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10º Simpósio de Metabolismo da Faculdade de Medicina da Universidade do Porto

– Palestras, Comunicações Orais e Posters

> SESSION I – METABOLIC REGULATION AND INFLAMMATION

Adipose tissue pollutants and metabolic inflammation

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The complex role of adipose tissue on energy metabolism is being the focus of intense research in the past few years with the recognition of its status as an endocrine organ and that its functions go well beyond energy storage. Obesity, an increasingly prevalent disease, is characterized by a low grade inflammatory status, that that is in tight relationship with the development of obesity comorbidities. Alongside with this, the mere increase in adipose tissue is not responsible for obesity-associated pathologies, which seem more dependent on location of adipose tissue accumulation, adipose tissue cellularity, and adipocyte dysfunction. Obesity is caused by the mismatch between ingested and expended calories, but many factors, including genetic, socioeconomical, cultural, psychological and environmental cues, contribute to this imbalance.
Recently, the accumulation of lipophilic environmental pollutants on the adipose tissue of humans has been highlighted. The adipose tissue serves not only as the main reservoir for these compounds, but it also constitutes one of the main targets of environmental toxicants actions. Either acting locally or being released from their adipose tissue reservoir, these chemicals, some of which have been demonstrated to possess endocrine-disrupting ability, impose pressure onto energy homeostasis regulation, predisposing to obesity and adipocyte dysfunction, the trigger of inflammatory activation in obesity.
Starting with an overview of the interplay between metabolic, inflammatory and immune processes in obesity, the presence of pollutants in the adipose tissue and their relationship with metabolic dysfunction will be documented, discussing their contribution to metabolic inflammation.

A role for UBXD8 in the regulation of adipose tissue homeostasis *in vivo*.

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The hairpin-containing protein UBXD8 acts as an adaptor to recruit the molecular segregase p97/VCP to ERAD complexes, presumably to mediate the dislocation of ERAD substrates into the cytoplasm, promoting their degradation in proteasomes.
Biochemical and cell biological studies suggest a role for UBXD8 in regulation of ERAD in general and in the dislocation of proteins implicated in metabolic control. These include the degradation of INSIG1, an important regulator of sterol biogenesis. In addition to its role at the ER, emerging evidence suggests a role for UBXD8 in mediating homeostasis of lipid droplets, by controlling dislocation of improperly lipidated ApoB-100, the major lipoprotein of low density lipoprotein (LDL). UBXD8 has also been reported to controls lipolysis by enforcing dissociation between the major triglyceride lipase ATGL and its activating cofactor, CGI-58.
To test the hypothesis that UBXD8 regulates metabolic homeostasis at the organismal level, we generated UBXD8 mutant mice. These animals exhibit striking defects in adipose tissue and have an altered metabolism.

The interplay between weight loss, inflammation and cardiovascular risk

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Introduction: The low-grade inflammatory state that characterizes obesity has been associated with iron deficiency and disturbances in erythropoiesis. The continuous inflammatory stimuli in obesity may trigger the expression of hepcidin, a major regulator of iron metabolism. There is also a close relationship between inflammation and dyslipidemia in obesity, increasing the risk for cardiovascular (CV) diseases. Current knowledge regarding the biology of high-density lipoprotein (HDL) points to different atheroprotec-

tive efficacy of HDL subpopulations. Concerning low-density lipoprotein (LDL) subpopulations, small dense LDL subfractions are considered more atherogenic than large LDL subfractions.
Aims: Review data concerning the impact of different weight loss strategies in iron metabolism, erythropoiesis, inflammation and dyslipidemia, and their relationships.
Methods: To study the impact of weight loss achieved through a physical exercise intervention program in overweight and obese children and adolescents[1], and through laparoscopic gastric banding surgery in adults patients with obesity, after 13-months of surgery [2,3], on markers of iron metabolism, erythropoiesis, inflammation and dyslipidemia, especially on HDL and LDL subfractions.
Results & Conclusion: Weight loss is associated with an improvement in inflammation, namely, with a reduction in interleukin-6 that by reducing hepcidin production, reduces the disturbances in iron status [1,2], improving iron availability for erythropoiesis, as showed by a more adequate erythrocyte hemoglobinization [2].
Besides the reduction in inflammation, weight loss, induces other atheroprotective changes, namely, on lipid profile, enhancing larger HDL, the more atheroprotective subfraction, reducing the less protective subfraction, small HDL, and reducing oxidized LDL (oxLDL) and oxLDL/LDL ratio [3]. The changes induced by weight loss are atheroprotective and, therefore, important to reduce CV risk.

References:

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> SESSION II – METABOLIC MARKERS AND SIGNALLING

Metabolic selection of tumor phenotypes

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Metabolic reprogramming plays a pivotal role in cancer progression. However, it is unclear if selective pressures, such as chemotherapy, imposed on tumor cells impact on the array of metabolic-adapted features that contribute for tumorigenesis and which signaling pathways orchestrate those adaptations. In order to elucidate this question, we established a mouse xenograft model of human acute myeloid leukemia (AML) that enabled chemotherapy-induced regression followed by lethal regrowth of more aggressive disease.
Human AML cells from terminally ill mice treated with chemotherapy (chemoAML) had higher lipid content, increased lactate production and ATP levels, reduced expression of PPARG coactivator 1α (PGC-1α), and fewer mitochondria than controls from untreated AML animals. These changes were linked to increased vascular endothelial growth factor receptor 2 (VEGFR-2) signaling that counteracted chemotherapy-driven cell death; blocking of VEGFR-2 sensitized chemoAML to chemotherapy (re-)treatment and induced a mitochondrial biogenesis program with increased mitochondrial mass and oxidative stress.
Collectively, this reveals a mitochondrial metabolic vulnerability with potential therapeutic application against chemotherapy-resistant AML.

