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11º Simpósio de Metabolismo da Faculdade de Medicina da Universidade do Porto

– Palestras, Comunicações Orais e Posters

> SESSION I – METABOLIC FITNESS AND AGEING

Skeletal muscle ageing: from a transcriptome and metabolome perspective

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Background: Age-related skeletal muscle loss (sarcopenia) increases the risk of frailty and mortality. Within the skeletal muscle, fast and slow muscles seem to be differently susceptible to age-related changes. Here, we set to understand how the transcriptome and metabolome change in pre-sarcopenic muscle types and how this relates to human muscle ageing.
Methodology: We extracted *Soleus* (slow, oxidative) and *Extensor Digitorum Longus* (EDL, fast, glycolytic) muscles from young (4 months old, n=6-8) and pre-sarcopenic (no muscle weight loss) old (25 months old, n=6-7) C57BL/6 mice and performed gene expression and metabolite analyses employing RNA-seq and UHPLC, respectively. Human RNA-seq counts were obtained from GTEx database (*Gastrocnemius*, fast muscle type), with age groups (in years) defined as 20-29 for young and ranging from 30-39, 40-49 and up to 70-79 for old depending on the analysis. Statistical analyses were performed using custom R scripts.
Results: Gene expression profiling revealed 229 differentially expressed genes (FDR<0.05) in ageing EDL (young vs old), and 131 genes were found to change (FDR<0.05) in ageing *Soleus*. Genes down-regulated in aged EDL were enriched for mitochondria-related processes, including "NADH dehydrogenase complex", "mitochondrial translation" and "mitochondrial gene expression". In *Soleus*, downregulation was related to extracellular matrix including "collagen trimer" and "focal adhesion". Differences in metabolite levels were also more pronounced in ageing EDL (192, p<0.05), enriched for "sphingolipid" and "nicotinamide" metabolism, than in ageing *Soleus* (132, p<0.05), enriched for "diacylglycerol metabolism". Overall, EDL seems to be more susceptible to age-related changes than *Soleus*.
Human data analyses revealed downregulated processes related to mitochondria in all age-group comparisons similar to what was observed for EDL. These age-related changes include detrimental, neutral and adaptative processes.
Conclusions: Different muscle types exhibited distinct age-related changes. Common to mice and humans were alterations related to mitochondria, which might be relevant to fast muscles atrophy.
Keywords: Ageing; Skeletal muscle; Transcriptome; Metabolome; Mitochondria

Nutritional and functional status in the elderly, a picture of the Portuguese elderly population

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Older adults present a higher risk of nutritional and functional status dysfunctions. The Nutrition UP 65 Project aimed to study the association between the nutritional and functional status of the Portuguese older population, based on anthropometric parameters, vitamin D, hydration status, handgrip strength (HGS) and gait speed (GS). This was a cross-sectional study that included 1,500 Portuguese older adults ≥ 65 years old, and the sample was representative of Portuguese older adults in terms of sex, age, educational level and area of residence. Results have shown that 44.3% of the elderly were overweight and 38.9% were obese. Approximately 14.8% were at risk of undernutrition and 1.3% were undernourished. In addition, among individuals identified at risk of undernutrition, more than 30% presented simultaneously overweight/obesity. Low values of HGS (<18 kgf in women and <30.3 Kgf in men) and of GS (>0.8 m/s) were observed.

Overweight, obesity and undernutrition risk were increasingly associated with slow GS. Individuals at risk of vitamin D inadequacy (29.4%, 30.0–49.9nmol/L) and of deficiency (39.6%, <30.0nmol/L) presented higher adjusted OR of lowest values of GS and HGS than those with adequate vitamin D levels (31%, ≥50.0nmol/L). The risk of hypohydration was directly associated with low HGS. Nutritional status dysfunctions were highly prevalent in this sample and were associated with low values of functional indicators.

Keywords: Elderly; Gait speed; Handgrip strength; Nutritional status; Undernutrition

Exercise and Ageing: the effects on cardiorespiratory fitness, muscle mass and function preservation

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Human ageing is associated with progressive declines in multiple physiological systems, with changes in the cardiorespiratory and neuromuscular systems being some of the most pronounced. Reduced levels of cardiorespiratory and muscular fitness, and skeletal muscle force production can severely limit physical performance and independence, and have been associated with increased mortality and morbidity. Improving these physical components offers the most effective strategy to reduce all-cause and cardiovascular mortality risk, and to help maintaining an independent living. Thus, these physical components are key targets for intervention.

It is well-established that both endurance exercise and resistance training can substantially improve physical fitness and health-related factors in older individuals.

The best exercise prescription is difficult to define, as it depends on the target outcomes, the individual characteristics, baseline fitness level, time available for training, among others. Aerobic training and resistance training are associated with wide-ranging improvements in multiple components of fitness. Consequently, combined or concurrent training are commonly prescribed and suggested to be a more effective than either endurance or strength training performed alone because of the potential to maximize performance. However, it can also expose the participants to overreaching or overtraining if training variables are incorrectly manipulated.

In summary, a structured training programs should be designed to improve the physiological function, particularly in this population. Older adults remain highly trainable into advanced age with substantial fitness improvements. However, special care should be taken when designing exercise intervention to older adults with chronic conditions, to accommodate their especial needs. Varying modalities, intensities, frequencies and volumes of training must be properly handled as they affect the physiological responses.

Keywords: Ageing; Combined training; Endurance training; Strength training; Physical fitness

> SESSION II – BIOCODEX SESSION – ENERGY HOMEOSTASIS AND AGEING

Slowing down the ageing process, targets and strategies. Role of sirtuins

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In Western societies the proportion of aged people has been increasing in the last decades, owning to the increase in life expectance associated with the improvement of socioeconomic conditions and easy access to medical care. Concomitantly with the increase of elderly people, the incidence of age-associated diseases, such as, cancer, cardiovascular disturbances, diabetes and neurological degeneration also increases. Indeed, the elucidation of molecular pathways involved in the physiology of ageing is of paramount importance for the implementation of strategies that counteract the onset of prevalent diseases in the elderly.

Ageing is a gradual process that conduces to the loss of function and death, not only of the cells in tissues, but also of the organisms. Senescent cells that accumulate in the tissues of aged organisms present a specific phenotype owning to the increase of oxidative environment, genomic instability, epigenetic drift, nutrient-sensing deregulation and loss of proteostasis. These cellular features stem from age-related changes of specific molecular pathways; AMP-activated protein kinase (AMPK) a key energy-sensitive enzyme that controls numerous metabolic and cellular processes; mammalian target of rapamycin (mTOR) another energy/nutrient-sensitive kinase that controls protein synthesis and cell growth; and sirtuins (1-7), a class of NAD+-dependent deacetylases that intervene in gene expression, epigenetics, DNA repair, energy regulation, autophagy, cell cycle control, inflammation and vascular function.

Among the interventions that improve lifespan and healthspan in mammals, energy restriction

is considered the main non-pharmacological intervention to protect organisms from age-associated modifications and several compounds that regulate the pathways of cellular response to ageing had been proposed to mimic its effects. These compounds termed energy restriction mimetics may be promissory in the prevention of age-associated diseases.

Keywords: Ageing; Age-associated diseases; AMPK; Energy restriction; Energy restriction mimetics; mTOR, Sirtuins

Proteostasis control during ageing: lessons from yeast

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Background: Proteostasis is a cellular housekeeping process that refers to the healthy maintenance of the cellular proteome. It is well recognized that the ability of cells to maintain proteostasis declines during ageing. Genetic or pharmacological enhancement of the proteostasis network has been shown to extend lifespan in a variety of ageing models.

