

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

– Palestras, Comunicações Orais e Posters



> SESSION I – METABOLIC FITNESS AND AGEING

Skeletal muscle ageing: from a transcriptome and metabolome perspective

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Background: Age-related skeletal muscle loss (sarcopenia) increases the risk of frailty and mortality. Within the skeletal muscle, fast and slow muscles seem to be differently susceptible to age-related changes. Here, we set to understand how the transcriptome and metabolome change in pre-sarcopenic muscle types and how this relates to human muscle ageing.

Methodology: We extracted *Soleus* (slow, oxidative) and *Extensor Digitorum Longus* (EDL, fast, glycolytic) muscles from young (4 months old, n=6-8) and pre-sarcopenic (no muscle weight loss) old (25 months old, n=6-7) C57BL/6 mice and performed gene expression and metabolite analyses employing RNA-seq and UHPLC, respectively. Human RNA-seq counts were obtained from GTEx database (*Gastrocnemius*, fast muscle type), with age groups (in years) defined as 20-29 for young and ranging from 30-39, 40-49 and up to 70-79 for old depending on the analysis. Statistical analyses were performed using custom R scripts.

Results: Gene expression profiling revealed 229 differentially expressed genes (FDR<0.05) in ageing EDL (young vs old), and 131 genes were found to change (FDR<0.05) in ageing *Soleus*. Genes down-regulated in aged EDL were enriched for mitochondria-related processes, including "NADH dehydrogenase complex", "mitochondrial translation" and "mitochondrial gene expression". In *Soleus*, downregulation was related to extracellular matrix including "collagen trimer" and "focal adhesion". Differences in metabolite levels were also more pronounced in ageing EDL (192, p<0.05), enriched for "sphingolipid" and "nicotinamide" metabolism, than in ageing *Soleus* (132, p<0.05), enriched for "diacylglycerol metabolism". Overall, EDL seems to be more susceptible to age-related changes than *Soleus*.

Human data analyses revealed downregulated processes related to mitochondria in all age-group comparisons similar to what was observed for EDL. These age-related changes include detrimental, neutral and adaptive processes.

Conclusions: Different muscle types exhibited distinct age-related changes. Common to mice and humans were alterations related to mitochondria, which might be relevant to fast muscles atrophy.

Keywords: Ageing; Skeletal muscle; Transcriptome; Metabolome; Mitochondria

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

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

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

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Background: Motor Imagery is a mental representation of movement without real execution, and it seems to promote the increase and/or the maintenance of balance in different health conditions. The aim of this study is to verify the influence of a motor imagery intervention program on the balance in older adults without neurological conditions.

Methodology: A randomized controlled trial, pre-post intervention design with a 4-weeks follow-up was performed. Sixteen subjects were randomized into experimental group (n = 7) and control group (n = 9). The selection was performed through a sociodemographic questionnaire, the Movement Imagery Questionnaire-3 and the Mini Mental State Examination. Subjects in the experimental group performed the motor imagery training, while the control group was listening a story (sham), both during 10 minutes, three-times-a-week for three weeks. Balance was assessed using the Single Leg Stance (SLS) and Functional Reach Test (FRT). To compare groups and moments within each group the Mann-Whitney test and the Wilcoxon test were used respectively, with a confidence level of 95%.

Results: There were differences between groups at follow-up in SLS (p=0.049). In the experimental group there was an improvement in SLS after intervention (p=0.018), that was maintained at follow-up. Moreover, during FRT the reach increased, however no statistical differences were found. In the control group no differences were found in all outcomes at all moments (p=0.197 and 0.104, in SLS and FRT respectively).

Conclusions: It was found that the motor imagery program increased the amount of time in single leg stance which remained at follow-up.

Keywords: Ageing; Limits of stability; Mental practice; Unipodal support

7 – Hepatic adaptations to high-fat diet

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Background: High-fat diets cause the accumulation of hepatic free fatty acids and triglycerides, which is pathognomonic for non-alcoholic fatty liver disease (NAFLD), which currently affects 25% of the worldwide population. Hepatic mitochondria are structurally and molecularly altered in NAFLD, and a decline in mitochondrial function may provoke metabolic disturbances and may potentially contribute to NAFLD progression. So, the aim of the current study was to gain a more comprehensive understanding of the hepatic responses to high-fat feeding in rats.

Methodology: Young male ZSF1 Lean rats were randomly divided and fed with control diet (Ctrl - 16.7% Fat; n=7) or a high-fat diet (HFD - 47.7% Fat; n=10) for 30 weeks. At this time, the liver was weighed and blood was collected for biochemical analysis. The liver was used for ultrastructural and

histopathological examination, as well as to evaluate mitochondrial function and acylcarnitine profile.

Results: HFD group showed the histological features of NAFLD, with the presence of steatosis and increased content of triglycerides in liver. HFD rats revealed a decreased in liver weight and reduced aspartate aminotransferase (AST) and alanine aminotransferase (ALT) serum levels when compared to control rats. Mitochondrial studies revealed an increased oxygen consumption (RCR, ADP/O and FCCP-uncoupling state) and an improved $\Delta\Psi$ (lag-phase) in HFD group. According to the results obtained, HFD group presented higher ATP levels, as well as acylcarnitine levels were consistently decreased in liver. Semi-quantitative and qualitative analyses from microphotographs displayed that liver mitochondria of HFD animals had lower density and circularity, but higher mitochondrial area.

Conclusions: In summary, high-fat feeding prevented the bioenergetics impairment induced by NAFLD, by increasing fatty acid oxidation and ATP production.

Keywords: Non-alcoholic fatty liver disease; Mitochondria; High-fat diet

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

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Background: Breast cancer is the second most diagnosed cancer and has one of the highest mortality rates worldwide for both sexes. Cancer cells have altered metabolic pathways to support their high proliferation rates, and these include an increased need of glucose (Warburg effect) and glutamine ('glutaminolysis'). Correspondingly, most cancer cells overexpress glucose and glutamine transporters (GLUT1 (SLC2A1) and ASCT2 (SLC1A5), respectively). In this study, the anticarcinogenic effect and the effect on glucose and glutamine uptake of several distinct phenolic acids (caffeic, ferulic, protocatechuic, p-coumaric and gallic acids) was assessed. This class of non-flavonoid polyphenols has yet to be extensively studied.

Methodology: The human breast adenocarcinoma cell line (MCF-7 cells) was exposed to three distinct concentrations of the phenolic acids (1, 10 and 100 μ M) for 24 h, and migration, proliferation, culture growth, viability and glucose (by using the glucose analogue ³H-deoxy-D-glucose (³H-DG)) and glutamine (by using ³H-L-glutamine (³H-GLN)) cellular uptake were determined.

Results: Ferulic acid stimulated cell migration, while protocatechuic and gallic acids decreased it; ferulic acid inhibited, and protocatechuic acid increased cell proliferation and caffeic, ferulic and gallic acids diminished culture growth, while protocatechuic acid augmented it. In the nutrient uptake experiments, protocatechuic acid reduced ³H-DG and ³H-glutamine uptake (in the presence of sodium), and caffeic and gallic acids also inhibited ³H-glutamine uptake, but in the absence of sodium. On the other hand, gallic and ferulic acids increased ³H-DG uptake, and caffeic acid increased ³H-glutamine uptake in the presence of sodium.

Conclusions: This study proved that these natural compounds can have several effects on MCF-7 cells, worth of being further studied as anticarcinogenic compounds.

Keywords: MCF-7; Breast Cancer; Phenolic Acids; Polyphenols

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