8º Simpósio em Metabolismo da Faculdade de Medicina da Universidade do Porto

– Comunicações Orais e Posters

> PROGRAMMING IN OBESITY

Maternal Obesity in Pregnancy: Consequences for Mother and Child

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One in five women in the UK is obese (body mass index BMI > 30kg/m^2) at antenatal booking. Maternal obesity is associated with complications for the mother including increased risk of developing gestational diabetes, pre-eclampsia and need for caesarean section. For the offspring short term complications include risk of macrosomia and need for admission to the neonatal unit. It is now apparent that the effects of maternal obesity for the offspring extend beyond the neonatal period with increased risk of obesity in childhood, adolescence and adult life. In a recent record-linkage study we demonstrated that maternal obesity is associated with increased risk of premature mortality and hospital admissions for cardiovascular events in her adult offspring. Animal models suggest the adverse effects of maternal obesity on offspring outcomes are 'programmed' in utero. We have been investigating potential underlying mechanisms in a cohort study of very severely obese pregnant women (BMI > 40kg/m^2) and key findings will be presented. Consideration of therapeutic interventions including lifestyle interventions and the findings of our recently published randomised controlled trial (EMPOWaR) in obese pregnant women using the insulin sensitiser metformin vs placebo will be discussed. We hope that by understanding the pathways to complications in obese pregnancy we may be able to intervene to improve outcomes for mother and baby.

Transgerational Effects of Obesogens: from the Gene to Environmental Health

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The presence of Endocrine Disrupting Chemicals (EDCs) in the environment is an issue of major concern worldwide since these compounds act as exogenous signals and can potentially disrupt hormone-controlled physiological processes of both vertebrates and invertebrates. In mammals, improper control of lipid metabolism may result in serious health problems such as obesity, increased risk of coronary disease, diabetes and related problems. Recent evidence suggests that many environmental pollutants may be involved in the increased prevalence of obesity. Moreover, recent studies suggest that xenobiotic compounds are able to induce effects in a transgenerational pattern. Here, we will address this issue focusing on the evolution of key molecular players of lipid homeostasis, as well as to the evidence linking epigenetic modulation as a key player in endocrine disruption processes.

On the Genetics and Epigenetics of Obesity

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The worldwide increased prevalence of common obesity and related co-morbidities has reached epidemic proportion becoming one of the major public health problems. While genetic factors are undoubtedly associated contributing to determine individual susceptibility risk to common obesity, several genetic variants have been identified explaining only part of this variation. Effectively, only 3% of all these genetic variants represent the BMI variation far from the 40-70% previously expected by using twin and adoption studies. This has led to growing interest in understanding the potential role that environmental factors can influence the genetic background contributing to the increase in individuals with an obese phenotype. Epigenetic mechanisms may act as a mediator of gene-environment interactions underlying the development of obesity and its associated co-morbidities which could help explaining the observed increase in obesity prevalence.
The advances in epigenetic methodologies combined with reduced cost of epigenome-wide association studies (EWAS) have led to a rapid increases of studies regarding the obesity complexity. Several of them have reported epigenetic differences between obese adults and healthy controls and epigenetic changes in association with other factors such as nutritional, weight loss, or exercise interventions. Based on both animal and humans studies some evidences have been found regarding the relationship between parental pre-conceptional and early in life environmental exposures with later risk of obesity mediated by epigenetic changes in the offspring. It could be a "critical period" where environmental conditions experienced *in utero* may have a lifelong effect on the propensity to develop the obese phenotype.
All these studies present promising data suggesting that he next decade promises to be a time of productive research into the complex interactions between the genome, epigenome, and nutrition referred to metabolic disease.

> MICROBIOTA AND INFLAMATION IN PROGRAMMING

Gut Microbiota Dysbiosis and Inflammation

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Gut microbiota has been considered a key factor for the maintenance of health. The homeostasis of the gut microbiota is dependent on host characteristics as age, gender, genetic background and, also diet habits. Dysbiosis occurs when the gut microbiota composition and functions are shifted from their normal beneficial state to another that is deleterious to the health of the host. There is evidence that the gut microbiota composition can be different between healthy and obese and type 2 diabetic patients. In agreement with that, microbiota has been studied as a key link between the pathophysiology of metabolic diseases and the inflammation present in obesity.
Several mechanisms are proposed linking events occurring in the colon and the regulation of energy metabolism, such as the energy harvest from the diet, the synthesis of gut peptides involved in energy homeostasis (GLP-1, PYY...), and the regulation of fat storage by the bacterial lipopolysaccharide (LPS). Fat feeding is associated with the development of metabolic endotoxemia in human subjects and participates in the low-grade inflammation.
Observational studies in obese patients, and more recently, experimental data in animals, suggest that the composition of the gut microbiota is a factor characterizing obese versus lean individuals (lower Bacteroidetes and more Firmicutes).
Diet composition (fat, carbohydrates, micronutrients, prebiotics, probiotics), have not only consequences on the gut microbiota quality, but may modulate the expression of genes in host tissues such as the liver, adipose tissue, intestine, muscle and brain. In fact, the changes in the gut microbes from obese patients can be reversed by dieting and related weight loss. In this regard, dietary interventions may play a role in reshaping the gut microbial community and enhance host microbial interactions to provide beneficial health effects. Thus, it would be useful to find specific strategies for modifying gut microbiota with impact on the occurrence of metabolic diseases.

> METABOLIC PROGRAMMING AND LIFESTYLE

Epigenetic Programming of Metabolic Health across the Lifecourse

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It is well established that patterns of early growth are associated with risk of metabolic diseases such as type 2 diabetes and obesity in later life, conditions which are major health care issues of the 21st century in both the developed and developing world. Strong evidence, from human and animal studies suggests that the early environment, including early diet, plays an important role in mediating these relationships. Permanent changes in epigenetic regulation of gene expression have emerged as an important contributing mechanism. Our findings at the key diabetes gene Hnf4 alpha demonstrated that changes in promoter-enhancer interactions induced by changes in epigenetic modifications represented a novel programming mechanism. Current work using state of the art epigenetic technology including promoter-capture Hi-C, is aimed at testing our hypothesis that modulation of promoter-enhancer interactions is a common mechanism underlying developmental programming. In this talk I will discuss critically the concept of an "epigenetic programme" during development, the implications of epigenetic dysregulation in the context of metabolic disease and the importance of studying 3D nuclear dynamics as a tool to unravel new regulators of adipogenesis and developmental programming.

Exercising the Future. An Epigenetic Approach

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Physical activity has been considered a gold-standard preventive and therapeutic tool against distinct pathophysiological conditions, including those related with metabolic constrains or deregulation. Different methodological approaches support the relevance of exercise has a strategy to mitigate the deleterious impact associated to an imbalance between energy intake and expenditure in several organs at distinct levels of cellular organization. Moreover, the metabolic alterations induced by physical exercise have pleiotropic effects. Numerous studies have shown that regular exercise affects not only the exercised skeletal muscle, but also other non-contractile organs over time significantly improving the quality of life. Recent data suggest that performing exercise during pregnancy could also mediate adaptive conditions favoring positive health outcomes in the next generation through epigenetic modifications. Whether different types, intensities and timings of exercise during pregnancy alter the potential epigenetic programming in different healthy and diseased cohorts is still worthy of investigation. Nevertheless, despite the scarcity of studies, the role of physical activity has a mean to positively regulate an epigenetic "healthy" landscape in the newborns is clearly an interesting and challenging issue both for the mothers and the researchers.