Methodology: Caloric restriction (CR) is a non-genetic intervention known to promote lifespan extension, linked to the modulation of the proteolytic systems, in several model organisms. Using the yeast *Saccharomyces cerevisiae* heterologously expressing the human aSyn (SNCA), the contribution of proteolytic systems and their crosstalk to the beneficial effects promoted by CR intervention during ageing were evaluated.

Results: Herein, the findings obtained with the chronological ageing model *Saccharomyces cerevisiae* will be presented. A review of the advances on ageing and age-related diseases research in yeast models will be made. Particular focus will be placed on key longevity factors, ageing hallmarks and interventions that slow ageing, both yeast-specific and those that seem to be conserved in multicellular organisms. Their impact on the pathogenesis of age-related diseases will be also discussed.

Conclusions: Mounting evidence suggests that maintenance of proteostasis is fundamental to delay ageing. However, part of the molecular mechanisms responsible for proteostasis network remodelling and the signals that promote the age-associated changes remain unknown. Therefore, simple organisms as yeast could provide insights on age-dependent repertoire of proteostasis network alterations as well as the signalling pathways responsible for those alterations.

Keywords: Proteostasis; Chronological ageing; *Saccharomyces cerevisiae*; Ubiquitin-proteasome system; Autophagy

The role of adipocyte lineagesin the development of age related disorders

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Obesity is a significant challenge to medical care systems worldwide. Thus, adequate treatment strategies are needed to counter overweight and its associated metabolic disorders. Ageing exacerbates the onset and progression of many of these complications, including the development of insulin resistance and the ectopic accumulation of adipocytes in atypical anatomical sites, such as the marrow cavities of long bones. These processes are thought to contribute to the impairment of normal tissue maintenance and also to inhibit regenerative tissue repair after injury. An important goal of our current research is therefore to decipher the cellular lineages involved in regeneration. Tissue-resident adult stem and progenitor cell populations that can give rise to mature fat cells are considered to be of the mesenchymal stem cell type, and their heterogeneous composition has not been assessed in much detail. We were recently able to show that mesenchymal cells of the bone marrow cavity are functionally diverse and give rise to ectopic fat but may at the same time contribute to the regulation of regenerative processes. These findings suggest that age-dependent changes of the cellular heterogeneity of the mesenchymal lineages may negatively affect tissue regeneration and health in aged individuals.

> SESSION III – CELLULAR MECHANISMS OF SENESCENCE

Biomarkers of tissue ageing and AGEs as modulators of ageing

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Background: Ageing is induced at least in part by accumulation of molecular damage. One damageing mechanism is protein glycation, the non-enzymatic reaction between reactive amino group side chains (e.g. lysine and arginine) and carbohydrates or dicarbonyls. The products of this reaction, the advanced glycation endproducts (AGEs), can induce tissue stiffening or after binding to receptors inflammation. There is an open question how endogenous and exogenous AGEs influence cell and organ function during ageing.
Methodology: Using the dicarbonyls glyoxal and methylglyoxal, AGE formation was induced in primary human endothelial cells. AGE accumulation in cardiac and skin tissue was analyzed as outcome predictors of heart surgery patients. AGEs from nutrition were prepared from bread crust to stimulate endothelial cells and the cellular responses were analyzed.
Results: AGE formation in cells can induce cellular senescence. The accumulation of AGEs in crosslinked collagen of the heart as well as in the skin are biomarkers of ageing and predictors of postoperative complication after heart surgery. In contrast, AGEs from nutrition can induce the protective nrf2 pathway and thereby protecting cells from stress induced cell death.
Conclusions: Whereas endogenous AGE formation seems to have a great impact on ageing and cardiovascular diseases, AGEs from nutrition can have positive effects as well.
Keywords: Advanced glycation endproducts; Ageing; Cardiovascular system; Nutrition

Bioactive sphingolipids, mitochondrial function and ageing: insights from yeast

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Sphingolipids are ubiquitous structural components of cell membranes and act as regulatory molecules in signal transduction pathways, e.g. through the modulation of protein kinases or phosphatases. Bioactive sphingolipids regulate numerous cellular processes, including cell growth, cell cycle, stress responses, autophagy and apoptosis, and have been implicated in ageing a wide range of diseases, including neurodegenerative disorders. Ceramide is the central core lipid in the metabolism of sphingolipids and constitutes a family of structurally distinct molecular species that can be generated by acylation of a long chain sphingoid base (LCB; sphingosine in mammals; dihydrosphingosine or phytosphingosine in yeast) or through hydrolysis of complex sphingolipids mediated by sphingomyelinases. Recent studies suggest that the interplay between sphingolipids and nutrient signaling pathways plays a key role in the regulation of mitochondrial function and ageing in yeast. Indeed, the Sch9/S6K protein kinases and the Sit4/PP6 protein phosphatase integrate sphingolipid signals with nutrient signals from TORC1 (Target of Rapamycin complex 1). Our studies have shown that downregulation of Isc1 (neutral sphingomyelinase) or Ncr1 (orthologue of NPC1, a protein associated with Niemann-Pick type C1 disease) impairs sphingolipid homeostasis and increases sphingolipid signaling through Sch9 and Sit4, leading to mitochondrial fragmentation and dysfunction. Notably, the deletion of *SCH9* or *SIT4* suppresses these phenotypes, increasing cell longevity. How the Sit4 protein phosphatase controls mitochondria by regulating protein phosphorylation will be discussed.
Keywords: Sphingolipids; Mitochondria; Ageing; Cell signaling

Insights into the contribution of copper to cellular ageing

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As a major concern of modern societies, ageing has been widely investigated in an attempt to find strategies able to delay age-related health deterioration. In this setting, the identification of potential contributors and the molecular mechanisms implicated in the ageing process is crucial. At the cellular level, ageing is characterized by the progressive accumulation of oxidatively damaged biomolecules that ultimately lead to cellular dysfunction. Age-associated alterations on metal homeostasis may favor this process. In fact, the accumulation of redox active metals within cells and tissues can promote the generation of reactive oxygen species (ROS) able to damage biomolecules. Copper levels were shown to increase with ageing and in age-associated diseases such as type II diabetes, atherosclerosis and neurodegenerative disorders, further supporting the contribution of this metal for oxidative stress and the overall age-related functional deterioration.
In vitro cellular models of senescence are a valuable tool to study molecular events involved in the ageing process. Besides the classical replicative senescence (RS) cellular model, stress-induced premature senescence (SIPS) may be achieved by the exposure of non-tumoral cells to oxidative stress inducing agents, such as hydrogen peroxide. Based on copper ability to mediate the formation of ROS, the establishment of a novel copper-induced SIPS model in human diploid fibroblasts will be documented. Further-

more, data on copper-induced activation of compensatory cellular responses aiming at reestablishing proteostasis will add further knowledge on the contribution of copper to cellular ageing.
Keywords: Cellular senescence; Copper; Cellular stress response; Proteostasis

