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# 5.º Simpósio em Metabolismo da Faculdade de Medicina da Universidade do Porto
## – Comunicações Orais e Posters


### > COMUNICAÇÕES ORAIS

**Exercise Throughout Life**

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The involvement of children and young people in sport has been to deserve increasing attention in different areas of research that support the sport sciences.

In spite of the interest in "children in exercise", we can find a"gap" between theory and practice, this means, a large contradiction betweenwhat one knows and what one understands.

For example, where is the evidence of the harmful effects of intense training in children? Its effects will bereversible orirreversible? Children are more susceptible to over stress injuries? Early specialization shortens the sporting career of an athlete? This set of questions could be widely extended, I would say, almost infinitely. Moreover, it is precisely the awareness that there is more ignorance than belief, the American College of Sports Medicine recently developed a framework aiming to the intensification of research in this age group.

The ethical limitations of research in children have been identified as strongly inhibiting the progress of knowledge in this topic.

And with regard to aging?

The decline in muscle strength that occurs with aging is one of the most widely studied phenomena relating to physiological changes induced by age.

In addition to observing the decrease in muscle strength with age, research has also been addressing the possible causes of these changes in functionality. The results obtained in several investigations of sarcopenia and aging led to the conclusion that there is a direct influence of age on muscle composition .Thus , in the age group of 24 years, the percentage of type I fibers was 49 % at 52 years was 52 % and 51 % at age 77. These results demonstrate an atrophy process induced by age and it can be concluded that the decrease in muscle mass is evenly distributed by the reduction in the number of fibers I and II .

Regarding the number of fibers, the literature has been unanimously considered aging as inducing muscle hypoplasia. The decrease in fiber area attributable to age, begins from 35-40 years progressively increasing loss of muscle mass until about the 5th decade , showing a very marked atrophy in individuals over 70 years .

The decrease in the area of type II fibers is about 26 % between 20 and 80 years. However, when evaluation is made on the basis of the subtypes of fibers II (IIa and IIb), the reduction is 14 and 25%, respectively, for IIa and IIb. In other words, there seems to be an increased susceptibility to atrophy of the fibers faster.

This preferential reduction in type II fibers will lead to the decrease in muscle mass and consequently in a decrease in muscle strength seen with aging.

In conclusion, we can say that we can find different metabolic needs in different ages in spite of the lack of sufficient and reliable results on these topics.

**Fetal Programming: How the Nine Months Before Birth Will Influence the Rest of Our Lifes**

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The *"developmental origins of adult disease"* hypothesis, also called the *"Barker hypothesis"* states that adverse influences early in development, and particularly during intrauterine life, can result in permanent changes in physiology and metabolism with increased disease risk in adulthood. Many studies provided evidence that reduced birth weight, probably reflecting impaired fetal growth, is related to the risk of developing coronary heart disease, diabetes, hypertension and stroke in adulthood. These relations are modified by patterns of postnatal growth. Despite initial concern that bias or residual confounding in the analyses had produced these associations; the findings have now been reproduced in different cohorts by independent investigators from all around the world.
The challenge now is to discover which mechanisms underlie these associations. The most widely accepted are those of fetal programming by nutritional stimuli or excess fetal glucocorticoid exposure. It is proposed that the fetus makes physiological adaptations in response to those stimuli, preparing itself for similar conditions in postnatal life. These changes may include epigenetic modifications of gene expression.

**Ageing: A Biological and Metabolic Perspective**

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Ageing is the result of multiple factors that lead to a progressive increase in damages to cells and tissues that are associated with the risk of disease and death. Within the different hypothesis to explain the mechanisms of ageing, it is well recognized that ageing is associated with the mitochondria production of reactive oxygen species leading to an oxidative stress [1]. Aged cells display a decrease in antioxidant defences that favours the accumulation of ROS that are able to damage cellular components contributing to cell ageing and death. A biomarker for ageing is the presence of oxidative modified molecules such as an increase of oxidised proteins and lipids. The importance of diet in the ageing process has been disclosed from the experiments showing that calorie restriction diet or down-regulation of nutrient signalling pathways increase mitochondrial fitness and longevity in different model organisms. Indeed, aged animals accumulated oxidised proteins that form aggregates and calorie restriction can decrease the levels of the damaged proteins either by slowing down protein synthesis or by stimulating protein degradation. This is in agreement with the fact that lowering mitochondrial reactive oxygen species production increases longevity. The molecular mechanisms associated with longevity are discussed namely the effect on cellular metabolism [2].

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**Drug Therapy and Food Interactions: Problems in Geriatrics**

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Drug-nutrient interactions are the consequence of the growing importance of drug therapy in modern medicine, of the obvious need for the human being to be fed and of the great complexity of biological systems in the human body. Thus, these interactions can be as simple as a mere chemical neutralization of two molecules, drug and nutrient, in the gut, or as complex as the interference of nutritional status in the pharmacokinetics of several compounds with pharmacological properties. The use of certain food supplements, considered broadly (and by EU regulations) as food products, also falls within the scope of drug-nutrient interactions, since not only some of them show pharmacological effects but also because there is a considerable amount of scientific information that reports their potential to interfere with drug therapy.
In the great majority of cases, these mutual interferences are harmful to the patients, either to the efficacy of the prescribed therapies or to the nutritional status. Therefore, it is of utmost importance to take these effects into account when considering the therapeutic strategies in each individual and this gains an even higher importance when we are dealing with the elder. Older individuals are more susceptible to this type of problems by several reasons, which include the higher probability of being polymedicated due to accumulation of chronic diseases throughout life, the progressive reduction of renal and hepatic function that result in a diminished capacity to metabolize and eliminate drugs and the often-observed degradation of the nutritional status.
Therapeutic groups such as gastric acid secretion inhibitors, antidiabeticbiguanides, anticoagulants such as warfarin, drugs acting in the central nervous system like antidepressants, antipsychotics or levodopa, statins, immunosupressants and antiretrovirals, among others, all share the property of either interfering with normal food intake or nutritional status or being affected in their actions by certain foods or nutrients.
A comprehensive review of data will be provided, underlining the paucity of robust scientific data on some of these issues and the importance of cooperation and true multidisciplinarity in patient's management to better avoid the negative impact of drug-nutrient interactions in the elderly.

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**Prevalence of Metabolic Diseases Throughout Lifespan**

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Obesity is the paradigm of the diseases of modern civilization. Few translate so clearly asymmetries and inequalities which characterize

the present world and that strike generally. Since the industrial revolution, the development model aimed at the creation of the industrial city that was synonymous with progress from 1850 to the late twentieth century. But the city changes its scale and expelled many activities of the center. The suburb becomes the city faceless and without references. Consumerism is the symbol of suburbia. The automobile, television and junk food are the exponents of this consumerism. From the most sensationalist elements of the media to researchers and medical associations, all classify the obesity condition as impending disaster. For the first time, the burden of obesity resulted in the reversal of the age-old tendency to increase the survival of mankind: today's children will die sooner than their parents. In addition to the more serious health problem of the XXI century, obesity is primarily a socio-political problem. Obese people are poorer, more disadvantaged and lower socio-cultural level. For this reason only corporate action can solve this problem. The training of all health personnel contributes to creating a mindset that accepts most difficult but probably more effective corporate action. Taxing energy-dense foods, restrict advertising of junk food and boost the frequency and intensity of physical education in schools are some of the most important steps to take. These sociopolitical measures require the collaboration of all partners inside but especially outside the health field.

**Evolution by Natural Selection or Pollution**. The life of the species "homo sapiens" has evolved immensely over its existence and particularly in the last 150 years. The first major change was the doubling of life expectancy: rose from about 40 years to the current 80. In terms of physical activity, we change from a work centered on the ability of the physically active man to the widespread use of machines for performing most of the tasks. From the nutritional point of view we move from hunter - gatherer to industrial food production, from the industrialization of agriculture to the food preparation industry. In all these years of evolution, we are witnessing an increase of the determinants of obesity.

