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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