> SESSION IV – FLASH SESSION 1

Glycemic control influence on sarcopenia risk in the elderly

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Background: Uncontrolled diabetes, largely characterized by inadequate insulin levels, chronic hyperglycemia and lipid abnormalities, causes decreases in muscle strength, which contributes to disease-related morbidity. Individuals identified as pre-diabetic (5,7%≤HbA1c≤6,4%) are at great risk to develop diabetes, whereas age is also recognized as an important risk factor for developing the disease. Sarcopenia is the age-related loss of skeletal muscle mass, strength and function. The main goal of this study was to correlate the prevalence of sarcopenia with glycemic control, estimated by HbA1c levels, in the elderly.
Methodology: A quantitative observational cross-sectional study was performed in a convenience sample of individuals aged over 60 years old recruited non-randomly. Main study variables were body composition (seca® mBCA515), glycemic control (HbA1c, cobas b101-Roche®), muscle strength (peak torque, Humac NORM isokinetic dynamometer), risk of falls (TUG test), and muscle function (LEFS).
Results: Our sample (n=36, 77,8% female, mean age 73 years old) was stratified into Normal (n=14, HbA1c<5,7%), Prediabetic (n=18), and Diabetic (n=4, HbA1c≥6,5%). Increasing levels of HbA1c positively correlated with increasing BMI ($p<0,01$), and average BMI from Normal was significantly lower ($p<0,05$) compared to Prediabetic/Diabetic (HbA1c≥5,7%). Although numerically unaltered between groups, quadriceps and hamstrings strength (peak torque), as well as free-fat mass %, negatively correlated with HbA1c levels, but only in Normal subjects ($p<0,05$). Muscle strength *vs* HbA1c showed similar trends in Prediabetes. TUG and LEFS were both HbA1c-independent.
Conclusions: Muscle strength negatively correlates with HbA1c in normal and pre-diabetic subjects, while BMI positively correlates with overall HbA1c levels. Glycemic control, even in the considered normal range, together with other age-related comorbidities, should be perceived as a sarcopenia risk factor in the elderly.
Keywords: Diabetes; Glycemic control; Sarcopenia; Ageing; Muscle function

> SESSION IV – FLASH SESSION 2

Uncovering the molecular mechanisms that tie autophagy and UPS during ageing

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Background: Ageing is a complex multi-factorial process that results in the progressive accumulation of molecular alterations and disruption of cellular homeostasis. Several hallmarks of ageing that represent age-common denominators in different model organisms have been proposed, including deregulated nutrient-sensing and loss of proteostasis; the latter is mainly caused by a decline on autophagy and the ubiquitin-proteasome system (UPS) activities. Caloric restriction (CR) is still the most effective non-genetic intervention known to promote longevity associated with the modulation of proteostasis. In the present work, we aim to understand the ties between UPS, autophagy and ageing under CR effects.
Methodology: Using the yeast model, we elicited proteotoxic stress by heterologous expression of human α-synuclein (aSyn). Proteasome catalytic activities and autophagy were monitored over several timepoints of chronological lifespan, with or without CR intervention.
Results: CR boosted UPS activity, reversing its aggravated decline in prematurely aged aSyn-expressing cells, while keeping autophagy at homeostatic levels. Autophagy inhibition upregulated UPS activity, however, UPS inhibition did not enhance autophagic activity.
Conclusions: The data suggests a compensatory mechanism between autophagy and UPS activities. Maintenance of autophagy at homeostatic levels appears to be relevant for UPS activity and for the mechanism underlying rescue of cells from aSyn-mediated toxicity by CR.
Keywords: Ageing; Chronological lifespan; Yeast, proteostasis; UPS; Autophagy

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> SESSION IV – FLASH SESSION 3

Testosterone and Estradiol: their relationship with musculoskeletal health in older adult

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Background: Age-related testosterone (T) and estradiol (E2) deficiency is associated with bone mineral density (BMD) loss and increase fracture risk; however the relative significance level of each one is still under debate. On the other hand, it is well known that muscle strength has a positive effect on attenuating age-associated BMD loss. Therefore, the aim of the present study was to analyse the association between both T and E_2, BMD and muscle strength in older adults.
Methodology: from an initial sample composed of 269 elderly recruited in Viana do Castelo, 58 participants (female, 79.3±6.2y and male, 79.53±5.89y)

were selected for determination of BMD by dual-energy X-ray absorptiometry (DXA). The values of BMD (BMC/bone area, g/cm2) and isometric knee extension strength were determined. The free T (pmol/L) and E2 (pmol/L) levels were quantified in serum. The T/E_2 ratio was also calculated.
Results: T-score statistic for the whole group showed that on an average there was no osteoporosis across +75y old. Male have higher levels of T and E_2 than female (14.82±9.57 *vs.* 2.78±2.09; 147.93±32.56 *vs.* 122.98±47.16; p<.01). The T/E2 ratio was higher in male than in female (0.10±0.06 *vs.* 0.02±0.01, p>0.05). In male, T/E_2 ratio showed a strong positive correlation with lower limb strength (p<.023, r=.683). Among female, T was positively correlated with total (p=0.05; r=.945) and proximal femur BMD (p=0.04; r=0.94) as well as T/E_2 ratio correlated with lower limb strength (p=.01; r=0.615).
Conclusions: In a community based sample of elderly individuals, free T associated with a higher BMD and muscle strength rather than E_2 levels, namely in female. Moreover, both T and E_2 could be important determinants for musculoskeletal health.

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> SESSION IV – FLASH SESSION 4

Beneficial effects of melanocortins on obesity-related metabolic dysfunction: a focus on the adipose tissue features of premature ageing

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Background: In obesity, the adipose tissue (AT) undergoes physiological and biochemical changes that features an accelerated ageing process defined by increased levels of intracellular oxidants and activation of stress-related signaling pathways. Recently, our group demonstrated that the melanocortin α-melanocyte stimulating hormone (α-MSH) ameliorates the metabolic profile of obese mice by stimulating AT "browning". The present work attempts to investigate if α-MSH also protect AT from obesity-related premature ageing, towards an improvement on the mechanisms of cellular stress responses.
Methodology: C57BL/6 mice fed with a high-fat diet for 10 weeks were intraperitoneally injected with α-MSH (150µg/Kg/day) or saline solution for 14 days. Inguinal white adipose tissue (ingWAT) was collected and used for the evaluation of oxidative stress, ER-stress and autophagy biomarkers, through qPCR and Western-blotting techniques. Lipid peroxidation was evaluated by the TBARS method.
Results: Our data revealed that α-MSH is capable of attenuating the three main ER-stress signaling pathways in ingWAT. Indeed, obese mice injected with α-MSH exhibited lower expression levels of PERK, lower phosphorylation levels of eIF2α and decreased *Xbp1* mRNA splicing rates. α-MSH treatment also improved ingWAT redox balance, since it reduced the expression of the enzyme SOD2 and the activation of NF-κB signaling, a key regulatory pathway in oxidative stress. More importantly, lipid peroxidation of ingWAT decreased significantly in α-MSH-treated animals and, accordingly, the mRNA levels of several autophagy biomarkers, namely, *Lamp-2*, *p62*, *LpL* and *Lipa*, were also diminished.
Conclusions: A novel therapeutic role for melanocortins on obesity-related metabolic dysfunction is unveiled in the current study, establishing that α-MSH protects obese mice against AT premature ageing through the attenuation of cellular stress signaling pathways.

Keywords: Ageing; Obesity; Adipose tissue; Melanocortin

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> SESSION IV – FLASH SESSION 5

HFE loss of function is a susceptibility factor for bone loss and early osteoporosis onset

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Background: Osteoporosis is a multifactorial disease that results from an unbalanced bone remodelling mechanism and is one of the most frequent complications associated with HFE-related hemochromatosis, a pathology associated with systemic iron overload. Recently several studies have shown a strong association between HFE-hemochromatosis and osteoporosis onset. We have previously found that Hfe-KO mice, when subject to iron enriched diet, showed an acceleration of bone loss phenotype and this was related to an increase of osteoclast activity which favoured bone resorption. This was likely the consequence of an increase in osteoblast death, possibly by ferroptosis, associated with an increase in osteoclast recruiting as a consequence of the iron overload related inflammation. In this study we aimed to compare the bone status of WT and Hfe-KO mice at 6 and 12 months of age in order to understand if older Hfe-KO mice showed higher susceptibility factors to bone loss phenotype.
Methodology: We have analyzed the bone status of WT and Hfe-KO mice by micro-CT method, followed by histomorphometric analysis of mineralized tibia sections of the mice to evaluate osteoclast resorption levels with Trap activity method and iron accumulation by Perls method.
Results: Both WT and Hfe-KO mice showed a degradation in bone quality from 6 to 12 months of age. However, in 12 months old Hfe-KO mice a significant degradation of bone quality was observed, in consequence of an increase in osteoclast activity due to generalized iron accumulation in bone trabeculae and resulting inflammation. Although also present, bone loss in aged WT mice was not so severe, with no statistical differences found in bone volume fraction, trabecular number and osteoclast activity.
Conclusions: The iron overload accumulated through time as a consequence of HFE loss of function is a susceptibility factor for bone loss phenotype and can explain the higher incidence of osteoporosis in HFE-hemochromatosis patients.
Keywords: Osteoporosis; Bone metabolism; Iron; HFE; Hemochromatosis