The thrifty genotype concept was originally developed by Neel from the study of inhabitants of Polynesia. During famine periods, mostly survived the inhabitants able to produce the same work to collect food and reproduction of species with a lower energy expenditure . During periods of starvation, less spenders are unable to survive. Individuals selected for their ability to store energy in grace periods, exposed to abundant food and a more sedentary lifestyle trigger obesity. Knowing that there is not only thrifty gene but a constellation polygenic pledge of this evolution, the concept has remained obstinately.

The importance of endocrine disruptors as causes of obesity has recently been recognized. Organochloride are lipo-soluble chemicals resistant to degradation, accumulated in the adipose tissue of virtually all planetary bodies including man. Obese individuals appear to have higher plasma concentrations of organochlorines than skinny. During weight loss, lipolysis and decreased fat mass result in higher concentrations of organochlorines in plasma and remaining adipose tissue. Organochlorides may decrease concentrations of T3, basal metabolism and lipid oxidation by skeletal muscle. Although requiring confirmation and clarification, these endocrine disruptors may limit or hinder weight loss.

**Extent of the Problem.** The prevalence of obesity in our country is high and has been increasing: when comparing the prevalence of obesity and pre-obesity from 1995 to 2005 an increase of 49.6 to 52.3 %, this increase is at the expense of pre- obese women passing from 35.2 to 38.4 %, and of men passing from 54 to 59.3%. Regarding abdominal obesity (AC) associated with increased risk of other risk factors (in women waist circumference > 80 cm in men and > 94 cm) the prevalence of 43.6 for women and 51.1 % for men. AC > 88 cm for women and 102 cm for men (very high risk) prevalence was 20.1 and 26.7 % respectively. If these figures are alarming, the gravity is extreme if we think of childhood obesity: Prevalence is 12.3 % for children from 2 to 5 a, 14.6 from 6 to 8, and 14 % for the 11 to 15 a. If we think of obesity and pre -obesity numbers are equally chilling: one third of children are obese and overweight: 2 to 5 years of 35.5 % (34.8 % for girls and 35.2 for boys), from 6 to 8 years and 42.2% of 11 to 15 years 33.9 % ( 32.7 % for girls and 35.3 for boys). The importance of the situation is echoed in diseases: diabetes prevalence is 12.9 %, and 14.2 in men and 9.55 in women. According to age distribution we have a prevalence of 2%, 12.7 and 27% respectively from 20 to 39 years, 40 to 59 years and from 60 to 79 respectively. When we think about costs, increased consumption of oral antidiabetic drugs increased 31.4 % from 2000 to 2007 clearly suggests that it is worth investing in the prevention of obesity to prevent diabetes, hypertension, dyslipidemia and last illness cardiovascular. In what concerns hypertension, the prevalence is 42.7% in PAP study. A similar figure was recently obtained in PHYSA study, with an increase with age. Regarding dyslipidemia, in WRisk study, the prevalence of hypercholesterolemia is 37.8%, 38.7% for men and 35.1% for women. The prevalence is bigger in man 21.1% compared with 13.3 in women. The overall prevalence is 17.4%. Both dyslipidemia increased with age.

Obesity is the *primum movens* of other civilization diseases.

Obesity, especially visceral, is associated with several cardiovascular risk factors including dyslipidemia, hypertension and hyperglycemia. Of course there are more than 80 years that the concept of this constellation has been recognized and has received various names, such as metabolic syndrome, syndrome X, insulin resistance syndrome, or deadly quartet. Not only the name but the criteria for the syndrome have been variable. In the VALSIM trial using NCEP-ATPIII criteria the prevalence in Portugal is 27.5%. The relevance is that fighting these diseases other passes in 1st place for the prevention of obesity.

## Sensitive Periods and Development of Healthy Eating Habits Early in Life

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Sensitive periods represent stages when the organism is particularly susceptible to effects of environmental influences. Evidence suggests that food habits acquired in early childhood may persist through adulthood. Therefore, tracking early experiences that may influence the development of healthy eating habits in infancy is important to improve dietary intake and the prevention of unhealthy eating.

Infancy is a period of rapid growth with important changes in nutritional requirements and feeding practices, changing from breast or formula milk, to a familial diversified diet.

In utero environment is important as a milieu that modulates infant growth and childhood obesity, and studies also focus on this developmental stage as a critical period for flavor learning. Birth weight and size at birth are often looked as the outcome of the environment that occurred in utero, being the extremes in birth weight associated with higher risk of obesity. Maternal characteristics such as high pre-pregnancy BMI and pregnancy weight gain, and inadequate maternal nutritional status may also increase the offspring risk of obesity.

The food environment is another important factor to shape children's

food experiences, preferences and knowledge about eating, and may depend on several characteristics such as food availability, feeding rules, role modeling, culture, and parents' food preparation skills.
In conclusion, understanding the role of children's early exposure to foods (when, what, and how) with different sensory and nutritional characteristics (eg, salt), encompassing in utero and nursing periods of growth, parenting styles and practices, will be crucial to provide the foundation for nutrition education and healthy eating.

**Generation XXI: Nature and Culture in Designing the Future**

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Early-life exposures may affect growth and development in fetal life and in childhood, and health across the life course. Identification of intrauterine and early life key causal exposures and the pathways through which they influence prognosis, as well as effective methods for preventing their adverse effects might benefit both the individual and the society. Developed countries commonly use birth cohort studies to understand most of such influences in adult diseases. Generation XXI (Geração XXI), designed to study birth and growth in the new millennium, and the only such study ever conducted in Portugal, provided: a) maternal and child health indicators, mainly those which are not available in a routine basis, as the proportions of smokers during pregnancy or breastfed children; b) the identification of genetic and environmental factors (and their interactions) playing a role on the fetal and early childhood development; c) estimates of the association between parental characteristics, prenatal and post-natal development and anthropometric and biologic childhood characteristics; d) information on the access, the use and the contents of the health care services and clues to their adequacy.
Women with a live birth (8495 women, 8647 children) were recruited from the five Portuguese public hospitals serving the region of Porto (April/2005–September/2006). The overall aim of this cohort was to identify characteristics during pregnancy and infancy witch influence development and health in later stages of life. Accompanying this cohort makes it possible to study the evaluation of health parameters (such as social, behavioral, organizational, biological) in order to discover an d understand the consequences of the prenatal phase of life as well as the first years of life on the health of the subjects during adolescence and adulthood.
Some examples:

1- The positive association between socio-economic position at 12 years of age and later cesarean rates suggests the influence of socioeconomic circumstances early in adolescence in the decision-making process concerning the mode of delivery, but only among women who experienced a previous cesarean section. Instead, among the other women, there was no evidence of a social gradient for cesarean rates. Accordingly, early-life socioeconomic circumstances drive cesarean rates but the effect can be modified by lived experiences concerning childbirth;
2- Overall, 47.4% of women who smoked ceased smoking and 41.7% reduced cigarette consumption during pregnancy. Four years after delivery, 32.1% of those who stopped smoking during pregnancy continued to abstain. Older women, first-time mothers, light smokers, those who were living with a partner at the time of follow-up, those who became pregnant again after the index pregnancy, those who breast fed for more than 52 weeks, and those with a child diagnosed with asthma and/or rhinitis were more likely to abstain from smoking;
3- Childhood growth is of interest in medical research concerned with determinants and consequences of variation from healthy growth and development. Linear spline multilevel modelling is a useful approach for deriving individual summary measures of growth, which overcomes several data issues to model individual trajectories of length/height and weight;
4- Parental child-feeding attitudes and practices may compromise the development of healthy eating habits and adequate weight status in children. Younger mothers, less educated, with poorer health perception, and with other sons, were associated with higher use of "pressure to eat". Maternal socioeconomic indicators and family environment were more often associated with perceived monitoring and pressure to eat, while maternal health-behavioral characteristics were mainly associated with restriction. The consumption of energy-dense foods at young ages is negatively associated with the diet quality of children a few years later. Consumption of energy-dense foods and weight status showed tracking in time, but energy-dense foods ingestion at 2 years was not associated with subsequent weight status.
5- Serum anti-H. pylori IgG in children between the ages of 4 and 5 years, and information on child day-care attendance since birth was collected. The prevalence of H. pylori infection was 30.6% (95% CI 27.9-33.6), and it increased significantly with the cumulative time of attendance in day-care centers/homes (from 13.2% among never attendees to 40.2% among those attending for >36 months; *P* for trend<0.001). The odds ratio was 4.88 (95% CI 2.55-9.35) among those attending these institutions for more than 3 years, in comparison with never attendees. Child day-care attendance increases the risk of infection, making this setting a target for preventive measures.

