytes leading to: atopic dermatitis and local bleeding. The most common sites where the mite settles are: interdigital space of hands and feet, armpits, wrists, elbows, penis for men, and inframammary folds for women. Initially there is the formation of small pustules that, when scratched lead the skin to become thicker, with crusted covering. The skin rash presents with red spots, and burrows dug by the mites are evident. The onset of scabies occurs primarily through contact with the infected person or contaminated object. Treatment includes different types of drugs and must often involve the family or community in which one lives to prevent a new infestation. Useful remedies against scabies include permethrin, which is useful for pest control in environments where the mite is present; cypermethrin, is a drug to be applied mainly at night; ivermectin, an oral drug, very effective, usually used for the more contagious crustose scabies and finally sulfur preparations, which are more widely used in developing countries given their low cost.

GUT-BONE AXIS: EFFECTS OF THE INTEGRATION WITH BIOFORTIFIED VEGETABLES ON BONE REMODELING AND METABOLISM

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Osteoporosis is a very common chronic disease that can affect people of all ethnic groups and particularly affect women and the elderly. Among the determinants of bone fragility, in skeletal microarchitecture, there is an insufficient supply of nutrients which favors the loss of bone mass and increased risk of osteoporosis during life. The gut peptides (GLP-1, GLP-2, GIP, PYY) are released following food intake and they can inhibit bone resorption. Recent evidence showed that the ingestion of molybdenum (Mo)-enriched lettuce improved glucose homeostasis by increasing, within physiological range, levels of PYY and GIP. Therefore, we hypothesized that bone turnover would be influenced by short-term nutritional intervention with biofortified molybdenum lettuce, and this would impact on the essential regulators of bone metabolism. Thus, the present study investigates if supplementation of lettuces enriched with Mo in a cohort of elderly subjects for 12 days affects bone homeostasis. Forty-two subjects (age range 54-74) were recruited and randomly assigned to a control group, a group that consumed lettuce biofortified with molybdenum and a group that consumed molybdenum in capsule. Blood samples were taken, before and after the intervention period, and markers for the state of bone turnover, C-terminal telopeptide (CTX) and osteocalcin, and metabolism, parathyroid hormone (PTH), calcitonin, albumin-corrected calcium, vitamin D, phosphate, and potassium were evaluated and compared. Supplementation of molybdenum in capsules did not lead to changes in the bone remodeling or metabolism of the participants. The intake of biofortified lettuce resulted in a significant decrease in CTX and PTH levels and an increase in vitamin D in all subjects. However, the values of calcitonin, calcium, potassium, and phosphorus did not change. The results obtained from the integration of biofortified cultures with molybdenum pose considerable prospects in the prevention of bone loss in elderly subjects. They suggest that the integration with a biofortified vegetable matrix with molybdenum could constitute an intervention for reduction of the depletion of bone mineral mass and in general of skeletal homeostasis in geriatric age unlike supplementation with molybdenum in capsules which has not conditioned bone turnover and metabolism. Consequently, the consumption of vegetable cultures enriched with molybdenum can represent a valid nutritional approach to favor the maintenance of bone homeostasis and counteract the degenerative processes in the elderly population.

Apis mellifera sicula AND ITS ENEMIES: POSSIBLE SOLUTIONS AGAINST *Vespa velutina nigrithorax*

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The Asian yellow-legged hornet *Vespa velutina nigrithorax* was introduced by mistake from China to other parts of the world and to Europe in the early 21st century, most notably in 2004. The invasion began in France and in recent years it has reached England and Italy. *Vespa velutina* represents a major problem for native pollinating insects in general, and for honeybees in particular. Hornets assault worker bees coming back from their food supply rounds, causing failure to return to the hive and lack of nourishment to it. Hornets may also enter such weakened hives, in order to raid on offspring and steal honey. In the long period, these actions lead to the death of the hive. Some species of wild pollinators have developed defensive systems against *Vespa velutina* while *Apis mellifera* seems totally defenseless against these predators. Given the ecological interest that *Apis mellifera* has as a pollinator and the economic interest linked to beekeeping, systems are being studied to eliminate or contain *Vespa velutina*. Such systems are primarily aimed to prevent the establishment of the hornet (which appears to be not feasible), monitoring its nests and containing its expan-

sion by destroying the nests. In France, the possible containment of *Vespa velutina* was studied evaluating the impact of its natural enemies, but the results were not very promising; in Spain the effectiveness of traps and baits was evaluated but this method too was rejected because not selective for hornets but rather a further source of danger for all insects. The only possible way therefore remains the protection of *Apis mellifera*, and recent research aims in fact to test systems for the protection and defense of beehives. In 2022 in Spain the effectiveness of the use of "electric harps" in reducing the predation of the Asian hornet on beehives was verified with promising results. In this study we analyzed the population of *Apis melllifera* present in Sicily with particular attention to the subspecies *Apis mellifera sicula*, currently endangered because it was put at risk in the past by uncontrolled introductions of other subspecies, and in more recent years by the invasion of *Vespa velutina*. The protection of this subspecies plays an important role in the preservation of biodiversity as well as being a priority for its economic value. Our study therefore aims to ensure that the Sicilian bee is protected. For this purpose we want to select genetically distant breeders in order to avoid imbreeding and the consequent weakening of the subspecies. At the same time, we are studying ways to ward off hornets with the possible use of selective olfactory calls or other traps.

LIFE MICROFIGHTER PROJECT: INNOVATIVE ZEO-BIOPESTICIDES, BASED ON USEFUL MICROORGANISMS, TO ELIMINATE THE USE OF COPPER-BASED PESTICIDES

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The massive use of copper compounds (Cu) in organic agriculture is no longer a sustainable practice, as copper tends to accumulate in the environment and soils. Even though the EU is restricting the use of copper-based pesticides these products are still widely used in many countries because there are no other alternatives with comparable efficacy. It is in this context that the LIFE MICROFIGHTER project was born, aiming to demonstrate in field the effectiveness of a new natural and ecologically sustainable Zeo-Biopesticide product and put it into practice to reduce/replace the use of copper-based pesticides for defense against the relevant pathogens. MAIN OBJECTIVES: 1) Demonstrate the effectiveness of a new natural and eco-sustainable Zeo-Biopesticide, composed of natural Italian K-chabasite zeolites and a specific mBCA (Pseudomonas sp. DLS65), as an alternative to copper-based products for the control of the most threatening grapevine diseases, tomato and olive tree on organic farms in Italy, Croatia and Spain. 2) Reducing the amount of copper in agricultural soils from an average of 4 kg/ha/year to an average of 2 kg/ha/year without affecting yields and food quality. 3) Demonstrate that cultivation with the new Zeo-Biopesticide will reduce the amount of copper accumulated in the soil by at least 0.7 ppm per year of experimental cultivation and increase soil biodiversity. SPECIFIC GOALS: 1) In all project areas (9 hectares for 9 farms in Italy, Croatia and Spain) the total copper input will be reduced from 4 kg/ha/year to 2.33 kg/ha/year, saving about 41.7% of Cu. 2) In all project areas it will be demonstrated that the thesis without the addition of copper will show a decrease in the accumulation of Cu in the topsoil layer (first 30 cm), equal to at least 0 .7 ppm/year. 3) In all project areas it will be demonstrated that the thesis with the addition of 50% copper will show a decrease in the accumulation of Cu in the topsoil layer (first 30 cm), equal to at least at 0.35 ppm/year. 4) Diseases (downy mildew, speckling, bacterial spot, olive mange and peacock eye) will be controlled with the innovative and sustainable treatment based on 50% copper and 50% ZBp to demonstrate that it has at least the same efficacy which is obtained using the 100% copper-based treatment. 5) Soil microbial biodiversity will be restored by reducing the use of Cu and applying ZBp. 6) The marketability and taste characteristics of the food products (olive oil, table tomato, tomato sauce and wine) will be guaranteed by the application of the Zeo-Biopesticide strategy to replace copper.

PROJECT N° 101074218 — LIFE21-ENV-IT-LIFE MICROFIGHTER.

DEVELOPMENT OF A PROTOTYPE FOR A BIOMONITORING DEVICE AIMED AT EVALUATING THE EFFECTS OF AIR POLLUTION ON A 3D MODEL OF HUMAN RESPIRATORY MUCOSA

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Air pollution has been identified as the leading environmental cause of disease and premature death worldwide (1). Smog causes both acute and chronic effects. The former can manifest themselves as acute bronchitis, asthmatic crisis, bacterial infections; the latter can result in respiratory disorders such as chronic obstructive pulmonary disease (COPD) and asthma, but they can lead to systemic inflammation that can cause cancer, stroke, cardiovascular and neurodegenerative diseases (2). In this regard, monitoring the air quality in urban and suburban zones has become extremely important. The aim of the presented study is to evaluate the effects of a direct exposure of the human respiratory mucosa reproduced *ex vivo* to air pollutants through the development of an environmental biomonitoring system. Biopsies of the nasal respiratory mucosa were collected and the fragments obtained from them were cultured inside PET membrane inserts to form a 3D culture that maintained the air-liquid interface (ALI) present *in vivo*. Complete differentiation of the various cellular elements is achieved after approximately 21 days of culture, at the end of which the Epithelial-Mesenchymal Trophic Unit (EMTU) model can be obtained (3). At the same time, in collaboration with a specialised company, a prototype of the environmental biomonitoring system was designed in order to allow the 3D cell cultures to survive and interact with the external environment. After the cell cultures were differentiated, the inserts were placed inside the prototypes in such a way that the epithelium was continuously in contact with all molecules and substances from the air through a continuous, non-selective exchange. After a week-long exposure to the environment, the cell cultures were fixed in 4% paraformaldehyde and included in paraffin. Hematoxylin-eosin staining was performed on the samples, and proteins such as pan cytokeratin and vimentin were searched for by immunohistochemistry. Preliminary results suggest differences between control cell cultures and those exposed to indoor pollution: the latter show a layer of keratinised

cells in the portion exposed to air, which appears to be twice as thick as the layer visible in controls. This suggests a possible defence mechanism of the respiratory mucosa against pollution. The ultimate goal is the long-term implementation of such a device to predict possible disease phenotypes that might arise in the population in different urban and suburban areas in response to air pollution.

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FORENSIC ENTOMOLOGY: EVALUATION OF DAMAGES MADE BY TERMITES ON THE ARCHITECTURAL CULTURAL HERITAGE

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The global termite fauna (Arthropoda: Insecta: Isoptera) is represented by ~3000 species (ten families), with two established genera in Europe *i.e.*, *Reticulitermes* and *Kalotermes*, and one genus under investigation, *Cryptotermes*. They belong to the xylophagous trophic group and feed by means of specialized chewing mouthparts mainly on wood, which they digest (lignocellulosic matter) thanks to endogenous enzymes and a microbiota consisting mainly of bacteria, fungi, and flagellated protozoa. The habitat optimum of these wood-dwelling insects is favoured by warm temperatures and humidity. Termites are eusocial insects with overlapping generations, and cooperative brood care. Colonies are hierarchical complex (castes) composed by kings and queens (fertile, reproductives, persistent or temporary, adults or neotenic), soldiers and workers (steriles, neuters). The latter (up to many thousands of individuals/colony) carry out a wide range of activities including defence, royals and larval care, collecting food supplies, building and enlarging nests. Individuals in castes deeply diverge for morphological traits *i.e.*, body size, colour, and macro- to micro-morphology. In Italy, the most assiduous native termites (family Rhinotermitidae) belong to several subterranean subspecies/strains of *Reticulitermes lucifugus* (Rossi, 1972), an economically important species, characterised by the absence of true, persistent royals. Colonies of *R. lucifugus* cannot be easily detected for the (a) small body size of adults (black, wingless ~6 mm, winged 10-12 mm in length) and soldiers/workers (whitish-yellowish, ~5 mm), (b) typical life style mostly nocturnal, and strictly sciaphilous related to the reduced compound eyes and long moniliform antennae well adapted as sensory organs in dark habitats, (c) cryptic subterraneous nests in the ground/cavities/ floors shaped as tunnels in wood, and also by external, descending or ascending ground tunnels made by rosume mixed to saliva. Knowledge in depth of termites' biology/ecology/ethology/taxonomy/genetics is essential to prevent their spread and to preserve the structural integrity of cultural heritage sites following eco-sustainable approaches presently focused in the framework of experimental applied research. This contribution aims to investigate the termites damaging on the materials discovered in the Etruscan Archeosite of Cerveteri. The detection of typical termites walkways, to define the topographic distribution and extension of the nest architecture, is based on a non-invasive fluorescence method by Luminol-like trackers. For this focused precious site in Tuscany, work planning and selection of the best control treatment technics are in progress.

PRO-AUTOPHAGIC ACTIVITY OF DIETARY INDICAXANTHIN IN INTESTINAL EPITHELIAL CANCER CELLS

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Autophagy is an evolutionarily conserved process critical in maintaining cellular homeostasis. Recently, the anticancer potential of autophagy inducers, including phytochemicals, was suggested (1). Indicaxanthin, a betalain pigment present in prickly pear fruit, possesses antiproliferative and pro-apoptotic activity in colorectal cancer cells, associated with epigenetic changes in selected methylation-silenced oncosuppressor genes (2). In this work, we demonstrate that indicaxanthin induces the up-regulation of the autophagic markers LC3-II and Beclin1 and increases autophagolysosome production in intestinal epithelial cancer cells. Methylomic studies show that the observed pro-autophagic activity of indicaxanthin is associated with epigenetic changes. In particular, in addition to acting as an ipermethylating agent at the genomic level, indicaxanthin also produces a strong differential methylation with respect to control cells in 39 out of 47 autophagy-related genes, especially those involved in the late stages of autophagy. Furthermore, *in silico* molecular modelling studies suggest a direct interaction of indicaxanthin with Bcl-2, able to influence the function of Beclin1, a key autophagy regulator. External effectors, including food components, can be able to modulate the epigenetic signature of cancer cells. This study demonstrates for the first time a pro-autophagic potential of dietary indicaxanthin in human colorectal cancer cells associated with epigenetic changes and contributes to define the potential healthy effect of this pigment in the pathophysiology of the gastrointestinal tract.

