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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 adaptative 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.

clude that TBH, insulin and INF-γ (MCF-7 cells) and TBH, insulin and leptin (MDA-MB-231 cells) stimulate GLUT1-mediated uptake of 1 mM ^{3}H-DG, but stimulate non-GLUT1-mediated uptake of 10 nM ^{3}H-DG. Na+-dependent ^{3}H-GLN uptake increased in the presence of INF-γ (in MCF-7 and MDA--MB-231 cells) and TBH (in MDA-MB-231 cells only); in contrast, Na^{+}-dependent ^{3}H-GLN uptake was reduced by insulin and leptin in both cell lines. By examining the influence of an inhibitor of the Na+-dependent carrier ASCT2 (GPNA 1 mM) on the stimulatory effect of these compounds, we conclude that INF-γ (in MCF-7 and MDA-MB-231 cells) and TBH (in MDA-MB-231 cells) stimulate ASCT2-mediated uptake of 5 nM ^{3}H-GLN, but stimulate non-ASCT2-mediated uptake of 0.5 mM ^{3}H-GLN.
Conclusions: Some obesity/T2DM biomarkers induce changes in glucose and glutamine transport that can contribute to breast cancer progression.
Keywords: Breast cancer; Obesity biomarkers; Glucose; Glutamine

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16 – Nutritional risk screening results using NRS 2002 in Orthopaedic and Gynecologist departments in VNGaia Hospital Center-Portugal

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Background: According to ESPEN, undernutrition is "a state resulting from lack of intake or uptake of nutrition that leads to altered body composition (decreased fat free mass) and body cell mass leading to diminished physical and mental function and impaired clinical outcome from disease". It causes many metabolic changes with decreased immune system's response with implications on treatment, difficulty in healing, increased number of complications, morbidity and mortality. In the literature, in 2015, 46% of hospitalized individuals in Portugal were at nutritional risk (NR).The aim was to evaluate NR and status of Orthopaedics' and Gynaecology's hospitalized patients in VNG Hospital Centre and study the association between patients undergoing scheduled (SC) and urgent surgery (US) and the effect of hospitalization using Nutritional Risk Screening 2002 (NRS2002).
Methodology: The NRS2002 protocol was used and anthropometric evaluation through direct and indirect measurements.
Results: This sample included 134 initial screening and 49 revaluations, from Orthopaedics' and Gynaecology's departments, aged between 20 and 99 years old. In the Orthopaedics' department (OD), the study showed a positive association between the type of surgery and the period of hospitalization ($p<0,01$). When comparing the last evaluation with the first results, the difference in the average score was statistically significant ($p=0,011$) with an increasing score in the last evaluation.
The results showed that 3,7% of patients were at undernutrition risk,60% from an US and 40% for a SC.
Conclusions: The NR screening tools are needed to easily allow the identification and control the NR and undernutrition. The patients with higher risk were those submitted to US because they stay longer in hospital and,according to the literature, the nutritional status is negatively affected by the time of hospitalization. We suggest that the type of surgery should be considered in the NR protocols, especially if it is to be applied in OD.
Keywords: NRS2002; Undernutrition; Urgent surgery; Schedule surgery

17 – Food intake of women with gestational diabetes *mellitus*, in accordance with two methods of dietary guidance: a randomised controlled clinical trial

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Background: Nutritional therapy is considered relevant for glycaemic control in pregnant women with gestational diabetes mellitus (GDM). The aim of this study was to evaluate the dietary intake of pregnant women with GDM according to two dietary guidance methods.
Methodology: Randomized controlled clinical trial conducted in consultation with the nutritionist, during prenatal care of adult pregnant women diagnosed with gestational diabetes mellitus (GDM) in a public maternity hospital in Rio de Janeiro, Brazil (2011-2014). The study population consisted of adult women diagnosed with GDM attending prenatal and delivery in the studied maternity (2011-2014). The control group (CG) received nutritional counseling by the traditional method and the intervention group (IG) was instructed on carbohydrate counting. Sugar intake, processed foods (FP) and ultra-processed foods (UPF) were evaluated.
Results: 286 pregnant women (145 in the CG and 141 in the IG) were evaluated. Of the pregnant women, 211/286 (73.8%) consumed sugar in pregnancy, 89/120 (74.2%). And 183/229 (79.9%) consumed PF daily in the second and third quarters, respectively. While 117/120 (97.5%) and 225/231 (97.4%) consumed UPF daily in the second and third quarters. There was no difference between groups regarding caloric or macronutrient intake. There was a higher consumption of PF per week among pregnant women who showed low adherence to diet at the second visit in the second trimester of pregnancy ($p = 0.04$). As for the comparative analysis of sugar consumption by the groups, there was no difference between them ($p = 0.78$) and sugar consumption did not influence the adequacy of weight gain ($p = 0.37$) and birth weight ($p = 0.11$).
Conclusions: The type of dietary orientation did not affect food intake, suggesting that both methods tested can be used to monitor the nutritional status of pregnant women with GDM.
Key words: Food intake; Gestational diabetes mellitus; Nutrition therap; Carbohydrate counting