> SESSION IV – FLASH SESSION 6

Specific antioxidant effect on ovarian ageing

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Background: The ovary is considered the main responsible for age-related infertility increase due to a progressive decay in follicle number and oocyte quality. This appears to be connected with an intracellular imbalance between reactive oxygen species (ROS) production and antioxidant mechanisms, that results in local oxidative stress (OS). OS is believed to increase inflammation and fibrosis, inevitably ending in ovarian tissue dysfunction. Thus, this work aims to evaluate whether these features are age-related and may contribute to the loss of reproductive function, and if specific antioxidant supplementation can ameliorate them.
Methodology: Young and aged mice were employed. Aged mice were treated with apocynin or TEMPOL in the drinking water. H&E, Sudan black and picrosirius red staining were used for ovarian histological examination. Protein carbonylation and nitration was evaluated by immunofluorescence. mRNA relative expression of collagen types, inflammation markers, matrix metalloproteinases (MMPs) and MMP tissue inhibitors (TIMP) was determined by qPCR. Statistical analyses were performed using Student's t-test. A $P<0.05$ was considered statistically significant.
Results: During ageing the ovary undergoes morphological and molecular changes related with a disturbance of redox homeostasis, evidenced by increased lipofuscin content, protein carbonylation and nitration, and collagen deposition. Along with these changes, there was an increase in inflammation and fibrosis, demonstrated by higher relative expression of pro-inflammatory and pro-fibrotic markers (cytokines, collagens, MMPs, TIMPs) and decreased anti-fibrotic miRNA 29c-3p expression. Antioxidant supplementation with apocynin, but not TEMPOL, was capable of ameliorating features of the ovarian ageing process.
Conclusions: Specific antioxidant supplementation can be seen as an important therapeutic way to ameliorate ovarian ageing.
Keywords: Ovarian ageing; Antioxidants; Inflammation; Fibrosis

> POSTERS

1 – Valuing an overlooked food-plant: phenolic profiling fruits and inflorescences of kitul and assessing their effects on diabetes-related targets

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Background: The fruits (FR) and inflorescences (INF) of kitul (*Caryota urens L.*) have long been valued as a traditional food in Asian countries. Despite of being claimed to be suitable for diabetic patients, there is no data concerning the effects of these plant materials on diabetes-related enzymes, their phenolic profile being also unknown. The aims of this work were to disclose the phenolic fingerprint of methanol extracts obtained from kitul FR and INF, and to screen their antidiabetic activity.
Methodology: HPLC-DAD-ESI/MS^n analysis was performed to characterize the phenolic profile of the extracts. Free cell systems were used to evaluate their scavenging properties against superoxide anion and nitric oxide radical, and their effect on α-amylase, α-glucosidase, and aldose reductase inhibitory activity, detailed kinetic studies being performed for the most promising target. In order to mimic the route of ingestion, the most effective extract was selected for evaluation on HepG2, AGS and Caco-2 cell lines.
Results: Nine caffeic acid derivatives were determined for the first time in the species. INF extract was particularly active against recombinant α-glucosidase (IC_{50}=1.44 μg/mL, ca. 100 times lower than acarbose), acting through a non-competitive inhibition mechanism. This inhibitory activity was further confirmed on human α-glucosidase obtained from Caco-2 cells (IC_{50} 64.75 μg/mL). This extract also displayed a strong inhibitory activity against α-amylase and aldose reductase (IC_{50} values of 50.51 and 55.69 μg/mL, respectively), as well as scavenging effects. Moreover, it did not caused significant changes on HepG2, AGS and Caco-2 viability.
Conclusions: The current study provides sustained evidence of kitul antidiabetic properties, contributing to their validation and encourageing further *in vitro* and *in vivo* studies.
Keywords: Diabetes; Functional food; Kitul; *Caryota urens*; α-Glucosidase

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2 – Metabolic and microbiome changes in the intestinal environment induced by fructose-feeding in rats is affected by chrysin

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Background: Intake of fructose-containing sugars is epidemiological and experimentally linked to metabolic syndrome (MS). We recently verified that the dietary polyphenol chrysin was able to abolish some of the metabolic changes induced by fructose-feeding in the rat. Because the role of the intestine upon fructose-induced MS is poorly understood, we decided to investigate the influence of fructose, in vivo, on the intestinal environment and the ability of chrysin to interfere with the putative observed changes.
Methodology: Adult male Sprague-Dawley rats were treated for 18 weeks as follows: (A) tap water (CONT), (B) tap water and chrysin (100 mg kg−1 day−1) (CHRY), (C) 10% fructose in tap water (FRUCT), and (D) 10% fructose in tap water and chrysin (100 mg kg−1 day−1) (FRUCT + CHRY).
Results: The relative expression of SGLT1 and GLUT2 mRNA were not affected by fructose-feeding and/or chrysin. In contrast, GLUT5 mRNA expression was markedly increased in fructose-fed animals, and this effect was reduced by chrysin. However, the apparent permeability to 14C-FRUCT was markedly and similarly decreased in FRUCT, CHRY and FRUCT + CHRY rats. Jejunal villus width and crypt depth were significantly higher in FRUCT and FRUCT + CHRYS rats, respectively.

Conclusions: Chrysin did not alter gut microbiota composition, but fructose significantly increased Lactobacillus and E. coli. Moreover, FRUCT + CHRY rats had an increase on the Firmicutes to Bacteroidetes ratio. This is the first report showing that chrysin is able to interfere with the effects of fructose at the intestinal level, which may contribute to the fructose-induced MS features.
Keywords: Metabolism; Chrysin; Intestine; Microbiota; Fructose

3 – Effect of a muscle strength exercise program on balance and risk of fall in institutionalized elderly

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Background: As a result of strength decreases and spatial orientation or proprioception loses occurring with ageing, elderlies show adverse clinical profiles in terms of balance. This is especially evident in institutionalized elderly. Therefore, strength exercise programs seem to be a key-point on the maintenance or improvement of balance, and consequent prevention of falls. The aim of this study was to investigate the influence of a strength exercise program (applied during 8 weeks) in terms of balance and risk of falls in institutionalized elderly.
Methodology: Ten participants with ages between 65 and 86 years old were included. Initially, the Berg Balance scale and the Timed Up and Go test were applied to assess static and dynamic balance, respectively. Then, the strength exercise program began, being composed by an initial phase training resistance and other of strength involving the major muscles groups of the body. The exercises were performed 3 times a week, with 45 min duration each. A final evaluation after the exposure to the 8-week program was performed using the same instruments for balance and risk of fall measures.
Results: From the initial to the final evaluation there was an improvement in static and dynamic balance, improving respectively 10,5 and 2,1 points (both p=0.005). Additionally, although 60% of participants were classified as having risk of falling in the beginning of the study (measured by the static balance scale), none of the participants were in the high-risk group. Consistent and similar results were observed for the dynamic balance with 50% decreases in the prevalence of high-risk group before-after the exercise program.
Conclusions: The muscular strength exercises program seems to have a positive effect on static and dynamic balance in institutionalized elderly. Consequently, these programs should be considered for the prevention of falls related with the ageing of populations.
Keywords: Older adults; Strength training; Static balance; Dynamic balance; Falls