## > POSTERS

**Familial Pseudoxanthoma Elasticum with Nephrocalcinosis: A Case Report**

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Pseudoxanthoma elasticum (PXE) is an autosomal recessive genetic disorder characterized by progressive calcification and fragmentation of elastic fibres. PXE commonly involves the reticular dermis, the Bruch membrane of the eye, and blood vessels. PXE is caused by mutations in the ABCC6 gene. More than 300 pathogenic ABCC6 mutations are known. Two of these mutations are common: p.R1141X in exon 24, with a prevalence of 30%, and the Alu-mediated deletion of exons 23 to 29 (EX23_29del; p.A999_S1403del) found in 10-20% of patients. Homozygosity is rare.
A 40-year-old female with a previous diagnosis of PXE was admitted in Nephrology Outpatient Clinic for nephrocalcinosis. She has two sisters,one of which also has a diagnosis of PXE and nephrocalcinosis. Physical examination revealed the presence of typical skin and ocular

abnormalities. Microscopic and gross hematuria was reported in both affected sisters. Abdominal ultrasound confirmed bilateral corticomedullar nephrocalcinosis. Calcium and phosphorus levels in blood and urine were normal. Hyperparathyroidism, renal tubular acidosis, hypervitaminosis D and hyperoxaluria were excluded. Renal biopsy showed only minor glomerular abnormalities. Medullary sponge kidney was identified by excretory urography.

Genomic DNA was used as a template for PCR amplification of the region spanning introns 22 to 29 of ABCC6 [Pfendner *et al*., J Med Genet 2007;44:621-8]. The oligonucleotide cocktail used generated a 552bp PCR product for the normal sequence, and a 652bp product for the deletion mutation.

Both sisters with PXE were homozygous for the EX23_29del mutation. The third sister did not carry this deletion.

There are occasional reports of diffuse visceral calcifications (testis, mammary gland) in PXE. PXE-associated nephrocalcinosiswas previously notedin four patients belonging to different families. This is the first report of familiar co-occurrence of PXE and nephrocalcinosis with medullary sponge kidney. These sisters' peculiar phenotype could be due to their unusual genotype, or other shared genetic and environmental factors.

### Type 1, but Not Gestacional Diabetes, is Associated With an Increase in Placental Oxidative Stress and Antioxidant Capacity

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Diabetes *mellitus* is one of the most prevalent metabolic disorders affecting pregnant women. Both gestational (GDM) and type 1 diabetes (T1D) are associated with perinatal complications and later in life metabolic diseases for the newborn (eg. obesity, type 2 diabetes and metabolic syndrome). Whilst oxidative stress has been recognized as a potential risk factor for the occurrence of these adverse pregnancy outcomes, it has been scarcely studied in human diabetic placentas. So, the aim of this work was to compare oxidative stress levels and antioxidant capacity in placentas obtained from T1D, GDM (diagnosed by the IADPSG 2010 criteria1) and uncomplicated (control) term pregnancies.

Placental villous samples were incubated in the absence or presence of the oxidative stress inducer tert-butyl hydroperoxide (tert-BOOH; 3 mM for 1 h) and levels of malonaldehyde (MDA), protein carbonyls, oxidized (GSSG) and reduced (GSH) glutathione, and the activity of Se-dependent glutathione peroxidase (GPx) were quantified.

Our results showed that biomarkers of oxidative damage to lipids (MDA) and proteins (carbonyls) were elevated in T1D, but not in GDM placentas, when compared to controls. Interestingly, third trimester fasting glycaemia in T1D women was positively correlated with MDA levels. Exposure to tert-BOOH induced an increase in MDA levels in all groups, although the relative increase was smaller in T1D (2x) than in control and GDM placentas (9 and 11x, respectively). Concerning antioxidants, the highest GSH and the lowest GSSG concentrations were found in T1D placentas, whereas both of these parameters were similar in GDM and control placentas. In the presence of tert-BOOH, the decrease in GSH levels was greater in T1D than in control and GDM placentas. Additionally, GPx activity was higher in T1D but unaltered in GDM placentas, in comparison to controls. Stratification for GDM therapy, mode of delivery and large-for-gestational age newborns did not alter oxidative stress and antioxidant levels in GDM placentas.

As a whole, we conclude that levels of oxidative stress and antioxidant biomarkers are increased in T1D, but unaltered, in GDM placentas. This suggests that T1D placentas may develop a protective antioxidant mechanism to overcome higher oxidative stress levels. Additionally, it is apparent that oxidative status in GDM and control placentas is very similar, supporting the concern that the new IADPSG criteria is likely to be classifying as GDM a large number of women that may not in fact be metabolically at high risk.

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### Modulation of VEGFR2, pERK, pAKT and ANG2-TIE2 Pathways by 8-Prenylnaringenin

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8-Prenylnaringenin (8PN) is a polyphenol and a powerful phytoestrogen with a binding affinity to estrogen receptors (ER) α three times higher than ERβ. Some studies have shown that 17β-estradiol stimulates angiogenesis. However, studies on the modulation of angiogenic process by 8PN are not consistent.

We aimed to evaluate the effect of 8PN on angiogenic pathways and verify whether or not the effect was dependent on the activation of the ER.

Human umbilical vein endothelial cells (HUVEC) were stimulated with vascular endothelial growth factor (VEGF) and treated with 10μM 8PN in the absence or presence of an ERα antagonist (MPP) or an ERβ antagonist (PHTPP). ERα and ERβ gene expression was evaluated by RT-PCR. The effect of the treatments on the expression of the receptor 2 of VEGF (VEGFR2), pAkt, pERK 1/2 and Tie2 was quantified by western blotting. Ang2 was evaluated by ELISA assay. The study of angiogenic pathways modulated by 8PN was subsequently confirmed *in vitro* by quantification of tubular structures formed by HUVEC cultivated in matrigel.

HUVEC only transcribed ERβ. 8PN, in the presence of VEGF, increased the expression of VEGFR2 (151.4 ± 23.3%), pAkt (116.1 ± 8.4%) and pERK (144.5 ± 23.9%) in a process dependent on ERβ. 8PN stimulated the release of Ang2 (122.3 ± 0.29%) and promoted the expression of its receptor, Tie2 (134.2 ± 11.2%), independently of ERβ. The matrigel assay showed a tendency to increase the formation of tubular structures after treatment with this polyphenol. The presence of PHTPP diminished this effect.

The results showed that 8PN has pro-angiogenic properties that may

be regulated by binding to ERβ, which can be interesting considering pathologies associated to poor angiogenesis, as myocardial ischemia, peripheral arterial disease and neurological diseases.

**Physical Exercise Antagonizes Heart Mitochondrial Dysfunction and Oxidative Stress in Sub-chronic Doxorubicin-treated Rats**

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The effects of two distinct chronic exercise models (endurance treadmill training – TM and voluntary free-wheel activity - FW) against alterations in cardiac mitochondrial bioenergetics, biogenesis, morphology, oxidative phosphorylation (OXPHOS) organization and activity, and oxidative stress induced by sub-chronic treatment of Doxorubicin (DOX) were analyzed.
Male young Sprague-Dawley rats were divided in six groups (n=6 per group): SAL+SED (saline sedentary), SAL+TM (12-weeks treadmill), SAL+FW (12-weeks voluntary free-wheel), DOX+SED (7-weeks of chronic DOX treatment 2mg.kg$^{-1}$ per week), DOX+TM and DOX+FW. Heart mitochondrial ultra-structural alterations, *in vitro* endpoints of heart mitochondrial function (oxygen consumption and membrane potential [ΔΨ]), SIRT3, p66shc(Ser$^{36}$)/p66shc, PGC1α, TFAM, UCP2, OXPHOS subunits, MDA, carbonyls and -SH, aconitase, Mn-SOD and in-gel OXPHOS complexes activity and organization were evaluated.
DOX affected mitochondrial morphology and function as seen by ultra-structural analysis, oxygen consumption and ΔΨ endpoints (DOX+SED *vs.* SAL+SED). Moreover, DOX compromised OXPHOS complex I activity and content, mitochondrial biogenesis (TFAM) and enhanced oxidative stress (MDA, carbonyls, aconitase, -SH, Mn-SOD, SIRT3 and p66shc(Ser$^{36}$)/p66shc ratio). TM and FW prevented DOX-induced impaired state 3 respiration, respiratory control ratio and ADP/O, ΔΨ, ADP-lag phase, OXPHOS complex I activity and content, as well as oxidative stress. DOX-induced decreased TFAM and SIRT3 expression were only prevented by TM.
In summary, both chronic models of physical exercise performed before and during the course of sub-chronic DOX treatment translated into an improved mitochondrial bioenergetic phenotype at least by regulating the enhanced oxidative stress and damage.