20 – Peripheral metabolism in rat with olanzapine subchronic treatment supplemented with acetyl-L carnitine

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Background: Olanzapine (Olz) have been reported to promote higher risk for metabolic side-effects, such as obesity, dyslipidemia, and diabetes. Moreover/On the other hand, acetyl-L-carnitine (ALC) has been reported to improve homeostasis. This study aims to explore if ALC could improve metabolic homeostasis due to Olz effects on a rodent model.
Methodology: We investigated the effects of subchronic administration of Olz and with the addition of ALC on body weight gain and glucose metabolism in Wistar rats.
Results: Mid-term Olz exposure in rats significantly increases blood glucose levels in fasting and after the overload of glucose compared to the sali-

ne-treated group ($p<0.05$). However, in rats mid-term treated with olanzapine plus acetyl-L-carnitine had no significant differences in fasting blood glucose levels compared to the saline-treated group.
Conclusions: The findings suggest that ALC added to Olz treatment appeared to be effective to improve glucose metabolism during olanzapine subchronic treatment
Keywords: Olanzapine; Acetyl-L-carnitine; Glucose homeostasis; Locomotor activity

21 – Maternal uterine ageing and placentation

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Background: Uterine redox imbalance may lead to deficient placentation and development of pregnancy-related complications, with increased incidence in older women. Thus, it was aimed to identify age-related oxidative modifications to uterine proteins and study their involvement in placentation.
Methodology: Uterine samples were collected after elective caesarean section. The protocol was performed with ethical approval and volunteers written consent. Protein carbonylation was detected by OxyBlot and albumin carbonylation was verified by immunoprecipitation. HTR-8/SV neo trophoblast cell line was used for *in vitro* studies. The effect of *in vitro* carbonylated albumin (CHSA) on cell viability, proliferation and adhesion was quantified with neutral red. Scratch assay was used to evaluate cell motility and collagen-coated transwells invasion. Signaling proteins involved in cell stress were analyzed by western blot. Statistical analysis was performed with Spearman correlation or ANOVA.
Results: At placental bed, carbonylated albumin correlated strong and positively with maternal age. *In vitro* results showed that CHSA did not affect cell viability or proliferation. However, at the concentration of 100 μM, CHSA reduced significantly cell motility, induced an upregulation of SOD2 expression and triggered unfolded protein response, as shown by an increase in PERK expression and phosphorylation of eIF2α. Additionally, CHSA cross-linked to a collagen matrix reduced significantly trophoblast adhesion and invasion capacity.
Conclusions: Maternal ageing is accompanied by selective albumin modifications, that may have a deleterious role in placentation.
Keywords: Maternal ageing; Placentation; Albumin; Carbonylated albumin.

22 – Leukocyte telomere length and *hTERT* genetic polymorphism rs2735940 influence renal cell carcinoma clinical outcome

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Background: The ageing process is a multifactorial process that varies among individuals. It is characterized by the decrease of physiological functions of the human body and described as one of the main risk factors for many age-related diseases, like cancer. Telomere length has been widely described as biological age biomarker. In cancer, the role of Leukocyte Telomere Length (LTL) as biomarker of prognosis was already suggested, but in Renal Cell Carcinoma (RCC) the results are controversial. Differences in LTL among individuals may be due to the occurrence of genetic polymorphisms like *hTERT-1327C>T*(rs2735940), that occurs in the promoter region of the telomerase gene. This study aimed to investigate the effect of *hTERT-1327 C>T*(rs2735940) in LTL and in RCC progression-free and overall survival.
Methodology: The study was conducted according to the Helsinki Declaration. Using leukocyte DNA samples of RCC patients and healthy individuals, LTL was measured by quantitative real-time PCR. Moreover, *hTERT-1327C>T* genetic polymorphism was analyzed by allelic discrimination using real-time PCR technique.
Results: LTL in RCC patients was shorter than in healthy individuals ($p<0.001$). Among patients, LTL was longer in patients with T3/T4 tumors ($p=0.044$) and in patients with tumors larger than 7 cm ($p<0.001$). CC homozygous presented reduced time-to-progression (HR=1.63, $p=0.048$) and lower overall survival (Log Rank test, $p=0.019$). The use of this genetic polymorphism increased the capacity to predict RCC progression's risk (c-index model: 0.770).
Conclusions: LTL seems to have a role in carcinogenesis and *hTERT-1327C>T* influences LTL, progression-free interval and overall survival. We propose that the assessment of *hTERT-1327C>T* may be useful, in the future, as potential prognosis biomarker in RCC. This reinforces the role of telomeres in ageing and in age-related diseases.
Keywords: Leukocyte telomere length; Ageing, Renal Cell Carcinoma; Genetic Polymorphism; Prognosis; Biomarkers