Establishing Eating Behaviours and Habits in Children: the Influence of Birth Weight and other Early Life Characteristics

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The evidence on how perinatal and postnatal exposures, such as birth weight or early parental feeding practices, influence later food consumption and eating behaviours, and how they could impact child's growth and adiposity, it will be presented and discussed.
Children eating behaviours may be influenced by biological pathways inherent to low or high birth weight. It has been described that low birth weight children have a compensatory accelerated growth, gaining more fat rather than muscle that leads to high fat mass later in life, and consequently to adverse metabolic outcomes. Low birth weight or pre-term children could also have more problematic eating behaviours at different stages of life, which in turn could be mediators to a worse health profile in future. Also environmental influences, namely eating patterns imposed by parents due to the low birth condition, could interact and help to explain these associations. Parents of low birth weight children or with children that lost an excessive amount of weight after birth, or grow slowly, may be more likely to implement feeding practices that result in higher later food intake.
Early parental feeding practices related with breastfeeding or weaning could also influence children food preferences and acceptance. The complementary feeding period is a 'window of opportunity' for the acceptance to a variety of foods with different flavours and textures. Also, some parental feeding practices could influence food intake regulation. For example parents who used 'Food as a reward' are more likely to have children (aged 3-6 years) who eat in the absence of hunger. When energy-dense foods are available freely after a meal, children eat in the absence of hunger and consume extra energy.
The effects of early feeding practices on a child's growth and obesity development have been more difficult to establish. However, it seems that weight trajectories could be influenced or influence eating habits in early stages of life. Based on data from Generation 21, the Portuguese birth cohort, we found that children in a "persistent weight

gain" trajectory had higher odds of following a dietary pattern rich in energy dense foods, at 4 years of age.
We also found that early consumption of energy dense foods tracks during early childhood and a dietary pattern high in this food group, followed at 4 years of age, increases later adiposity in girls. Protein intake at 4 years is positively associated with BMI in both girls and boys at 7 years of age, but is associated with increased serum insulin only in boys. Also, dietary glycaemic load is positively associated with adiposity only in boys, in whom it seems to interact with protein intake enhancing increased adiposity. This finding may be reflecting the role that both amino acids and glucose (along with growth factors such as insulin) play in signalling pathways that regulate cell growth, and may be related to higher sensitivity to central insulin in boys.

> ORAL COMMUNICATIONS

1 – Body Mass Index is Associated with Metabolic Reprogramming in Visceral but not in Subcutaneous Adipose Tissue

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Adipose tissue (AT) is a dynamic organ that besides storing energy to be mobilized as needed, influences whole body metabolism and has a crucial role in the pathogenesis of dysmetabolism. Regional fat distribution and functioning may contribute to obesity related metabolic disorders and adverse health outcomes. Though specific fat depots were suggested to possess unique biological properties, the specific molecular metabolic profiles of subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) remain largely unknown.
Our aim was to characterize the VAT and SAT depots metabolism, and their correlation with body mass index (BMI). With that purpose, AT of patients (n=12) with female predominant gender distribution (F:M, 9:3), a mean age of 46 years (26-83) and a body mass index (BMI) average of 29.6 kg/m^2 (18-37 kg/m^2) were used. VAT and SAT explants were obtained during elective laparoscopy, either cholecystectomy for uncomplicated cholelithiasis or gastric bypass for severe obesity. AT explants were placed in insulin free cell culture media for 72 hours and their metabolic profile established by a metabolomics-based approach. Our data showed that AT explants display a glucose and pyruvate consumption and acetate production that is region-dependent according with the patients BMI. In VAT, glucose consumption was positively correlated with BMI, while alanine and lactate production were negatively correlated with BMI, whereas in SAT the patients BMI did not affect the AT secretome.
In conclusion, our results show that insulin independent glucose uptake in VAT increases with raising BMI. Targeting the BMI induced metabolic remodeling may represent a novel therapeutic target to counteract the increased health risks derived from visceral adiposity.

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2 – Modulation of Cardiac Structure by Cardiac Visceral Adipose Tissue

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Introduction: Diastolic heart failure (DHF) is recognized an important cause of cardiovascular mortality and morbidity reaching approximately 50% of heart failure cases. Several risk factors, such as obesity, are associated with its development. Adipose tissue is now considered an 'endocrine organ' that secretes numerous bioactive peptides, termed adipokines. In obesity, due to adipocyte hypertrophy and dysfunction, there is an increased secretion of proinflammatory adipokines. These adipokines produced by cardiac visceral adipose tissue (CVAT) can act in a paracrine manner directly on the myocardium and influence their structure and function. In this work we aim to characterize the profile of CVAT under conditions of DHF and to evaluate their possible changes in cardiac structure.
Methods: CVAT of 20-weeks-old lean and obese ZSF1 rats was collected for: 1) separation of proteins to mass spectrometry identification, 2) adipokines' expression, 3) adipocytes fibrosis and cross-sectional area assessment and 4) for 24h DMEM incubation to obtain conditioned media. Successively, organotypic cultures were prepared from 7 days-old Wistar Kyoto cardiac explants and incubated for 24h with the conditioned media previously obtained from both groups. After incubation, cross-section area of cardiomyocytes and fibrosis were evaluated.
Results: In CVAT of the obese ZSF1, the results presents a decrease of 3-ketoacyl-CoA thiolase protein enzyme as a compensatory mechanism in order to inhibit fatty acid oxidation and an increase of lumican and collagen-alpha-1(I) proteins suggesting a link between inflammation caused by obesity and increases of adipose tissue extracellular matrix. The histological and molecular studies of CVAT revealed hypertrophy of adipocytes in obese animals (1505 ± 80.01 µm^2 vs. 7595 ± 265.5 µm^2, $p<0.0001$) without fibrosis, as well as a significantly increase in expression of several adipokines. Among these overexpressed adipokines are visfatin (0.42 ± 0.18 AU vs. 1.4 ± 0.33 AU, $p<0.05$), leptin (0.12 ± 0.032 AU vs. 0.93 ± 0.18 AU, $p<0.0001$), apelin (0.08 ± 0.03 AU vs. 0.24 ± 0.04 AU, $p<0.05$) and chemerin (0.33 ± 0.096 AU vs. 0.90 ± 0.16 AU, $p<0.05$) that are involved in fibrosis and hypertrophic pathways. In organotypic cultures, conditioned media from obese ZSF1 CVAT rats triggered a significant increase in the cross-sectional area of cardiomyocytes (100.7 ± 18.98 µm^2 vs. 111.25 ± 24.02 µm^2, $p<0.05$) and in fibrosis (3.48 ± 1.51% vs. 4.79 ± 1.53%, $p<0.05$) compared to the conditioned media from lean rats ZSF1.
Conclusions: Obesity stimulates alterations in CVAT profile which alter the myocardial structure, inducing collagen deposition and cardiomyocyte hypertrophy that are important determinants for cardiac remodeling.

3 – Arachidonic Acid Prevents the Adverse Impact of the Polyphenol Xanthohumol upon Placentation – an Approach to Fetal Nutritional Programming of Metabolic Syndrome

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Placentation is a continuous and highly regulated process that begins after fertilization and ends only after delivery. It is performed by extravillous trophoblasts (EVTs), which are fully specialized trophoblasts exhibiting an invasive and proliferative phenotype. EVTs perform the anchorage of the chorionic villi into the uterine wall and actively regulate the process of uterine spiral arteries remodelling, a process that ends up with the establishment of the utero-placental blood flow. The long-chain polyunsaturated fatty acid (LC-PUFA) arachidonic acid (ARA) is essential for the development of fetal nervous, visual, immune and vascular systems. LC-PUFAs act as ligands for the intracellular receptors PPAR-γ, and several studies point to the involvement of PPAR-γ in the regulation of cell invasion, migration and proliferation of EVTs. [(1)] Our research team recently showed that the dietary polyphenol xanthohumol (XH) impacts the placentation process by affecting viability, proliferation, culture growth and migration of the HTR-8/SVneo human first-trimester EVT cell line in a glucose-dependent manner. [(2)] The aim of this study was to investigate the putative modulation ARA uptake by XH, in HTR-8/SVneo cells, and to study its relationship with the effects of XH upon cell viability, proliferation, culture growth, migration and apoptosis.