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TOXICOLOGICAL EVALUATIONS OF GLYPHOSATE IN ZEBRAFISH EARLY-LIFE STAGE

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The glyphosate is now considered the most widely used herbicide in the world. It is a systemic, non-selective, post-emergency herbicide and it operates via 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme. This enzyme is critical in the shikimate pathway. Its use in agriculture determines the destruction of unwanted weeds. The high persistence and its widespread determine the presence of traces of this herbicide

increasingly frequently in soil, water, and air, as well as in food (Zoller *et al.*, 2018), and for this becoming a growing concern for human health. Indeed, although shikimate pathway is not found in humans, it is typical in bacteria, including those in the human gut microbiota (Mesnage *et al.*, 2021). In addition, glyphosate and glyphosate-based herbicides can also be toxic for aquatic organisms. Ecotoxicology studies based on water organisms as experimental models are particularly useful, as glyphosate-based herbicides and their metabolites are often found in rivers and other water ecosystems (Gasnier *et al.*, 2009). A distinctive feature of some of these water environments, particularly those highly polluted, is the low water oxygen concentration. This leads to the development of hypoxic environments which represent a stress factor for the living organisms. For this reason the present study aimed to evaluate the glyphosate potential effects on the zebrafish early-life stages development. The research focused on the evaluation of glyphosate acute toxicity by the Fish Embryo Acute Toxicity (FET) tests. In order to reproduce the environmental conditions the glyphosate was associated to hypoxia induced by $CoCl_2$. These results confirmed the developmental toxicity of glyphosate, $CoCl_2$ and $CoCl_2$ glyphosate mixture in zebrafish early stages. The LD_{50} for glyphosate and $CoCl_2$ are respectively: 122,9 mg/L and 30,4 mM. The tested mixture concentrations did not permitted to calculate the LD_{50}. The sub-lethal alterations are: - glyphosate: yolk sac edema, pericardial edema, impaired blood flow, blood stasis and smaller head. - $CoCl_2$: yolk sac edema, pericardial edema, impaired blood flow, blood stasis, deformed head and delayed hatching rate. - mixture: yolk sac edema, pericardial edema and blood stasis. Moreover, the preliminary data show how hypoxia can exert a protective effect against exposure to some toxic substances as already demonstrated in the literature. Then, the aim of future studies is to evaluate the long-term effects of this herbicide.

VALORIZATION OF FOOD WASTE: RASPBERRY SEED POWDER (*Rubus idaeus* L.) AS A FUNCTIONALIZING AGENT OF A BAKERY PRODUCT

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In addition to the role of macronutrient and micronutrients in replenishing energy and material losses, the functional properties of some foods are due to the presence of specific bioactive molecules. In this context, the term "functional food" describes a particular food that, beyond basic nutrition, naturally possesses or is enriched by bioactive components. Plant kingdom represents the main source of bioactive molecules, and it is widely acknowledged that a diet rich in plant foods produces positive health outcomes (1). Nowadays, through sustainable approaches in line with a circular economy model, agri-food waste and by-products are also exploited as a source of functional ingredients to improve nutraceutical value of foods. Red raspberry is the fruit of *Rubus idaeus*. Due to its profitability, its production has significantly increased in recent years in Italy. In addition to being used for fresh consumption, because of their pronounced perishability, much of the harvest is industrially processed. Seeds are the main waste from this processing and strategies for their valorization have been investigated. Cold pressing of raspberry seeds is exploited to obtain a very valuable oil (2). Nevertheless, this process produces also an agro-industrial waste (Waste Raspberry Seed Powder, WRSP), which has not yet found potential applications. We previously showed that WRSP is an extraordinary source of bioactive molecules, proanthocyanidins, and possess antimicrobial, antiproliferative and antioxidant activity in several experimental models (3). In this work we explored the suitability of WRSP as a functional ingredient of bread, a common dietary food worldwide with a very low nutraceutical value especially when prepared from refined flours. Our data revealed that the replacement of a small percentage (5 or 10%) of wheat flour with WRSP significantly increases the functional value in term of content of polyphenolic compounds and antioxidant activity of the functionalized dough and consequently of the final product. We have recorded that the addition of WRSP allows to obtain a final product that has a polyphenolic content almost double (5%) or triple (10%) than the value of control bread. Furthermore, comparison of the t-PAC value of the control bread with that of the functionalized breads shows an increase in proanthocyanidins content by a factor equal to 10 in the 5% bread and equal to 20 in the 10% bread. Regarding, instead, the contribution of fortification on the total antioxidant activity, the good correspondence between the expected and experimental data, especially for 5% bread, would indicate a good stability of the antioxidant components of WRSP at high temperatures. Suggesting, therefore, a potential use of this agro-industrial waste for the functionalization of other bakery products. Finally, rheological, microbiological, and sensory studies performed on functionalized breads indicate that WRSP did not negatively affect the technological parameters and organoleptic characteristics of the product. In conclusion, the obtained results suggest that WRSP may find application in nutraceutical field as an effective functionalizing agent.

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BIOLOGICAL EFFECTS OF NON-INTENTIONALLY ADDED SUBSTANCES (NIAS) IN HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS

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Plastic materials are frequently employed in food packaging due to their good functional properties. However, food contact material (FCM) may release different substances that could migrate into food posing human health risk. Among these contaminants, intentionally added substances (IAS) are normally added during the manufacturing of plastics to increase their performances while non-intentionally added substances (NIAS), are generated as side products in one or more steps of plastic production process. They are often unknown in terms of chemical composition and toxicity and Countries have not yet adopted specific policies to monitor and regulate NIAS in commercial foods for human consumption [1]. Therefore, our objective was to investigate the cytotoxic effects of NIAS from two high-density polyethylene (HDPE) films, differing in NIAS composition, in resting and mitogen activated human peripheral blood mononuclear cells (hPBMCs). The two HDPE blown films, named F48 and F56, were produced using two different extrusion temperatures and screw speed, starting from granules added with IRGAFOS 168,

or tris(2,4-di-tert-butylphenyl)phosphite, an antioxidant IAS commonly used to improve the stability of polyolefins (POs) and approved for food contact. During processing, IRGAFOS 168 can degrade by oxidation and hydrolysis forming two NIAS: tris (2,4-ditertbytylphenyl) phosphate (I168-0x) and 2,4-di-tert-butylphenol (2,4 DTB) respectively; the extrusion conditions can affect the extent of IRGAFOS degradation and subsequent NIAS formation. By gas chromatography/mass spectrometry analysis we found that F48 contained 2,4 DTB and 168-ox at the concentration of 220 µg/g and 66 µg/g, while F56 showed a higher content of 2,4 DTB and I168-ox (342 µg/g and 150 µg/g respectively). To study *in vitro* cytotoxicity, hPBMCs from five unrelated human healthy donors were stimulated or not with phytohemagglutinin (PHA) and seeded in 96-well plates containing increasing concentrations (from 37 mg/ml to 296 mg/ml) of conditioned culture media previously exposed to F48 and F56 for 48 hours or 18 days to mimic a condition of NIAS production and release from POs. Cells were treated with these conditioned media and then MTT assay was used to evaluate the cytotoxic activity after 24 and 48 hours. Results showed that the treatment did not affect cell viability in both stimulated and unstimulated hPBMCs. However, an increase of cell viability at lower doses tested was observed at both 24 and 48 hours of treatment. In order to verify if this increase could be due to a general metabolic activation or was the consequence of the induction of a proliferative activity, a BrdU-based proliferation assay was carried out on PHA stimulated hPBMCs treated as in the MTT assay. Results have shown a marked (about 30 %) increase of cell proliferation compared to controls at lower concentrations. In summary, these preliminary results show that NIAS produced during F48 and F56 manufacturing do not induce any cytotoxic effects in differentiated human lymphocytes while improving cell metabolism. However, tested NIAS may alter the normal control of proliferation in lymphocyte precursors that are physiologically present in lymphoid organs. This might suggest that a chronic exposure to these substances, alone or in combination with other environmental pollutants could interfere with normal activity of human lymphocytes potentially representing a human health risk.

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BROILER CHICKENS AND THEIR HEALTH STATUS IN FARM: WINE WASTE, AN ADDED VALUE ON FEEDING

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Various key economic sectors of the National and Sicilian economy, in addition to contributing to the economic growth also cause a considerable production of waste which today are a problem both for companies then for the environment. For this reason, there is considerable interest in this waste and in the possibility of their reuse to extract bioactive molecules with added value, all in accordance with the objectives of the Blue Economy. In this regard, it has already been demonstrated that from the production waste of various economic sectors it is possible to obtain bioactive molecules with anticancer, antimicrobial, cosmetic, antioxidant and nutraceutical properties (Mauro *et al*., 2022). If we focus on farm animals and the feed used, it is known that the latter can be a source of antioxidants which help the body to prevent the harmful effects of free radicals and metabolic products. In this context, polyphenolic compounds (*e.g.* flavonoids) showed important antioxidant activities *in vitro*, although it seems that these are poorly absorbed in the intestine and their tissue concentrations are too low to contribute to the antioxidant defense (Surai, 2013). In light of all this, within the SMILING Project a study concerned the possibility of reusing wine production waste (pomace and grape seeds, the cause of the production of considerable quantities of waste) rich in fatty acids, triglycerides and polyphenols (Di Stefano *et al*., 2021; 2022) in the feeding of broiler chickens by evaluating the possible effects of these diets on the animal's health status also in terms of antioxidant capacity. Three experimental sets were carried out and concerned the administration of three different types of diets: Grape marc (0%, 3% and 6%), Grape seeds (0%, 3% and 6%) and a mix of grape marc and grape seed (0 and 3%). At the end of the experimental times, biomarkers typical of the evaluation of the state of health of the organisms were used for the preliminary analyzes of the blood and meat samples. Significant effects were observed in all diets depending on the concentration administered. Probably the phenolic content of the flours used contributes to the improvement of the health of the farmed animals.

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ANTITUMORAL ACTIVITY OF *Asparagus officinalis* EXTRACTS AGAINST BREAST CANCER

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Breast cancer (BC) is the most frequent cancer among women and high rate of resistance and loss of response to treatment enhance the attention in novel drug research, mainly from natural origins. *Asparagus officinalis* (Asp) is reported to possess therapeutic proprieties, both from edible and inedible portions. We characterized the chemical content of new aqueous extracts

derived from the non-edible portion of the plant and we assayed the biocompatibility and bioactivity *in vitro* on normal fibroblasts and on cellular models of BC. Results showed no interference with fibroblast viability, while a significant reduction in the proliferation rate associated with significant G1/S cell cycle arrest and low levels of apoptosis, was specifically observed in breast cancer cells. Asp extracts were also shown to significantly inhibit cell migration after 24 hours treatment in BC cells, in a dose-dependent matter. Additional investigation showed that Asp extracts exert specific pro-oxidant activity against tumoral cells, and, notably, that their combination with menadione resulted in a significant increasing of oxidants production compared with menadione alone only in in breast cancer cells (but not in normal cells). Our results make the aqueous Asp extracts very attractive for further investigation in breast cancer research. The selectivity of action on tumoral cells and the easiness of their preparation make them good candidates to test role as co-adjuvant agents of clinical drug therapies.

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BIOFORTIFIED VEGETABLES AND METABOLYC SYNDROME: A POSSIBLE TOOLS TO PREVENT UNHEALTHY MICRONUTRIENTS REDUCTION IN LIFE

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Metabolic syndrome is defined as a pathological condition characterized by abdominal obesity, insulin resistance, hypertension and hyperlipidemia. This pathology has become the main danger to global health since can influence the develop of diseases as diabetes, cardiovascular, neurodegenerative disease and also cancer. Micronutrients as iodine and molybdenum are known to be key element in hepatic and lipid pathway and therefore playing a role in metabolic syndrome. The present study aims to evaluate, on heathy population, the effect of nutritional intervention using biofortified vegetables with Iodine and Molybdenum under the form of potassium iodate and sodium molybdate. A cohort of 69 volunteer subjects were recruited and divided into four experimental groups who ate fortified vegetables for 12 days: IODIUM GROUP (biofortified lettuce with iodine), MOLIBDENUM GROUP (biofortified lettuce with molybdenum), IODIUM+MOLYBDENUM GROUP (biofortified lettuce with iodine) and molybdenum), CONTROL GROUP (non-biofortified lettuce). We looked at blood and urine analysis at baseline (Time 0) and after 12 days (Time 1) of fortified vegetables administration. We analyzed, among others: aspartate aminotransferase (AST), alanine aminotransferase (ALT), triglycerides (TG), insulin (INS), LDL cholesterol, HDL cholesterol, LDL/HDL ratio, total cholesterol/LDL ratio; and the incretins GLP-1 (Glucagon-like peptide-1) and GIP (Gastric Inhibitory Peptide), GLP-2 (Glucagon-like peptide-2), PYY (Peptide Tyrosine Tyrosine). The results showed that after 12 days of biofortified vegetables the IODIUM+MOLYBDENUM GROUP showed a significative reduction of AST and ALT levels and increased HDL and PYY levels. This might shed light on a possible use of biofortified vegetables supplementation in order to prevent unhealthy micronutrients reduction occurring along different stage of life especially those related to metabolic syndrome.

EXPERIMENTAL ONCOLOGY

DIAGNOSTIC AND PREDICTIVE BIOMARKERS IN GLIOBLASTOMA

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Glioblastoma multiforme (GBM) is the most common tumor of the central nervous system and has a bad prognosis, with poor response to treatment and high frequency of relapse. Few GBM molecular biomarkers are available, and their usefulness is limited because of interpatient and intratumoral heterogeneities. This highlights the need to elucidate the mechanisms underlying tumor initiation-progression-relapse and, thus, identify biomarkers useful for diagnosis, prognostication, and patient monitoring[1]. We have assessed the molecular chaperones Hsp27, Hsp60, Hsp70, and Hsp90, and the vascular endothelial growth factor receptors VEGFR1, VEGFR2, and VEGFR3 by immunohistochemistry and immunofluorescence in GBM tumor tissue and derived primary and secondary cell lines. In addition, our research aims to identify biomarkers in liquid biopsies from GBM patients. Liquid biopsy is a minimally invasive diagnostic tool that can be used to monitor patients with GBM[2]. We characterized extracellular vesicles (EVs) isolated from plasma (liquid biopsy) of patients with GBM for morphology, size, concentration, and protein profile. We also assessed Hsp70 and Calcitonin (CT) receptor in EVs obtained from GBM patients before and after surgery. Hsp levels were higher in GBM compared with healthy controls. After surgery, a significant drop in plasma EVs concentration occurred, but Hsp70 and CT Receptor in EVs from patients were increased as compared to controls. These results point to biomarkers that should be measured in many patients to uncover molecular signatures that will allow the classification of patients into groups with distinctive prognostic outlook and therapeutic preferences.