4 – Metabolic Reprograming during Zebrafish Caudal Fin Regeneration

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Background: Regeneration is the capacity to fully restore structure and function of an organ or limb, upon damage or injury. One of the animal models used to study tissue regeneration is the zebrafish, *Danio rerio*, having the capacity to regenerate several organs and appendages, such as the caudal fin.
Previous results from our laboratory indicate that, during caudal fin regeneration, the formation of the blastema depends on the dedifferentiation of osteoblasts. It also suggests that during this dedifferentiation process, osteoblasts change their metabolic profile. Considering the importance of metabolism on several cellular processes, we aim to investigate the role of this metabolic reprograming during the initial steps of caudal fin regeneration, mainly osteoblast dedifferentiation and blastema formation.
Methodology: We performed qPCR analysis to measure gene level expression, and we used specific glycolytic inhibitors to observe how regeneration progresses when glycolysis is impaired.
Results: Our results indicate that at 6 hours post-amputation (hpa), and until at least 24hpa, there is a major upregulation of the expression of enzymes related to glycolysis and lactate formation. To assess if this glycolytic increase is crucial for regeneration, we inject amputated fish with 2DG, a glycolysis inhibitor, during the first 48hpa. Both dedifferentiation and blastema formation were affected, with immature osteoblasts marker, *osterix*, diminished, showing indeed the importance of metabolism in early regeneration, mainly in the formation of osteoblast populations. We also observed an upregulation of genes related to mitochondrial fission, a process commonly associated with increased glycolysis, and we observed that the number of mitochondria per cell increases during regeneration.
Conclusions: Here, we provide novel insights demonstrating the importance of metabolic reprogramming during regeneration.
Keywords: Glycolysis; Metabolism; Mitochondria; Osteoblast; Regeneration

5 – Mitochondrial Dynamics in Thyroid Cancer: Unravelling the Role of DRP1

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Background: Cancer cells prefer to metabolise glucose by glycolysis instead of oxidative phosphorylation (OXPHOS), even in the presence of O_2. Mitochondria produce energy trough OXPHOS and adapt their structure and function by mitochondrial dynamics. Mitochondrial fission provides a proper number of mitochondria to support growth and division, being DRP1 its key component. Earlier, our group showed that DRP1 is overexpressed in thyroid oncocytomas.
MAPK pathway is frequently activated in thyroid cancer (TC). BRAF (a protein of MAPK pathway) was related with higher expression of DRP1 suggesting a crosstalk between both pathways.
We aim to assess the relevance of DRP1 and to evaluate a possible interaction between mitochondrial fission and MAPK signalling, in TC.
Methodology: We assessed the effects of Mdivi-1 (a DRP1 inhibitor) and Dabrafenib (a BRAF inhibitor) in TPC1, C643, 8505C and XTC-1 cell lines regarding viability, apoptosis, colonic potential and cell cycle profile, as well as effects on relevant proteins for both signalling pathways.
Results: Our results show that Mdivi-1 decreases cell viability, colonic potential and proliferation, in all cell lines. Moreover, Mdivi-1 induces cell death and increases the percentage of cells in G0/G1 in a dose-dependent manner for all cell lines, except for XTC-1. Dabrafenib showed a time-dependent effect in cell viability. It also induced cell death, but in a less extent than Mdivi-1. Dabrafenib increased the percentage of cells in G0/G1 and decreased the percentage in G2/M. Drugs combination increased the effect on colonic potential and cell death. Mdivi-1 and Dabrafenib decrease phospho-ERK and DRP1 expression in 8505C. Dabrafenib seems to increase NIS mRNA expression in XTC-1.
Conclusions: Mitochondrial dynamics and DRP1 can be relevant for TC. MAPK signaling seems to cooperate with DRP1. Mdivi-1 and Dabrafenib can be promisor drugs as complement treatment to conventional TC treatments.
Keywords: DRP1; Thyroid cancer; MAPK

6 – Motor imagery program effect on the balance in older adults

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Background: Motor Imagery is a mental representation of movement without real execution, and it seems to promote the increase and/or the maintenance of balance in different health conditions. The aim of this study it to verify the influence of a motor imagery intervention program on the balance in older adults without neurological conditions.
Methodology: A randomized controlled trial, pre-post intervention design with a 4-weeks follow-up was performed. Sixteen subjects were randomized into experimental group (n = 7) and control group (n = 9). The selection was performed through a sociodemographic questionnaire, the Movement Imagery Questionnaire-3 and the Mini Mental State Examination. Subjects in the experimental group performed the motor imagery training, while the control group was listening a story (sham), both during 10 minutes, three-times-a-week for three weeks. Balance was assessed using the Single Leg Stance (SLS) and Functional Reach Test (FRT). To compare groups and moments within each group the Mann-Whitney test and the Wilcoxon test were used respectively, with a confidence level of 95%.
Results: There was differences between groups at follow-up in SLS (p=0.049). In the experimental group there was an improvement in SLS after intervention (p=0.018), that was maintained at follow-up. Moreover, during FRT the reach increased, however no statistical differences were found. In the control group no differences were found in all outcomes at all moments (p=0.197 and 0.104, in SLS and FRT respectively).
Conclusions: It was found that the motor imagery program increased the amount of time in single leg stance which remained at follow-up.
Keywords: Ageing; Limits of stability; Mental practice; Unipodal support

7 – Hepatic adaptations to high-fat diet

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Background: High-fat diets cause the accumulation of hepatic free fatty acids and triglycerides, which is pathognomonic for non-alcoholic fatty liver disease (NAFLD), which currently affects 25% of the worldwide population. Hepatic mitochondria are structurally and molecularly altered in NAFLD, and a decline in mitochondrial function may provoke metabolic disturbances and may potentially contribute to NAFLD progression. So, the aim of the current study was to gain a more comprehensive understanding of the hepatic responses to high-fat feeding in rats.
Methodology: Young male ZSF1 Lean rats were randomly divided and fed with control diet (Ctrl - 16.7% Fat; n=7) or a high-fat diet (HFD – 47.7% Fat; n=10) for 30 weeks. At this time, the liver was weighed and blood was collected for biochemical analysis. The liver was used for ultrastructural and histopathological examination, as well as to evaluate mitochondrial function and acylcarnitine profile.
Results: HFD group showed the histological features of NAFLD, with the presence of steatosis and increased content of triglycerides in liver. HFD rats revealed a decreased in liver weight and reduced aspartate aminotransaminase (AST) and alanine aminotransferase (ALT) serum levels when compared to control rats. Mitochondrial studies revealed an increased oxygen consumption (RCR, ADP/O and FCCP-uncoupling state) and an improved ΔΨ (lag-phase) in HFD group. According to the results obtained, HFD group presented higher ATP levels, as well as acylcarnitine levels were consistently decreased in liver. Semi-quantitative and qualitative analyses from microphotographs displayed that liver mitochondria of HFD animals had lower density and circularity, but higher mitochondrial area.
Conclusions: In summary, high-fat feeding prevented the bioenergetics impairment induced by NAFLD, by increasing fatty acid oxidation and ATP production.
Keywords: Non-alcoholic fatty liver disease; Mitochondria; High-fat diet

8 – Assessment of the effects of dietary phenolic acids on carcinogenic traits and glucose and glutamine uptake in breast cancer MCF-7 cells