^{14}C-ARA (500 nM) uptake by HTR-8/SVneo cells was found to be time-dependent, saturable, acidic pH-stimulated, inhibited by the fatty acids palmitic acid (PA), linoleic acid (LA) and γ-linolenic acid (γ-LNA) (with the following ranking order of potency: LA>γ-LNA>PA) and markedly inhibited by the long-chain acyl-CoA synthetase (ACSL) inhibitor triacsin C. Uptake of ^{14}C-ARA (100 nM) was concentration-dependently inhibited by both a short-term (26 min) and a long-term (24h) exposure to XH. XH (24h; 5 μM) was found to be an uncompetitive inhibitor of ^{14}C-ARA uptake (as it significantly reduced both V_{max} and K_m of uptake) and the mammalian target of rapamycin (mTOR), tyrosine kinases (TK) and c-Jun N-terminal kinases (JNK) intracellular signaling pathways were involved in this effect. Interestingly enough, the effects of long-term exposure to XH (24 h; 5 μM) (a decrease in cell viability, proliferation, culture growth and migration and an increase in cell apoptosis) were completely prevented by high extracellular ARA (10 and 100 μM). Moreover, XH (24h; 5 μM) potently decreased ACSL1 gene expression (mRNA) levels, and this effect was also prevented by high extracellular ARA. On the other hand, the PPAR-γ agonist rosiglitazone did not alter the effect of XH upon cell viability, proliferation, culture growth, cell migration, apoptosis and ^{14}C-ARA uptake.

We thus conclude that XH inhibits ARA cellular uptake and that its effect upon cell proliferation, culture growth, migration and apoptosis is dependent on inhibition of ARA uptake but independent on PPAR-γ activation. Because maternal undernutrition during critical periods of fetal development can program the fetus for metabolic syndrome (MetS) later in life, [(3)] especially when postnatally challenged with a hypernutritive diet, [(3)] our results suggest that XH can have long-term consequences upon the developmental programming of MetS, which can be prevented by ARA.

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4 – Modulation of VEGF-B Signaling by DDE Exposure – Effect on Fatty Acid Accumulation

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Introduction: Recently, it was demonstrated that vascular endothelial growth factor (VEGF)-B signaling, through an increase in the fatty acid transport protein (FATP) 3 and FATP4 expression can modulate the fatty acid uptake into organs such as adipose tissue (AT), liver and heart. [(1)] The *p,p'*-dichlorodiphenyldichloroethylene (*p,p'*-DDE) is a persistent organic pollutant, that present endocrine disruptor properties. [(2)] It is present in blood circulation and is mainly accumulated in AT, leading to metabolic dysfunction and contributing to obesity. [(3)]
Objective: To verify if *p,p'*-DDE exposure, in different diet contexts, can change VEGF-B signaling and thus modulate the uptake of fatty acid, across the endothelium, to different tissues.
Methodology: Thirty Wistar rats were divided into four treatment groups during 12 weeks: Standard diet (St), St with DDE (St+DDE, 100 μg/kg/day), High-fat diet (HFD) and HFD with DDE (HFD+DDE, 100 μg/kg/day). At the end of the treatment the expression of *Vegfb*, *Vegfr1*, *Fatp3* and *Fatp4* genes was quantified by qRT-PCR in mesenteric adipose tissue (mAT), liver and heart. Tissue lipid content was measured and plasma biochemical profile was analyzed. Statistical analysis included two-way ANOVA followed by Turkey's multiple comparison test.
Results: Rats fed with HFD and exposed to DDE manifested a more pronounced dyslipidemia and an increase in plasma markers of tissue dysfunction. However, HFD alone increased rats' body, mAT and heart weight. In mAT, DDE exposure and HFD increased the expression of *Vegfb*, *Vegfr1* and *Fatp3* genes. Moreover, HFD increased in 157.39% the area of adipocytes and decreased in 54.66% the number of adipocytes per area of mAT. In liver, DDE exposure increased the expression of *Vegfb*, *Vegfr1*, *Fatp3* and *Fatp4* genes. Consequently, lipid content was higher in rats feed with HFD and exposed to DDE. In heart tissue, DDE exposure increased the expression of *Vegfb*, *Vegfr1*, *Fatp3* and *Fatp4* genes. Rats fed with HFD increased heart triglyceride concentration in 241.46%, compared to St diet group.
Conclusion: The results obtained allowed to conclude that DDE exposure, in combination with HFD, promoted tissue dysfunction and dyslipidemia. DDE exposure modulated *Vegfb*/*Vegfr1* signaling and thus the *Fatp3* and *Fatp4* genes, promoting fatty acid accumulation in several tissues. So, it is possible that DDE exposure can modulate fatty acid transport proteins and contribute to metabolic disorders and obesity.

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5 – Xanthohumol Restores Hepatic Glucolipid Metabolism Balance in Type 1 Diabetic Wistar Rats

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Diabetes is a major public health problem worldwide. Beer-derived xanthohumol exhibits anti-inflammatory, anti-angiogenic and anti-apoptotic effects, three processes exacerbated in diabetes. Thus, we propose to identify molecular mechanisms associated with hepatic glucolipid metabolism imbalance in type 1 diabetic Wistar rats and to evaluate the effect of xanthohumol-enriched stout beer consumption in these pathways.
Eight week-old healthy male Wistar rats were divided into five groups (n=6 each): diabetic rats drinking water; treated with 5% ethanol; treated with stout beer; treated with stout beer supplemented with 10mg xanthohumol/L; and healthy rats drinking water (control group). Type 1 diabetes was induced by an intraperitoneal injection of streptozotocin. After 48h the diabetic condition was confirmed by measuring blood glucose content (>250mg/dL). The animals were euthanized 5 weeks later.
Hepatic H&E, PAS, Reticulin and Sirius Red histological staining were performed and quantified using ImageJ®. Expression of pACC/ACC ratio and FAS levels were evaluated by Western blotting.
Diabetic rats showed liver glycogen reduction, and increased apoptosis and fibrosis compared to control group. Xanthohumol consumption attenuated these three parameters to control values.
Lipogenesis was assessed by pACC/ACC and FAS expression. Increased pACC/ACC ratios and decreased FAS levels were found in diabetic rats. Xanthohumol reverted the expression of these two metabolic enzymes.
Xanthohumol consumption interfered with liver catabolic state seen in type 1 diabetes, reverting glycogen depletion, enhancing lipogenesis and attenuating apoptosis and fibrosis, implying an effect in inflammation. Altogether these findings reveal that xanthohumol can be a therapeutic agent against liver metabolic changes in type 1 diabetes, playing a possible role in the insulin receptor pathways.

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6 – Effects of Voluntary Physical Activity and Endurance Training in Cardiac Mitochondrial Function of High-fat Diet-fed Rats.