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SPE AND HPLC-UV METHOD FOR THE QUANTIFICATION OF VENETOCLAX IN HUMAN PLASMA

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Venetoclax (ABT-199) is an orally bioavailable BH3 mimetic drugs; it is a specific inhibitor of B-cell leukemia/lymphoma-2 signalling within the cell and an inductor of the TP53-independent apoptotic pathway. Venetoclax has been introduced in treatment of chronic lymphocytic leukemia, indolent Non-Hodgkin lymphoma, acute myeloid leukaemia (AML) and multiple myeloma. Drug maximum plasma concentration is reached 5–8 hours and the elimination half-life ranges between 14 and 18 hours. Venetoclax is metabolised by the CYP3A pathway and through the hepatic-faecal system. Moreover, the drug is a P-glycoproteins (p-gp) substrate. A fixed dose is administered regardless of body surface area or weight when venetoclax is administered concurrently with moderate or CYP3A inhibitors, anticancer medications, and anticancer medications. At a 400-mg dose, venetoclax has a mean steady-state maximum plasma concentration (Cmax) of 2.1 1.1 g/mL, with significant inter-individual variability. It is advised that the dose of venetoclax be decreased to 100 mg when combined with potent CYP3A inhibitors such voriconazole. Therefore, therapeutic drug monitoring with personalized dose may improve in the safe and efficient optimization of clinical treatment. Our aim was to obtain a simple, fast and reliable method for the quantification of venetoclax for clinical routine use. We here describe the development and validation of an analytical method for drug determination in human plasma by high pressure liquid chromatography coupled with ultraviolet detection (HPLC-UV). Solid phase extraction (SPE) was performed whit Oasis MAX 30um 100mg, conditioned with 1ml of acetonitrile/water, 1/10. One ml of plasma was diluted with 2.0 ml of deionized water. Ten ul of the internal standard solution was added to the sample (1:10 of the stock solution in ACN + 0.1% v / v TFA). The pretreated sample was dispensed in the SPE column, washed with 500 ul of NH4OH 5% v / v and with 750 ul of acetonitrile/ water, 2/10 and then eluted with 250 ul of ACN 0.1% TFA. Eventually, 150 ul of the eluate was transferred into vials by adding 50 ul of diluent solution (acetonitrile / water, 60/40 + 0.1% TFA) and inject into HPLC. The calibration curve is performed on four points above zero: 0-1.0-2.5-5.0-10.0 ug/ml. The method was then applied on real samples from AML patients treated with venetoclax, assessing its eligibility for the routine use. Observed data suggest the usefulness of investigating intracellular and cerebrospinal fluid concentration of the drug. Moreover, further studies are necessary to test the correlation of venetoclax pharmacokinetics with treatment outcome and toxicity.

circSMARCA5 IS AN UPSTREAM REGULATOR OF THE EXPRESSION OF MIR-126-3P, MIR-515-5P AND THEIR mRNA TARGETS INSULIN LIKE GROWTH FACTOR BINDING PROTEIN 2 (*IGFBP2*) AND NRAS PRO-TO-ONCOGENE, GTPASE (*NRAS*) IN GLIOBLASTOMA

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The involvement of non-coding RNAs (ncRNAs) in Glioblastoma Multiforme (GBM) pathogenesis and progression has been ascertained, but their cross-talk within GBM cells remains elusive. We previously demonstrated the role of circSMARCA5 as a tumor suppressor (TS) in GBM. In this paper we explore the involvement of circSMARCA5 in the control of microRNA (miRNA) expression in GBM. By using TaqMan® Low Density Arrays, the expression of 748 miRNAs was assayed in U87MG overexpressing circSMARCA5. Differentially expressed (DE) miRNAs were validated through single TaqMan® assays in: (i) U87MG overexpressing circSMARCA5; (ii) 4 additional GBM cell lines (A172; CAS-1; SNB-19; U251MG); (iii) 38 GBM biopsies; and (iv) 20 biopsies of unaffected brain parenchyma (UC). Validated targets of DE miRNAs were selected from the databases TarBase, miRTarbase, and from the literature and their expression was inferred from the GBM TCGA dataset. Expression was assayed in U87MG overexpressing circSMARCA5, GBM cell lines and biopsies through real-time PCR. TS miRNAs 126-3p and 515-5p were upregulated following circSMARCA5 overexpression in U87MG and their expression was positively correlated with that of circSMARCA5 (*r*-values = 0.49 and 0.50, *p*-values = $9*10^{-5}$ and $7*10^{-5}$, respectively) in GBM biopsies. Among targets, *IGFBP2* (target of miR-126-3p) and *NRAS* (target of miR-515-5p) mRNAs were positively correlated (*r*-value = 0.46, *p*-value = 0.00027), while their expression was negatively correlated with that of circSMARCA5 (*r*-values = -0.58 and -0.30, *p*-values = 0 and 0.019, respectively), and miR-126-3p (*r*-value = -0.36, *p*-value = 0.0066) and miR-515-5p (*r*-value = -0.34, *p*-value = 0.010), respectively. Our data identified a new GBM subnetwork controlled by circSMARCA5, which regulates downstream miRNAs 126-3p and 515-5p and their mRNA targets *IGFBP2* and *NRAS*.

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MODULATION OF MCF7 BEHAVIOR BY REAC TECHNOLOGY: INVOLVEMENT OF CELLULAR ENDOGENOUS BIOELECTRICAL ACTIVITY

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Human breast adenocarcinoma is a form of cancer that tends to metastasize to other tissues, including bone, lung, brain, and liver [1]. Several chemotherapeutic drugs are used to treat breast cancer. Their combination is used to simultaneously target different mechanisms involved in cell replication [2]. The radio electric asymmetric conveyer (REAC) technology is a novel technology, used both *in vitro* and *in vivo* to induce cellular reprogramming and counteract senescence processes [3]. This technology exploits the ability to interact with and manipulate the endogenous cellular bioelectric activity (EBA). Within this context, we exposed MCF7 cells to the radioelectric fields emitted by REAC for a period between 3 and 10 days. We then maintained in culture for additional 3 to 10 days and analyzed cell viability by trypan blue and MTT assays and gene and protein expression by real time-qPCR and confocal microscopy, respectively. We also detected the levels of the main proteins involved in tumor progression, DKK1 and SFRP1, by ELISA and cell senescence by the b-galactosidase assay. Our results showed the ability of REAC to counteract the proliferation of MCF7, inducing senescence and probably autophagy through the upregulation of LC3-IIand Beclin1 and the modulation of DKK1 and SPFR1 levels. Also cell morphology appears to be influenced by the bioelectric fields emitted by REAC, as well as the expression of the main markers related to cell cycle progression. Our results seem promising for future application of REAC technology in *in vivo* experiments as a potential tool to aid the therapeutic strategies usually applied for the treatment of breast cancer.

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ANTIPROLIFERATIVE ACTIVITY TOWARD HUMAN MUCOEPIDERMOID CARCINOMA CELLS OF COPPER (II) COMPLEX OF 3-(2'-PYRIDYL)5-(PHENIL)-1,2,4-OXADIAZOLE

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The oxadiazoles are heterocyclic compounds applied in different scientific fields. The coordination with the transition metals has been shown to improve the intrinsic characteristics of these organic ligands. Based on these considerations, our research group has synthetized and characterized several oxadiazole compounds, which are efficient DNA ligands, with potential anticancer properties. Among them, the Copper(II) complex of 1,2,4-oxadiazole has the higher affinity with DNA than the corresponding isolated ligands (1). Therefore, we tested it on an *in vitro* model of a pulmonary mucoepidermoid carcinoma (NCI-H292 cells) and, to assess the optimal concentration of the compound, we performed MTT assay. We observed that the Copper oxadiazole compound reduces the cell viability in a dose and time dependent manner and induces cell apoptosis (2). We also evaluated the biological activity of the Copper oxadiazole compound assessing the expression levels of the 60 kDa Heat Shock Protein (Hsp60), a mitochondrial protein that, in cancer cells, have either pro- and anti-apoptotic effects, depending on it accumulates in the cytosol with, or without, mitochondrial release, respectively (3). In conclusion, we think this complex could be a good candidate not only in

conventional anticancer therapy but also in new applications, like the use of engineered extracellular vescicles as a vehicle to confer greater specificity to the complex, to reduce characteristic toxic effects of anticancer therapy.

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ROLE OF THE NOVEL NF-KB INHIBITOR SEMBL [(S)-β-SALICYLOY-LAMINO-α-EXO-METHYLENE-γ-BUTYROLACTONE] IN THE INHIBITION OF MIGRATION AND INVASIVENESS IN BREAST CANCER CELL LINES

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Breast cancer (BC) is a heterogeneous disease and represents the second leading cause of cancer-related mortality in women. One major challenge in the field of breast cancer is that of identifying and exploiting useful therapeutic targets for its most aggressive and problematic forms. NF-kB is a transcription factor that promotes the expression of many inflammatory cytokines, adhesion molecules, anti-apoptosis proteins. and metastasis-promoting proteins such as VEGFs and matrix metallo-proteinases (MMPs). However, its excessive activation often induces inflammation and contributes to cancer progression. There is growing evidence that aberrant activation of nuclear factor NF-kB signaling is a frequent feature of breast cancer and is associated with its epithelial-mesenchymal transition (EMT) and a high propensity for early metastasis (1), thus, NF-kB is considered to be an attractive target for drug discovery. An NF-kB inhibitor, DHMEQ (dehydroxymethylepoxyquinomicin), was previously designed and synthesized based on the structure of epoxyquinomycin C. DHMEQ covalently binds to a specific cysteine residue of Rel family proteins, inhibits the nuclear traslocation of NF-kB and has shown good antitumor effect in different *in vitro* and *in vivo* tumor models (2-6). However, since DHMEQ is relatively unstable in the body, a new, more stable analog has been designed and synthesized, the (S)-β-salicyloy-lamino-α-exo-methylene-γ-butyrolactone (SEMBL). This compound was found to be more potent as a cytotoxic drug, inhibited the constitutively activated NF-kB activity and reduced MMP-2-mediated metastatic process in a cellular model of ovarian carcinoma (7). We evaluated the effects of SEMBL in three breast cancer cell lines, MCF-7 and its multidrug-resistant (overexpressing ABCB1) and estrogen-independent variant MCF-7R and the triple-negative breast cancer (TNBC) cell line, MDA-MB 231 characterized by lack of estrogen (ER), progesterone (PR), and HER2 receptors. The effect of SEMBL on cell viability in the three breast cancer lines was evaluated by MTS assay at different concentrations. SEMBL induced cell growth reduction in an equivalent and concentration-dependent manner in the three cell lines. The inhibitory activity was found to be significantly stronger than that of DHMEQ. We therefore investigated the effect of SEMBL on cellular invasiveness. The results of the Matrigel Invasion assays showed that the invasive ability was significantly impaired by SEMBL in all cell lines. SEMBL reduced cell invasion at concentrations below the IC_{50}, showing higher activity than DHMEQ, used at 10-fold higher doses. Next, the scratch Wound Healing assay results revealed that SEMBL significantly suppressed also cell migration. The evaluation by Western Blot assays of the main proteins involved in the invasive capacity acquired by tumor cells, E-cadherin, Vimentin and MMP-2, confirms that SEMBL determines a strong and significant reduction of Vimentin and a simultaneous increase of E-cadherin in all three cell lines. SEMBL also resulted in significant inhibition of MMP-2 in MCF-7 and MCF-7R cells. Overall, these results deserve further study, but they demonstrated that SEMBL is able to counteract the invasion and migration of breast cancer cells of different histotypes, supporting the idea of its role in regulating tumor growth and metastasis. Therefore, SEMBL has the potential to be a candidate for a new anticancer agent.

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GLUCOSE DEPRIVATION IN A HEPATOCELLULAR CARCINOMA CELL MODEL ALTERS CYTOCHROME C OXIDASE SUBUNIT 4

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Accumulating evidence suggests that tumor cells exhibit a high degree of metabolic flexibility that allows them to adapt to changes in nutrient availability. Most cancer cells generate ATP through fermentation (the Warburg effect) in the presence or absence of O2. From this standpoint, fermentation can be considered a form of reversion to an ancestral unicellular-like phenotype. The aim of this study was to investigate the adaptive response of cancer cells to nutrient deprivation (low levels of glucose) after acute (3 h and 24 h) and chronic exposure (15 days) and to assess its impact on energy metabolism. In particular, we tested whether glucose restriction alters energy metabolism and the activity of the OXPHOS using two hepatocellular carcinoma cell lines (HepG2 and Huh7). To this end, the expression of the last enzyme in the mitochondrial electron transport chain, cytochrome c oxidase, COX subunits 1 and 4, was also tested. Cellular respiration was measured by oxygen consumption assays, while the expression of COX1 and two subunits of COX4 (COX4-1 and -2) was quantified by RT-PCR. We demonstrated that cellular respiration (basal, ATP-dependent, and respiratory reserve) increases compared to control cells after 24 h and chronic exposure to low glucose, whereas it did not occur after only 3 h of exposure to low glucose. Furthermore, we found that COX4-2 was only upregulated in Huh7 cells (3.7-fold change), but downregulated in HepG2 (0.04-fold change). Correspondingly, COX1 was slightly upregulated or unchanged in Huh7 and downregulated in HepG2 cells. Our findings show that the glucose restriction affects COX4-2 expression. It remains to be elucidated if it occurs through direct or indirect effects on certain transcription factors. Our evidence provides the basis for further understanding of how COX regulatory subunits are affected by an unfavorable cellular microenvironment, and further data could identify potential targets that could be exploited for therapeutic benefit.

DETECTION AND MAPPING OF THE CCT CHAPERONIN IN NERVOUS SYSTEM TUMOR CELLS

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Chaperonin-containing TCP-1 (CCT) is composed of nine subunits and assists the folding of essential proteins of normal (actin and tubulin), and tumor cells (oncoprotein cyclin E, Von Hippel-Lindau tumour suppressor protein, cyclin B, and p21ras (1)). Increased expression of CCT subunits occurs in advanced stages of breast, liver, gastrointestinal, and lung cancers; and acute myeloid leukemia; and correlates with invasiveness and poor patient survival (2). Glioblastoma multiforme (GBM) and neuroblastoma are the most common primary tumors in adults and children, respectively, with the former occurring in the brain and the latter in extra-brain locations. The CCT6A subunit is implicated with survival, increased migration and invasion of GBM cells and with epithelial-to-mesenchymal transition (3). Silencing of CCT2 in SK-N-AS and IMR-32 neuroblastoma cell lines correlates with decreased cell migration and is considered a useful marker for detecting neuroblastoma cells in blood (4). We determined the levels and the subcellular localization of CCT subunits in the LAN5 neuroblastoma cell line and in primary GBM cell lines. The CCT subunits occurred at different levels with CCT5 being higher in the LAN5 than in the GBM cells. We found CCT subunits in the cell cytosol and nucleus in GBM biopsies and LAN5 neuroblastoma cells. The data indicate that measuring CCT subunits in the tumor cells is a promising research avenue to elucidate the chaperone's role in pathogenicity and, thus, reveal candidates for biomarkers useful in diagnosis and patient management.