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Background: Breast cancer is the second most diagnosed cancer and has one of the highest mortality rates worldwide for both sexes. Cancer cells have altered metabolic pathways to support their high proliferation rates, and these include an increased need of glucose (Warburg effect) and glutamine ('glutaminolysis'). Correspondingly, most cancer cells overexpress glucose and glutamine transporters (GLUT1 (SLC2A1) and ASCT2 (SLC1A5), respectively). In this study, the anticarcinogenic effect and the effect on glucose and glutamine uptake of several distinct phenolic acids (caffeic, ferulic, protocatechuic, p-coumaric and gallic acids) was assessed. This class of non-flavonoid polyphenols has yet to be extensively studied.
Methodology: The human breast adenocarcinoma cell line (MCF-7 cells) was exposed to three distinct concentrations of the phenolic acids (1, 10 and 100 μM) for 24 h, and migration, proliferation, culture growth, viability and glucose (by using the glucose analogue ^{3}H-deoxy-D-glucose (^{3}H-DG)) and glutamine (by using ^{3}H-L-glutamine (^{3}H-GLN)) cellular uptake were determined.
Results: Ferulic acid stimulated cell migration, while protocatechuic and gallic acids decreased it; ferulic acid inhibited, and protocatechuic acid increased cell proliferation and caffeic, ferulic and gallic acids diminished culture growth, while protocatechuic acid augmented it. In the nutrient uptake experiments, protocatechuic acid reduced ^{3}H-DG and ^{3}H-glutamine uptake (in the presence of sodium), and caffeic and gallic acids also inhibited ^{3}H-glutamine uptake, but in the absence of sodium. On the other hand, gallic and ferulic acids increased ^{3}H-DG uptake, and caffeic acid increased ^{3}H-glutamine uptake in the presence of sodium.
Conclusions: This study proved that these natural compounds can have several effects on MCF-7 cells, worth of being further studied as anticarcinogenic compounds.
Keywords: MCF-7; Breast Cancer; Phenolic Acids; Polyphenols

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9 – Cell culture models for bone metabolism

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Background: A variety of conditions can affect the bone tissue, including osteoporosis and imbalance on inflammatory and hormonal systems. *In vitro* approaches are useful models to study bone metabolism, allowing the evaluation of bone cells proliferation, differentiation and functional activity for research purposes in a variety of experimental settings. The immortalized cell lines allow the study of particular stages of osteoblast phenotype and has several advantages to the primary cells. The aim of this study was to assess the behavior of Saos-2 and MG-63 osteoblastic human cell lines in basal and osteogenic conditions for future studies.
Methodology: Saos-2 (ATCC® HTB-85™) and MG-63 cell lines (ATCC® CRL-1427™) were maintained on McCoy's 5a and E-MEM medium respectively, supplemented with 10% FBS, 100 IU/mL penicillin, 100 µg/mL streptomycin and 2.5 µg/mL amphotericin B (basal medium). Cells were seeded at 5 x 10^3 cells/cm^2 (Saos-2) and 2.5 x 10^3 cells/cm^2 (MG-63) density in basal or osteogenic medium (basal medium supplemented with 50 µg/mL ascorbic acid, 10 mM sodium β-glycerophosphate and 10 nM dexamethasone) for 10 days. MTT assay, total protein, alkaline phosphatase (ALP) activity were performed, as well as histochemical staining for ALP and collagen.
Results: The proliferation ratio of MG-63 cells was higher than that of Saos-2 cells. In both cell lines, the osteogenic medium significantly increased proliferation at days 3 and 7 when compared to basal. Regarding cell differentiation, Saos-2 cells presented much higher ALP activity compared to MG-63 cells and levels were similar in osteogenic and basal media. However, the osteogenic medium significantly induced ALP activity on MG-63 cells from day 3 onwards.
Conclusions: The selection of an appropriate cell model is crucial for responding to a research question. Saos-2 and MG-63 cell lines have different cell proliferation rates and ALP activity and they respond in a different way to the osteogenic stimulus.
Keywords: *In vitro* cell models; Osteoblastic cell culture; bone metabolism

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10 – Characterization of the Metabolic Requirements in *Drosophila* Neural Stem Cells

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Background: To form the correct cell type and number at a specific time of animal development, stem cells must be tightly regulated in time and space. The Drosophila central nervous system is formed by neural stem cells called neuroblasts (NBs), that divide asymmetrically to generate all neurons and glial cells of the adult fly. At pupal stages, NBs exit cell cycle and disappear. It was found that the timely disappearance of these stem cells is regulated by a metabolic shift, which increases the levels of oxidative phosphorylation. These results suggest that metabolism has an important role in stem cell regulation. However, how fate and metabolism are interconnected is still poorly understood.
Methodology: In this work, we use NBs to explore the role of metabolism in the regulation of stem cell fate and proliferation. We combine whole brain live imageing and fluorescent metabolite biosensors to assess neuroblast metabolic state.
Results: We are developing and optimizing several genetically encoded fluorescent biosensors for key metabolites which will allow us to follow metabolism with high spatiotemporal resolution. Taking advantage of such genetic tools we are characterizing how metabolism changes throughout NB lineage to understand whether cells with different cell identities have different metabolic profiles. Furthermore, we are exploring how metabolism is altered in tumors using a *Drosophila* brain tumor model. Preliminary results showed that tumor-like neuroblasts have altered levels of ATP, when compared with normal neural stem cells.
Conclusions: Overall, we expect that this approach will help reveal how metabolism is interconnected with cell fate changes in the developing brain and will in addition allow us to understand what is the role of metabolism in several disease contexts, as in tumors.
Keywords: Metabolism; Stem cell; *Drosophila*; Biosensors

11 – Cardiovascular responses in elderly hypertensive women after a resistance training session with different movement speeds

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Background: The purpose of the present study was to compare the cardiovascular responses after a resistance training session (RT) with different velocities of movement; slow movement velocity (SMV) and traditional movement velocity of (TMV) and to analyze the rate perceived exertion (RPE) at both contraction velocities in elderly hypertensive women.
Methodology: Eleven elderly women (66.5 ± 4.8 years) active and hypertension controlled by medication after a week of familiarization with the exercises and tests for maximum load prediction were randomly submitted for a RT session with SMV or traditional. The sessions consisted of three sets of ten repetitions with 60% of 1RM. Systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR), mean arterial pressure (MAP) and frequency-pressure product (PFP) were measured pre-exercise, immediately, 5, 10, 15, 35, 45 and 60 minutes after the sessions. Finally, the rating of perceived exertion (RPE) was made at the end of each series of RT.
Results: Both protocols caused similar increases in cardiovascular responses immediately at the end of exercise, post-exercise supervision showed more pronounced reduction in SBP, HR, MAP, and PFP ($p < 0.05$) in SMV compared to baseline values, however there were no significant differences in post-exercise hypotension (PEH) between contraction velocities ($p > 0.05$. VLM training induced a greater RPE ($p < 0.05$).
Conclusions: We conclude that SMV and TMV promoted post-exercise hypotension in elderly hypertensive women under medication.
Keywords: Ageing; Resistance training; Post-exercise hypotension; Hypertension.

12 – Nutritional status, readmission and death in elderly octogenarian patients admitted to intensive care units

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Background: Over the past decades had an increase in the proportion of elderly people. Older age is a risk factor for the occurrence of several diseases, especially chronic noncommunicable diseases, furthermore, this group occupies most of the hospital beds, present a higher risk of complications, hospital readmissions and malnutrition. The aim of this study was to evaluate the nutritional status, readmission rate with death in octogenarian critically ill elderly patients.
Methodology: Retrospective clinical study, initially performed with 1315 elderly patients, of which 318 (24%) were ≥80 years old and were part of the study. Outcome variables were: nutritional status at admission and outcome, ICU readmission rate with death of the population studied. To as-

sess nutritional status, the Global Subjective Assessment (SGA) was used. Readmission was considered to be those patients coming from the hospital itself, from home care or transferred from another hospital unit. It was evaluated the association between nutritional status and readmission rate with the occurrence of death.
Results: The average age was 85.4 ± 4.5 years, of which 200 (62.4%) were female. The data showed that there was a worsening in nutritional status in the percentage of severe malnourished patients during hospitalization (24.2% *vs.* 31.4%; p <0.001). The readmission and death rates were 33% (n = 115) and 30.4% (n = 96), respectively. Severe malnourished elderly people were twice more likely to die than those without this nutritional status (61.4% *vs.* 38.5%; OR 2.17 95% CI 1.3 - 3.55; p = 0.002). The data also showed a significant increase in the chance of death in those patients who were readmitted (39% *vs.* 25.8%; OR 1.8 95% CI 1.12-3.02; p = 0.016).
Conclusions: About a quarter of critical octogenarian elderly hospitalize severely malnourished, the readmission rate was 33% and there is an increased chance of death for these severely malnourished and readmissioned patients.
Keywords: Octogenarians; Intensive care units; Critically ill patient