Lipid metabolism in macrophages activation and function

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Alternatively activated macrophages are involved in important physiological and pathophysiological processes as diverse as containing tissue parasites, contributing to the maintenance of adipose tissue homeostasis and insulin sensitivity and promoting an immune tolerant microenvironment that allows tumor growth and metastasis. Alternative macrophage activation, triggered by interleukin 4 (IL-4), is associated with a metabolic remodelling requiring increased lipid and glucose utilization.
We identified a role for sterol regulatory element binding protein 1 (SREBP1) in the control of macrophage alternative activation. SREBP1 is activated by IL-4 in an AKT-dependent manner, resulting in upregulation of the *de novo* lipogenesis (DNL) program. DNL consumes NADPH, decreasing NADPH availability for antioxidant defences.
Thus, by indirectly reducing antioxidant defences in macrophages, activation of SREBP1 leads to accumulation of reactive oxygen species, which are essential for macrophage alternative polarization and parasitic helminth immunity. Mice unable to activate SREBP1 are more susceptible to helminth infection.

mTOR as a metabolic regulator in macrophages controlling tissue homeostasis

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Macrophages are central for the maintenance of tissue homeostasis and quickly responds to local or systemic perturbations by pathogenic or sterile insults. This rapid response is metabolically supported to allow cell migration and proliferation and to enable efficient production of cytokines and lipid mediators.
The presentation focuses on the role of mechanistic target of rapamycin (mTOR) in controlling and shaping the effector responses of macrophages. mTOR reconfigures the cellular metabolism and polarization of macrophages.
A detailed understanding of how mTOR metabolically coordinates effector responses in macrophages will provide important insights into granulomatous diseases and cancer.

> SESSION III - GUT MICROBIOTA

Microbiota and Insulin Resistance

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Obesity has turned an epidemic problem, increasing the prevalence of associated metabolic disturbances, including type 2 diabetes (T2DM). Both result from the balance between genetic and environmental factors. A new model, based on emerging discoveries, proposed the metagenome hypothesis that takes into account the overall bacterial genome presented in Humans.
Humans host 10^{14} bacteria in the gut, representing more than 150 fold their eukaryotic nuclear genome. It seems that gut microbiome perform a variety of physiological functions, including the regulation of glucose homeostasis and lipid metabolism. Germ-free mice colonized with gut microbiota from obese animals showed an increase in body fat mass, as well as an increase in insulin resistance. Some studies explain these changes by intestinal dysbiosis, present in obese mice, which is characterized by a greater proportion of *Firmicutes* and a reduced level of *Bacteroidetes*.
Some mechanisms have been proposed to further understand the crosstalk between microbiota, regulation of fat storage and development of obesity-related diseases: a) the capacity of the "obese microbiome" to harvest energy from food – the storage hypothesis; b) the contribution of lipopolysaccharides (LPS), a component of the cell wall of gram-negative bacteria, to trigger an inflammatory state, described as metabolic endotoxemia– the metainflammation hypothesis; c) the role of microbioma to interact with intestinal cells, modifying intestinal permeability.

In obese humans, it has been described shift toward higher relative abundance of *Bacteroidetes* and decreased number of *Firmicutes* after a low--calorie diet inducing weight loss. Furthermore, experimental data showed the role of gut microbiota in energy metabolism, by modulation of nutrient absorption, maintenance of gut barrier integrity, lipogenesis and hormonal status, leading to an increasing interest in shaping human gut microbiota composition in order to prevent and treat obesity and restore glucose homeostasis.

> ORAL COMMUNICATIONS

Analysis of TRIB2 expression levels in insulin-sensitive tissues to unraveling TRIB2's role in metabolism

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Introduction: The incidence of Type 2 Diabetes (T2D) and insulin resistance is increasing at an alarming rate, greatly associated with obesity. Therefore, identifying novel molecular mechanisms related to metabolic diseases is very important. Our laboratory discovered TRIB2, a member of Tribbles pseudokinase family, as a suppressor of FOXO proteins that mediate insulin action on key functions involved in cell metabolism, growth and aging. We hypothesize TRIB2 might play an essential role in insulin sensitivity and cellular metabolism.
Objectives and Methods: The main goal of this work was unraveling TRIB2's potential metabolic function, by analyzing TRIB2's expression levels in selected insulin-sensitive tissues, using GEO profiles available at public data repository.
Results: In human liver, there was 30% decreased TRIB2 expression in obese compared to lean individuals (p=0,006). However, hepatic TRIB2 levels remained unchanged in T2D patients. In response to high fat/methionine--choline deficient diet there was a ~2-fold increase in hepatic TRIB2 (p=0,001), partially blunted when mice were simultaneously metformin--treated (p=0,004). In contrast to liver expression, analysis of adipose tissue from obese individuals showed 34% increase (p=0,029). T2D non-obese patients showed 7% decrease (p=0,003). Intriguingly, analysis of fat from mice undernourished in utero, but permitted catch-up growth during suckling, showed 48% decreased TRIB2 (p=0,027). In skeletal muscle, human studies revealed no differences in TRIB2 levels in conditions such as T2D, insulin resistance, obesity. In murine models, 34% increase was observed in muscle from mice fed high-fat diet for 3 days, compared to low-fat diet (p=0,05), and 6% increase was verified in low capacity runners trained rats when compared to sedentary (p=0,014).
Conclusion: Our results uncovered TRIB2 differential expression in obesity, in liver and adipose tissue, although in opposite directions, where TRIB2 might have a role in fat accumulation and inflammation. Understanding TRIB2 function under these circumstances is critical to potentially recognize TRIB2 as pharmacological target for metabolic disturbances.

Ancestry influence in host-*Helicobacter pylori* interaction: human transcriptome assessment of co-cultures from controlled ancestry backgrounds

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Introduction: *Helicobacter pylori* (*H. pylori*; abbreviated as *Hp*) is recognized as a type I carcinogen for gastric cancer, however, it has been challenging to understand under which circumstances the bacterium causes disease. The *Hp*-human interaction dates to more than 100,000 years, meaning that the bacteria have travelled together with humans in the out--of-Africa migration. As a consequence, *Hp* genomes are distinct among continents, similarly to host genomes. Recent epidemiologic evidence has pointed out for a co-evolution phenomenon, as mismatched *Hp*-human ancestries increased significantly the risk to develop gastric disease, in comparison to matched ancestries.
Objectives: We aimed to investigate the differences in molecular host responses in mismatched *vs.* matched *Hp*-human ancestries.
Methods: We selected African (J99) and European (26695) *Hp* strains of identical virulence capacity, and performed 24h co-culture assays of gastric cell lines of African (NCI-N87) and European (Hs746T) origins, under matching and mismatching *Hp*-human ancestries. Then we evaluated the human transcriptomes of these four co-culture conditions, using an Ion AmpliSeq Transcriptome Human Gene Expression kit (Thermo Fisher Scientific, Waltham, MA, USA).
Results: Preliminary results show that in mismatched conditions there is an increase in the expression of genes involved in the nuclear transcription machinery, proliferation, and inflammatory response. In contrast, in matched conditions, only few genes were overexpressed, in particular those involved in epithelial-mesenchymal transition, metabolism, extracellular matrix ligands, and oxidative stress. We are currently conducting functional assays to investigate the extent to which ancestry accounts for differences in proliferation, apoptosis and metabolism.
Conclusions: The mismatch of *Hp* and human ancestries seems to lead to a more profound alteration of the human cellular program, while matched conditions only change particular specialized pathways. These results support the co-evolution phenomenon, by which matched ancestries will be adapted and less virulent to the host, leading to a disruption of this relationship when they are mismatched.