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ISOLATION AND CHARACTERIZATION OF PRIMARY CELL LINES FROM FRESH HUMAN GLIOMAS TISSUE FOR THE GENERATION OF 3D SPHEROIDS

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Gliomas are the most common primary malignant brain tumors in adults (1). Several experimental models, such as *in vitro* cultures and xenograft, have contributed to the current understanding of biological mechanisms underlying gliomas pathogenesis (2). Among them, 3D spheroids recapitulate the tumor mass spatial complexity and represent a useful system for phenotypic, molecular, and genetic analyses, as well as facilitate drug screening and biomarker identification (3). The aim of this study is to contribute to strengthening the knowledge of gliomas biology, generating 3D models of these tumors for use in the search for novel biomarkers and for elucidating molecular mechanisms of pathogenesis. To this end, gliomas cells were isolated and cultured from fresh tissue after surgery. Tumoral aggressiveness was estimated by determining cell duplication time, growth kinetics, and cell migration patterns. In addition, to demonstrate the establishment of primary glioma cell lines, immunomorphological analyses were performed. 3D spheroids were prepared by the hanging drop method, which consists of a cell suspension cultured as a "hanging drop" on the inverted lid of a culture dish, was used. The data thus far confirm that the spheroids reproduce the spatial arrangement of the tumor tissue and offer an experimental tool to investigate key issues pertaining to carcinogenic mechanisms, diagnosis, and prognostication, and to the development of novel treatments directed to well defined molecular targets.

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LONG NON-CODING RNA H19 ENHANCES THE PRO-APOPTOTIC ACTIVITY OF HISTONE DEACETYLASE INHIBITOR ITF2357 (GIVINOSTAT) IN COLORECTAL CANCER CELLS

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Histone deacetylases inhibitors (HDACi) are a new class of anticancer agents, targeting the aberrant activity of histone deacetylases (HDACs), which promotes cell proliferation, angiogenesis, and escape from apoptosis in cancer cells. Among HDACi, ITF2357 (Givinostat), is a hydroxamic acid-

based pan-HDAC inhibitor whose antitumor potential is under current investigation in clinical trials. Recent scientific papers suggest that ITF2357 induces apoptosis in leukemic, melanoma, and glioblastoma cells. Here, we investigated the potential use of ITF2357 for the treatment of colorectal cancer (CRC). Our data obtained in HCT-116 cell line showed that the drug is able to induce cell death in a dose-dependent manner. Transcriptional analyses were performed in order to identify the molecular pathways involved in ITF2357-induced cell death. Surprisingly, among the genes induced by the treatment, we recognized the long non-coding RNA H19 (lncH19) whose role as a non-coding oncogene has been largely documented. In fact, lncH19 overexpression is known to play a key role in the onset, progression, and chemotherapy resistance in CRC. In order to identify a possible correlation between lncH19 expression and ITF2357 activity, we stably silenced HCT-116 cells for lncH19. Interestingly, silencing of lncH19 decreased the pro-apoptotic action of the drug, supporting the hypothesis that lncH19 could be necessary for ITF2357 to promote cell death. This hypothesis was confirmed by western blot analyses demonstrating that ITF2357 treatment increased the levels of the apoptotic markers cleaved PARP, Caspase 3, and Caspase 9. The drug was not able to induce the same effects in H19-silenced cells. To our knowledge, these data indicate for the first time a synergistic relationship between lncH19 and HDACi. However further studies are needed to dissect the molecular mediators of this interaction. Finally, it is interesting to observe that 5-Fluoruracil (5-FU) resistant HCT-116 cells express high levels of lncH19 and respond well to ITF2357 treatment. Overall, the expression of the oncogenic lncH19 might represent a new putative marker for the screening patients to be subject to possible HDACi treatment.

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MICRO- AND NANOVESICLES IN BIOMEDICINE

LIQUID BIOPSY AS POSSIBLE DIAGNOSTIC TOOLS

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Liquid biopsies could be considered an excellent diagnostic tool, in different physiological or pathological conditions. The possibility of using liquid biopsies for non-invasive clinical purposes is quite an old idea: indeed many years ago it was already being used in the field of non-invasive prenatal tests (NIPT) for autosomal fetal aneuploidy evaluation. In 1997 Lo *et al.* had identified fetal DNA in maternal plasma and serum, showing that about 10–15% of cfDNA in maternal plasma is derived from the placenta, and biologic fluid represents an important and non-invasive technique to evaluate state diseases and possible therapies. Nowadays, several body fluids, such as blood, urine, saliva, sinovial fluid and other patient samples, could be used as liquid biopsy for clinical non-invasive evaluation. These fluids contain numerous and various biomarkers and could be used for the evaluation of pathological and non-pathological conditions. In this poster we will analyze the different types of liquid biopsy, their potential role in clinical diagnosis and the functional involvement of extracellular vesicles in these fluids as carriers.

DEVELOPMENT OF A METHOD OF ENGINEERING TUMOR-DERIVED EXTRACELLULAR VESICLES FOR DELIVERY OF CHEMOTHERAPEUTIC COMPOUNDS IN MALIGNANT PLEURAL MESOTHELIOMA (MPM)

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First-line treatments in the handling of malignant pleural mesothelioma (MPM) are not resolutive in most cases, driving the patient into an ineluctable relapse of the disease after a few years from the surgery and/or chemotherapy. Various tested solutions are trying to exploit the tumor microenvironment (such as the usage of anti-angiogenic factors) and/or specific surface markers of the neoplasm (such as PDGF receptor or Mesothelin). None of the strategies above seem to be effective enough to be used in the clinic. The MPM has biomolecular characteristics that offer few possible targets to be exploited for selective targeting. In this context is a realistic possibility of the utilization of a "Trojan horse" self-produced by the tumor itself as a paracrine communication mechanism. The use of appropriately engineered extracellular vesicles (EVs) has been investigated to exploit a consolidated paracrine communication network in most solid tumors to select delivery chemotherapy compounds directly to the target site. In this context, we thought we could investigate the suitability of EVs as possible therapeutic tools to be exploited for handling MPM. From this starting point, we assessed an extraction and engineering protocol of the MPM-derived EVs and deepened the possibility to exploit EVs as nanocarriers for selective delivery to the tumor site. In this preliminary study, we completed the isolation and characterization of tumor-derived vesicles. In addition, we completed the engineering of vesicles by comparing *in vitro* the efficiency of drug-loaded vesicles as a possible nanocarrier through MTT assays and immunofluorescence.

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DEVELOPING LIVER SPHEROIDS AS A MODEL TO INVESTIGATE THE ROLE OF COLORECTAL-CANCER DERIVED SMALL EXTRACELLULAR VESISCLES IN METASTATIC NICHE ESTABLISHMENT

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Colorectal cancer (CRC) is the third most diagnosed malignancy worldwide both in men and women. Despite being a disease with a long survival when prematurely detected, more than 50% of patients develop metastases, mainly in the liver, which represent one of the leading causes of CRC-related deaths. The establishment of metastases consists of many steps, including the formation of a metastatic niche (MN), a supportive and receptive microenvironment for metastatic cells colonization. Several data in literature have highlighted the crucial role played by small extracellular vesicles (SEVs) in initiating MN. Our previous study demonstrated that CRC-derived SEVs (CRC-SEVs) induce in healthy hepatocytes (Heps) the epithelial-mesenchymal transition, early event leading to liver fibrosis evolution. In the light of the great leverage that liver fibrosis has in determining liver MN establishment, our goal is to better investigate the pro-fibrotic effects of CRC-SEVs in Heps, for the purpose of outlining the events underlying MN establishment. Liver fibrosis is the result of an impaired interaction within cells, together with an excessive accumulation of extracellular matrix proteins. However, the traditional bidimensional model lacks both these crucial aspects, thus failing in properly describing the complexity of this process. In view of this, we developed a tridimensional (3D) model of human healthy hepatocytes spheroids (h-HeSphs), which were characterized by dimension and other physical parameters, to better investigate the effects that CRC-SEVs can elicit on Heps. SEVs isolated from CRC cell line SW480, were used to treat the h-HeSphs, obtained by seeding human healthy hepatocytes (THLE-2) in ultra-low

attachment 96 well plates. After evaluating the CRC-SEVs uptake by the h-HeSphs, we assessed the CRC-SEVs ability to alter the expression of hepatocytes' structural and functional markers, such as apolipoprotein E, albumin and cytokeratins 8/18. Moreover, by co-colturing h-HeSphs with SW620-GFP cells, a metastatic counterpart of SW480 cells expressing green fluorescent protein, we revealed that the treatment with the CRC-SEVs enhanced the invasiveness of SW620-GFP cells, suggesting that CRC-SEV-conditioned Heps can actively support colorectal cancer colonization of the liver. Taken together, these results, beyond showing the suitability of HeSPHs model to study the role of tumor-derived SEVs in modulating target cell behavior, revealed that CRC-SEVs can affect Heps properties inducing them to acquire an active role in shaping the liver metastatic niche.

UNRAVELING THE ROLE OF EXTRACELLULAR VESICLES IN THE CROSS-TALK BETWEEN ADIPOSE TISSUE AND OVARIAN CANCER

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Ovarian cancer (OC) is still the most lethal gynecologic tumor, due to the rapid and silent development of omental metastasis. Thus, a deeper understanding of the mechanisms regulating OC progression may have crucial impact on the outcomes of this deadly disease. There is consistent evidence of an association between obesity and increased OC aggressiveness. As omentum is rich in adipocytes, a key pro-tumor role for visceral adipose tissue has been postulated. In this regard, a cross-talk between OC and omental adipose cells has been demonstrated; however, the study of this dialog has been limited to metabolites and adipokines, although recent findings point to a key role of extracellular vesicles (EVs) in the control of tumor evolution. In the present study, we found that EVs derived from adipocytes could affect OC cell traits, inducing increased proliferation, migration and invasion. Furthermore, conditioning of OC cell lines with adipocyte-released EVs resulted in lower sensitivity to cisplatin, with reduced phosphatidylserine externalization and decreased caspase 3 and PARP cleavage. In particular, these alterations were paralleled by an Akt/SREBP-mediated increase in fatty acid synthesis and oxidation, as well as by an enrichment in ABCG2+ cancer stem cells. Of note, pretreatment of OC cells with amiloride and dynasore successfully counteracted the above EV-related effects, suggesting that both macropinocytosis and dynamin-dependent endocytosis are involved in the vesicular uptake. Collectively, these findings demonstrate that an EV-mediated crosstalk exists between adipocytes and OC, driving tumor aggressiveness. Further studies will be performed to identify the adipocyte EV molecular cargo responsible for the modulation of this dialog.

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THE ROLE OF EXTRACELLULAR VESICLES SECRETED BY COLON CANCER CELLS IN MEDIATING THE NUCLEAR TRANSLOCATION OF PD-L1 IN A MODEL OF HUMAN HEALTHY HEPATOCYTES: NEW INSIGHTS ON IMMUNE CHECKPOINT PATHWAYS

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Evasion of immune surveillance is a key process in tumour progression regulated by different molecular mechanisms. Among these, the dysregulation of immune checkpoints, such as CTLA-4 and PD-L1/PD-1 interaction, plays a fundamental role. It is well known that the ability of tumour cells to evade Immune response is due to the high expression of PD-L1 leading to T cell exhaustion and anergy. The use of PD-1/PD-L1-targeted inhibitors, as human monoclonal antibodies, have improved clinical outcomes in a broad range of malignancies, and for this reason the immunotherapy is considered a promising cancer treatment strategy. However, only a minority of patients show positive response to PD-1/PD-L1 blockade therapy. Interestingly, it has been reported that the presence of liver metastases is often associated with resistance to PD-1/PD-L1 inhibitors. To understand the mechanisms underlying this primary or acquired resistance is a significant challenge to improve the immunotherapy strategies. Recent evidence correlates the lack of efficacy of PD-1/PD-L1-targeted inhibitors to the alternative cellular localization of PD-L1. In fact, emerging data in the literature show that, in addition to being a cytoplasmic and surface protein, PD-L1 is also present in a nuclear form (nPD-L1), which can act as a crucial effector in transducing intrinsic signals responsible for tumor progression. This nuclear sequestration of PD-L1 can realistically represent a resistance mechanism, through which tumour cells become insensitive to target therapy specifically designed for the surface form of this protein. These data suggest that the molecular mechanisms underlying the immune checkpoints are not completely understood. A study previously performed by our research group has demonstrated that small extracellular vesicles derived from colorectal cancer (CRC-SEVs) significantly upregulate the expression of PD-L1 in M0 macrophages, mediating the inhibition of the local immune system. Data here reported for the first time demonstrate that CRC-SEVs can have a role in affecting the immunomodulatory properties of the liver pre-metastatic niche by inducing in hepatocytes (Heps) the increase of PD-L1 expression, promoting its nuclear translocation. Interestingly, according to data present in literature, this observation was associated with (i) the increase of GAS6 expression and TAM receptor-mediated release of TGF-β1, a master regulator of epithelial to mesenchymal transition (EMT) and fibrosis, and (ii) of PD-L2 and VISTA, two other immunoregulatory proteins which could have a role in regulating the liver-mediated immune tolerance during the metastatic process. Finally, we propose that the increase of the released GAS6 could have a paracrine effect on other liver cell components. Indeed, we found that the conditioned medium of CRC-SEV-treated Heps lead the macrophage M2 polarization, as demonstrated by the significant induction of the expression of IL-10, TNFα and TGF-β1. The obtained results highlighted that CRC-SEVs elicit in Heps alternative activities of PD-L1. These data could open new avenues to better understand the mechanisms of the immune checkpoint pathways, in order to develop new potential therapies for the early treatment of liver metastatic disease and improve the response rate to immunotherapies.

PLANT-DERIVED NANO VESICLES AS siRNA DELIVERY VEHICLES

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Nano Vesicles (NVs) are lipoproteic particles released by organisms belonging to different kingdoms and are considered mediators of cell-to-cell communication. NVs contain various biomolecules, such as proteins, nucleic acids, lipids, and metabolites. Recently, NVs have been purified from different plant species attracting the attention of the scientific community for the therapeutic opportunities they offer. Our research group has purified NV-like structures from different citrus fruits. NVs have demonstrated unique advantages in serving as nanocarriers for drug delivery, however, loading drugs into NVs is a challenging procedure. Currently, sonication, electroporation, transfection, incubation, saponin-assisted loading, and thermal shock have been applied to load various drugs into NVs. The electroporation approach is one of the most often utilized ways for loading siRNAs into NVs. In the present study, we have isolated and characterized NVs from tangerine juice (TNVs) and we have tested the possibility to use those vesicles as exogenous RNA delivery vehicles. TNVs have been isolated from tangerine juice by differential centrifugation, filtration steps, and ultracentrifugation. The vesicles were characterized at dimensional (Nanosight), morphological (TEM), and protein levels. Electroporation approaches have been tested for loading siRNAs in TNVs. Fluorescent quantification and confocal microscopy analysis have been carried out to evaluate the electroporation loading efficiency and cellular internalization. Preliminary results demonstrated that TNVs possessed a heterogeneous size distribution, with a mean of 254.7 nm±17.5 nm and a mode of 180.2 nm±14.0 nm and are positive for the Heat Shock Protein 70. Metabolomics analysis revealed the presence of several lipids and metabolites characteristic of the citrus fruit. Through electroporation protocol, we obtained an average oligonucleotide loading efficiency of 13%. TNVs loaded with the siRNA are internalized by colon cancer cells leading to the decrease of the specific target. In conclusion, the data obtained from these preliminary investigations confirm that it is possible to isolate NVs from tangerine juice and that they can be loaded with siRNA; future functional studies will allow us to define if they can serve as a drug delivery vehicle in colon cancer treatments.