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We here investigate the effects of two distinct chronic exercise modalities voluntary free wheel training (FW) and the endurance treadmill training (Tm) in animals fed with isocaloric diets different in energy derived from fat and carbohydrates: standard (SD) and high fat diet (HFD) on cardiac mitochondrial bioenergetics and oxidative stress. Male Sprague-Dawley rats were divided into standard-diet sedentary (SD + SED, n = 20), standard-diet free wheel (SD + FW, n = 10), high-fat diet sedentary (HFD + SED, n = 20) and high-fat diet free wheel (HFD + FW, n = 10) groups. After 9-weeks, half (n = 10) of SD + SED and HFD + SED groups were engaged in a Tm program (8 wks, 5 d/wk, 60 min/day; SD + Tm and HFD + Tm respectively). *Ex vivo* cardiac mitochondrial function endpoints were assessed under normal oxygenation conditions and anoxia-reoxygenation. Semi-quantification of oxidative phosphorylation subunits, lipid peroxidation and the glutathione redox status were also measured. 17-weeks of HFD treatment, did not affect cardiac mitochondrial function neither the redox state (HFD + SED vs. SD + SED) Importantly, Tm exercise improved mitochondrial respiratory activity in both diet regimens before and after anoxia-reoxygenation. FW running increased OXPHOS complexes IV and V subunits in both diet treatments and improved mitochondrial antioxidant capacity (GSH/GSSG), particularly in HFD group. Although 17 weeks of HFD did not induced mitochondria bioenergetics impairments, Tm training had a stronger effect in overall mitochondria oxygen consumption, whereas FW exercise seems to positively modulate the antioxidant machinery. Overall, we conclude that chronic exercise may constitute an effective strategy to increase cardiac mitochondrial functionality in a context of obesity and HFD.

Keywords: exercise; heart; bioenergetics; mitocondria; obesity.

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> POSTERS

1 – Quercetin, Apigenin and Chrysin Can Interfere with the Intestinal Absorption of Fructose – Possible Influence on Metabolic Syndrome

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Introduction: Metabolic Syndrome (MS) increases the risk for atherosclerotic cardiovascular disease and type 2 Diabetes Mellitus. Fructose consumption has been associated with MS development and, interestingly enough, a substantial increase in both consumption of this sugar and MS incidence has been observed during the last 30 years. [1-4] Dietary polyphenols have been largely studied due to their human health benefits. [5] Several polyphenols are known to interfere with the intestinal absorption of glucose, but little is known concerning the effect of these phytochemicals on fructose intestinal absorption. [6]
Aim: To investigate if the polyphenols quercetin, chrysin and apigenin can interfere with fructose intestinal absorption.
Methods: We tested both the acute (26 min) and the chronic (24h) effect of quercetin, chrysin and apigenin (100 µM) on the uptake of ^{14}C-fructose (100 nM) by Caco-2 cells (a cell line mimicking the human intestinal epithelium). Moreover, we tested (by RT-qPCR) for mRNA expression of fructose transporters (GLUTs) after chronic treatments with these polyphenols.
Results: Acutely, ^{14}C-fructose uptake was inhibited by chrysin only (±20%). In contrast, chronically, quercetin, chrysin and apigenin (100 µM) caused a ±25% decrease in ^{14}C-fructose uptake. Their inhibitory effect was not related to a cytotoxic effect (as determined with the MTT and the LDH assays). GLUT5 is the main carrier involved in the apical uptake of fructose by enterocytes. [7] By using a specific inhibitor of GLUT5 (L-Sorbose-Bn-OZO 10 µM) we could conclude that these polyphenols do not appear to interfere with this transporter. On the other hand, by using phloretin (1 mM), an inhibitor of GLUT2, we could conclude that these polyphenols appear to interfere with this transporter. Moreover, ^{14}C-fructose uptake appears to be dependent on p38 MAPK, PI3K and PKA intracellular signalling pathways, but the polyphenols do not act through these signalling pathways. Furthermore, the polyphenols were able to markedly (>80%) decrease the mRNA expression of GLUT2 and GLUT5.

Conclusion: Quercetin, apigenin and chrysin were found to be effective inhibitors of ^{14}C-fructose uptake by Caco-2 cells and they appear to interfere with GLUT2-mediated ^{14}C-fructose uptake. Moreover, they are stunning inhibitors of GLUT2 and GLUT5 gene expression. This suggests that these compounds might decrease the intestinal absorption of fructose, with beneficial effects on type 2 diabetes/MS.

Acknowledgements and Funding: Professor Arnaud Tatibouet of the University of Orleans, France, for having provided the inhibitor of GLUT5 and CAPES – Brazilian Federal Agency for Support and Evaluation of Graduate Education within the Ministry of Education of Brazil, for financing this project – PN: 10103/13-9.

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2 – Pediatric Obesity – Reality of Nutritional Consultation in a Hospital

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Introduction: Childhood obesity, is reaching alarming proportions in many countries, representing an urgent and serious challenge of the XXI century, when we talk about public health. [1, 2] In Portugal, 31,6% of the children are overweight 13,9% are obese.
Objectives: To evaluate the evolution of the anthropometric measures of children/adolescents that are attending nutritional consultation in a hospital. To verify which eating habits they have in a subsequent nutritional consultation.
Methods: This study was conducted from 22nd of April to 1st of July, 2016, in 72 children (who attending nutritional consultation since February, 2009) in whose a questionnaire was administered indirectly for benchmarking the variables of children/teenager lifestyle (regular physical activity, daily spent on electronics and eating habits) and education and anthropometric measures of parents and children/adolescents. The anthropometric measures of children/adolescents used in the study were obtained in the first nutritional consultation and in the subsequent nutritional consultation when the questionnaire was applied. We defined overweight when the percentile of body mass index (BMI) is superior of 85 and less than 95 for age and sex and obesity like percentile of body mass index over 95 for age and sex according to tables of Center for Disease Control and Prevention, 2000. It was calculated the reference weight according to the percentile of height, age and sex in the first consultation and in the subsequent consultation. The overweight it was calculated by the difference between the real weight and the reference weight and it was divided into classes.
Results: Our sample it was made by 48 children/teenagers from female gender and 24 children/adolescents from male gender with average age in the first consultation of 9 ± 3 years and with average age in the subsequent consultation of 12 ± 4 years. We verify that the BMI more prevalent in both genders was obesity. Relatively to overweight, the bigger percentage of children/adolescents have more than 15kg in female gender (35,6% first consultation versus 47,7% subsequent consultation) and in male gender (48% first consultation versus 54,2% subsequent consultation). When we evaluate the scholarity, the bigger percentage of children/adolescent (50%) attended the 5th to 9th grade and 54,2% of mothers and 59,7% presented the same scholarity. In result of maternal and paternal BMI, we found more prevalent the class "25-29,9kg/m^{2}" (52,8% (n = 38) and 38,9% (n = 28), respectively). The practice of physical activity extra school between 30-60 minutes was performed by 69,5%. The most common frequency was twice a week (40%). Only 18% practice daily. The time spent in electronic devices was divided in "watch television" and "play electronic games". The results show that 55 children/adolescent (76,4%) play daily and the most prevalent category was 60minutes (n = 18; 32,7%). Relatively to "watch television" we assess that 59 children/ adolescents (81,9%) do it daily and the most prevalent category was 60minutes (n = 22; 37,3%). The eating habits of products with higher energetic density (chocolate, cakes, sugary cookies, gum, juice/soft drinks and fried products) collected on the subsequent consultation, was gums (55,6%), sugary cookies (52,8%) and chocolates (51,4%) the products with higher percent of children/ adolescents that not consumption them. The products with higher frequency of consumption was cakes (70,8%) and juice/soft drinks (63,9%). In this group, the daily frequency of consumption was higher (9,7% versus 4,2%). Fried products were also consumed in larger quantities (70,8%). When we asked about the heathy habits, we verify that soup in lunch was consumed by 80,6% and in dinner by 91,7%. The higher frequency of consumption was daily. Soup wasn't consumed only by 19,4% in lunch and this percentage decreased in dinner until 8%. Fruit wasn't consumed by 5,6% of children/adolescent and at dinner this percentage increased to 12,5%. The higher percentage of consumption daily was at lunch (n = 38, 52,8%).
Conclusions: Consultations were positive because a big percentage of children and teenagers start eating soup and fruit daily. However, they not abandoned the habit of consuming products with high energy density. We already, verify that they continue to have sedentary lifestyle.