Browning effect of the melanocortins among mice different adipose tissue depots

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Background: Transdifferentiation of white into brown-like/beige adipo-

cytes is currently considered a promising approach for obesity treatment. Alpha-melanocyte stimulating hormone (α-MSH), a melanocortin neuropeptide, implicated in the regulation of food intake, was recently highlighted to also have a role on this browning phenomenon. Recently we observed that browning of the subcutaneous adipose tissue induced by α-MSH is accompanied by mice weight loss and amelioration of their metabolic status.
Objectives: With this in mind, the present study aims to provide a characterization of the browning capacity of α-MSH in other adipose tissue depots, namely mWAT – mesenteric, eWAT – epididymal and rpWAT – retroperitoneal, in both obese and non-obese mice.
Methods: For this purpose, α-MSH (150µg/kg), saline and CL-316,243 (1µg/kg, as a positive control) were intraperitoneally injected throughout 14 days in C57BL/6 lean mice (standard diet) and obese mice (high-fat diet, 10 weeks). After euthanasia, adipose tissue depots were collected for the qPCR analysis of the expression of browning-related genes or processed for functional (mitochondrial respiration rate) and morphological studies (Hematoxylin & Eosin).
Results: In obese animals, α-MSH promotes a two-fold upregulation of *Ucp1* expression in both mWAT and eWAT while having no effect in rpWAT. In eWAT, *Cited1*, another beige-related gene, was also found to be increased 2,5-fold with α-MSH treatment. In agreement, the area of adipocytes from eWAT was reduced. Beta-adrenergic stimulation with CL-316,243, however, has a more pronounced effect on browning of eWAT and mWAT, increasing the expression levels of other beige-related genes and improving both basal and uncoupled respiration rate of the latter depot. Curiously, in lean mice, α-MSH have an opposite role as it decreases the expression level of several beige-related genes in eWAT, but most significantly in rpWAT, without affecting mWAT.
Conclusions: These results demonstrate that α-MSH has a dissimilar browning effect in the diverse adipose tissue depots among obese and non-obese animals.

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The dietary polyphenol chrysin attenuates metabolic disease in the rat induced by fructose feeding

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Introduction: Metabolic Syndrome (MS) is a major public health issue worldwide. Fructose consumption has been associated with MS development and a substantial increase in both consumption of this sugar and MS incidence has been observed during the last 30 years. Recently, we verified that the dietary polyphenol chrysin is an effective inhibitor of fructose uptake by human intestinal epithelial cells. So, our aim was to investigate if chrysin interferes with the development of MS induced by fructose in an animal model.
Methods: Adult male Sprague-Dawley rats (220-310 g) were randomly divided into 4 groups: a) tap water (Control), b) tap water and a daily dose of chrysin (100 mg/kg) by oral administration (Chrysin), c) 10% fructose in tap water (Fructose), and d) 10% fructose in tap water and a daily dose of chrysin (100 mg/kg) p.o. (Fructose+Chrysin). All groups were fed *ad libitum* with standard laboratory chow diet and dietary manipulation lasted 18 weeks.
Results: Fructose-feeding for 18 weeks induced a significant increase in energy consumption, liver/body, heart/body and right kidney/body weight ratios, serum proteins, serum leptin and liver triacylglycerols and these changes were not affected by chrysin. In contrast, the increase in serum triacylglycerols, insulin and angiotensin II levels and in hepatic fibrosis induced by fructose did not occur in the presence of chrysin. Moreover, the increase in both systolic and diastolic blood pressure which was found in fructose-fed animals from week 14th onwards was not observed in fructose+chrysin animals.
Conclusions: Chrysin was able to protect against some of the MS features induced by fructose-feeding.

Metabolomic study of quercetin-mediated metabolic reprogramming of human macrophages

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Introduction: The ability of macrophages to change between pro-inflammatory (M1) and anti-inflammatory (M2) phenotypes makes their modulation an attractive therapeutic strategy to mitigate excessive and/or chronic inflammation. Bioflavonoids are considered potent immunomodulatory compounds with promising application in the treatment of inflammatory disorders. However, knowledge about their molecular effects on human macrophages remains scarce. Energy metabolism has recently been uncovered as a central axis of macrophage phenotypic and functional regulation. Therefore, investigating the metabolic effects of bioflavonoids may shed light into their mechanisms of action and potentiate their possible therapeutic use as immunomodulators.
Objectives: The general aim of this work was to reveal the effects of quercetin (a flavonoid abundant in fruits and vegetables) on the metabolism of human macrophages, both unstimulated and after pro-inflammatory activation.
Methods: In vitro-cultured macrophages differentiated from human THP-1 monocytes were treated with quercetin (6, 24, 48 hours), both in the uncommitted state (M0) or after pre-polarization with LPS/IFN-γ (M1). Treatment with IL-4/IL-13 (M2) was also carried out for comparison. Cells were solvent-extracted to obtain the polar metabolite fractions, which were subsequently analysed by 1H NMR spectroscopy.
Results: Multivariate and quantitative spectral analyses revealed marked changes in the metabolic profile of quercetin-treated cells compared to controls. Major alterations suggested decreased glycolytic activity, increased glutaminolysis and disturbances of the TCA cycle (e.g. high citrate, low succinate). Modification of the cellular redox state and osmotic balance was also apparent. Notably, most quercetin effects were dependent on the initial macrophage activation state and clearly distinct from those induced by LPS/IFN-γ, while showing some similarities with the metabolic profile of M2 macrophages.
Conclusions: The flavonoid quercetin induced time-dependent metabolic reprogramming of human macrophages, affecting major pathways of glucose metabolism and energy generation. Overall, we may conclude that metabolism appears to play a key role on the immunomodulatory action of quercetin.