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**The Poliohenol Xantohumol Reduces Glucose Uptake and Affects Viability, Proliferation and Migration of a First Trimester Trophoblast Cell Line (HTR8/SVneo Cells)**

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Glucose is a major substrate for fetal and placental energy metabolism. Since gluconeogenesis in the feto-placental unit is negligible, the supply of glucose from the maternal circulation is determinant for the process of placentation.Despite such importance, glucose homeostasis in first trimester trophoblasts is still largely unexplored. So, the aim of this work was to characterize the uptake of $^3$H-2-deoxy-D-glucose ($^3$H-DG)by the HTR8/SVneo human first-trimester extravilloustrophoblast cell line, to study the effect of some xenobiotics(dietary compounds, therapeutic agents and drugs of abuse) upon this process, and to investigate the relationship between inhibition of $^3$H-DG uptake and the effect upon cell proliferation, viability and migration.
Uptake of $^3$H-DG (10 nM) by HTR8/SVneocells was: 1) time-dependent and linear for the first 10 min of incubation; 2) saturable ($K_m$= 2.9±0.5 mM and $V_{max}$=63±4.8 nmol.mg prot$^{-1}$.10 min$^{-1}$); 3) inhibited by cytochalasin B (50-100 μM), phloretin (0.5 mM) and phloridzin (1 mM); 5) insulin-insensitive; and 6) sodium-independent. This indicates that $^3$H-DG uptake by HTR8/SVneo cells is mediated by members of the GLUT family of glucose transporters (most probably GLUT1 and/or GLUT3).
Acutely (30 min), quercetin (30-1000 μM), epigallocatechin-3-gallate (EGCG; 30-1000 μM), xantohumol (XH; 1-500 μM) and resveratrol (RESV; 1-500 μM) decreased $^3$H-DG uptake. All the polyphenols were shown to inhibit both GLUT and non-GLUT-mediated $^3$H-DG uptake. XH was found to be the most potent inhibitor of $^3$H-DG uptake by HTR8/SVneo cells, with an $IC_{50}$ of 3.55 (1.37-9.20) μM. Moreover, XH (100 μM) significantly reduced the $V_{max}$ (from 63.0±4.8 to 40.5±2.8 nmol.mg prot$^{-1}$.10 min$^{-1}$) while not affecting the $K_m$ (2.9±0.5 and 2.8±0.4 mM for control and XH, respectively) of $^3$H-DG uptake. Chronically (24h), XH (5 μM) reduced $^3$H-DG uptake and decreased cell viability (lactate dehydrogenase assay), proliferation ($^3$H-thymidine and SRB assays) and migration (injury assay). The effects of XH upon cell viability and integrity were mimicked by low extracellular glucose conditions and reversed by high glucose extracellular conditions. Lastly, XH (5 μM; 24h) induced an increase in extracellular lactate levels over time, suggesting an inhibition of MCT-mediated lactate reuptake. So, this polyphenol might interfere with HTR8/SVneo cell metabolism.
In conclusion, XH potently inhibits glucose uptake by HTR8/SVneo cells and this effect is associated with a decrease in cell viability, proliferation and migration. This could have consequences in terms of glucose delivery to the fetus and upon the process of placentation.

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**Exercise Improves Liver Mitochondria Phospholipidomic Profile and Mitochondrial Activity in Non-alcoholic Steatohepatitis**

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Mitochondrial membrane lipid composition is a critical factor in non-alcoholic steatohepatitis (NASH) pathogenesis. Exercise is the most prescribed therapeutic strategy against NASH and a potential modulator of membrane architecture. Thus, we aimed to analyze whether physical exercise exerted preventive (voluntary physical activity-VPA) and therapeutic (endurance training-ET) effect on NASH-induced mitochondrial membrane structural changes. Thirty-six male Sprague-Dawley rats were divided into standard-diet sedentary (SS, n=12), standard-diet VPA (SVPA, n=6), high-fat diet sedentary (HS, n=12) and high-fat diet VPA (HVPA, n=6). After 9 weeks of diet-specific feeding, half of SS and HS group were engaged in an ET program for 8 weeks, 5d/week and 1h/day. Liver mitochondria were isolated for the measurement of *in vitro* oxygen consumption and transmembrane-electric potential. Mitochondrial phospholipid classes and fatty acids were quantified through thin layer chromatography and gas chromatography, respectively, while cardiolipin molecular profile was determined by electrospray mass spectrometry. In parallel with histological signs of liver damage, high-fat diet decreased PI, CL and PC/PE ratio, whereas PE and PA content were increased in sedentary animals. A decrease in linolelaidic acid, monounsaturated fatty acids content and an increase in saturated fatty acids (SFAS) was also observed in this group. These phospholipidomic alterations (PC/PE, SFAS) along with an improvement of mitochondrial function (respiratory control ratio, FCCP-uncoupled respiration and FCCP-oligomycin activity index) were counteracted by both exercises regimens. In conclusion, both exercise types used with preventive (VPA) or therapeutic (ET) roles preserved liver mitochondrial membrane phospholipidic profile, which is very likely to maintain mitochondrial function under high-fat input.

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**The Genetic Suppression of NRF2 Promotes Hepatic Fibrosis in a Mouse Model of Hereditary Hemochromatosis (Hfe Knock-Out)**

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HFE-associated hereditary hemochromatosis (HH) is the most common genetic disorder of iron overload among Caucasians. When untreated, it can lead to iron accumulation in tissues, especially in the liver, with secondary organ damage attributed to oxidative stress. HH patients have increased risk of developing hepatic fibrosis, cirrhosis and hepatocellular carcinoma, with symptoms often starting only in the fourth decade of life. While most HH patients are homozygous for the C282Y mutation in the HFE gene, the low penetrance of the mutation indicates that C282Y homozigosity is a necessary but not sufficient factor for disease development [1]. Organ disease develops only in a minority of C282Y homozygotes, as a result of alcohol abuse or of other genetic or environmental modifying factors that remain unidentified [2]. We hypothesized that resistance to oxidative stress may be a modifier of disease progression in HFE–HH. Transcription factor NRF2 plays a key role in adaptation to oxidative stress by regulating the induction of antioxidant/cytoprotective genes [3]. We have recently reported that iron activates NRF2 in mouse primary hepatocytes and that $Nrf2^{-/-}$ mice develop marked liver injury when fed a diet containing an excessive amount of iron [4]. The aim of this work was to evaluate if the genetic suppression of NRF2 predisposes the $Hfe^{-/-}$ mouse, a model of HH where iron deposition occurs spontaneously and gradually with age, to the development of liver damage.
Female C57BL/6 (wild-type), $Hfe^{-/-}$, $Nrf2^{-/-}$ and double knock-out (Hfe/$Nrf2^{-/-}$) mice (n=4-8) were provided standard rodent diet and water *ad libitum*. Animals were sacrificed at the age of 6, 12 or 18 months. The experimental protocol was approved by the IBMC ethical committee and by the Direcção Geral de Veterinária. We found no differences in body weight, relative liver weight, serum alanine transaminase activity, hepatic glutathione or antioxidant enzyme activity between the age-matched experimental groups. Serum iron and transferrin saturation were equally elevated in the $Hfe^{-/-}$ and Hfe/$Nrf2^{-/-}$ groups when comparing with wild-type and $Nrf2^{-/-}$ animals of the same age, and these parameters showed no variation with age. The hepatic non-heme iron content of $Hfe^{-/-}$ and Hfe/$Nrf2^{-/-}$ animals was elevated from the sixth month of age when comparing with wild-type and $Nrf2^{-/-}$ mice, and increased further until the twelfth month, after which it appeared to reach a plateau. Whilst the hepatic iron build up of aging $Hfe^{-/-}$ and Hfe/$Nrf2^{-/-}$ mice was similar, the pattern of iron distribution in the liver showed remarkable differences. $Hfe^{-/-}$ mice stored iron exclusively within the liver parenchyma, whereas in Hfe/$Nrf2^{-/-}$ animals we found an increasing accumulation of iron in macrophages of liver sinusoids and connective tissue with age. Non-parenchymal iron accumulation was associated with an increase in the number of apoptotic (TUNEL-positive) cells. In 18 month old Hfe/$Nrf2^{-/-}$ mice, there was an abundance of large aggregates of heavily iron laden sinusoidal macrophages ('siderotic nodules'). Histological and ultrastructural analysis revealed a substantial deposition of collagen fibers by myofibroblasts that were recruited towards the siderotic nodules. Hepatic fibrosis developed in Hfe/$Nrf2^{-/-}$ mice in an age-dependent manner, as supported by significant increases in fibrotic area and in the mRNA levels of collagen type I alpha 1 and alpha-smooth muscle actin. In conclusion, Nrf2 modulated the distribution of hepatic iron in $Hfe^{-/-}$ mice, as evidenced by the formation of siderotic nodules in aged Hfe/$Nrf2^{-/-}$ animals. We demonstrate that these nodules represent pre-fibrotic lesions and suggest that NRF2 status may be a modifier of disease progression towards hepatic fibrosis in HH patients.