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3 – Hyperuricemia in Obesity

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**Equally contributed to this work.*

Background: The prevalence of obesity is growing worldwide. Hyperuricemia is associated with the metabolically unhealthy obesity phenotype (MUHO) and is an independent predictor of cardiovascular diseases. [1] We aimed to evaluate the circulating uric acid levels in Brazilian individuals presenting either the metabolically healthy obesity (MHO) or the MUHO phenotype. [2]
Material & Methods: A descriptive cross-sectional study was conducted with obese individuals [body mass index (BMI) ≥35.0 kg/m²] of both sexes, aged 21-63 years. Anthropometric data [weight, height and waist circumference (WC) besides BMI] and metabolic parameters [blood pressure and blood glucose, HDL-cholesterol, triglycerides (TG) and uric acid] were obtained. Cut-off points for hyperuricemia were ≥6.8 and ≥5.4mg/dL, respectively, for men and women. [3] Individuals were classified as MHO or MUHO, according to the NCEP/ATP III definition. [4]

Results: Of the 232 subjects evaluated, 40.1% were classified as MHO (n = 93) and 59.9% as MHUO (n = 139). Age (years), weight (Kg), BMI and WC (cm) mean (± standard deviation) values were 39.6 ± 11.1/43.6 ± 10.1, 117.5 ± 17.1/117.8 ± 20.0, 42.9 ± 4.5/42.4 ± 4.9 and 118.0 ± 11.9/120.7 ± 14.2 for the MHO and MHUO groups, respectively. Accordingly, in these groups, circulating uric acid concentrations (mg/dL) were 5.0 ± 1.1 and 5.4 ± 1.4 (p = 0.033), respectively. Hyperuricemia was present in 30.1% (n = 28) and 34.5% (n = 48) of the MHO and MHUO groups (6.2 ± 0.7 and 6.8 ± 1.3, respectively; p = 0.042). Among the features included in the MHUO definition, WC, TG and glucose were positively correlated with uric acid (r = 0.275/p<0.001; r = -0.199/p = 0.002 and r = 0.156/p = 0.017, respectively) while HDL-c was negatively correlated (r = -0.188/p = 0.004). Circulating uric acid concentrations increased with the increasing number of the features of the MHUO definition (B = 0.153; p = 0.045).
Conclusion: In our Brazilian population, hyperuricemia was present in both obesity phenotypes, but higher in the MHUO. In this phenotype, as individuals had more components of the MHUO definition, the circulating uric acid levels were higher.

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4 – Metabolic Syndrome and Inflammation: Is There a Microvascular and an Incretin System Impairment in the Gastrointestinal Tract?

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Introduction: Metabolic syndrome is a multifactorial disorder characterized by increased plasma levels of glucose, cholesterol and triglycerides, but also overweight and obesity promoted by increase of body fat mass, alterations in oxidative stress, chronic low grade inflammation and resistance to insulin leading to risk of cardiovascular diseases. [(1)] The stomach and the intestine have an essential role in metabolism with functions of digesting food and absorption of nutrients. Also, the intestine produces incretin hormones, such as GLP-1 which regulates glucose metabolism and processes of the gastrointestinal tract. [(2)]
Objectives: We aim to evaluate the inflammatory status, blood and lymphatic microvasculature and the incretin system in the intestine and stomach in animals exerting metabolic syndrome.
Methods: It was assessed the expression of IL-6, 3-NT, CD31, LYVE-1 and GLP-1 receptor on tissues from the stomach and intestine of C57BL/6 mice, divided in two groups, a high fat diet and a normal diet group.
Discussion and Conclusion: The results suggest that metabolic syndrome promotes alterations in the inflammation status, vascularization and in the incretin system of gastrointestinal tract. In high fat diet mice, it was observed higher levels of inflammation, no alteration in expression of 3-NT, lower blood microvascular density, lower number of lymphatic vessels and decreased expression of GLP-1 receptor. Further studies are important to understand other molecular mechanisms involved in metabolic syndrome and their possible influence in the gastrointestinal tract.

Keywords: metabolic syndrome, inflammation, incretin system, microvasculature, gastrointestinal tract

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5 – Is the MTHFR C677T Polymorphism Associated with Obesity Risk? – a Meta-Analysis.

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Background: Overweight and obesity are a major worldwide health problem and its incidence is increasing every year. [(1)] Methylenetetrahydrofolate reductase (MTHFR) plays an important role in folate metabolism and as a regulator of DNA methylation, synthesis, and repair. MTHFR gene is polymorphic at nucleotides 677 (C→T) and 1298 (A→C). MTHFR C677T polymorphism results in alloenzymes with decreased activity and several studies have pointed to association between the MTHFR C677T polymorphism and overweight/obesity risk. [(2)]
Objectives: The present study aims to contribute to the elucidation of the impact of any C677T overweight/obesity association through a meta-analysis study of published case control studies.
Material and Methods: Pubmed, Google Scholar, Elsevier and Cochrane trials databases were searched for case control studies of associations between MTHFR C677T polymorphism and overweight/obesity. Odds ratios (ORs) with 95% confidence intervals (CIs) were estimated to assess the association.
Results: Our results suggest that MTHFR C677T polymorphisms may modify the association between intracellular folate levels and overweight/obesity risk. Homozygous individuals for the MTHFR 677T polymorphism may have a significantly increase of obesity risk.
Conclusion: Our results suggest an association between MTHFR C677T polymorphism and risk of being overweight/obese.

Keywords: MTHFR, C677T, polymorphism, obesity, risk.

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6 – Polyphenols Modulate Type 2 Diabetes: Relevance to Angiogenic Paradox

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Background: Diabetes *mellitus* (DM) is responsible for metabolic de-

regulation leading to inflammation and oxidative stress, causing angiogenic derangements. [1] In fact, DM is a paradoxical disease regarding vascular complications with distinct angiogenic patterns in different organs, namely an increase in kidney neovascularization and the opposite in the left ventricle (LV). [2] Nevertheless, the mechanisms governing this angiogenic paradox remain unclear. DM associated vascular complications are highly dependent on organ-specific metabolic disturbances. [3] Beer polyphenols, as xanthohumol (XN) and 8-prenylnaringenin (8PN) modulate angiogenesis, [4] being promising candidates for further development to treat chronic diseases namely metabolic syndrome and diabetes.

Aim: This study aimed to verify if polyphenols consumption affects angiogenic paradox and if this is related with metabolic changes in diabetic mice.

Methods: For this purpose, C57Bl/6 mice were divided in 5 groups treated with: standard diet, high fat diet (HFD), HFD and ethanol, HFD and XN, and HFD and 8PN, during 20 weeks. Kidney and LV were collected to evaluate microvessel density (MVD) and the expression of angiogenic receptors and related pathways. Statistically significance was assessed by ANOVA followed by Bonferroni test.