> POSTERS

1 – Potent cytotoxic, antiproliferative, pro-apoptotic and anti-migratory effect of a Catechin:Lysine complex in pancreas cancer cell lines

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Introduction: Pancreatic cancer is recognized as a highly malignant and incurable disease worldwide, with a 5-year overall survival rate of only 8%[1]. Polyphenols, abundantly found in plants, display anticarcinogenic properties[2].
Objectives and Methods: We aimed to compare the effect of a Catechin:Lysine complex (Cat:Lys) on cell proliferation rates, culture growth, cell viability, apoptosis rates, migration and metabolism in two human pancreatic cancer cell lines (PANC-1 and AsPC-1) and in a non-tumorigenic human pancreatic cell line (H6c7).
Results: PANC-1, AsPC-1 and H6c7 cells were exposed to Cat:Lys (0.01-1 mM) for 24h. Cat:Lys induced a concentration-dependent decrease in cell proliferation, culture growth and cell viability in the two cancer cell lines (PANC-1 and AsPC-1). In contrast, in the noncancerous cell line H6C7, a concentration-dependent reduction in proliferation was found but culture growth and viability were only affected by higher concentrations of Cat:Lys (0.1-1 mM). Furthermore, in the cancer cell lines (PANC-1 and AsPC-1), Cat:Lys (0.01-1 mM) caused a concentration-dependent increase in the apoptotic index and a reduction in the migratory capacity, but no significant effect of Cat:Lys on these two parameters was observed in the non-cancer cell line, H6c7. The pro-apoptotic effect of Cat:Lys (0.1 mM) is JAK/STAT signaling pathway-dependent in PANC-1 cells and WNT signaling pathway-dependent in AsPC-1 cells, and the antimigratory effect is JAK/STAT signaling pathway-dependent in AsPC-1 cells. Moreover, Cat:Lys concentration-dependently decreased ^{3}H-deoxy-D--glucose uptake by H6c7 cells, but had no inhibitory effect on ^{3}H-DG uptake by the cancer cell lines. Also, Cat:Lys reduced ^{3}H-lactate uptake by the cancer cell lines (PANC-1 and AsPC-1 cells) but increased ^{3}H-lactate uptake by H6c7 cells. Finally, Cat:Lys decreased lactate production in PANC-1 and AsPC-1, but increased it in H6c7 cells.
Conclusions: Our results show that Cat:Lys has a potent cytotoxic, antiproliferative, antimigratory and pro-apoptotic effect in pancreas cancer cells, and a much more limited effect in non-cancer epithelial pancreas cells.

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2 – Pathways of estrogen metabolism underlying the association between *Schistosoma haematobium* and bladder cancer

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Introduction: Squamous cell carcinoma (SCC) is a malignant, poorly differentiated neuroendocrine neoplasm. SCC is the common form of bladder cancer in rural Africa where *S. haematobium* is prevalent. In contrast, the majority of bladder cancer in developing countries and regions not endemic for urogenital schistosomiasis is transitional cell carcinoma (TCC) that arises from the transitional epithelium lining of the bladder. The parasite eggs trapped in the bladder wall release antigens and other metabolites (presumably evolved to expedite egress to the urine, and hence to the external environment). However, the phenomenon leads to haematuria and to chronic inflammation, in turn increasing risk of SCC of the bladder. In addition to the hormone--like effects of the parasite estradiol-related molecules on the endocrine and immune system of the host, in relation to cancer initiation metabolites of estrogens can be also considered as carcinogenic chemicals.
Methods: For the purpose of this study we used cell lines (CHO and HCV29), animal models, immuno(histo)chemistry and RT-PCR.
Results: We observed hormonal imbalance caused by estrogen-like molecules produced by schistosomes. These molecules are catechol estrogen-3,4-quinones, the major carcinogenic metabolites of estrogens. We also observed down-regulation of estrogen receptor by schistosomes in urothelial cells and bladders of CD-1 mice and estrogen metabolism-associated CYP2D6 and IL6-174G/C polymorphisms in *S. haematobium* infected patients.
Conclusion: Accordingly, the hypothesis that underpins our work is that metabolism of estrogens and production of depurinating estrogen-DNA adducts leads to parasite metabolite-promoted host cell DNA damage, and ultimately urogenital schistosomiasis associated SCC.

3 – Role of the MITOchondrial fission protein Drp1 as a prognosis and predictive biomarker in the treatment of differentiated thyroid cancer (ROMITO-DRP1)

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Introduction: Differentiated thyroid cancer (DTC) is the most common endocrine cancer. Prognosis relies on clinical and histopathological factors and treatment is based on surgery and radioiodine (131I). Despite an overall good prognosis, 10-15% of those will eventually recur, become 131I-refractory and/or evolve to distant metastases. A pattern of dysregulation of the mitochondrial fission protein DRP1 (Dynamin-related protein 1) has been described in different tumour models, where its expression has been implicated in chronic mitochondrial fission, mitochondrial dysfunction,cancer cell invasion and migration,and resistance to targeted therapies.
Objectives: We hypothesize that DRP1 may have a role in the progression of DTCand therefore be a relevant target in delaying or overcoming treatment resistance.
Methods: In the first part of our project, we evaluated the relationship between DRP1 expression(by immunochemistry) and clinico-pathological features in a series of 259 cases follicular cell-derived thyroid carcinomas.
Results and conclusions: DRP1 expression was positive in 90.3% of the cases, and was significantly associated withpapillary and oxyphilichystotypes, non-capsulated tumors,tumor capsule and thyroid capsule invasion,and higher number of 131I treatments.The expression of DRP1 was lower among patients who presented with lymph node metastases and with distant metastases, although not statistically significant. The significant association between DRP1 expression and thyroid capsule invasion, and to a lesser extent, extrathyroidal invasion, may indicate that DRP1 is required for the invasiveness properties of tumor cells in earlier stages of the tumorigenesis. Whether DRP1 may also play a role in lymph node invasion and/or distant metastization remains unclear. If any such association exists in DTC, it seems to fall in the opposite direction.The association between higher mean DRP1 expression and predictors of poor prognosis will be further elucidated by the investigation of the role of DRP1 in TC cell lines with different genetic backgrounds.