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**Vascular Impairment Associated with Diabetic Condition**

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Diabetes *mellitus* (DM) is a common metabolic disease, affecting about 170 million people worldwide. DM is characterized by micro and macrovascular alterations impairing vascular homeostasis. Angiogenesis, the formation of new blood vessels from preexisting ones, appears as a deregulated mechanism in DM, with distinct angiogenic patterns in different organs. The same patient can present an enhancement in microvessel density (MVD) in the kidney, and a reduction in the number of vessels in heart.
This study aimed at investigating the molecular mechanisms underlying the DM related angiogenic paradox.
For the analyses, C57Bl/6 mice were randomly divided into 2 groups: the control group (fed with a standard diet), and the diabetic group (fed with a high fat diet) during 12, 16 or 20 weeks. After this period, animals were euthanized and the blood was collected to assess inflammatory and biochemical markers. Kidney, left ventricle and liver were collected for western blotting analyses to evaluate the expression of specific angiogenic receptors and related pathways; or for histological analyses. Three-micrometer-thick tissue sections were used for histological and immunohistochemistry analysis to evaluate tissue microvessel density. Each determination was performed in at least three independent experiments. Statistical significant differences between groups were evaluated by ANOVA followed by the Bonferroni test. A difference between experimental groups was considered significant with a confidence interval of 95%, whenever $p \leq 0.05$.
According to the obtained results, a slight increase was observed in MVD in the kidney of HFD animals, as well as a tendency for a reduction of the neovascularization in the left ventricle. In addition, an increase in VEGFR2 phosphorylation (p-VEGFR2) was observed in kidney both over time and in HFD-fed animals. Conversely, in the left ventricle, animals fed with a HFD had a reduction in p-VEGFR2. These results were accompanied by an imbalance in several biochemical markers and in IL-1β plasmatic levels.
These findings confirm the presence of the angiogenic paradox in diabetic animals. The identification of these angiogenic molecular pathways and the clarification of this paradox in DM associated with metabolic profile is the first step for development of therapeutic strategies against DM complications.

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**Anti-angiogenic Effects of New Di(hetero)rylthioethers 1,3-diarylureas in the Thieno[3,2-B]pyridine Series. Role in VEGFR2 Inhibition**

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The vascular endothelial growth factor receptor-2 (VEGFR-2) is a tyrosine kinase receptor involved in the growth and differentiation of endothelial cells that are implicated in tumor-associated angiogenesis. Angiogenesis is a crucial step in the growth, progression, and metastasis of cancers, since it enables the growing tumor to receive oxygen and nutrients [1]. VEGFR-2 plays an important role in tumor angiogenesis, so anti-angiogenesis approaches targeting VEGFR-2 has been considered as an important strategy for cancer therapy. Small molecules may act as inhibitors by competing for the ATP-binding site of the VEGFR2 intracellular tyrosine kinase domain, thereby preventing the intracellular signaling that leads to angiogenesis [3]. Herein, we report the synthesis using rational design of new 1-aryl-3-[3-thieno[3,2-b]pyridin-7-ylthio)phenyl]ureas **(1a-c)** as VEGFR2 tyrosine kinase inhibitors. The compounds presented, with the arylurea in the meta position to the thioether and with F or a Me group, showed very low IC50 values (11-28 nM) in enzymatic assays as predicted by molecular docking.
Cellular assays were performed to examine the activity of compounds **1a-f** in endothelial cells, and to indentify the angiogenic steps targeted by these compounds. VEGF-stimulated (60 ng/mL) Human Umbilical Vein Endothelial Cells (HUVECs) were cultured in the absence or presence of each compound at different concentrations. A remarkable reduction in the proliferation of HUVECs, using the BrdU incorporation assay, was observed for all compounds at 1 μM, being the antiproliferative effect statistical significant, for compounds 1b, d and e, at 0.5 μM, without affecting the cell viability.
To understand the molecular mechanism of compounds-mediated anti-angiogenic properties, we examine whether these molecules affect the expression of VEGFR2 (active and total form) using western blotting assays. We conclude that the compounds significantly inhibit the phosphorylation of the receptor at 1 μM.
Given the established role of VEGFR2 in proliferation and migration of endothelial cells, these molecules are promising anti-angiogenic agents that can be used for therapeutic purposes in pathological conditions where angiogenesis is exacerbated, such as cancer.

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**Endurance Training Mitigates Mitochondrial Alterations-induced by a High-fat Diet in Visceral Adipose Tissue**

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Obesity-related modulation in visceral adipose tissue (VAT) mitochondria has been reported. However, the role of exercise against the mo-

difications induced by high-fat diets on VAT mitochondria are poorly understood.
We aimed to analyze the influence of an endurance training (ET) program on markers of mitochondrial function and biogenesis in epididymal adipose tissue from rats fed with a high-fat diet-induced obesity.
Adult male Sprague-Dawley rats were randomly assigned into sedentary (SED, n=6) and trained groups (ET, n=6) fed with two isocaloric diets, a standard and a high-fat diet (35% or 70% fat-derived Kcal, respectively) as follows: SED35, ET35, SED70 and ET70. After 9-weeks of high-fat diet regimen, animals from ET groups were submitted to an 8-weeks treadmill-training program while maintaining dietary treatments. Trained animals were sacrificed 48 hours after the last training bout. Epididymal adipose tissue were excised and weighted. Subunits of the oxidative phosphorylation system (OXPHOS), adenine nucleotide translocator (ANT), uncoupling protein 2 (UCP2), and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) levels were determined by Western Blot.
Eight weeks of ET decreased epididymal fat pad weight and visceral adiposity index (total visceral fat-to-body weight ratio). No alterations were found in spleen-to-body weight ratio. Animals fed with the high-fat diet showed a decrease in subunits of OXPHOS complexes III, IV and V levels (SED70 vs. SED35). ET did not alter the expression of complex I protein content; however increased complex II in ET35 animals. Subunits of OXPHOS complexes III, IV, V and ANT levels increased significantly with ET although no alterations were observed in UCP2 levels. ET also increased significantly PGC-1alpha levels in ET35 group and a tendency to increase was also found in ET70 animals, although without statistical meaning.
Our results suggest that ET positively modulate the expression of several mitochondrial proteins in visceral adipose tissue in rats fed with a high-fat diet.