Results: An increase in kidney and a reduction in LV microvessels of diabetic C57Bl/6 mice were observed. XN consumption reduced angiogenesis, demonstrated by a reduction in MVD, in VEGFR-2 activation and in the expression of its downstream effectors (AKT and Erk) as well as by the reduction in kidney VEGF-A content. 8PN had the opposite effects in diabetic LV. These findings were accompanied by tissue and plasma reduced levels of VEGF-B and its receptors, VEGFR-1 and neuropilin-1, by both polyphenols.

Conclusion: XN and 8PN modulated T2DM angiogenic paradox in a tissue-dependent manner. We also reported for the first time that both polyphenols decrease VEGF-B pathway, which is implicated in endothelial-to-tissue lipid metabolism. Thus, nutritional supplementation with polyphenols that exert beneficial effects in the cross talk between angiogenesis and metabolism render them putative tissue specific-target agents, potentially allowing a better resolution of T2DM-related complications.

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7 – Identifying Risks: Metabolic Syndrome Prevalence on Two Units of Primary Care

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Introduction: Metabolic syndrome (MS) designs a constellation of multiple cardiovascular risk factors that tend to occur together. There is no universal consensus on the definition but, in 2009, a new definition was published considering metabolic syndrome is observed when at least 3 of the following 5 criteria occur: elevated abdominal waist circumference (Portuguese cut off: >102cm in males and >88cm in females); triglyceride level ≥ 150 mg/dL or use of fibrates, niacin or high dose ω3 fatty acids; low c-HDL (< 40mg/dL in males and < 45 mg/dL in females) or use of fibrates or niacin; high blood pressure (systolic ≥ 130 and/or diastolic ≥ 85 mmHg) or use of antihypertensive drugs; fasting glucose > 100 mg/dL or use of antidiabetic drugs. The prevalence of metabolic syndrome is about 20-30% in the majority of countries. Prevention and detection is crucial to reduce mortality and mobility related to cardiovascular events.

Objective: Calculate metabolic syndrome prevalence between patients with dyslipidaemia from two different units of primary care.

Material and methods: Observational retrospective descriptive study. Population: adults from two units of primary care with active T93 diagnosis (dyslipidemia), on September 2016. Data were collected from MIM@UF® and SClínico® about age, sex, body mass index, abdominal waist circumference, c-HDL and triglyceride levels, hypertension and fasting glucose. Antidyslipidaemic drugs were registered. Data were registered on an Excel® sheet and descriptive statistics were calculated.

Results: 4106 patients were identified with dyslipidaemia and 27% (n = 1106) of them meet criteria to metabolic syndrome. 759 patients (18%) were excluded because of the lack of data. Mean age of the metabolic syndrome group was 72 years old and female represented 56% (n = 620) of the affected individuals. Raised fasting glucose and hypertension was observed on 68% (n = 755) and 86% (n = 954) of the patients respectively. Metabolic syndrome was observed on 54% (n = 519) of obese patients (n = 953). The overall prevalence of metabolic syndrome was 7,6% (1106 out of 14486 patients).

Discussion: The overall prevalence of metabolic syndrome in these two units of primary care is lower than the majority of studies published. The lack of information seems to be a determining factor to this result. Investigation of risk factors and registration of them on clinical record is crucial to be made by family doctors so that a higher number of patients can be diagnosed and intervention can be made. Considering the methodology applied (selection of dyslipidemic subjects), there can be a significant number of patients that were not included but whom could have metabolic syndrome (if the other 3 criteria were present). Having in mind the high prevalence of metabolic syndrome between obese patients, these group should merit physician attention.

8 – Adipose Tissue Adaptations Triggered by Left Ventricular Chronic Pressure Overload

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Introduction and Aims: The progression of chronic pressure overload (CPO) is associated to cardiac cachexia as a consequence of insufficient energy supply. Additionally, some studies demonstrated that the heart is able to modulate the adipose tissue (AT) structure and function by the cardiokines secreted. In this study we investigate the effects of CPO in the AT during the early stages of the disease.

Methods: Wistar rats were submitted to aortic banding (Ba group) or sham procedure (Sham group). After 8 weeks, left ventricular (LV) function and structure (echocardiography and invasive hemodynamic) was evaluated and samples collected (LV, AT and plasma). Visceral AT from normal rats was incubated with B-type natriuretic peptide (BNP; 0.27 and 0.47ng/ml) for 24h and then collected for molecular studies.

Results: Eight-weeks of banding increased systolic pressure and triggered cardiac remodeling with cardiomyocytes' hypertrophy and fibrosis when compared to the Sham animals. The same group was at a compensated stage of the disease with higher ejection fraction, however a stiffer myocardium was observed with increased end diastolic pressure-volume relation and passive force of isolated cardiomyocytes. Despite similar adiposity between the 2 groups, aortic constriction triggered adipocyte atrophy as well as AT increased fibrosis and dysfunction, as observed by overexpression of pro-in-

flammatory adipokines. The incubation of AT from normal rats with BNP confirmed that the Ba group increased circulatory levels of BNP were able to induced higher expression of pro-inflammatory adipokines by AT.
Conclusions: We demonstrated that elevated circulatory levels of BNP promoted by LV CPO are able to induce remodeling of the AT and overexpression of pro-inflammatory adipokines.

	Sham	Ba
LV systolic pressure (mmHg)	110 ± 3.6	153 ± 10.5 *
Heart/tibial length (g/cm)	2.3 ± 0.05	3.3 ± 0.30 *
LV cardiomyocyte cross-sectional area (μm^2)	382 ± 23.6	484 ± 33.6 *
LV fibrosis (%)	4.2 ± 0.52	6.3 ± 0.94 *
LV ejection fraction (%)	78 ± 0.9	89 ± 1.9 *
LV end diastolic pressure volume relationship	0.04 ± 0.006	0.11 ± 0.031*
LV passive force at 2.2μm (mN/mm^2)	3.3 ± 0.29	4.4 ± 0.57
Plasma BNP (ng/ml)	0.27 ± 0.048	0.47 ± 0.080 *
AT/tibial length (g/cm)	7.9 ± 0.88	7.5 ± 0.25
AT fibrosis (%)	7.2±0.31	8.7±0.61 *
Adipocyte CSA (μm^2)	1659±103.8	1287±85.1 *
AT TNFα (AU)	0.03±0.013	0.06± 0.018 *
AT IL-1β (AU)	0.04 ± 0.01	0.28 ± 0.15 *
TNFα (AU) after incubation with BNP	26380 ± 1428	31125 ± 1455 *
IL-1β (AU) after incubation with BNP	9038 ± 678	12221 ± 1086 *

Data presented as mean± SEM. * p<0.05 vs Sham.