4 – The effect of oxidative stress induced by *tert*-butylhydroperoxide (TBH) upon *in vitro* intestinal sugar transport

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Introduction: The pathogenesis of various gastrointestinal diseases, including gastrointestinal cancers and inflammatory bowel disease, is associated with increased oxidative stress levels. We decided to investigate the effect of oxidative stress on the intestinal uptake of glucose and fructose.
Methods: We evaluated the effect of oxidative stress induced by tert-butylhydroperoxide (TBH) on the uptake of ^{3}H-deoxy-D-glucose (^{3}H-DG) and ^{14}C-fructose by the human intestinal epithelial Caco-2 cell line.
Results: TBH (500 µM; 24h) increased lipid peroxidation (TBARS) levels and was not cytotoxic. TBH (500 µM; 24 h) increased uptake of both low (SGLT1-mediated) and high concentrations (SGLT1- and GLUT2-mediated) of ^{3}H-DG, but did not affect absorption of ^{14}C-fructose (GLUT2- and GLUT5-mediated). The polyphenol chrysin abolished the increase in TBARS levels and the increase in uptake of both low and high concentrations of ^{3}H-DG induced by TBH. On the other hand, TBH blocked the inhibitory effect of chrysin on ^{14}C-fructose uptake. ^{3}H-DG uptake, but not ^{14}C-fructose uptake, was sensitive to the sweet taste receptor (STRs) inhibitor lactisole. The inhibitory effect of lactisole in relation to uptake of ^{3}H-DG (10 nM) (SGLT1-mediated), but not in relation to uptake of ^{3}H-DG (50 mM) (SGLT1- and GLUT2-mediated), was abolished in the presence of TBH. So, the stimulatory effect of STRs on SGLT1-mediated transport is dependent on oxidative stress levels.
Conclusion: This work shows that uptake of both ^{3}H-DG and ^{14}C-fructose is sensitive to oxidative stress levels. Moreover, it suggests that the three distinct transporters involved in the intestinal absorption of glucose and fructose (SGLT1, GLUT2 and GLUT5) have different sensitivities to oxidative stress levels, SGLT1 being the most sensitive and GLUT5 the least.

5 – Morning cortisol secretion, physical functioning and mental health in older adults

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Background: The aging process is associated to physical functional limitations associated to performing daily activities and psychological distress. This situation can lead to changes in serum level of many stress hormones, including cortisol.
Aims: We aimed to evaluate physical functional limitations, mental health and morning cortisol secretion.
Methods: in this cross-sectional study, 269 non-institutionalized older than 70 years (female, 77.3±5.1 years and men, 77.7±4.8 years) were randomly recruited from north of Portugal. Based on the General Physical Activity Questionnaire, older adults were divided into i) physically active (PA; n=196) for at least 150 min of moderate physical activity per week and ii) non-physically active (N-PA) groups. Anthropometric variables, such as body mass index (BMI) and waist circumference (WC) were determined. Self-reported physical functioning and mental health were assessed by SF-36 health survey. Blood samples were used for cortisol levels determination.
Results: PA group showed significantly lower BMI (28.9 ± 4.1 *vs.* 30.0 ± 5.1; $p<0.01$) than N-PA group while WC (96.2 ± 10.5 *vs.* 97.2 ± 12.2; $p>0.05$) remained unchanged. Physical functioning (70.08 ± 23.02 *vs.* 51.80 ± 29.11; $p=0.03$) and mental health (66.97 ± 23.86 *vs.* 52.6 ± 25.42; $p=0.02$) scores were both higher in PA group than N-PA group. The mean of morning serum cortisol levels was 16.3 ± 5.9 µg/dL although were lower in PA group (16.1 ± 6.4 *vs.* 18.8 ± 7.2; $p=0.03$) than N-PA group.
Conclusions: Data suggest that old adults physically active showed highest physical functioning and mental health scores. Although individuals have cortisol levels within the reference range, those are physically active showed lower diurnal cortisol levels. In addition, this data strengthen the relevance of exercise programs to improve physical functioning-related performing daily activities in aged population.

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6 – Inhibitory activity of flavonoids on digestive enzymes α-amylase and α-glucosidase

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Introduction: After food ingestion, α-amylase is responsible for the initial hydrolysis of starch into shorter oligosaccharides, being these sugars finally breakdown to glucose by the intestinal brush border α-glucosidase. Glucose is then absorbed, leading to post-prandial hyperglycaemia (PPHG), which is impaired in type 2 diabetes *mellitus* (DM). Acarbose is the most prescribed α-amylase and α-glucosidase inhibitor. However, it is associated with gastrointestinal adverse effects. Thus, the search and development of new antidiabetic agents targeting these two important digestive enzymes, is a hot research topic.
Objectives: The purpose of the present work was to evaluate the inhibitory activity of a panel of synthetic flavonoids against α-amylase and α-glucosidase activity. Flavonoids with -OH, -OMe and/or -OBn groups in different positions of A, B and C rings, were studied, in order to establish a reliable structure-activity relationship.
Methods: An *in vitro* spectrophotometric screening assay was used to measure the enzyme-catalyzed hydrolysis of the substrate (selected according the enzyme under study) by monitoring the absorbance of the product formed.
Results: The obtained results suggest that the presence of -OMe groups at 7- and 8- positions of A ring, as well as the presence of -OH groups at 3'- and 4'- positions of B ring and at 3- position of C ring in the flavonoids' structure significantly increased the α-amylase inhibition. Concerning the inhibition of α-glucosidase, it was found that the presence of -OH groups at 7- and 8- positions of A ring, at 3' and 4' positions of B ring, and at -3 position of C ring, clearly increased the flavonoids' inhibitory activity of α-glucosidase.
Conclusion: These promising preliminary results highlight the therapeutic value of flavonoids in the management of PPHG in type 2 DM, as different structural features define their selectivity to α-amylase or α-glucosidase, potentially diminishing the most common gastrointestinal adverse effects.

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7 – Inadequacy of vitamin d nutritional status associated with high body adiposity in individuals with obesity classified according to *Edmonton Obesity Staging System*

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Background: Inverse correlation between vitamin D serum concentrations and growing adiposity has been observed. Edmonton Obesity Staging System (EOSS) is a 5-point ordinal classification system, for patients with obesity, that takes into account severity of comorbidities and functional limitations.
Aim: To apply EOSS to individuals with extreme obesity and evaluate the nutritional status of vitamin D and body adiposity across stages of EOSS.
Methods: Study was conducted with individuals with obesity, both gender, aged 21-59 years (n=232). Anthropometric data [weight, waist circumference (WC), ratio waist-to-height (WtHR) and body fat (%)] and vitamin D (25(OH) D) were obtained. The cut-off points for 25(OH)D deficiency and insufficiency were ≤20 and 21-29 ng/mL, respectively. Individuals were categorized as either EOSS stage 0 (no risk factors), stage 1 (subclinical risk factors), stage 2 (obesity-related chronic disease), stage 3 (established end-organ damage/ significant functional limitations) and stage 4 (severe disabilities/limitations).
Results: Sample was composed 76.6% (178) by women. The distribution of EOSS stages was 1.7% (4), 21.6% (50), 62.5% (145), 14.2% (33), in Stages 0, 1, 2 and 3, respectively. Individuals did not found in stage 4. Higher mean of weight (120.6±16.9 Kg; p=0.041), WC (120.7±11.2 cm; p=0.033) and body fat (48.3±9.6%; p=0.014) were observed in stage 3. Inadequacy of vitamin D was 84% in the over sample, being 40% of deficiency and 44% of insufficiency. Across stages of EOSS 0, 1, 2 and 3, means of 25(OH)D (ng/mL) were 24.8±7.8; 21.7±8.2; 21.3±7.8; 18.8±4.7, respectively [p=0.019]. 25(OH)D nutritional status showed highest deficiency (45.4%-15.3±4.6 ng/mL) [p=0.032] and insufficiency (42.4%-25.0±2.1ng/mL) [p=0.044] in stage 3, when compared with others stages.
Conclusion: In individuals with extreme obesity, high prevalence of inadequacy of vitamin D serum concentration with lowest mean, either deficiency as insufficiency, was identified in EOSS stage 3, among subjects with highest weight, WC and body fat percentage.