EXTRACELLULAR VESICLE-DERIVED BIOMARKERS IN THYROID CANCER

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Thyroid cancer (TC) is a type of cancer that affects the thyroid gland. Women are four times more likely than men to be diagnosed with thyroid cancer (1). Routine diagnosis of thyroid nodules usually relies on a fine-needle aspirate biopsy, which is an invasive diagnostic method and often inaccurate, therefore repeated aspirations are needed due to plausible false-negative results. Thus, it is necessary to identify a novel non-invasive approach for the detection of biomarkers for early tumor diagnosis, prognosis, and disease monitoring. Extracellular vesicles (EVs) could be considered one kind of promising tool for liquid biopsy, they are nanosized bilayer particles that are secreted by all kinds of cells and that carry a specific cargo representative of the producing cell, including proteins, lipids, DNA, mRNAs and microRNAs (miRNAs) (2). EVs are secreted into all body fluids, such as blood, urine, saliva, and breast milk. Cancer-derived EVs play a crucial role in intercellular communication, promoting cell growth and survival, modulating the cancer microenvironment and increasing invasive and metastatic activity (3). Moreover, it has been demonstrated that heat shock proteins (Hsps), which are proteins involved in many physiological mechanisms in normal cells, are also expressed inside EVs secreted by cancer cells. Hsps released by cancer-derived EVs are involved in human cancer development and immune system stimulation (4), and their expression may be modulated by specific miRNAs (5). In this scenario, miRNAs in circulating EVs have been suggested to be the most potential candidates as useful biomarkers for TC diagnosis and prognosis, since they are a class of molecules dysregulated in a wide range of human cancers and because of their abundance and stability (6). Liquid biopsies were obtained from patients, initially diagnosed with thyroid disorders, before and after thyroidectomy. Samples were collected from each patient, and EVs were isolated and characterized. The aim of the current study is to provide different miRNAs expression levels between patients with thyroid cancer and a control group with benign goiter, with the final purpose of identifying specific biomarkers within EVs.

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CREATION AND VALIDATION OF A NEW ANTICACHEXIA DRUG BASED ON HSP60 AND NANOVESICLES (EXERCISE MIMICKING)

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Cachexia is a multifactorial inflammatory syndrome with a high prevalence in cancer patients. It is characterized by metabolic chaos culminating in a dramatic reduction in body weight, mainly due to skeletal muscle and fat depletion. The etiology of cachexia has not been fully elucidated, however it appears that chronic systemic inflammation is present in the vast majority of patients [1]. There is currently no standard intervention for cachexia, but it is believed that a proactive approach should be applied early in the disease course to maintain or slow the loss of physical function [2]. Increasing evidence demonstrates that exercise, used as a tool to counteract systemic inflammation, in combination with an adequate diet, may represent an alternative for the prevention/mitigation of tumor cachexia. Unlike resistance exercise, this can be performed without equipment, it is well tolerated by patients and an anti-inflammatory effect can be observed even at low intensity [3]. The present study aims to evaluate *in vivo* the effects of Physiactisome® [4], a mixture of extracellular vesicles released by murine myoblasts (C2C12 cells) containing the HSP60 protein, which *in vitro* seems to show the effects of exercise on cachexia. Sixty-four 10-weeks-old male mice (BALB/c AnNHsd), subcutaneous inoculated with a fresh fragment of C26 colon carcinoma were divided into eight different groups: sedentary-inoculated-placebo (SED/I/PLA), sedentary-inoculated-Physiactisome (SED/I/PHY), sedentary-placebo (SED/PLA), sedentary-Physiactisome (SED-PHY), training-inoculated-placebo (TR/I/PLA), training-inoculated-

Physiactisome (TR/I/PHY), training-placebo (TR/PLA) and training-Physiactisome (TR/PLA). The mice underwent 6-week endurance training with progressively increasing time and speed: 15 minutes and 3.2 m/min (week 1), 30 minutes and 3.2 m/min (week 2), 30 minutes and 4 m/min (week 3), 45 minutes and 4 m/min (week 4), 60 minutes and 4 m/min (week 5) and 60 minutes and 4.8 m/min (week 6). Through the administration of Physiactisome® to a limited group of animals, we will evaluate - in association with standard physical exercise protocols (endurance training) validated by the research group [5] - the ability of the drug to mimic (adjuvant) physical exercise, to date the only system to combat cachexia. The study will also verify whether the intramuscular administration of Physiactisome® increases muscle mass and is able to improve muscle functions, decrease inflammatory markers and increase oxidative and regenerative capacity. It will be possible to clarify some of the molecular mechanisms through which muscle strength, activity and mitochondrial biogenesis are regulated as well as the function of muscle progenitor cells.

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NEUROSCIENCE

THE EFFECT OF NON-INVASIVE BRAIN STIMULATION ON THE PROLIFERATION OF NEURONAL-LIKE CELLS *IN VITRO*

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Non-invasive brain stimulation (NIBS) is used for learning physiology and physiopathology of the central nervous system (CNS), for instance, the role of specific structures in brain function [1,2]. NIBS is also applied to treat several deficits that impair life quality in aging people, such as alterations in speaking, swallowing, movement, and cognition. One potentially advantageous characteristic of NIBS as a clinical tool is its protracted effect on the patient. Consequently, two NIBS forms, *i.e.*, transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS), are applied for managing certain neuropsychiatric diseases [3–7]. These two non-invasive procedures show online effects and offline after-effects that are related to long-term potentiation and long-term depression-like mechanisms [8]. Despite the abundance of clinical data, the neurobiological pathways underlying NIBS' efficacy are poorly understood. Current data suggest that not only changes in neural excitability, but also other phenomena participate in causing the long-term effects of NIBS [9]. In this regard, research on the NIBS effects on cellular properties promises to provide a solid scientific basis to the procedure and, thus, open new avenues for development and applications. What are the modifications of nervous tissue cells caused by NIBS? We are studying the effects of NIBS (TMS and tDCS) on the proliferation of nervous system cells *in vitro*, and its impact on the expression of selected genes involved in the production of neurotrophic factors, including components of the chaperone system.

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AGONIST OF GROWTH HORMONE-RELEASING HORMONE IMPROVES THE DISEASE FEATURES OF SPINAL MUSCULAR ATROPHY MICE

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Spinal Muscular Atrophy (SMA) is a neurodegenerative disease characterized by motor neuron (MN) loss due to the reduction of survival motor neuron (SMN) protein levels. As a result, it determines a progressive atrophy of skeletal muscles. Nowadays the available therapies show several limitations (difficult administration, side effects and high costs) and therefore the investigation of SMN-independent treatments is spreading ever more in order to bypass them. Here we investigated the efficacy of the growth hormone-releasing hormone (GHRH) agonist MR-409 that showed anti-apoptotic effects and reduced proteolytic events in a cellular model of muscle atrophy. Therefore, from postnatal day 2 (P2) to P12, we daily administered vehicle or MR-409 (1mg/Kg and 2mg/Kg) to SMNdelta7 mice, a severe model of SMA. From a phenotypical point of view, we observed a progressive gain of weight, more pronounced with the highest dose, as well as a significant improvement of motor performances in SMA mice. According to these positive outcomes, histological analysis revealed a dose-dependent increase in muscular fiber dimensions and neuromuscular junction maturation with an enhanced monoinnervation and a reduced denervation of the endplates, in both quadriceps and gastrocnemius. Moreover, at molecular level, we observed an increased expression of several myosin heavy chain isoforms (Myh1, Myh2, Myh7 and Myh8) and of markers of myogenesis and muscular damage repairing (Myog and Myod1), as well as a significant downregulation of apoptosis markers correlated with muscular atrophy (MuRF1 and Atrogin-1) in the same muscles. Finally, further analysis conducted on CNS revealed that the highest dose of MR-409 seemed to reduce MN loss in lumbar spinal cord and to decrease astrogliosis rate with a downregulation of proinflammatory cytokine (TNFα, IL-1b and IL-6) release in the same district. Thus, MR-409 could be considered a promising therapeutic approach for SMA treatment, maybe in combination with SMN-dependent therapies.

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PITUITARY ADENYLATE CYCLASE-ACTIVATING POLYPEPTIDE (PACAP) COUNTERACTS GLIOBLASTOMA MULTIFORME AGGRESSIVENESS

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Glioblastoma multiforme (GBM) represents the deadliest form among brain cancers. This tumor is characterized poor prognosis due to high rate of cells migration and invasion. The uncontrolled proliferation of cancer cells produces hypoxic niches in tumor mass. The hypoxic microenvironment triggers many signaling cascades downstream the activation of hypoxia inducible factors (HIFs), such as uncontrolled cell proliferation and vascular endothelial growth factor (VEGF) release. This growth factor is directly responsible to aberrant neovascularization characterizing GBM [1]. All these factors conduce to cancer development by promoting malignant progression and recurrence. Assumed the heterogeneity of tumoral mass, the actual therapeutic approach consists in a multimodal treatment comprising in surgery, radiation and chemotherapy with different molecules. It has previously demonstrated that pituitary adenylate cyclase-activating polypeptide (PACAP) is involved in GBM since it interferes with the hypoxic microenvironment through the modulation of HIFs via PI3K/AKT and MAPK/ERK pathways inhibition [2]. Considering that hypoxic tumor microenvironment is strictly linked to epithelial-mesenchymal transition (EMT), in the present study, we have investigated the PACAP ability to regulate this process by using GBM frozen sample and human U87MG glioblastoma cells exposed to hypoxic mimetic agent, DFX. The immunolocalization analysis, conducted by using confocal microscopy, have revealed that PACAP and its related receptor PAC1R are expressed in cells with different phenotypes in GBM tissue. The peptide treatment decreases the expression of mesenchymal markers such as vimentin, matrix metalloproteinase 2 (MMP-2) and matrix metalloproteinase 9 (MMP-9) as well as simultaneously increases ZO- 1 expression that represents a marker of epithelial cells. Moreover, PACAP exogenous administration significantly reduces the migratory ability of mesenchymal cells exposed to DFX-induced hypoxia, as demonstrated by the reduced expression of CD44 and vimentin. Although additional investigations are warranted to determine PACAP role in GBM malignancy, in this study we have point out new insight on modulatory action exerted by PACAP in GBM.

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DIET- AND PROBIOTIC-MEDIATED MODULATION OF THE MATERNAL GUT MICROBIOME ON EARLY-LIFE PROGRAMMING OF OFFSPRING NEURODEVELOPMENT AND BEHAVIOR

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Traditionally, genetic alterations were considered the sole driver of neurodevelopmental disorders, including autism spectrum disorders (ASD); however, consensus is growing behind a double-hit model in which environmental factors can significantly increase disease risk in genetically predisposed individuals. Among environmental exposures, those altering the maternal gut microbiome during pregnancy are emerging as key regulators of offspring neurodevelopment and behavioral outcomes. Specifically, dysbiosis of the maternal gut microbiome is associated with neurodevelopmental impairments[1]. In previous work, we showed that, in mice, maternal high-fat diet (MHFD) determines dysbiosis of the gut microbiome, social dysfunction, and associated synaptic plasticity deficits in male offspring (F_1)[2]. Here, we studied whether MHFD also induces dysbiosis in female offspring (F_1). We postulated that this would repropose the detrimental in utero environment endured by the F_1 males and the resulting social deficits in the F_2 generation, even in the absence of a direct exposure to MHFD. 16S rRNA gene sequencing data showed a considerable decrease in microbial richness in the gut microbiome of female F_1 MHFD offspring, with a specific reduction of short-chain fatty acid (SCFA)-producing taxa. While bacterial richness was recovered in the F_2 generation male and female mice, their social behavior was persistently impaired, suggesting a role for the F_1 female gut microbiome in offspring social dysfunction. Intriguingly, post-weaning treatment with probiotic *Limosilactobacillus (L.) reuteri* was sufficient to rescue social deficits in F_2 mice. Surprisingly, *L. reuteri* differentially impacted gut microbiome composition of control versus MHFD-descendant F_2 offspring, implying a certain instability of the microbiome in the MHFD lineage. This phenomenon was particular evident among females[3]; this previously unknown increased sensitivity of the female gut microbiome to probiotics suggested a new therapeutic approach: probiotic-mediated targeting of maternal gut microbiome during gestation to prevent offspring social deficits. Based on this, we designed a multi-strain probiotic mixture (patent pending) consisting of immunomodulatory taxa to be administered to regular and HFD-fed dams from conception to offspring weaning. Ameliorating the maternal gut microbiome was sufficient to restore normal social behavior in the F_1 MHFD male and female offspring. Our data show a robust link between maternal lineage HFD, gut microbiome alterations and impaired social behavior across multiple generations. Importantly, they emphasize the potential for therapeutic targeting of the maternal gut microbiome to ensure normal neurodevelopment and prevent behavioral alterations in descendants.

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REDUCING VISUOSPATIAL PSEUDONEGLECT IN HEALTHY SUBJECTS BY ACTIVE VIDEOGAMING

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Pseudoneglect phenomenon refers to a condition in which healthy subjects tend to perceive the left side of exactly bisected lines as being slightly longer than the right one. However, behavioral data showed that athletes practicing open-skill sport display less pseudoneglect phenomenon than the general population. Given the fact that the so-called exergames are platforms designed to fully mimic sport activity, here we investigate whether and how a one-week training period of virtual open skill sport can determine a similar decrease in pseudoneglect. Fifteen sedentary participants responded to a visuo-spatial attention task to evaluate pseudoneglect error and a control memory task in basal conditions (t0: Pre-game) and after a short period (one week, one hour/day) of virtual tennis exergaming (t1: Post-game). In the Post-game condition, subjects made significantly fewer leftward errors compared to the Pre-game condition. Additionally, a control group was also evaluated within the same conditions but using a virtual non-exergame. Our findings suggest that a daily training of virtual tennis exergame can improve visuo-spatial attention isotropy reducing leftward errors, whereas non-exergame does not modify performance.