9 – Age-related Metabolic Biomarkers of Testicular Function: NMR-based Metabolomics

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Ageing is characterised by a general decline in cellular function, usually attributed to oxidative stress and metabolic dysfunction. Although alterations in fatty acid and glucose metabolism were found in liver and muscle of healthy mouse, [(1)] little is known about the influence of age related processes on molecular mechanisms of male fertility and reproductive system functioning. [(2)] In this study a nuclear magnetic resonance (NMR) based global metabolomics analysis of testicular extracts was used in order to identify new metabolic biomarkers of ageing in the animal model and to correlate them with the known changes in the age related testicular function. Wistar rats were divided into 3 groups and sacrificed after 3, 6, and 24 months (n = 24). The animals had free access to standard animal diet. After the sacrifice, testes samples were collected, homogenized (pulverized) and metabolites extracted using methanol/water/chloroform system. Hydrophilic metabolites were re-suspended in D_2O buffer solution and ^{1}H NMR spectra recorded (600 MHz Varian spectrometer). Multivariate statistical analysis (MVA) was applied on the processed NMR spectra. Principal component analysis (PCA) was used to provide qualitative information on the observed data set by grouping the samples with similar metabolite profiles and identifying possible outliers. [(3)] Although scattering was observed within the 24-month old group, clear separation was observed between all age groups indicating distinct metabolic profile related to each age. Partial least square discriminant analysis (PLS-DA) was used to identify the main metabolites that contribute to the class discrimination. MVA was complemented with the quantitative assessment of metabolite variations between groups through spectral integration. Changes were observed in various classes of metabolites during ageing. Increase in amino acids (Val, Leu. Ile, Phe, Tyr, Asp) continued until the older age (except Arg). Compounds related to the metabolism of lipids (choline, phosphocholine, glycerophosphocholine, ethanolamine, glycerol, carnitine, and myo-inositol) were also affected. Nucleotides like IMP/AMP, CMP or ATP were observed in significant quantities only at young age (3 months). In comparison, concentration of nucleosides (adenosine, uridine) and free bases (adenine, uracil) start to rise with age (6 and 24 months).

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10 – Childhood Obesity: the Role of Intestinal Microbiota

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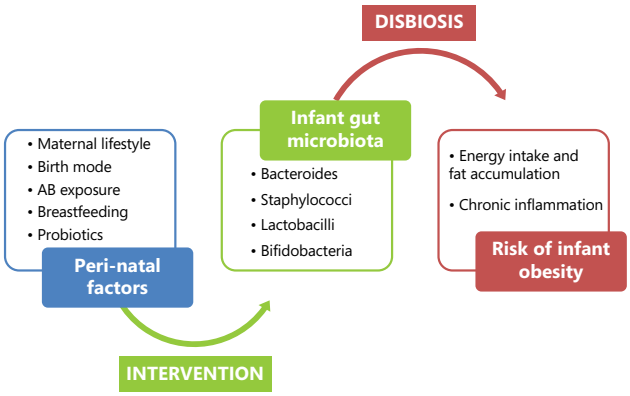
Childhood obesity is associated to incremented risk of developing diseases such as diabetes cardiovascular diseases or cancer, later in life.
Gut microbiota plays an essential role on obesity and its development is performed until 3-5 years old. Peri-natal period is crucial to define quantity and diversity of a healthy intestinal microbiota. Maternal diet/BMI, delivery mode, antibiotic exposure and breastfeeding are some of the processes that will determine a favorable gut microbiota.
Functions of gut microbiota, mostly by producing short-chain fatty acids as metabolites, include regulation of metabolism and immune system of the host, which may be compromised in case of disbiosis.
This review communication pretends to evaluate the state of the art concerning infant obesity and the role of gut microbiota.

Conclusions:



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11 – Differential Imune Response to Vitamin A in B16-F10 Malignant Melanocytes

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Introduction: Melanoma is an aggressive form of skin cancer with a poor prognosis, due to its refractory behavior to radiation and chemotherapy. Although the diagnosis is straightforward, there are many disagreements regarding its treatment and surveillance. [1, 2, 3] In order to surpass some of the limitations addressed to the treatment, preventive methods like antioxidant vitamins are nowadays a relevant field of research, as well as immunostimulation by external agents. [4] Despite the knowledge about melanoma biology, pathogenesis and developed therapies, [2] is important to understand the effect of vitamin A in order to suggest alternatives to conventional therapies, [5] which are known to be ineffective against melanoma.

Objectives: The main goal of the present project was to create and develop an *in vitro* model that could be used to address the use of antioxidant vitamins as therapeutic contributors. In particular, we wanted to understand the redox effect of vitamin A in a melanoma cell line (B16-F10) as well as to understand its role in the immune system by assessing the activation of naïve macrophages (Raw 264.7).

Materials and Methods: All experiments were performed on melanoma cells, B16-F10, and macrophage-like cells, Raw 264.7. B16-F10 cells were treated with different concentrations of hydrogen peroxide (H_2O_2), different concentrations of vitamin A and with a mixture of H_2O_2 + vitamin A. Raw 264.7 cells, in co-culture with B16-F10, were also treated with different concentrations of vitamin A. The metabolic activity was measured by MTT assay.

Results and Discussion: Lower doses of vitamin A resemble an enhancement of the cytotoxic activity of macrophages, whereas higher concentrations could have the opposite effect.

Conclusion: The co-culture experiment allowed the study in a more complex environment showing that the possible therapeutic effect of vitamin A was inversely correlated to the results of melanoma cell cultures alone. However, further studies are needed in order to validate the proposed results.

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12 – Mitochondrial DNA Depletion Re-programs the Expression of Fatty Acid Metabolism-related Genes and Leads to Lipid Droplet Accumulation

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Metabolism and the Warburg effect are considered cancer hallmarks. The last few decades revealed the importance of lipid metabolism and lipid droplets (LDs) in cancer biology. Although mitochondria play a central role in overall cellular metabolism, the influence of mitochondrial dysfunction (particularly due to mitochondrial DNA (mtDNA) pathogenic mutations) in lipid metabolism and cytoplasmic LD accumulation in cancer remains unclear.

In this work, we aimed to link mitochondrial dysfunction with lipid metabolic re-programming, with focus on fatty acid (FA) metabolism. Specifically, we aimed to understand if pathogenic mutations in mtDNA lead to cytoplasmic LDs alterations (number and/or size) and to identify key genes involved in alterations of FA metabolism.

To do so, we characterized the lipidic profile of seven cybrid cell lines (osteosarcoma derived cell lines), as a model of mitochondrial dysfunction due to the presence of mtDNA alterations. Our data demonstrates that LDs content, as well as LDs number and size, are influenced by mtDNA depletion. Concomitantly, we showed that mtDNA depletion induces profound alterations in FA metabolism by altering the expression of different metabolic genes, and identified three genes potentially involved in LDs accumulation and/or lipid metabolic re-programming.

Additionally, mtDNA mutations and mutation load differently impact FA metabolism by modulating expression of genes involved in this pathway.

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13 – Gestational Diabetes and Cognitive Performance at 10 Years of Age

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Background: Gestational diabetes is one of the most common pregnancy complications worldwide and it has been associated with impaired fetal neurodevelopment. However, the long term impact of intrauterine hyperglycemia on children's cognitive performance remains unclear. The main aim of this study was to evaluate the effect of intrauterine exposure to gestational diabetes on cognitive performance of 10 years old children.
Methods: Generation XXI birth cohort were assembled in 2005/2006, at all public units providing obstetrical and neonatal care in Porto, Portugal. Information was collected by face-to-face interview and additionally from clinical records, including gestational diabetes. Cognitive performance was assessed using the Wechsler Intelligence Scale for Children (WISC-III) conducted by trained psychologists as part of the 10 year follow-up examination. The WISC–III comprises 4 dimensions (perceptual organization index, processing speed index, verbal comprehension index, performance index) that sum to produce a full-scale IQ. Regression coefficients (β) and 95% confidence intervals (CI) were computed using generalized linear models using data from 2122 10 years-old children. Multivariate model were adjusted for maternal education.
Results: Of the 2122 children, 144 were offspring of gestational diabetic mothers (GDM) and 1978 were offspring of non-GDM mothers. In crude models, cognitive scores were similar in both groups of children. When adjusted for maternal education, offspring of GDM had statistically significant higher scores than offspring of non-GDM for processing speed index (β = 2.804; 95% CI: 0.429 to 5.179; p = 0.021) and for full scale IQ (β = 2.669; 95% CI: 0.139 to 5.200; p = 0.039).
Conclusions: Exposure to maternal diabetes in utero did not seem to impair children's cognitive performance.