8 – Relationship of body adiposity with nutritional status of vitamin a in women with of childbearing age

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Introduction: The accumulation of visceral fat is associated with a higher metabolic risk and mortality in general. Evidence indicates that vitamin A plays an important and regulatory role in body fat reserves.
Aim: To evaluate the nutritional status of vitamin A (retinol and β-carotene) and its relation with body adiposity in women of childbearing age.
Methods: A descriptive cross-sectional study constituted of 82 women, aged 20 to 49 years. Serum retinol ≥ 1.05 µmol/L and β-carotene values between 50 µg/dL and 250 µg/dL were considered adequate. Body adiposity was evaluated through Body Mass Index (BMI), Waist Circumference (WC) and Waist-height Ratio (WhtR).
Results: The means of BMI (kg/m²), WC (cm) and WhtR were 25.93 ± 4.34; 94.54 ± 21.32; 0.59 ± 0.12, respectively. 100% of women classified as overweight and with obesity had inadequate β-carotene (43.52 ± 5.86/33.37 ± 5.01; p=0.004); and 57.1% of overweight and 94.7% with obesity had inadequate retinol (1.04 ± 0.27/0.70 ± 0.34, p = 0.049), respectively. The increase in the inadequacy of serum β-carotene and retinol concentrations was observed, as the BMI increased. It was also observed that 100% of individuals classified as very high WC (> 88cm) had inadequate β-carotene (35.76 ± 6.92; p = 0.000) and 79.5% had inadequate retinol (0,78 ± 0.35; p = 0.000). 94.4% of the individuals classified as having high WhtR (> 0.5) had inadequate β-carotene (39.67 ± 12.31; p = 0.000) and 75% presented inadequate retinol (0.86 ± 0.39; p=0.000). Both BMI, WC and WhtR were significantly increased in subjects with vitamin A deficiency (VAD) (p = 0.000).
Conclusion: Lower serum concentrations of β-carotene and retinol were observed as there was an increase in body adiposity. And the anthropometric variables evaluated (BMI, WC and WhtR) were increased in the presence of VAD.

9 – The influence of natural mineral waters intake on blood pressure and associated regulatory mechanisms

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Introduction: Hypertension is a global public health issue that leads to premature death and disability, also being a major risk factor for cardiovascular and renal diseases as well as retinal hemorrhage and visual impairment. According to the World Health Organization, ischemic heart disease and stroke were the 2 most important global causes of death in 2016, highlighting hypertension as an important preventable cause of death. [1-6]
Objective: To review the contribution of natural mineral water consumption on blood pressure and associated regulatory mechanisms.
Methods: The literature review was performed on Pubmed, Scopus and Google Scholar databases, up to 17th September 2018. An additional filter for English and Spanish languages was applied. The following keywords were used: hypertension, blood pressure, mineral water, bicarbonate mineral water, natural mineral water, mineral intake and/or mineral water intake. Thirteen studies were selected so far: 2 carried out in rodent models and 11 in humans. Natural mineral waters were tested in diverse protocols in terms of type of water and amount consumed, study type and/or duration of intervention. Human studies were performed in populations with different sizes and characteristics (the same for rat strains). Several parameters were evaluated, alone or in distinct combinations: tissue catechol-O--methyltransferase activity, resting and ambulatory systolic and diastolic blood pressure, heart rate, circulating minerals/electrolytes, renin activity, atrial natriuretic factor, aldosterone and catecholamines, circulating and urinary uric acid, glomerular filtration rate, urinary acid-base status and urea and minerals/electrolytes excretion.
Results: Six studies found a significant beneficial effect of natural mineral water intake on blood pressure while 5 studies found no significant effect. No negative effect on blood pressure was reported. No putative physiological regulatory mechanisms were consistently observed as targets of natural mineral water action.
Conclusion: The consumption of natural mineral waters may exert a positive modulation on blood pressure. Further studies are needed to clarify the underlying physiological regulatory mechanisms involved in natural mineral water effects.

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10 – Possible combination therapies to overcome resistance to MAPK inhibitors in *BRAF* mutant melanoma cell lines

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Introduction: Melanoma accounts for only 1% of all skin malignant tumours, however it is the deadliest form of skin cancer. Current therapeutic approaches for melanoma include surgical resection, chemotherapies, immunotherapies and targeted therapies. Melanoma has the highest mutation rate from all types of cancer and the main cause is the ultraviolet exposure. Considering the genetic mutations that originate this disease, some drugs were developed and approved for melanoma treatment, such as vemurafenib, an oral selective BRAF inhibitor, used for the treatment of unresectable or metastatic melanomas harbouring $BRAF^{V600}$ mutations. In $BRAF^{V600}$ melanoma patients, alterations in the melanoma cells can originate mechanisms of resistance to vemurafenib, which may be related with deregulation of MAPK and PI3K/AKT/mTOR pathways. To overcome this undesired outcome, the combination of vemurafenib and cobimetinib, an oral selective MEK inhibitor, was approved for melanomas harbouring $BRAF^{V600}$ mutations. Despite the approved therapies for melanoma, the overall survival of patients did not change significantly, and the development of new therapies is still crucial.
Objectives and Methods: Considering the genetic and molecular alteration and the presence of the Warburg effect in melanoma cells, in order to overcome the resistance to MAPK inhibitors, in this study, a vemurafenib-sensitive melanoma cell line, with $BRAF^{V600E}$ mutation, and a derived vemurafenib-resistant melanoma cell line were tested in response to vemurafenib (BRAF inhibitor), cobimetinib (MEK inhibitor), everolimus (mTOR inhibitor) and dichloroacetate, a metabolic modulator, alone or in combination.
Results: Our data suggest that the combination of cobimetinib and everolimus is a more appropriate therapy than the approved combination for $BRAF^{V600E}$ melanoma patients, vemurafenib and cobimetinib.
Conclusions: Our results point that, in melanoma, targeting two crucial pathways, MAPK and PI3K pathways, is more effective than using two different inhibitors to target the MAPK pathway.