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**Modulation of the Uptake of Critical Nutrients by Breast Cancer Cells by Lactate: Impact on Cell Survival, Proliferation and Migration**

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Breast cancer is the most common cancer among women, both in developed and developing regions of the world. Although the survival is improving, it is expected that the burden of breast cancer will increase globally. Folate and glucose are critical nutrients for cancer cell proliferation and their cellular uptake and metabolism are good targets for anticancer therapies. Lactate, a by-product of cancer cell metabolism, may also serve as a nutrient for cancer cells and thus play a role in cancer progression.
In this context, the present work aimed to study the effect of lactate metabolites upon the transport of folic acid and glucose and upon the viability, proliferation and migration capacity of T47D cells (a model of human breast cancer cells).
First, we studied the uptake of deoxy-D-glucose (2-[1,2-$^3$H(N)] ($^3$H-DG)) by T47D cells. It was found to be: a) time-dependent, b) optimum at physiological pH, c) substantially inhibited by the facilitative glucose transporter (GLUT) inhibitor cytochalasin B and less inhibited by the sodium-dependent glucose co-transporter (SGLT1) inhibitor phloridzin, d) sodium-independent and e) slightly insulin-stimulated. Consequently, we conclude that $^3$H-DG uptake by T47D cells is mediated by members of the GLUT family (most probably GLUT1, given its characteristic expression in breast cancer cell lines, with a possible contribution of the insulin-responsive glucose transporters, GLUT4 and GLUT12) and it does not involve SGLTs.
The modulation of $^3$H-folic acid ([3',5',7,9-$^3$H] sodium salt($^3$H-FA)) and $^3$H-DG uptake in T47D cells by lactate metabolites and the effect of these compounds upon the viability, proliferation and migration capacity of T47D cells were also evaluated.
Acute exposure (26 min) of T47D cells to lactic acid (LA) (but not to sodium lactate (SL)) caused a significant stimulatory effect on $^3$H-FA uptake. This effect was abolished in the presence of an inhibitor of the monocarboxylate transporter 1 (MCT1) (sodium propionate), indicating that the stimulatory effect of LA upon $^3$H-FA uptake by T47D cells involves MCT1. This effect was also observed in relation to $^3$H-DG uptake by T47D cells, although it was less expressive.
Chronic exposure (24 h) of T47D cells to LA or to SL increased $^3$H-FA uptake, while not affecting $^3$H-DG uptake.
Chronic exposure of T47D cells to either LA or SL significantly decreased cell viability, whereas chronic LA, but not SL, decreased cellular proliferation. Additionally, we observed that chronic exposure to either LA or to SL tended to stimulate T47D cell migratory capacity.
In conclusion, these results show a surprising effect of lactate on breast cancer cells, which deserves further research in the future to dissect the molecular mechanisms involved.

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**Fibroblast-endothelial Co-culture System as an *In Vitro* Dermal Angiogenesis Model in Wound Healing**

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Diabetic chronic foot ulceration represents a major medical, social, and economic problem. Key features of the non-healing ulcer in diabetic patients are persistent inflammation and impaired blood vessel regeneration (angiogenesis). Angiogenesis is a complex physiological process that requires normal functions and properly orchestrated interaction between fibroblasts and endothelial cells (EC). In diabetic chronic ulcer, those cellular activities and functions are impaired.The fibroblasts-endothelial cell interactions in impaired angiogenesis are poorly understood, primarily due to the lack of an *in vitro* wound-healing angiogenesis model. In order to improve the knowledge of this issue in the present work human outgrowth endothelial cells (OECs) and mature ECs were co-cultured with different types of human fibroblasts.

Experimentally, OECs were isolated from human umbilical cord blood samples and characterized by immunofluorescence, western blot and imaging flow cytometry. Two types of human dermal fibroblasts were used (neonatal human foreskin fibroblasts – HFF-1 – and juvenile human dermal fibroblasts – HDF), being characterized in terms of the expression of podoplanin (PDPN) and transglutaminase-2 (TG2), markers of dermal fibroblasts, as well as alpha smooth muscle actin (α-SMA), a marker of fibroblast activation. Co-culture systems were established using either human umbilical vein ECs (HUVECs) or OECs with HFF-1 or HDF. Several culture time points were evaluated, namely 7, 14 and 21 days. The formation of capillary-like structures was assessed by immunocytochemistry against CD31 and vWF proteins and by confocal microscopy. Parameters such as the number of tubular structures, their length, thickness and branching points were evaluated. The presence of ECM components, such as collagen types I and IV, laminin and fibronectin, was assessed in all cell types and in co-cultures by immunofluorescence. HFF-1 exhibited a higher expression of TG2 than that observed for HDF, while HDF expressed higher amounts of PDPN and α-SMA. Indeed, the formation of capillary-like structures was only observed in co-cultures with HDF and not with HFF-1 fibroblasts. In addition, in the co-culture system, HUVECs/HDF formed a highly branched capillary-like network to a higher extent than that observed for OECs/HDF. In terms of ECM formed, HUVECs were found to secrete collagen type IV, fibronectin and laminin to the extracellular media, whereas in OECs these proteins were only detected intracellularly. These findings suggest that HDF is a preferential cell source for used an *in vitro* dermal angiogenesis model in wound healing.

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### Kaempferol Inhibits Glucose Uptake in a Human Breast Cancer Cell Line: Relationship to its Anticarcinogenic Effect

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Cancer cells present an altered metabolism, with an increased rate of glucose uptake and lactate production instead of oxidative metabolism (the Warburg effect) [1]. Dietary polyphenols are known to possess cancer preventive and anticancer effects [2]. Recently, our group verified that the polyphenols quercetin (a flavanol) and epigallocatechin-3-gallate (a flavan-3-ol) inhibited glucose uptake by the MCF-7 breast cancer cell line [3]. So, we decided to investigate if other polyphenols could also interfere with glucose uptake by these cells. The polyphenols tested were other flavanols (myricetin and kaempferol) and flavan-3-ols ((+) catechin and (-) epicatechin), a flavone (chrysin), an isoflavone (genistein), a chalchone (xanthohumol) and a stilbene (resveratrol).
Uptake of $^3$H-deoxy-D-glucose ($^3$H-DG) by MCF-7 cells was time-dependent and saturable ($K_m$=6.5±0.5 mM and $V_{max}$=63.6±2.3 nmol/mg prot).
Acutely (26 min), myricetin (10-100 μM), chrysin (100 μM), genistein (10-100 μM), resveratrol (10-100 μM), kaempferol (10-100 μM) and xanthohumol (10-100 μM) inhibited $^3$H-DG uptake. By contrast, (+) catechin and (-) epicatechin slightly (by 10-15%) increased it. The effect of the polyphenols was not related to changes in cell viability. Kaempferol was found to be the most potent inhibitor of $^3$H-DG uptake by MCF-7 cells, with an $IC_{50}$ of 4.0 (1.6-9.8) μM. Kaempferol (100 μM) behaved as a mixed-type inhibitor, since it simultaneously increased the $K_m$ (to 15.6±2.4 mM) and the $V_{max}$ (to 106.9±10.6 nmol/mg prot).
Chronically (24h), kaempferol was also able to inhibit $^3$H-DG uptake ($IC_{50}$ of 13.6 (2.8-66.9) μM). This effect was associated with a 40% decrease in GLUT1 gene transcription and, although not statistically significant, it was accompanied by a decrease in GLUT1 protein amount. We also verified that exposure of cells to kaempferol induced an increase in extracellular lactate levels over time (to 731±32% of control after a 24-h exposure; *n*=4), suggesting that this flavanol inhibits MCT1-mediated lactate cellular reuptake.
Additionally, kaempferol (100 μM) revealed antiproliferative (sulforhodamine B (SRB) and $^3$H-thymidine incorporation assays) and cytotoxic (extracellular lactate dehydrogenase activity determination) properties. These effects of kaempferol, obtained at 5.56 mM extracellular glucose, were mimicked by low extracellular (1 mM) glucose conditions and were reversed by high extracellular (20 mM) glucose conditions.
In conclusion, kaempferol potently inhibits glucose uptake by MCF7 cells, apparently by blocking members of the GLUT family of transporters (most probably GLUT1). This effect may contribute to the antiproliferative and cytotoxic effect of this dietary compound in these cells.