14 – Unveiling Metabolic Cues in Type 1 Diabetes Vascular Complications. The Endotelial Cell as a Target

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Introduction: Type 1 Diabetes *Mellitus* (T1DM) is metabolic disorder characterized by chronic hyperglycaemia state associated with micro and macrovascular complications in several organs. [(1)] The vascular endothelium is regarded as an important factor in the pathogenesis of T1DM, [(2)] since it embraces the so-called "angiogenic paradox". Accordingly, angiogenesis the formation of new blood vessels from pre-existing ones, [(3)] is often exacerbated (e.g. in the retina and kidney) as well as impaired, namely in wound healing and compromised formation of collateral arteries, [(4)] in the same patient.
Objectives: To examine angiogenesis and metabolic-associated gene expression profile in T1DM mice kidney and heart endothelial cells.
Methodology: C57Bl/6 mice were administered streptozotocin to induce T1DM. Kidneys and hearts were harvested ten weeks after diabetes development. Endothelial cells (ECs) were isolated by fluorescence-activated cell sorting (FACS), and subjected to Angiogenesis and AMPK Signaling PCR Arrays following total RNA extraction. Gene expression and statistical analysis were assisted by the RT [(2)] Profiler PCR Array Data Analysis Web Portal (Qiagen).
Results: Upregulation of 5 genes were found upon AMPK Signaling PCR Array analysis: Adra1a, Cpt1a, Pfkfb2, Strada and Rb1cc1 in the kidney ECs. Inversely, Cab39, Akt2, Rps6kb2, Adra2c, Pnpla2, Prkacb transcripts were down-regulated in heart ECs. The analysis of the Angiogenesis PCR Array showed Tgfb2 and Timp2 down-regulated in the kidney, while in the heart Notch ligand, Jag1, was downregulated whereas Smad5 expression was upregulated.
Conclusion: Imbalances in mTOR, Akt and PI3K signaling, as well as in growth factors involved in angiogenesis were found in ECs from the two organs, implying lipid, carbohydrate and protein metabolism changes. Elucidating the crosstalk between endothelial metabolism-vascular complications will enable novel therapeutic approaches.

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15 – Ligand-bound Glucocorticoid Receptor Inhibits TGF-β1 Induced Epithelial to Mesenchymal Transition in Mammary Cells. Does Diabetes-associated Alostasis Disruption with Impaired Stress Responsiveness Promote Tumor Metastasis?

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A massive black hole impairs the biology field concerning the role of stress upon cancer progression and metastases dissemination. More than 90% cancer-related deaths are the result of distant metastases onset [(1)] and diabetes, for example, as a multisystem disease that in-

creases the allostatic load due to constant homeostatic challenges impacting both interoceptive and exteroceptive neural pathways (the latter expressing more marked interpersonal dynamic variability) involved in perception of stressful events predisposes to an unfavourable outcome with a significant increase in the mortality of breast cancer patients. [2] In a model of streptozocin induced diabetes in mice, stress responsiveness was impaired with generation of cortisol, ACTH and CRH plateau curves across time with a obviously decreased magnitude of secretion of the endogenous glucocorticoid per comparison with healthy controls, after induction of restraint. [3] Albeit the association between flattened diurnal cortisol rhythmicity with poor prognosis in breast cancer patients, [4] only recently Lauriola and colleagues have shown that ligand-bound glucocorticoid receptor is able to downregulate signalling downstream to EGFR, with the retrospective analysis of biological specimens of breast cancer patients revealing that those ones with decreased glucocorticoid receptor expression were associated with a poorer prognosis. [5]

Herein, taking altogether in account, we established a protocol to address the role of glucocorticoid signalling upon TGF-β_1 induced transient epithelial to mesenchymal transition (EMT) in mammary cells (MCF10A), concerning that interestingly, TGF-β_1 plasma levels are higher in diabetics in comparison with healthy controls. Pathological EMT is associated with metastatic dissemination, involving the loss of epithelial phenotype and acquisition of mesenchymal characteristics. [6] Control excipient treated cells were compared with TGF-β_1 standalone, TGF-β_1 + 100nM dexamethasone, TGF-β_1 + 100 nM dexamethasone + 5 µM RU486 (potent glucocorticoid receptor antagonist) and 100nM dexamethasone singly. Our immunofluorescence analysis showed that MCF10A cells treated with TGF-β_1 acquired a fibroblastoid morphology with loss of epithelial markers (E-cadherin, β-catenin) and up-regulation of mesenchymal ones (vimentin). Controls displayed a cortical pattern of F-actin disposition, with TGF-β_1 treated ones revealing a reorganization of the cytoskeleton with abundant stress fibres. Once again, simultaneous treatment with TGF-β_1 and dexamethasone partially reversed the phenotypical transdifferentiation with a marked decrease in stress fibres. In all the experiments, treatment with TGF-β_1 + dexamethasone + RU486 exacerbated the phenotypical switch, and in regard to the cytoskeleton, rather than stress fibres, the lamellipodia and filopodia extension was also clearly noted. Generically, dexamethasone reverted the ability of TGF-β_1 to induce the phenotypical switch and also the topological alterations observed with the EMT phenomenon.

In synthesis, our results support a cross-regulation between glucocorticoid receptor and TGF-β_1 signalling pathways. Lifelong increase in allostatic load (such as in diabetes) and impaired response to stress may act as a contributing factor for pathological EMT and, thus, tumor metastasis.

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16 – Consumption of Sweetened Beverage in Children and its Relation with Metabolic Syndrome: Needs Assessment and Health Education

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Introduction: The intake of sugar-sweetened beverage (SSB) has been associated with obesity, type 2 diabetes, cardiovascular diseases and dental caries. Thus, an increased consumption of SSB may potentiate metabolic syndrome (MS). [1] The consumption of SSB has increased amongst children. [2] The promotion of health education at parents/tutors level may lead to a decrease in SSB intake by children, with an improvement in their quality of life in medium-long term and, consequently, diminished costs for public health. [3]

Objectives: The objective of the present study was to study the intake of SSB in children. Additionally, we aim to sensitize parents/tutors for the risks of high SSB intake, namely in the development of MS.

Materials and Methods: In this study participated 24 parents of 29 children from a primary school in Northern Portugal. The children's dietary intake was measured using a food frequency questionnaire completed by the parents. The questionnaires were used to assess the consumption habits of SSB. Additionally, the questionnaires collected demographic (age, gender, marital status, education level, number of children) and socio-economic (annual household income) data.

Results: In this study, the data showed that 29% of children consume fruit-flavored drinks without sweeteners, 38% fruit-flavored drinks with sweeteners, 92% soft and 13% diet drinks. Among the SSB, coca-cola and ice tea were identified as the most consumed. Our results demonstrate that 46% of our participants consume such drinks between 2-4 times a week. Regarding to the period of consumption, drinks are consumed on a weekly basis, especially during the afternoon.

Discussion/Conclusion: The occurrence of metabolic disorders is starting at an earlier age, leading to an increase of MS in children and young adults. Our results showed that nearly 50% of the participants consumed frequently SSB. Thus, a health education approach directed to children and parents/tutors may reduce the consumption of SSB and, consequently, diminish the incidence of MS in children and young adults.

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