11 – Scavenging of hypochlorous acid and modulation of neutrophil's oxidative burst by methoxylated and hydroxylated chalcones

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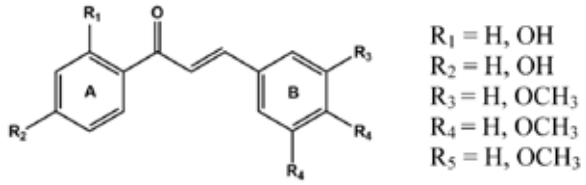
Introduction: Neutrophil's oxidative burst is an important event of the inflammatory response against invading pathogens. (1) One of the main reactive oxygen species produced during this event is hypochlorous acid (HOCl), which may cause deleterious effects on the surrounding tissues if sustainably overproduced, as it may happen in chronic inflammation. (2) Thus, inflammation research requires the development of new anti-inflammatory drugs with HOCl scavenging and oxidative burst inhibitory properties.
Objectives: The aims of the present study were to evaluate the ability of methoxylated and hydroxylated chalcones to scavenge HOCl and to modulate the human neutrophils oxidative burst, establishing the respective structure-activity relationship, whenever possible.
Methods: *In vitro* microanalysis methodologies were applied. Fluorimetric detection was used to measure the ability of HOCl to oxidize the non-fluorescent probe dihydrorhodamine (DHR) to fluorescent rhodamine 123. (3) Chemiluminescent detection was used to investigate the modulation of oxidative burst by the studied chalcones. Human neutrophils were isolated and stimulated by phorbol 12-myristate-13-acetate with the subsequent measurement of the ROS produced, using luminol as a probe. (4) Results: The results obtained indicate that the presence of two hydroxyl groups at 2' and 4' positions of the aromatic ring A seems to be favourable to the studied activities.
Conclusions: Hydroxylated chalcones were shown to present a favourable scaffold to be further studied and synthetically developed leading to the discovery of new and alternative anti-inflammatory drugs.



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12 – Brazil nut consumption reduces DNA damage in patients with type 2 diabetes mellitus probably through changes in oxidative status

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Introduction: Type 2 diabetes mellitus (T2DM) is a metabolic disease, occurring largely due to lifestyle changes. There is a strong link between T2DM and oxidative stress, that leads to damage to lipids, proteins and DNA. Dietary interventions are essential for the treatment and prevention

of T2DM-related complications. Brazil nuts (*Bertholletia excelsa, H.B.K.*) are the richest source of selenium in nature, and this mineral presents health benefits, including improve of antioxidant system and maintenance of genomic stability.
Objectives: This work aimed to assess the effects of Brazil nut consumption on biochemical and oxidative stress parameters, as well as genomic instability in T2DM patients.
Methods: Seventy-four T2DM patients (registered in the Integrated Clinics, University of Southern Santa Catarina) consumed one Brazil nut a day (210 µg of selenium) for six months. Blood and exfoliated buccal cells samples were collected at the beginning and at the end of treatment. Were evaluated the glycemic, lipid, renal and hepatic profiles; levels of DCF, MDA, nitrites, total thiols, protein carbonylation, GSH and selenium; GPx and CAT activity; and DNA damage.
Results: The data presents an increase in fasting glucose levels, HDL- and LDL-cholesterol, and GGT levels. The insulin levels and triglycerides/HDL--cholesterol ratio were decreased. Six-month Brazil nut consumption proved to be enough to significantly increase selenium and GSH levels, and GPx and CAT activity, improving the antioxidant system of patients. Relative to oxidant production, DCF and nitrites levels were decreased. A reduction of proteins and lipids oxidized were observed by increase in total thiols, and a decrease in protein carbonyl and MDA levels. Relative to genomic instability, basal and oxidative DNA damage, and micronuclei frequency were significantly decreased after Brazil nut consumption.
Conclusions: Taken together, the results indicate that Brazil nut consumption could be an ally to module the genomic instability in T2DM patients, probably through changes in redox balance.

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13 – Plasma concentration of vitamin d is associated with physical activiy in a elderly population

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Introduction: we aimed to analyze the physical functional capacity and 25-hydroxyvitamin D [25 (OH)D] levels in physically and non-physically active old adults.
Methods: In this cross-sectional study, a sample of 255 older than 70 years [female (n=187) 77.9 ± 5.2 years and men (n=68) 78.0 ± 4.9 years] were randomly recruited from north of Portugal. Based on the General Physical Activity Questionnaire, older adults were classified in i) physically active (PA; n=173) for at least 150 min of moderate physical activity per week and ii) non-physically active (N-PA, n=82) groups. The weight and height were determined and the body mass index (BMI) was then calculated. The physical functional capacity was evaluated by a submaximal exercise test 6 minutes walking test (6MWT). Blood samples were used for 25-Hydroxyvitamin D3 [25(OH)D3] levels determination.
Results: PA group showed showed significantly lower BMI (28.9 ± 4.1 *vs.* 30.0 ± 5.1; p<0.01) than N-PA group while WC (96.2 ± 10.5 *vs.* 97.2 ± 12.2; p>0.05) remained unchanged. The distance covered in the 6-minute test was higher in PA group than N-PA group (445.3 ± 138.6 *vs.* 309.7 ± 134.2; p>0.01). Considering US Endocrine Society Vitamin D levels, 98,3% of the participants were considered to have an insufficient level (below 30 ng/dl), with only 1.7% within sufficient values. The mean of vitamin D levels was 18.5 ± 6.8 ng/mL in PA group and 13.0 ± 6.4 ng/mL in N-PA group. The correlation between distance traveled and vitamin D was also significant (p<0.001).
Conclusion: our data suggest that old adults physically active showed highest concentration of vitamin D and its correlation with physical functional capacity suggest that physical activity at an advanced age is crucial to improve overall health status.