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### Adipocyte-Released Factors Enhance Melanocyte's Proliferation And Motility

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**Background:** Obesity, favored by the modern lifestyle, acquired epidemic proportions nowadays. Obesity has been associated with various major causes of death and morbidity including malignant neoplasms. Cutaneous melanoma incidence rates have also been increasing during the last four decades in several countries. Obesity involvement in melanoma etiology has been recognized, but the implicated mechanisms remain unclear.
We propose to address the above relationship and investigate the mechanism interplaying between obesity and an increased risk of melanoma onset.
**Methods:** 3T3-L1 pre-adipocytes and B16F10 melanocytes were cultured in DMEM. 3T3-L1 pre-adipocytes were differentiated into ma-

ture adipocytes using an established cocktail of 500 µM 3-isobutyl-1-methylxanthine, 250 nM dexamethasone and 10 µg/mL insulin. Subsequently, B16F10 cells were exposed to conditioned medium obtained from the mature adipocyte cultures, for a 24 hours period. Later on, treated B16F10 cells viability, migration, proliferation and apoptosis were accessed by MTS, Injury, BrdU and TUNEL assays, respectively. Melanin content of cells was determined by spectrophotometry.

**Results:** After a 24 hour exposure to adipocyte conditioned medium, melanoma cells show an increase of 48% in their viability. The percentage of proliferating cells was also increased in the presence of adipocyte-released factors. Adipocyte conditioned medium simultaneously decreased B16F10 programmed cell death by approximately 50% and the melanin content per melanocyte was also significantly reduced. In the injury assay, melanocytes motility was highly increased when exposed to adipocyte conditioned medium.

**Conclusion:** Adipocyte-released factors play a dual role in increasing melanocytes survival; both by enhancing melanoma cell's proliferation and simultaneously decreasing melanocyte apoptosis. B16F10 cell motility was also improved by exposure to adipocyte conditioned medium, suggesting that adipocyte secretome might be able to increase melanoma cell invasiveness. The observed decrease in melanin content, a differentiation marker in melanoma cells, might disclose a more dedifferented phenotype of melanocytes.

The preliminary results obtained in the present study are good indicators of the possible deleterious effects of adiposity on melanoma aggressiveness. The adipocyte-mediated increased survival and invasive phenotype of melanocytes support our intentions of additional *in vitro* assays and future *in vivo* animal models to further scrutinize the mechanisms that predispose obese individuals to cutaneous melanoma.

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### Physical Exercise and Doxorubicin Treatment Modulate Hepatic Mitochondrial Bioenergetics, Oxidative Stress and Apoptotic Signaling

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Doxorubicin (DOX) is a potent chemotherapeutic agent widely used in cancer treatment causing dose-limiting toxicity in non-target organs including the liver through the generation of free radicals and mitochondrial dysfunction. Physical exercise is increasingly advised in patients receiving chemotherapy due to the afforded cardioprotection and the limiting effects on physical fatigue. We here aimed to analyze the effects of physical exercise performed during the course of sub-chronic DOX treatment on liver mitochondrial function, oxidative stress and apoptotic signaling.

Male adult Sprague Dawley rats were divided into four groups: sedentary saline (SED+SAL), sedentary with sub-chronically treated with DOX (SED+DOX), 12-wks of endurance training treated with DOX (ET+DOX) and 12-wks of voluntary free wheel activity treated with DOX (VPA+DOX). *In vitro* liver mitochondrial function [oxygen consumption and membrane potential (ΔΨ)] was evaluated. Mitochondrial and tissue oxidative stress (aconitase, shcp66 and shcp66(Ser36)), apoptotic signaling (caspases 3, 8 and 9, Bax, Bcl-2 and CypD), OXPHOS complex subunits and ANT content were assessed.

Administration of DOX in SED and VPA groups resulted in mitochondrial dysfunction and increased oxidative stress. An increase in mitochondrial state 4 respiratory rate and a consequent decrease in RCR were observed in these groups. Aconitase activity was decreased, and caspases activity, shcp66(ser36) and complexes I and III contents were increased in SED+DOX. Interestingly, ET+DOX condition limited the increased shcp66(ser36), complex I and complex III, and also showed higher aconitase activity and lower shcp66 content, indicating decreased oxidative stress. Decreased Bax and, to a lesser extent, Bcl-2 contents (lower Bax/Bcl-2 ratio) in ET+DOX *vs.* SED+DOX suggests a decrease in apoptotic signaling, although caspases activity were unaltered.

These results indicate that, when performed during the course of DOX treatment, ET, and not VPA, induces positive adaptations and has a potential protective effect against sub-chronic DOX-induced hepatic mitochondrial toxicity, suggesting that the modulation of liver mitochondrial defense systems by chronic exercise may be type- and intensity-dependent.

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### Handgrip Strength and Associated Factors in Hospitalized Patients

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**Background:** Handgrip strength (HGS) is a marker of nutrition status [1,2]. Many factors are associated with HGS. Age [3,4], height [4], body massindex [5], number of diagnoses [3], and number [3] and type of drugs [5] have been shown to modify the association between undernutrition andHGS. Nevertheless, other patient characteristics that could modify this association and its joint modifier effect have not been studied yet.

**Objective:** To evaluate the association of inpatients' HGS and undernutrition considering the potential modifier effect of cognitivestatus, functional activity, disease severity, anthropometrics, and other patient characteristics on HGS.

**Methods:** A cross-sectional studywas conducted in a university hospital. Sex, age, abbreviated mental test score, functional activity score, Charlson index, number ofdrugs, Patient-Generated Subjective Global Assessment (PG-SGA) score, body weight, mid-arm muscle circumfe-

rence, adductor pollicismuscle thickness, body height, wrist circumference, hand length, and palm width were included in a linear regression model to identifyindependent factors associated with HGS (dependent variable).

**Results:** The study sample was composed of 688 inpatients (18–91 yearsold). All variables included in the model were associated with HGS (β, –0.16 to 0.38; $P \leq 0.049$) and explained 68.5% of HGS. Age, functional activity decline, Charlson index, number of drugs, PG-SGA score, body weight, and wrist circumference had a negativeassociation with HGS. All other studied variables were positively associated with HGS.

**Conclusion:** Nutrition status evaluated by PG-SGAwas still associated with HGS after considering the joint effect of other patient characteristics, which reinforces the value of HGS asan indicator of undernutrition.

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## Pupillometric Evaluation of Autonomic Nervous System Dysfunction in Women with Metabolic Syndrome

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The metabolic syndrome (MetS) has a major impact on public health and is highly prevalent in Western societies (in Portugal, estimates suggest a prevalence between 24.0% and 27.5%, according to NCEP-ATP III criteria, and 41.9%, according to IDF criteria). MetS is clinically useful to classify patients according to risk of developing cardiovascular disease and type 2 diabetes, enables the inclusion of abdominal perimeter in the initial evaluation of the patient and proposes a treatment plan targeting the "syndrome" (with special focus on insulin resistance and hypertension).

Autonomic nervous system (ANS) dysfunction is a landmark characteristic of diabetes, even in early stages, and has been associated with the MetS or its individual components. Previous studies suggest that sympathetic nerve hyperactivity precedes insulin resistance and the onset of diabetes. On the other hand, overall decreased sympathetic nervous system (SNS) and parasympathetic nervous system (PSNS) tone was associated with obesity. By assessing the systemic ANS, others have proposed a characteristic pattern of increased SNS and decreased PSNS for MetS. The efferent local ANS in the eye is easily accessed by the non-invasive pupillometry and, despite being extensively studied in several diseases, including diabetes, it has been understudied in MetS.

We aimed to assess the autonomic nervous system in women with MetS, with the aid of a portable pupillometer.

This cross sectional study was approved by the local Ethics Committee and took place in a Primary Care setting. The study included a total of 44 women, and by using clinical and laboratorial parameters two groups were formed: 25 healthy volunteers and 19 subjects with MetS. The efferent ANS function was assessed by pupillometry under mesotopic conditions. Age-adjusted analyses were carried out using SPSS and a p-value of <0.05 was considered statistically significant.

Measures of pupillary dilation (initial diameter, average dilation velocity) and of pupillary constriction response (maximum constriction velocity) were significantly reduced in women with MetS, respectively indicating a decreased sympathetic and parasympathetic response. Furthermore, there was a significant reduction of the minimum pupil diameter in the MetS group, reflective of both SNS and PSNS decreased activity.