13 – Low skeletal muscle function, but not mass, is associated with the presence of type 2 Diabetes

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Background: The pathophysiology of type 2 Diabetes mellitus (T2DM) is intimately connected to the skeletal muscle (SkM). SkM affects insulin resistance and is, in turn, affected by the metainflammation, microvascular disease and ectopic fat deposition of T2DM. SkM mass can be inferred by the waist-to-calf ratio (WCR) and its function by the Short Physical Performance Battery (SPPB). The aim of this study was to determine the association between SkM mass and function with T2DM in patients with Metabolic Syndrome (MetS).
Methodology: Patients with MetS, aged 18 to 75 years-old, attending an outpatient clinic from April 15th to September 30th 2019, were consecutively included. Exclusion criteria comprised type 1 Diabetes, secondary hypertension, active neoplasia, autoimmune disease, HIV or hepatitis virus B or C infection and end-stage renal disease and/or liver disease. History and anthropometric data were collected, including weight, height, waist circumference (WC) and WCR; the SPPB was applied.
Results: A total of 81 patients were included, of which 58.0% had T2DM; most patients were female (55.6%) and the median age was 65 (interquartile range 16.5) years. Patients with T2DM were older (64.1 *vs.* 56.5 years, p=0.001) and more likely to have concurrent hypertension (96% *vs.* 65%, p<0.001) and dyslipidemia (96% *vs.* 56%, p<0.001). In univariate analysis, WC [odds ratio (OR) 1.1, 95% confidence interval (CI) 1.0-1.1), WCR (OR 146.2, 95% CI 9.9-2159.0) and SPPB (0.6, 95% CI 0.4-0.8) were associated with T2DM. In multivariate analysis, only SPPB maintained its association (OR 0.65, 95% CI 0.44-0.97).
Conclusions: Poorer muscle function, as determined by the SPPB, was associated with the presence of T2DM, even when considering body composition, per WCR. Longitudinal and mechanistic studies are warranted to best characterize this relationship.
Keywords: Diabetes; Sarcopenia; Muscle function; Muscle mass

14 – Acute effect on glycolytic involvement after resistance training sessions differing in set configuration

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Background: Resistance training (RT) is currently recommended by health organizations as a significant component of a healthy fitness lifestyle and many prevention programs for several diseases[1]. Loading parameters can be manipulated in different ways, providing different effects. In order to prescribe RT with safety and maximal efficiency, the effects of loading parameters should be clarified. A parameter that could modulate the RT effects is the set configuration. Set configuration refers to the repetitions actually performed with regard to the maximum possible number of repetitions in a set[2]. The aim of this study was to analyze the acute effects on glycolytic involvement of two RT sessions differing in set configuration.
Methodology: 41 sport science students (29 males, 12 females) performed in a random order two RT sessions (leg extension, leg curl, lat pull, bench press and parallel squat).Experimental sessions were equated for intensity, volume and total resting time (40 repetitions with the 15RM load with 360 sec of total resting time between sets for each exercise) and differed by set configuration: 4 sets of 10 repetitions with 2 min-rest between sets (Traditional Session: TS) and 8 sets of 5 repetitions with 51 sec-rest between sets (Cluster Session: CS). In both training sessions, 3 min-rest between exercise were performed. Before and after the sessions, capillary blood lactate concentration (BL) was obtained and a two-way repeated-measures analyses of variance (ANOVA) was used to evaluate the effect of session (TS or CS) and time (Pre-Post) on lactatemia.
Results: For BL, main effects of time and session and time x session interaction were found (p<0.001). After TS and CS, higher values were observed compared with baseline values. For comparison between values after RT, TS showed higher values in comparison with CS (p<0.001).
Conclusions: These data shown that both RT sessions increased BL. However, the BL increments are mitigated by CS. In conclusion, set configuration can modulate the glycolytic response after RT sessions.
Keywords: Set configuration; Cluster configuration; Resistance training; Glycolytic involvement; Blood lactate

15 – Obesity/type 2 diabetes *mellitus* biomarkers induce changes in nutrient transport that can promote breast cancer progression

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Background: Obesity and type 2 diabetes mellitus (T2DM) associate with increased incidence and mortality from many cancers, including breast cancer. We investigated the effect of obesity/T2DM biomarkers (hyperinsulinemia, hyperleptinemia, and increased levels of inflammation and oxidative stress) upon uptake of two important nutrients (glucose and glutamine) by breast cancer cells.
Methodology: Breast cancer cells (MCF-7 and MDA-MB-231) were exposed to high levels of the oxidative stress inducer *tert*-butylhydroperoxide (TBH; 0.5-2.5 μM), insulin (1-50 nM), leptin (10-500 ng/ml) and pro-inflammatory cytokines (TNF-α or INF-γ; 1-100 ng/ml) for 24 h, and 3H-deoxy-D-glucose (^{3}H-DG) and ^{3}H-glutamine (^{3}H-GLN) uptake (6 min) were then quantified.
Results: TBH, insulin and INF-γ induced a concentration-dependent increase in 3H-DG (10 nM) uptake in both cell lines, and leptin only in MCF-7 cell line. By examining the influence of a selective GLUT1 transport inhibitor (BAY-876 500 nM) on the stimulatory effect of these compounds, we con-

clude that TBH, insulin and INF-γ (MCF-7 cells) and TBH, insulin and leptin (MDA-MB-231 cells) stimulate GLUT1-mediated uptake of 1 mM ^{3}H-DG, but stimulate non-GLUT1-mediated uptake of 10 nM ^{3}H-DG. Na+-dependent ^{3}H-GLN uptake increased in the presence of INF-γ (in MCF-7 and MDA--MB-231 cells) and TBH (in MDA-MB-231 cells only); in contrast, Na^{+}-dependent ^{3}H-GLN uptake was reduced by insulin and leptin in both cell lines. By examining the influence of an inhibitor of the Na+-dependent carrier ASCT2 (GPNA 1 mM) on the stimulatory effect of these compounds, we conclude that INF-γ (in MCF-7 and MDA-MB-231 cells) and TBH (in MDA-MB-231 cells) stimulate ASCT2-mediated uptake of 5 nM ^{3}H-GLN, but stimulate non-ASCT2-mediated uptake of 0.5 mM ^{3}H-GLN.
Conclusions: Some obesity/T2DM biomarkers induce changes in glucose and glutamine transport that can contribute to breast cancer progression.
Keywords: Breast cancer; Obesity biomarkers; Glucose; Glutamine

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16 – Nutritional risk screening results using NRS 2002 in Orthopaedic and Gynecologist departments in VNGaia Hospital Center-Portugal