NEUROPROTECTIVE EFFECTS OF VITAMIN C VIA THE MAPK-INDUCED GSK3B INACTIVATION

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Neuroinflammation is a defense mechanism finalized to the preservation of the brain in response to inflammatory stimuli. By contrast, a long-lasting inflammatory status, as well as oxidative stress, creates a conducive environment for the onset and progression of neurodegenerative disorders. Vitamin C (Vit C) has both anti-inflammatory and antioxidant properties and, although its neuroprotective effects are clear, the underlying molecular mechanisms remain to be clarified. Glycogen synthase kinase 3β (GSK3β) is a serine/threonine kinase having a role in regulating the inflammatory response, suggesting it may be a potential target for the treatment of neurodegenerative diseases. In this study, we investigated the effects of Vit C on GSK3β by using a well-known 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced animal model of Parkinson's disease (PD) and LPS-treated BV2 cells as cellular model for neuroinflammation. We found that Vit C alleviates the inflammatory responses and improves the antioxidant responses both *in vitro* and *in vivo* by promoting the pp38 MAPK-induced inactivation of GSK3β. In addition, the anti-inflammatory M2 phenotype appeared to be dominant on the M1 pro-inflammatory phenotype and the microglial reactivity reduced in response to Vit C.

OXYGEN-OZONE THERAPY AND COGNITIVE FRAILTY: A NON-PHARMACOLOGICAL APPROACH TO POTENTIALLY RESOLVE IMMUNE AND INFLAMMATORY DYSFUNCTIONS

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As the world's population ages, Cognitive Frailty (CF) is becoming one of the most serious health problems and elucidating its biological mechanisms along with prevention and treatments becomes increasingly important also considering the associated health costs. We thus performed a clinical randomized trial where CF subjects received a non-pharmacological therapy based on the regenerative properties of ozone (O_3) known to act on immune/inflammation processes, strongly altered in CF. A cohort of 75 patients was stratified in non-, mildly- or severely frail rate and treated with placebo, oxygen (O_2) or O_2-O_3. The serum levels of 27 peculiar pro- and anti-inflammatory cytokines and chemokine cell signalling molecules were measured by using the Bio-Plex Pro Human Cytokine 27-plex immunoassay. The student's t-test and analysis of variance (ANOVA) followed by Tukey's post hoc test were used for comparison of means between the groups. Preliminary analyses evidenced the implication, at different levels, of some molecules in relation to the frailty rate. Noteworthy, we observed modulations of immune (i.e interleukin, IL-9) and inflammation (i.e IL-1β) biomarkers at baseline and after treatment. Correlations between clinical CF profiles and peripheral levels of the considered biomarkers are ongoing in order to predict the response to O_2-O_3 therapy. Although preliminary, these results confirm that the immune-inflammation systems are involved in the aetiopathogenetic mechanisms of CF, and that the related molecules could be potential therapeutic targets/biomarkers for the O_2-O_3 therapy. These data will further permit to validate a potential new non-pharmacological treatment approach for this condition.

IMPACT OF TRPV1 CHANNELS AND NITRIC OXIDE-MEDIATED SIGNALLING IN CANNABINOID MODULATION OF HIPPOCAMPAL EXCITABILITY

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Transient receptor potential vanilloid type 1 (TRPV1) are ionic channels modulated by cannabinoids that are involved in the transduction of a plethora of stimuli and trigger fundamental downstream pathways in the pre- and post-synaptic sites. Importantly, TRPV1 possess a nitric oxide (NO)-sensitive gate modulating its activation and NO is considered a mediator for several cannabinoid receptor type 1(CB1) effects. Nonetheless, the linkage between these modulatory systems remains elusive Exploring hippocampal transmission is aimed at individuating putative targets for CB-mediated neuromodulation in terms of potential repercussions on synaptic efficiency and excitability-related phenomena. Electrophysiological patch-clamp recordings on CA1 pyramidal neurons unveiled that TRPV1 and NO are involved in the synaptic modulation of excitatory transmission exerted by cannabinoids. Indeed, the manipulation of TRPV1 and the inhibition of neuronal NO synthase (nNOS) impair the release of glutamate (GLU) and subsequently GLU post-synaptic cascades, but also these systems influence CB1-mediated modulation of GLU transmis-

sion. Our data could implicate a putative attenuation of hippocampal tendency to abnormal discharge and influence homeostatic phenomena. In this light, it was outlined that the inhibition of NO production in different models of temporal lobe epilepsy (TLE) reinforces the antiepileptic effects of CB1 on behavioural symptoms and electrophysiological correlates, highlighting TRPV1 role in this mechanism. Pharmacological manipulation of nitrergic pathway in an *in vivo* rat model showed that the blockade or the promotion of the NO production modified oppositely the influence of TRPV1 activation on cannabinoid antiepileptic activity. The overview of these research findings encourages speculations on an unbalance of CB1/TRPV1 and nitrergic systems that could be associated with bioelectrical alterations, influencing homeostatic regulation of synaptic processes and epileptic conditions.

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LYSOSOMAL-DRIVEN REDUCTION OF α-SYNUCLEIN AGGREGATES IN NEURON-LIKE MODEL OF PARKINSON'S DISEASE

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Presence of α-synuclein insoluble aggregates is a typical hallmark of different neurodegenerative disorders characterised by the presence of Lewy bodies, particularly Parkinson's disease (PD). Clearing such aggregates, or preventing their formation, is a crucial test for any therapy aiming to reverse, or stop, the progression of PD. Boosting lysosomal activity is a promising route to clear synuclein aggregates since, physiologically, lysosomes are the cellular organelle responsible of clearing proteins and peptides. Lysosomal biogenesis is finely regulated by transcription factor EB (TFEB), which is normally detected in the cell cytoplasm in its inactive form. Upon signal transduction TFEB is translocated into the nuclei, where it activates a series of pathways responsible for lysosomal biogenesis. A well know mechanism for TFEB translocation into the nuclei is PIKfyve inhibition, which prevents mTORC1-dependant phosphorylation of TFEB. To test whether PIKfyve inhibition results in a lysosomal-driven reduction of α-synuclein aggregates, we exploited a mutant neuroblastoma cell line overexpressing an aggregation prone form of synuclein and treated it with PIKfyve inhibitor YM201636. Our findings reveal a significant reduction ($P<0.001$) of α-synuclein aggregates as early as 24h post-treatment compared to the untreated group. Following this result, we investigated whether the reduction would still be present in a neuron-like model, we used BDNF and retinoic acid to differentiate the 3K-synuclein expressing cells and treated them with YM201636. PIKfyve inhibition proved to be able to significantly ($P<0.01$) drop α-synuclein aggregates in differentiated cells as well. Finally, to investigate whether YM201636 effects were lysosomal driven, we have co-treated cells with bafilomycin to disrupt proper lysosomal function. Impairment of lysosomes prevented the YM201636 reduction of α -synuclein in both model tested. These results suggest that PIKfyve inhibition could be exploited as target to reduce toxic α-synuclein aggregates.

MELATONIN EFFECTS ON NEURAL DIFFERENTIATION OF HUMAN ADIPOSE-DERIVED MESENCHYMAL STEM CELLS

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Adipose Derived mesenchymal Stem Cells (ASCs) are multipotent stem cells, being able to differentiate not only into cells of the mesodermal lineage, but also into elements of endodermal and ectodermal origin, such as neurons and glial cells. The easy procedures for their harvesting, isolation and expansion have prompted a plethora of investigations in the field of cell-based therapeutical strategies for the treatment of numerous human pathologies, including degenerative diseases, for which pharmacological approaches still provide poor results. The aim of this work was to test ASC neural differentiation by using glial conditioned media and melatonin. ASCs were obtained from the stromal vascular fraction in the lipoaspirate from several healthy donors. After their expansion in basal growth medium, ASCs underwent neural differentiation procedures. As previously reported, conditioned media obtained from glial Olfactory Ensheathing Cells (OEC-CM) and from Schwann Cells (SC-CM) were used. In some samples, 1 μM of melatonin was added. After 1 and 7 days of culture, samples were examined by immunocytochemistry and western blot analysis, to evaluate neural marker expression in the different conditions. The results confirmed that a successful neural differentiation was observed by OEC-CM and SC-CM treatment, whereas addition of melatonin alone did not induce appreciable changes. Instead, when melatonin was combined with conditioned media, ASC neural differentiation was enhanced, especially after 7 days of treatment, as demonstrated by a further improved neural marker expression. In conclusion, data obtained confirm that melatonin neurogenic differentiation ability can be usefully applied to obtain neural-like differentiated ASCs for a potential alternative therapeutic tool.

BRAIN IRON DEPOSITION DURING AGING AND NEURODEGENERATIVE DISEASES

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Iron accumulation occurs during aging with a preferential distribution in specific brain areas: the cerebral cortex and the hippocampus, which are more vulnerable to age-dependent neurodegeneration. However, apart from its deposition, the comprehension of iron regulation in the Central Nervous System (CNS) remains scarce. We investigated iron metabolism in the brain of C57BL/6 mice during physiological aging in order to answer two unsolved questions: what is the mechanism regulating iron accumulation during brain aging? Which role do astrocytes and neu-

rons play in the maintenance of brain iron homeostasis? In our study we confirmed that during brain aging the permeability of the blood–brain barrier (BBB) is altered and this could favour iron overload that we found as selectively localized in the cerebral cortex and hippocampus. Iron overload is accompanied by inflammation and oxidative stress, demonstrated by increased inflammatory genes' expression and reactive astrocytes and microglia. Interestingly, we found that Hepcidin (Hepc), a peptide produced mainly by hepatocytes and acting as regulator of iron amount and availability, is active in the cerebral cortex and hippocampus. In details, we found that Hepc expression increases in the aged brain and it binds the iron exporter Ferroportin1 (Fpn1), causing its degradation and, as a consequence, iron retention within neurons. Indeed, we found by immunocytochemistry that the expression of Fpn1 in the brain is cell-specific and that neurons and astrocytes respond differently to iron excess: Fpn1 colocalizes mainly with astrocytes, while the iron storage protein ferritin light chain (FtL) accumulates in cortical and hippocampal neurons. We demonstrated for the first time in the brain that iron accumulation within neurons is due to the activation of "ferritinophagy", an evolutionarily conserved degradation pathway mediated by NCOA4. Altogether, our data highlight new key regulators of brain iron homeostasis that we could target in order to prevent oxidative damage, stress, and neurodegeneration. This study adds insights about the mechanism regulating brain iron metabolism in response to iron overload, a typical pathological feature not only of aging but also of several neurodegenerative disorders, including Alzheimer's disease.

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HIIT TRAINING IMPROVES FUNCTIONAL MOVEMENT TESTS WITH A POSITIVE EFFECT ON THE BONE METABOLISM IN MULTIPLE SCLEROSIS PATIENTS: A PILOT STUDY

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Multiple sclerosis (MS), is a neurodegenerative disease, chronic in nature, affecting the central nervous system and resulting in large and varied symptomatology[1]. Subjects with this disease have impaired motor function and associated altered bone metabolism, with vitamin D deficiency and consequently risk of osteoporosis[2]. Physical activity could play a key role in improving these aspects. However, to date, it is unclear whether physical activity is as effective in these patients and what exercise would be best suited to reduce the risk of osteoporosis, improve motor function, and contribute to the reduction of chronic fatigue characteristic of the disease[3]. The purpose of our study is to understand whether high-intensity interval training can be effective in improving motor performance and bone metabolism. A first group of 10 subjects diagnosed with MS (EDSS 1.17±0.56) was recruited for our pilot study. At the beginning of the study (T0) blood sample was collected for each subject in order to analyze bone markers and tests of strength, balance, reaction time, and mobility were administered. In addition, we performed a postural evaluation to investigate anteroposterior and later-lateral oscillations and body weight distribution at the feet. After the initial assessments, the subjects began the 6-week of HIIT (HR between 60% and 80% of HRmax) three times a week, each session lasting about 30 minutes. at the end of the training period (T1), subjects performed all assessments again. Our results showed a better performance after the training period in the lower limb strength tests: 5 times sit to stand (s) (T0:12.54±; T1:10.89±; 0.03) and Wall squat test (s) (T0: 27.19±0.9; T1: 41.68±1.02; p:0.00); in the lower limb reaction time tests: foot-reaction time test dominant limb (s) (T0: 0.52±0.01; T1:48.2±0.02 p:0.05) foot-reaction time test non-dominant limb (s) (T0:0.55±0.04; T1: 0.48±0.02; p:0.03); in the mobility test cervical range of motion with an improvement in the total cervical inclination (°) (T0:81.93±5.7; T1:99.66±6.6; p:0.01) and in the total cervical rotation (°) (T0:127.96±8.9; T1:162.53±5.2;p:0.00) regarding the balance tests an improvement in performance was found only in the time up and go test (s) (T0:7.66±0.4; T1: 6.34±0.4; $p < .001$). There were no significant changes in strength and reaction times tests for the upper limbs. As regards bone metabolism, the results show an increase of the bone formation marker Osteocalcin (µg/L) (T0:23.11±8.24; T1: 25.4±8.04; p:0.025) and the Vitamin D (µg/L), started below normality values (<30 micrg/L) before the training period, increased after the six weeks (T0:20.44±6.9; T1:26.44±2.7; p:0.07). However, this difference does not appear to be statistically significant. No statistically significant differences were found between T0 and T1 for the markers CTX ($p>0.05$) and PTH ($p>0.05$) In conclusion, this training had positive effects on lower limb function by increasing strength, reaction time, and gait stability. In addition, HIIT has an effect on bone metabolism as evidenced by increased markers of bone formation. However further studies with different training times and number of subjects are needed.