Our observations suggest that a pattern of decreased SNS and PSNS efferent activity in the pupil is characteristic of women with MetS, indicating autonomic dysfunction. These results contradict previous findings and underpin the importance of local ANSs.

## Exploring some Myths about a Portuguese Hypersaline Sodium-rich Naturally Sparkling Mineral Water Ingestion

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Natural mineral-rich waters that originate underground are protected from chemical and microbiological contamination and are characterised by highly bioavailable mineral content (for example, magnesium and calcium). In general, their consumption is associated with beneficial health effects. However, the general public and some health practitioners believe natural mineral-rich waters can have negative effects on blood pressure and bone mineral density when they have high sodium and/or carbonate levels, respectively. Pedras-Salgadas® is a Portuguese hypersaline sodium-rich naturally sparkling mineral water that belongs to UnicerBebidas, SA. It is rich primarily in sodium and bicarbonate, and contains higher potassium, calcium and magnesium content than tap water. Additionally, it has low chloride content.

In order to scientifically explore if the two concerns mentioned above could apply to PedrasSalgadas®, UnicerBebidas, SA, established aresearch protocol with the Biochemistry Department, from the Faculty of Medicine of Porto. In a first experimental protocol, a crossover and non-blinded study, seventeen normotensive adult individuals (nine female and eight male) were randomly allocated in two groups and had their blood pressure evaluated when ingesting 500 mL/day of Pe-

drasSalgadas® or Vitalis® (hyposaline natural mineral water), for seven weeks. PedrasSalgadas® ingestion had no effect on blood pressure (Santos *et al* 2010). In a second experimental protocol, twenty-four female Sprague-Dawley rats subjected to ovariectomy ingested tap water or PedrasSalgadas®, with or without fructose (n=6/group), for twelve weeks. At the end of the protocol, all ovariectomized females had their bone mineral density decreased as compared with sham-operated control animals (n=6; ingesting tap water). PedrasSalgadas® did not induce additional bone loss, with or without fructose co-ingestion. Considering these results and the published data regarding the beneficial effects of natural mineral-rich water ingestion on blood pressure and plasma lipid profile, insulin and glucose homeostasis and redox status, in healthy humans and/or animals, a third experimental protocol was developed in order to evaluate if the ingestion of PedrasSalgadas® could be protective against Metabolic Syndrome (MS) induction, using fructose-fed male Sprague-Dawley rats (n=21). MS is a cluster of interrelated metabolic abnormalities, including hypertension, dyslipidemia, glucose intolerance/insulin resistance and visceral obesity, which increases the risk of cardiovascular disease and type 2 Diabetes *Mellitus*. After eight weeks, fructose-containing tap water (n=7) increased systolic blood pressure *vs.* controls (n=7; tap water without fructose). Fructose-containing PedrasSalgadas® (n=7) did not intensify this effect and, interestingly, delayed its onset. Fructose-containing tap water increased plasma triacylglycerols and insulin levels and reduced the plasma insulin sensitivity index. Fructose-containing PedrasSalgadas® reduced the magnitude of these effects. Regarding some of the mechanisms that may contribute to MS induction, fructose-containing tap water induced alterations on the hepatic redox status and on glucocorticoid and insulin signalling pathways both in liver and adipose tissue, which were minimized by fructose-containing PedrasSalgadas® and adding to the induction of some protective pathways.
In conclusion, PedrasSalgadas® does not seem to have negative effects on the parameters evaluated and seems to have potential to prevent MS induction.

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**The Role of Voluntary Physical Activity and Endurance Training on the Hepatic Insulin Signaling Pathway in High-fat Diet-induced Obesity**

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Non-alcoholic steatohepatitis (NASH), a pathological condition characterized by steatosis and inflammation,has been related with excessive high-caloric food consumption and increased sedentary life style [1]. Moreover, steatosis and insulin resistance are also closely associated [2]. Therefore, we aimed to analyse the effect of voluntary physical activity (VPA: free wheel for 16 weeks) and endurance training (ET: treadmill for the last 8 weeks of the dietary intervention) programs on hepatic insulin signalling in an animal model of high-fat diet-induced obesity.

Male Sprague-Dawley rats were divided into six groups (4<n<7): standard diet sedentary (SS), standard diet + voluntary running (SV), standard diet + endurance training (ST), high-fat diet sedentary (HS), high-fat diet + voluntary running (HV) and high-fat diet + endurance training (HT). The diagnosis of NASH was confirmed through histological analysis, using the NASH score proposed by Kleiner *et al*, 2005 [3]. The protein expression of IRS1, p-IRS1 (Ser 307), IRS2, JNK 1/2/3, p-JNK 1/2/3 (Thr 183 and Tyr 185), AKT and p-AKT (Ser 473) was evaluated by Western blot. Plasma insulin and glucose levels were also quantified and the insulin sensitivity index was calculated.
Histological analyses confirmed that sedentary animals fed with the high-fat diet developed NASH (HS *vs.* SS), which was significantly decreased by ET (HT *vs.* HS).
Insulin and glucose tended to decrease in HT, while insulin sensitivity index was higher in ST and HT compared to SS and HV, respectively. The ratio of inhibitory IRS1 phosphorylation to total IRS1 was higher in HS *vs.* SS; howeverboth VPA and ET prevented this increase. In both diet regimens, trained animals presented lower levels of p-AKT expression compared to sedentary animals (ST *vs.* SS and HT *vs.* HS). HS presented a tendency to increase total AKT expression compared to SS; however HV and HT strongly reduced total AKT expression when compared to HS. The ratio of AKT activation decreased in HT *vs.* HS (with a similar tendency for HV). Total JNK expression was not altered with the nutritional regimen (HS *vs.* SS) although a reduction was observed with VPA and ET in the animals fed with the high-fat diet. Likewise, p-JNK expression decreased in HV and HT *vs.* HS. Accordingly, a significant reduction in JNK activity was found in HV and HT *vs.* HS, although not in the ratio of JNK activation. Total JNK and p-JNK expression decreased in HT *vs.* ST and HV *vs.* SV, respectively. Total ERK expression decreased in HV and HT *vs.* HS.
Taken together, our results suggest that endurance training reduced the NASH score evaluated by histological analysis and mitigated the insulin signallingimpairment in a high-fat dietanimal model.

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**ER Stress Response Activation in Cellular Senescence**

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The aging process is associated with a progressive accumulation of damaged biomolecules, such as proteins, usually as a result of the increased oxidative stress that accompanies cellular senescence. These age-related features might lead to ER homeostasis disruption and consequently to the activation of ER stress response. It is hypothesized that the increased oxidative stress that accompanies senescence in human cellular models of Replicative Senescence (RS) and Stress-Induced Premature Senescence (SIPS) would favor the occurrence of ER stress

and disturb the ER chaperoning mechanisms leading to UPR pathways activation. Protein and gene expression levels of key ER chaperones/enzymes as well as IRE1-, ATF6- and PERK- mediated ER stress response pathways activation were assessed in RS, hydrogen peroxide-SIPS and copper sulfate-SIPS cellular models. For the first time it is shown the existence of moderate ER stress in human cellular models of senescence. ER molecular events occurring in copper-SIPS cells are similar to the processes taking place in RS. In these two models, the levels of BiP, calnexin, PDI and Ero1 are adjusted in an attempt to restore proteostasis. Also, protective ER stress responses mediated by IRE1, ATF6 and PERK are activated to promote cell survival. However, hydrogen peroxide-SIPS model does not exhibit IRE1 and ATF6 pathways activation, but a PERK-mediated upregulation of CHOP suggesting the occurrence of CHOP-induced autophagy to promote survival and avoid apoptosis. Regarding proteostasis and ER stress response, $CuSO_4$-SIPS cellular model mimics better the molecular events occurring during RS than the most commonly used $H_2O_2$-SIPS cellular model. Disruption of copper homeostasis has been involved in several age-related diseases; consequently, these copper-induced senescent cells are a valuable tool for researchers to identify potential targets for pharmaceutical interventions, aiming to optimize cellular stress responses during aging, in order to fight against age-related health deterioration.