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Background: According to ESPEN, undernutrition is "a state resulting from lack of intake or uptake of nutrition that leads to altered body composition (decreased fat free mass) and body cell mass leading to diminished physical and mental function and impaired clinical outcome from disease". It causes many metabolic changes with decreased immune system's response with implications on treatment, difficulty in healing, increased number of complications, morbidity and mortality. In the literature, in 2015, 46% of hospitalized individuals in Portugal were at nutritional risk (NR).The aim was to evaluate NR and status of Orthopaedics' and Gynaecology's hospitalized patients in VNG Hospital Centre and study the association between patients undergoing scheduled (SC) and urgent surgery (US) and the effect of hospitalization using Nutritional Risk Screening 2002 (NRS2002).
Methodology: The NRS2002 protocol was used and anthropometric evaluation through direct and indirect measurements.
Results: This sample included 134 initial screening and 49 revaluations, from Orthopaedics' and Gynaecology's departments, aged between 20 and 99 years old. In the Orthopaedics' department (OD), the study showed a positive association between the type of surgery and the period of hospitalization ($p<0,01$). When comparing the last evaluation with the first results, the difference in the average score was statistically significant ($p=0,011$) with an increasing score in the last evaluation.
The results showed that 3,7% of patients were at undernutrition risk,60% from an US and 40% for a SC.
Conclusions: The NR screening tools are needed to easily allow the identification and control the NR and undernutrition. The patients with higher risk were those submitted to US because they stay longer in hospital and,according to the literature, the nutritional status is negatively affected by the time of hospitalization. We suggest that the type of surgery should be considered in the NR protocols, especially if it is to be applied in OD.
Keywords: NRS2002; Undernutrition; Urgent surgery; Schedule surgery

17 – Food intake of women with gestational diabetes *mellitus*, in accordance with two methods of dietary guidance: a randomised controlled clinical trial

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Background: Nutritional therapy is considered relevant for glycaemic control in pregnant women with gestational diabetes mellitus (GDM). The aim of this study was to evaluate the dietary intake of pregnant women with GDM according to two dietary guidance methods.
Methodology: Randomized controlled clinical trial conducted in consultation with the nutritionist, during prenatal care of adult pregnant women diagnosed with gestational diabetes mellitus (GDM) in a public maternity hospital in Rio de Janeiro, Brazil (2011-2014). The study population consisted of adult women diagnosed with GDM attending prenatal and delivery in the studied maternity (2011-2014). The control group (CG) received nutritional counseling by the traditional method and the intervention group (IG) was instructed on carbohydrate counting. Sugar intake, processed foods (FP) and ultra-processed foods (UPF) were evaluated.
Results: 286 pregnant women (145 in the CG and 141 in the IG) were evaluated. Of the pregnant women, 211/286 (73.8%) consumed sugar in pregnancy, 89/120 (74.2%). And 183/229 (79.9%) consumed PF daily in the second and third quarters, respectively. While 117/120 (97.5%) and 225/231 (97.4%) consumed UPF daily in the second and third quarters. There was no difference between groups regarding caloric or macronutrient intake. There was a higher consumption of PF per week among pregnant women who showed low adherence to diet at the second visit in the second trimester of pregnancy ($p = 0.04$). As for the comparative analysis of sugar consumption by the groups, there was no difference between them ($p = 0.78$) and sugar consumption did not influence the adequacy of weight gain ($p = 0.37$) and birth weight ($p = 0.11$).
Conclusions: The type of dietary orientation did not affect food intake, suggesting that both methods tested can be used to monitor the nutritional status of pregnant women with GDM.
Key words: Food intake; Gestational diabetes mellitus; Nutrition therap; Carbohydrate counting

20 – Peripheral metabolism in rat with olanzapine subchronic treatment supplemented with acetyl-L carnitine

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Background: Olanzapine (Olz) have been reported to promote higher risk for metabolic side-effects, such as obesity, dyslipidemia, and diabetes. Moreover/On the other hand, acetyl-L-carnitine (ALC) has been reported to improve homeostasis. This study aims to explore if ALC could improve metabolic homeostasis due to Olz effects on a rodent model.
Methodology: We investigated the effects of subchronic administration of Olz and with the addition of ALC on body weight gain and glucose metabolism in Wistar rats.
Results: Mid-term Olz exposure in rats significantly increases blood glucose levels in fasting and after the overload of glucose compared to the sali-

ne-treated group ($p<0.05$). However, in rats mid-term treated with olanzapine plus acetyl-L-carnitine had no significant differences in fasting blood glucose levels compared to the saline-treated group.
Conclusions: The findings suggest that ALC added to Olz treatment appeared to be effective to improve glucose metabolism during olanzapine subchronic treatment
Keywords: Olanzapine; Acetyl-L-carnitine; Glucose homeostasis; Locomotor activity

21 – Maternal uterine ageing and placentation

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Background: Uterine redox imbalance may lead to deficient placentation and development of pregnancy-related complications, with increased incidence in older women. Thus, it was aimed to identify age-related oxidative modifications to uterine proteins and study their involvement in placentation.
Methodology: Uterine samples were collected after elective caesarean section. The protocol was performed with ethical approval and volunteers written consent. Protein carbonylation was detected by OxyBlot and albumin carbonylation was verified by immunoprecipitation. HTR-8/SV neo trophoblast cell line was used for *in vitro* studies. The effect of *in vitro* carbonylated albumin (CHSA) on cell viability, proliferation and adhesion was quantified with neutral red. Scratch assay was used to evaluate cell motility and collagen-coated transwells invasion. Signaling proteins involved in cell stress were analyzed by western blot. Statistical analysis was performed with Spearman correlation or ANOVA.
Results: At placental bed, carbonylated albumin correlated strong and positively with maternal age. *In vitro* results showed that CHSA did not affect cell viability or proliferation. However, at the concentration of 100 μM, CHSA reduced significantly cell motility, induced an upregulation of SOD2 expression and triggered unfolded protein response, as shown by an increase in PERK expression and phosphorylation of eIF2α. Additionally, CHSA cross-linked to a collagen matrix reduced significantly trophoblast adhesion and invasion capacity.
Conclusions: Maternal ageing is accompanied by selective albumin modifications, that may have a deleterious role in placentation.
Keywords: Maternal ageing; Placentation; Albumin; Carbonylated albumin.

22 – Leukocyte telomere length and *hTERT* genetic polymorphism rs2735940 influence renal cell carcinoma clinical outcome

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Background: The ageing process is a multifactorial process that varies among individuals. It is characterized by the decrease of physiological functions of the human body and described as one of the main risk factors for many age-related diseases, like cancer. Telomere length has been widely described as biological age biomarker. In cancer, the role of Leukocyte Telomere Length (LTL) as biomarker of prognosis was already suggested, but in Renal Cell Carcinoma (RCC) the results are controversial. Differences in LTL among individuals may be due to the occurrence of genetic polymorphisms like *hTERT-1327C>T*(rs2735940), that occurs in the promoter region of the telomerase gene. This study aimed to investigate the effect of *hTERT-1327 C>T*(rs2735940) in LTL and in RCC progression-free and overall survival.
Methodology: The study was conducted according to the Helsinki Declaration. Using leukocyte DNA samples of RCC patients and healthy individuals, LTL was measured by quantitative real-time PCR. Moreover, *hTERT-1327C>T* genetic polymorphism was analyzed by allelic discrimination using real-time PCR technique.
Results: LTL in RCC patients was shorter than in healthy individuals ($p<0.001$). Among patients, LTL was longer in patients with T3/T4 tumors ($p=0.044$) and in patients with tumors larger than 7 cm ($p<0.001$). CC homozygous presented reduced time-to-progression (HR=1.63, $p=0.048$) and lower overall survival (Log Rank test, $p=0.019$). The use of this genetic polymorphism increased the capacity to predict RCC progression's risk (c-index model: 0.770).
Conclusions: LTL seems to have a role in carcinogenesis and *hTERT-1327C>T* influences LTL, progression-free interval and overall survival. We propose that the assessment of *hTERT-1327C>T* may be useful, in the future, as potential prognosis biomarker in RCC. This reinforces the role of telomeres in ageing and in age-related diseases.
Keywords: Leukocyte telomere length; Ageing, Renal Cell Carcinoma; Genetic Polymorphism; Prognosis; Biomarkers