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POSSIBLE CORRELATION BETWEEN STRESSORS AND AMYOTROPHIC LATERAL SCLEROSIS ONSET AND PROGRESSION: *IN VITRO* PRELIMINARY RESULTS

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Nowadays, our body is constantly subjected to many stressors, due to physical, social and environmental events. These conditions can trigger several cellular alterations that also characterize the neurodegenerative diseases, such as Alzheimer disease, Parkinson disease and Amyotrophic Lateral Sclerosis (ALS). More in detail, ALS is a motor neuron (MN) disease determining the progressive degeneration of both upper and lower motor neurons, characterized by weakness, muscle atrophy and premature death. The ALS physio-pathological mechanisms include cytotoxicity, oxidative stress and neuroinflammation, which are cellular processes also activated by stressor exposure. The purpose of the study is clarifying the contribution of

different stressors on ALS onset and progression, since many mechanisms are still unclear. In order to simplify the first analyses, an *in vitro* experimental model of stress on ALS has been set-up using NSC-34 cells expressing hSOD1 gene under the control of a doxycycline-inducible promoter. On the grounds of cell viability results, cells have been differentiated in MN-like cells with 20μM of retinoic acid (RA) for 4 days. Concerning the expression of hSOD1, 5μg/ml of doxycycline for 24h have been added into culture medium and the protein expression was observed by Western Blot, in all the experimental conditions. Then, to mimic a stress condition, cells underwent oxygen and glucose deprivation: CoCl2 was used as hypoxic agent and its toxicity was measured by MTT assay. Different concentrations of CoCl2 were evaluated in both high and low glucose medium, suggesting 100μM CoCl2 in low glucose as optimal stress condition. Cell damage was then studied by evaluating mitochondrial activity (using MitoTracker Red/MitoTraker Green) and UCP4 protein expression. Finally, the gene expression analysis has been performed in order to study several genes related to stress and ALS, using pre-designed plates by real-time PCR and preliminary results seems to suggest a possible cell damage under stress conditions. Additional experiments are needed to confirm and extend these results, however we have set-up the conditions for the next analyses, to evaluate genetic/epigenetic mutations and cellular/molecular alterations, and to clarify *in vivo* the stressor impact on the onset and progression of ALS.

MONITORING BENEFITS OF 6 WEEKS OF A HIGH-INTENSITY INTERVAL TRAINING PROTOCOL THROUGH POLAR UNITE SYSTEM IN MS PATIENTS: A PILOT STUDY

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For many years, patients with multiple sclerosis (MS) have been advised to avoid exercise due to the risk of increased neurological damage [1]. Over the years, the literature has evaluated how physical activity can instead improve physical condition. Is already known that high-intensity interval training (HIIT) could have potentially greater effects on fitness and cognition than moderate continuous exercise [2]. HIIT decreases many MS symptoms and can positively counteract the disease progression even if is performed in a short time compare with continuous training. Patients with MS may have a dysfunction of cardiovascular regulation together with respiratory involvement, which can impair aerobic capacity and thus lead to high fatigue. While aerobic physical activity is known to be beneficial in people with MS, more research is needed to explore the effectiveness of HIIT in people with progressive MS and those with higher levels of disability. This is the start point of our study focused to investigate the effects of a 6-week HIIT workout, three times a week, 30' each session the improvement of cardiovascular function and physical performance and decrease average heart rate. Five subjects with MS (age 39.8±6.3) were enrolled in the study. We analyzed and recorded the training sessions with Polar Vantage V2 Unite, a smartwatch with integrated GPS that allow to evaluation of the differences between the cardiovascular parameters. The results showed a statistically significant decrease, after 6 weeks, of the minimum peak heart rate ($p = 0.043$), which leads to an improvement in diastolic function. Considering that in the literature, subjects with MS often show diastolic dysfunction due to drug intake such as Novantrone ® (Meda Pharma) [3]. Statistically significant increases were also found in calories consumed between the first and last training session ($p=0.038$) suggesting that patients were able to achieve higher work intensity during the training session: higher exercise intensity leads to higher calories expenditure. We can therefore infer that the HIIT protocol, in MS patients, undergoes an improvement in physical performance and training performance. Lack of physical activity tends to increase muscle weakness and fatigue, as well as perceived fatigue during daily activities and training. Since several studies have already shown that physical training can benefit people with MS, exercise capacity, health-related quality of life, and reduced fatigue, we suggest a HIIT protocol to positively affect cardiovascular functionality, improving diastolic function and decreasing the perception of fatigue.

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FROM TYPE 2 DIABETES TO DEMENTIA: STUDY OF THE CELLULAR AND MOLECULAR MECHANISMS INVOLVED, FROM THE PERIPHERY TO THE CNS

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Dementia is the progressive degeneration of the brain, rendering patients no longer able to utilize the simplest of cognitive functions. Dementia incidence exponentially increases with age, doubling approximately every 5 years. Alzheimer's disease (AD) accounts for 60%-70% of all cases of dementia, becoming a global public health priority. The AD International World Alzheimer Report 2015 estimated about 47 million people, becoming a global public health priority. The 2017 Lancet Commission on Dementia Prevention, Intervention and Care found that approximately 35% of dementia is attributable to potentially modifiable lifestyle risk factors. Among them, diet and nutrition may offer potential for nonpharmacological prevention in AD as demonstrated by epidemiological findings showing the relationship between adherence to specific dietary patterns and association with a lower risk of AD. Furthermore, there is an accumulating body of evidences suggesting that chronic diabetes may accelerate and promote onset of neurodegeneration leading to dementia with a mechanistic link between the two. Nevertheless, little is known about the cellular mechanism linking 2TM diabetes and dementia. Recently, evidences suggest that hyperglycemia and insulin resistance are key contributors to neurodegeneration, disrupting the functional activity of many proteins and protein complexes within the neuronal environ-

ment. AD has been described as "type III DM" due the discovery that insulin resistance is closely related to AD-induced neurodegeneration in the brain. This discovery has prompted the start of an increasing amount of research regarding insulin signaling within the CNS. In the CNS, insulin plays crucial regulatory roles, while chronic hyperglycemia leads to formation and accumulation of advanced glycation end products (AGEs). AGEs are the major contributor to insulin resistance in diabetic cells, due to their regulatory role on sirtuin expression. The relationship between peripheral glycaemia and cognition is the goal of this study. Herein, we investigated the intersection of type 2 diabetes, different diets, peripheral glycaemia and glycation pathway, on recognition memory, cerebellum skills and locomotor performances, through a longitudinal study in a mouse model. After diabetes chemically induction, animals followed three different diets: a low glycemic diet, the animal house diet (standard diet), and a high glycemic index diet. We performed a battery of behavioral tests to assess recognition memory, in both the knowledge and the remember component, and cerebellum skills, monitoring the development of frailty, through a frailty index, as previously published (Roda *et al.* 2021; Roda *et al.* 2022). In parallel, we monitored glycaemia, methylglyoxal and glycated albumin level in plasma. Preliminary data suggest a direct link between peripheral metabolic condition and the central nervous system, in both hippocampus and cerebellar component. In near future, we will assess the role of sirtuin and other key molecules in the link among diabetes, diet, recognition memory, cerebellar functions.

ALTERATION OF PROJECTION NEURONS IN A MURINE MODEL OF SPINAL MUSCULAR ATROPHY SUGGESTS A REMODELLING IN CORTICAL CYTOARCHITECTURE DUE TO SMN LACK

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Spinal Muscular Atrophy (SMA) is a severe neurodegenerative disease of the early childhood, caused by the mutation/deletion of the survival motor neuron (SMN1) gene. The lack of functional SMN protein induces the selective degeneration of spinal motor neurons (MNs), resulting in progressive skeletal muscle denervation and atrophy. However, little is known about the effects of SMN deficiency in the brain and only recent studies in both animal models and human patients highlighted, interestingly, that also the brain is affected by SMN loss. For instance, functional alterations in cerebral neural networks have been reported in SMA models, and imaging studies on patients suggested the occurrence of a reorganization of cortical grey matter, due to the progressive neurodegeneration in the spinal cord. Thus, we focused on the sensorimotor cortex of the severe SMA model SMAΔ7 mice, to investigate the effect of SMN deficiency on the cortical cytoarchitecture. We analysed early (postnatal day 5) and late (P11) symptomatic animals, comparing SMA mice with their wild-type (WT) littermates. We investigated projection neuron distribution by immunofluorescence analysis, using different markers to identify specific neuronal subtypes. We confirmed a lower cell density in layer V of SMA cortex. Indeed, looking at different projection neuron subtypes, we found that both corticospinal (Ctip2-positive) and callosal (Satb2-positive) neurons are reduced at P11 in SMA cortex by about 40%, suggesting that SMN reduction could affect the upper MNs as well. These neuronal cell types in SMA also show alteration in some morphological traits, as observed by morphological analysis using retrograde tracers. Overall, we found a remodelling in cortical cytoarchitecture in SMA which could contribute to the etiopathology of the disease. Knowing the involvement of cerebral cortex in SMA will contribute to unravelling the dynamics of progressive degeneration occurring in the disease and will be also useful in designing new comprehensive treatments strategies, for better clinical outcomes.

NEURO-ACTIVE EFFECTS OF "GOLDEN" TOMATO CONSUMPTION IN A HIGH-FAT DIET-FED RAT MODEL OF METABOLIC SYNDROME

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"Golden" tomato (GT) represents an intriguing source of neuroactive phytonutrients, obtained by fruits harvested at an incomplete ripening stage with respect to red tomato at full maturation. Previous evidence unveiled the potential of GT consumption in oxidant-driven, metabolic dysfunction. The goal of this research is to explore the influence of GT on behavioural and cognitive impairment induced by Metabolic Syndrome (MetS) in male Wistar rats. To do so, animals were fed with High-Fat Diet (HFD) for 8 weeks to induce the typical dysmetabolism of MetS, as evidenced by evaluation of biometric and metabolic parameters such as body weight gain, glucose and lipid intolerance. Afterwards, GT was orally administered to rats for one month (HFD-GT group), at a daily dosage of 200 mg/kg body weight by two control groups, one fed with HFD (HFD group) and the other with Normal Pelletized Diet (NPD group), were administered with vehicle. At the end of the GT treatment, all groups underwent specific tests to: i) assess their behavioural reactivity, locomotor activity and exploratory behaviour by an Open Field Test (OFT); ii) evaluate their eating behaviour and stress response by means of a Novelty-Suppressed Feeding Test (NSFT) and iii) declarative memory system by an Object Recognition Test (ORT). The behavioural assessment highlighted that HFD induced a profound impairment in cognitive function that was ameliorated by GT consumption. The behavioural reactivity was indeed improved in HFD-GT groups as emerged by OFT. Besides, our data outlined that rats consuming GT for one month have a lower hesitation to consume highly palatable food in a novel environment in the NSFT, considered a measure of both anxiety and anhedonia. Furthermore, GT consumption managed to rescue the learning and memory impairment determined by HFD in the ORT, recovering to basal recognition index of NPD group. Lastly, the neuroprotective effects exerted by GT in MetS rats were correlated with neurotrophic factors and relative signalling investigated in several brain areas. In conclusion, this study provides evidence of the importance of food supplementation with GT in the prevention and management of cognitive alterations associated with MetS.

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AUTOANTIBODIES AGAINST NEURONAL PEPTIDES IN THE SERUM OF POST-COVID-19 AND VACCINATED SUBJECTS: MAY MOLECULAR MIMICRY BE THE CULPRIT OF NEUROVEGETATIVE SIGNS AND SYMPTOMS?

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Many individuals who suffered from severe forms of COVID-19 experienced a series of neurovegetative signs and symptoms after their recovery (e. g. postural orthostatic tachycardia syndrome, POTS), suggesting a possible imbalance of the sympathetic-parasympathetic activity of the autonomic nervous system, which may last for a long time (weeks/months) after respiratory symptoms stop [1]. It was well established that autonomic disorders such as POTS are associated with autoantibodies, for example to α-/β-adrenoceptors and muscarinic receptors. Therefore, an underlying autoimmune component could explain the post-COVID-19-associated autonomic dysfunction (dysautonomia) [1]. Our bioinformatic and alignment analysis revealed that 3781 human proteins, including 22 proteins expressed in vagal nuclei and ganglia, share peptides of at least six amino acids (≥ 6 mer) with SARS-CoV-2 proteins and can generate a T or B-cells autoimmune response likely through a molecular mimicry mechanism [2]. Among them, the corticotropin-releasing factor receptor-2 (CRHR2) is expressed both in the nucleus ambiguus and in cardiomyocytes, and its ligand may elicit cardiac effects directly or via the vagus nerve [3,4]. To confirm its predicted ability to induce an autoimmune response following SARS-CoV-2 infection, we performed a Dot Blot analysis searching for the presence of autoantibodies against this receptor in sera from post-COVID-19 subjects, individuals vaccinated against the SARS-CoV-2, and controls (neither infected nor vaccinated). The obtained preliminary results seem to confirm the presence of autoantibodies against CRHR2 in both sera from post-infected and vaccinated subjects, suggesting a possible cross-reactive event, which could explain post-COVID-19 autonomic disorders like POTS. However, further experiments are in progress to confirm this observation and to better understand the mechanisms underlying post-COVID-19 manifestations affecting the autonomic nervous system.

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SCIENCE COMMUNICATION

VIRTUAL AND AUGMENTED REALITY: NEW OPPORTUNITIES FOR SCIENCE COMMUNICATION

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The growth of Virtual Reality (VR) and Augmented Reality (AR) technologies is opening an entire new spectrum of possibilities in science communication. While VR recreates complete environments through the use of 3D computer-generated graphics, AR overlays digital geolocated information and objects on top of the real world. Both technologies are characterized by a high level of engagement as they provide an immersive first-person view of the surrounding environment. Digitally created objects are interactive, can be moved in space and further analyzed inside. Virtual environments allow to experience unprecedented visual perspectives by simulating worlds that would result impossible to explore: from micro to macro scale, from the present to the past and to the future. Some relevant examples and applications are presented to show opportunities and emerging trends.

TRANSLATIONAL APPROACHES TO EXPERIMENTAL BIOLOGY

THE IMPORTANCE OF PRESERVING ANCIENT BOOKS FROM *Lepisma saccharina* INFESTATIONS AND RELATED METHODS OF PEST CONTROL

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Books, along with all other cultural goods, have always represented ways of transmitting to posterity the culture and history of different nations, and therefore constitute exceptional resources to be preserved and safeguarded over time for future generation, the value of which is not only cultural but also economic. However, antique books have not always been properly preserved: very often they arrive to the present day damaged by the action of time but also by insects such as woodworms and silverfish. It often happens because those who handled them were not aware of the possible infestations they might have encountered, but also because they did not have the necessary means to prevent such phenomena. Silverfish belong to the order Thysanura. They are small, wings free insects up to 20 mm long. They have a sharp, carrot-shaped body covered with shiny scales. They possess long, threadlike antennae and three long, segmented tails at the posterior end. They are very primitive insects with a simple chewing apparatus. Metamorphosis in these insects is an inconspicuous phenomenon, the main change being the appearance of scales after the first few molts; one can count at least six stages and often many more. The silverfish can frequently be found in homes, where it feeds especially on books, glue, and flour; in fact, it is common to see a silverfish escape from some flour piled up in the pantry, or from inside a book when it is opened. Today, we are able to recognize a silverfish infestation and, in some cases, preserve the integrity of books if there is already an infestation in place, thanks to well-established and widely used pest control methods. Among the methods used to combat silverfish infestations we have cryo - disinfestation, with a direct application of liquid nitrogen stored at -176 °C, through special dispensers. Liquid nitrogen has an immediate cryo - burn power, capable of killing any type of insect at any life stage (egg, larva, juvenile, adult). For administration on infested volumes, we start from the outer binding in both corners of the book and then go through the cover. Nitrogen, as a primary component of air, in a percentage of 78%, makes it possible to carry out pest control that is non-toxic to humans. This type of intervention is particularly effective against silverfish because of the percolation effect: the nitrogen, in fact, penetrates the fabrics without wetting and brings the temperature considerably below zero reclaiming the treated material. At least every two years then, through the protective shield obtained by spraying water diluted Fenthrin insecticide, which is non-toxic to humans and highly toxic to adult crawling insects, lasting protection of antique books is ensured.