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14 – Physiological role of Transthyretin in glucose metabolism at the liver

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Background: Transthyretin (TTR), a 55 kDa evolutionarily-conserved protein, presents altered levels in several conditions, including malnutrition, inflammation, diabetes and Alzheimer's Disease. It has been shown that TTR is involved in several functions, such as insulin release from pancreatic β-cells, recovery of blood glucose and glucagon levels of the islets of Langerhans, food intake and body weight.
Objectives: We aimed at assessing the involvement of TTR in glucose homeodynamics in plasma and liver cells and at unraveling the underlying mechanism, and at evaluating the role of TTR in mitochondrial function and lipid peroxidation.
Methods: We studied the levels of glucose in plasmas from mice with different TTR genetic backgrounds and in the medium of primary hepatocytes derived from those mice, and analyzed the expression of glucose transporters in mice livers and in HepG2 cells. Further, the effect of TTR was studied on the expression of the liver glycolytic enzyme, Pyruvate Kinase (PKM), using qRT-PCR, and on the production of glucose-derived metabolites, using ^{1}H NMR. We then assessed mitochondrial density by immunofluorescence, liver expression of mitochondrial complexes by western blot and levels of lipid peroxidation using slot blot.
Results: We showed for the first time that TTR insufficiency leads to higher glucose in both plasma and hepatocyte media and to lower expression of the influx glucose transporters, GLUT1, GLUT3 and GLUT4. TTR insufficiency decreases PKM levels in mice livers, but it does not affect the hepatic production of the studied metabolites. We demonstrated that TTR increases mitochondrial density in HepG2 cells and that TTR insufficiency triggers higher degree of oxidative phosphorylation and lipid peroxidation in the liver.
Conclusions: Altogether, these results indicate that TTR contributes to the homeostasis of glucose by regulating the levels of glucose transporters and of the PKM enzyme, and by protecting against mitochondrial oxidative stress and lipid peroxidation.

15 – Effects of AICAR, Compound C and Metformin administration in metabolic and angiogenic behavior in human microvascular endothelial cells (HMECs)

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Introduction: An important feature of type 1 diabetes (T1D) is the existence of the so-called "angiogenic paradox", a phenomenon in which the same organism presents exacerbated vascularization in certain organs (e.g. kidney) and impaired angiogenesis in others (e.g. heart). AMPK is an energy sensor that targets carbohydrate and lipid metabolism by modulating gene expression and is an insulin sensitizing molecule, rendering this kinase an ideal therapeutic target.
Objectives: To study the effects of administration of AICAR, Compound C and Metformin on the angiogenic behavior of human microvascular endothelial cells (HMEC-1), as well as, to analyze the possible changes in expression of genes related with metabolism.
Methodology: HMEC-1 were cultured in with two different glucose concentration: 5.5mM D-Glucose (low glucose (LG)) or 20mM D-Glucose (high glucose (HG)) and treated with AICAR, Compound C or Metformin to evaluate angiogenic behaviour by proliferation, migration and tube capillary formation assays. Alterations of genes related with metabolism and angiogenesis were verified by q RT-PCR.
Results: All treatments induced a decrease in proliferation, migration and tube formation in HMEC-1 when subjected to both LG and HG. The analysis of metabolic and angiogenic gene expression showed some alterations: Kdr, Pfkfb2, Tgfb2, Timp2 and Jag1 gene expression were increased when treated with all compounds *vs* control group; Smad5 reduced with AICAR treatment, but increased with Compound C and Metformin in LG condition. Timp2 expression was increased with AICAR and Compound C treatment and decreased with Metformin administration when compared to LG control.
Conclusion: Administration of AICAR, Compound C and Metformin alters the angiogenic behavior of HMEC-1 and can modulate the expression of important genes related to angiogenesis and cellular metabolism. These preliminary results bring new insights in the crosstalk between endothelial metabolism and angiogenic behavior and may be useful to develop new therapeutic approaches to counteract diabetic complications.

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16 – Endothelial metabolism impacts on vascular behaviour in type 1 diabetic mice

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Introduction: "Angiogenic paradox" is a vascular phenomenon common in type 1 Diabetes (T1D), a metabolic disorder characterized by chronic hyperglycaemia associated with micro and macrovascular complications in several organs. Accordingly, in the same patient, angiogenesis is exacerbated in some organs (e.g. in the retina and kidney) as well as impaired in others (e.g. heart).
Objectives: Analyze the expression profile of genes associated to metabolism and angiogenesis in renal and cardiac endothelial cells of T1 diabetic mice.
Methodology: T1DM was induced in C57Bl/6 mice by administration of streptozotocin. Kidneys and hearts were isolated ten weeks after T1D development and endothelial cells (ECs) were isolated by FACS. Total RNA samples were submitted to Angiogenic and AMPK Signaling PCR Arrays. Microvessel density (MVD) in kidney and heart tissue were quantified by immunohistochemistry with CD31 staining.
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, Kdr and Timp2 down-regulated in the kidney, while in the heart Notch ligand, Jag1, was downregulated whereas Smad5 expression was upregulated. MVD analysis showed a significantly increased of the number of CD31-positive ECs in kidneys of diabetic mice when compared to healthy animals, whereas in heart there was the slight reduction.
Conclusion: Imbalances in mTOR, Akt and PI3K signaling, as well as growth factors involved in angiogenesis were found in ECs from the two organs, implying metabolic changes. Elucidating the crosstalk between endothelial metabolism-vascular complications will enable novel therapeutic approaches.

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17 – Role of sex hormones in the innate immunity against prostate cancer cells

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Background: Innate immunity and inflammation may increase the risk of prostate cancer. Sex hormones influence the state of inflammatory immune responses, previous studies show that hormonally active androgens are anti-inflammatory, whereas estrogens are pro-inflammatory thus sex hormones can modulate macrophage response. Chronic inflammation can be a major contributor to prostatic cancer and the link between them of major importance to the development of novel therapeutical approaches via molecular targeting of inflammatory mediators and immunotherapy-based approaches.
Objectives: In the present study we investigated the effects of testosterone and estradiol on macrophage activation of the innate immune response using macrophages-like cells (RAW 264.7).
Methods: Induced cytotoxic and antitumor response of RAW 264.7 to M1 phenotype by exposure to E. coli lipopolysaccharide. After M1 phenotype induction macrophages were exposed to sex hormones. This will trigger various activation rates and different outcomes in prostate cancer cell line (PC3).
Results: It was observed an increase in the activation status of RAW 264.7 with a concentration of $1x10_{-6}$M both in estradiol and in testosterone. Although in co-culture there aren't significant differences when compare with the control. It seems that hormonal supplementation overlaps to the immunotoxic response.
Conclusions: This cellular model represents a good study model to future *in vitro* studies in immunotherapies, especially in combine therapies.