PRECLINICAL MODELS IN BIOMEDICAL RESEARCH: A GLOBAL 3Rs APPROACH

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Biomedical research needs preclinical models able to discriminate among the consequences of various stimuli acting on the organs of the body, in order to distinguish more specifically their mechanisms of action (doi: 10.1038/s41569-019-0178-1). In the present communication it will be described how a global 3Rs approach (Refinement, Reduction and Replacement of animal use) to our preclinical models could lead to important observations and to "technological spillovers", ensuring the welfare of the animals and their partial replacement in certain experimental steps. Pain and stress in laboratory animals dramatically affect physiology leading to unnecessary suffering and to bias in results, with a consequent loss in the data translation from bench to bedside. Several physiological alterations are present in suffering animals, making essential a pain/stress quantification. In the last decade, our group applied a global 3R approach in several preclinical models, Refining surgery with new analgesic strategies, Reducing the number of animals by observing mice and rats already scheduled for medical investigations, and partially Replacing some *in vivo* procedures by introducing IVTech Bioreactor System®. To quantify pain/stress, effective methods have been used. The published results: • showed that new analgesic strategies had a greater efficacy than those commonly used; • described typical pain signs associated with the applied surgeries; • showed a correlation between Brown Adipose Tissue (BAT) temperature and pain; • showed an impact of handling and restraint on parameters like BAT temperature, weight and neuroinflammation. Working on pain/stress, the issue of finding a model to investigate the organ crosstalks alteration activated by suffering arose. Animals are too complex and cells in a petri dish too simple. The development of the IVTech Bioreactor System® allowed to explore crosstalks in a way impossible with classic *in vivo*/*in vitro* models. In a recent work, awarded with AAALAC International Global 3Rs Award 2022 (doi: 10.1371/journal.pone.0242627), we assessed a simplified model of nervous-cardiovascular crosstalk by using IVTech System, finding that coronary-artery and neuroblastoma cells connected under flow conditions started a dialogue triggering the activation of PKCβII/HuR/VEGF pathway after angiotensin II treatment. This activation was not present when cells were subjected to flow, but not connected with each other. In conclusion, a global 3Rs approach to our preclinical models allowed experiments quality improvement, thus reducing bias, and enabled to find partial Replacement methods, important to answer to clinical and physiological questions not addressable with current *in vivo*/*in vitro* models, and potentially useful to test therapeutic strategies before the eventual *in vivo* phase. (doi: 10.1371/journal.pone.0095913 doi: 10.1371/journal.pone.0224337 doi: 10.1371/journal.pone.0259938).

FORENSIC ENTOMOLOGY AND DEPLETION OF VALUABLE GOODS: THE CASE OF WOODWORMS AND OLD WOODS IN A SOUTH TYROL ABBEY

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Anobium punctatum (De Geer, 1774) (Artropoda: Insecta: Coleoptera) is the main xylophagous insect species responsible for biodegradation and alteration on wooden objects such as statues, frames, antique furniture artworks and many other valuable objects items. Also known as "the common furniture beetle", this insect attacks and develops at the expense of hard

woods *e.g.*, walnut, mahogany and ebony but it can also affect the soft woods *e.g.*, conifers. The nickname "woodworm" is related to a long-term life cycle phase when the soft, white, moisture-loving, vermiform larvae dig tunnels in the wood, degrading cellulose/ starch, leaving deposits of wood fibres and faecal pellets (rosume). The main track of their presence is evident only after the reddish-brown adults flick from the wood, through the characteristic exit holes (1-1.5 mm in diameter). This case study focuses on the damage caused by woodworms on old wooden statue located in the Augustinian Novacella Abbey (Neustift Abbey, Vahrn), one of the most important monasteries in the South Tyrol area.The *in situ* investigative protocol to find tracks of xylophagous insects in the artworks was based on: (a) visual survey to assess the presence/absence of exit holes, fine sawdust and adults/larvae/pupae on the wood surface or exposed tunnels with the aid of a magnifying glass; (b) photographs of the wooden material and specimens; (c) sampling ; (d) worklab for Light Microscopy identification of larvae/adults/pupae on the basis of diagnostic morphotraits. The last step is the planning and application of standard protocols of pest control in the focused scenario. *A. punctatum* is the main beetle pest of the wooden goods heritage in Italy. Applied research is in progress to standardize sustainable approaches for a green pest control.

EFFICACY OF THE SORAFENIB TREATMENT IN A 3D HEPATIC CANCER MODEL BY COMPARING STATIC AND DYNAMIC CULTURE CONDITIONS

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Drug development is a long and highly expensive process. During the preclinical research phase both *in vitro* and *in vivo* models are used but with some limitations. The classical human *in vitro* setup fails to recapitulate the complexity of systemic biology, while animal models do not exactly reproduce human biology. In the last years, ethical concerns about the wide use of animals for research purposes have emerged. This promoted the development of *in vitro* models that could better mimic the complexity of tissues and biological systems. 3D cell structures developed in the last years represent very promising models for both healthy and pathological setups. For the study of tumors, the use of 3D spheroids or organoids allows the establishment of cell-to-cell interactions, in a more realistic tumor microarchitecture when compared to the 2D tumor cell cultures. We developed a reusable platform to generate thousands of 3D spheroids, from the human hepatocellular carcinoma HepG2 cell line, in a faster way. The spheroids are homogenous in shape and size and remain viable. Moreover, we can easily retrieve the 3D spheroids from the platform and use them for further experiments. We are currently developing a system to test in physiological-like conditions the cytotoxicity of Sorafenib, a reference drug used in the treatment of hepatic cancer. The spheroids are inserted in a commercially available bioreactor (MIVO^{R,} React4Life) in which we can reproduce the systemic administration of the compound by using a dynamic flow. The bioreactor has already been used to show the efficacy of the Cisplatin treatment in an ovary cancer cell model with a prediction that closely matches the *in vivo* results, requiring a much shorter experimental time [1]. Our preliminary results show a difference in the cytotoxic effect on the Sorafenib-treated HepG2 spheroids by comparing static and dynamic treatment conditions. This approach can thus become a reliable method to speed up the preclinical research phases and contribute to the reduction of the use of animals.

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ADIPOSE DERIVED STEM CELL BEHAVIOR IS INFLUENCED BY MCF-7 EXHAUSTED MEDIUM

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Stem cells are an excellent tool for regenerative medicine. They can be isolated from various tissues, including adipose tissue. Adipose tissue is used in reconstructive medicine, as after mastectomy, to restore a natural appearance following surgery. Adipose derived stem cells (ADSCs) are able to differentiate into different cell lines after different type of stimulation. The cellular environment is able to influence the fate of stem cells residing in the tissue. In this work, we investigated how an exhausted breast cancer cell line (MCF-7) medium is able to influence stem cell differentiation. Cells were exposed to an exhausted medium harvested at different time points. After treatments, we evaluated the expression of stemness genes, adipogenic and osteogenic differentiation-related genes. To confirm the gene expression data, the oil red and alizarin red colorimetric assay was performed. The data reported here demonstrate that stem cells exposed to the differentiation medium maintain a stemness phenotype with high proliferation rate.

CELL CYCLE BLOCK BY p53 ACTIVATION REDUCES SARS-COV-2 RELEASE IN INFECTED ALVEOLAR BASAL EPITHELIAL A549-hACE2 CELLS

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The two last decades have shown great sanitary emergencies due to the pandemic diffusion of SARS-CoV-2 that has presented a new scientific challenge for the search of effective therapies against infection, replication and spreading. Among the intracellular targets of the virus, p53 is one of the targets that plays an important role both in the mechanisms of innate immunity as well as in the control of the cell cycle and other pathways that regulate cell replication, damage repair, apoptosis and metabolism. SARS-CoV viruses adopt several strategies to silence p53, including the stabilization of its inhibitor, MDM2,

and the interference with its transcriptional activity, indicating that p53 has a central role in controlling its proliferation in the host. For these reasons, the aim of the project was to evaluate a new approach against the virus, by using MDM2 inhibitors to effectively raise p53 levels and activate p53-dependent pathways including cell cycle inhibition. Experiments setting was done in the alveolar basal epithelial cell line A549-hACE2 expressing $TP53^{wild\text{-}type}$ and the SARS-CoV2 receptor ACE2. Cells were treated with several concentration of Nutlin-3 or RG-7112 at the time points of 24 and 72 hours post treatment for the instauration of a cell cycle block steady-state condition before and during SARS-CoV-2 infection, and for the evaluation of p53 activation and impact on virus release and related innate immune events.The results of the project suggest that Nutlin-3, as well as RG-7112, significantly reduced SARS-CoV-2 replication in A549-ACE2 cells and promoted a complete inhibition of IL-6 expression, associated with inhibition of NF-kB and interferon-lambda, important mediators of inflammation. These data indicate that p53 represents an efficient target for new therapies against the virus and that MDM2 inhibitors can be a realistic therapeutic option.

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IMPACT OF PROGRAMMED CELL DEATH PROTEIN 1 VARIANT ON LIVER TRANSCRIPTOME AND NONALCOHOLIC FATTY LIVER DISEASE PROGRESSION

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Nonalcoholic fatty liver disease (NAFLD) accounts for a growing proportion of cases of chronic liver disease, liver decompensation and hepatocellular carcinoma (HCC), and it represents an emerging indication for liver transplantation. Evidences support a role of immune response in promoting nonalcoholic steatohepatitis (NASH), the inflammatory progressive form of NAFLD, leading to fibrogenesis and development of HCC. The programmed cell death protein 1 (PD1) is an immune checkpoint surface receptor, which plays a prominent role in maintaining the immune tolerance and, moreover, it is involved in immune escape from cancer. Programmed cell death protein 1/programmed death-ligand 1 (PD-1/PDL-1) axis has been reported to modulate liver inflammation and progression to HCC in patients with NAFLD. Recent data, in experimental models and in clinical samples, suggest that PD1 is expressed by a subset of CD8+ cells with exhausted phenotype, which accumulate during NASH inducing a non-antigen restricted killing of steatosis hepatocytes. The exhausted T cells facilitate the development of inflammation leading to disease progression and promoting hepatic carcinogenesis. The aim of our study was to evaluate, in a wide multicentric cohort, the impact of *PDCD1* gene variants, rs13023138 G>C, on NASH patients, who have a potential to develop HCC. Moreover, hepatic transcriptome was examined by RNASeq in a subset of patients. Transcriptomic and deconvolution analysis were performed to identify biological pathways modulated by the risk allele. Our results reported that the frequency distribution of PDCD1 rs13023138 CC, CG and GG genotypes was 38.5%, 45% and 16.5%, respectively. Genetic frequencies fitted Hardy–Weinberg equilibrium. Moreover, rs13023138 G allele was linked to higher hepatic representation of M1 macrophages, together with the upregulation of pathways related to inflammation and higher expression of C-X-C Motif Chemokine Receptor 6 (CXCR6). In our cohort, the *PDCD1 rs13023138* G allele was independently associated with severe steatosis, NASH, advanced fibrosis and HCC, suggesting a role of this polymorphism in the monitoring of NAFLD progression and HCC development.

MORPHO-FUNCTIONAL MODIFICATIONS OF GUT BARRIER PROPERTIES INDUCED BY SURFACE LAYER PROTEINS (S-LAYER) FROM *Lactobacillus helveticus* ATCC® 15009™ IN A CO-CULTURE OF CACO2/HT-29 CELLS AS A MODEL OF HUMAN INTESTINAL EPITHELIUM

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Healthy gut barrier (GB) can be improved by specific probiotic strains, even if their reliability and safety concerns in clinical practice are largely discussed. Paraprobiotics and postbiotics can represent a valid alternative due to their intrinsic higher stability and preservation in food matrices. Among probiotic species, Lactobacilli constitute an important group of microorganisms able to stimulate host's immune system and act as an effective therapeutic alternative for the treatment of gut inflammation, obesity, and chronic degenerative diseases. The present study investigated the effects of surface-layer proteins (S-L) of the dairy strain *Lactobacillus helveticus* ATCC® 15009™ (*Lh* ATCC® 15009™) on the morpho-functional modulation of GB in comparison to live or heat killed *Lh* ATCC® 15009™ in a Caco-2/HT-29 70/30 co-culture cells. Live or heat-killed *Lh* ATCC® 15009™ (100 CFU/cell and 1000 CFU/cell) negatively affect transepithelial electrical resistance (TEER) and paracellular permeability, resulting in an altered distribution of tight junction (TJ) and protein Claudin-1, stained by immunofluorescence (IF). Conversely, the addition of S-L, in amounts present into the doses of *Lh* ATCC® 15009™ administered to cells, improves TEER, and decreases permeability in physiological conditions only when basal TEER registered in co-cultures established by Caco-2 and HT-29 parental cell lines with at least 40 and 21 sub cultivation passages respectively is minor than 50 ohm*cm^2. This experimental condition may suggest the presence of a physiologically leaky gut as it occurs in old people. Transmission electron microscopy (TEM) and IF analyses suggest that S-L induces a structural TJ rearrangement and desmosomes' formation and stability. S-L is also able to restore TEER and permeability of GB in the presence of lipopolysaccharide (LPS), but not of pro-inflammatory cytokines (TNF-α plus IFN-γ). IF analysis shows an increase in Claudin-1 staining when LPS and S-L were co - administered, suggesting that the downstream Toll-Like Receptor- mediated signaling (TLR4 for LPS, TLR2 for S-L) may result in junctional apparatus remodeling, such as increased desmosomes' protein complexes transcription or TJ protein phosphorylation and redistribution. In addition, S-L can counteract the reduction of alkaline phosphatase detoxification activity and the enhancement of pro-inflammatory interleukin-8 (IL-8) release both induced by LPS. Altogether, these data obtained in a model of

injured intestinal epithelial barrier, either physiological or induced, corroborate the supposed paraprobiotic role of S-L from *Lh* ATCC® 15009™ as a possible candidate for therapeutic and prophylactic uses in alternative to viable microbiota, in conditions related to gastrointestinal health and extra-intestinal disorders, some of which are correlated with gut dysbiosis.

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