A leucine supplementation after an immobilization-induced atrophy in old rats enhanced protein anabolism but failed in muscle mass recovery

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Metabolic pathways and local progenitor activation to regulate skeletal muscle mass

ROLE OF VITAMIN D IN THE PATHOGENESIS OF MUSCLE WASTING IN CANCER CACHEXIA

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Cancer cachexia is a syndrome characterized by loss of skeletal muscle protein, depletion of lipid stores and hormonal perturbations. Vitamin D (VitD) has been recently proposed as a potential regulator of skeletal muscle tissue since several studies described a relationship between muscle weakness/wasting and vitamin D deficiency.
Preliminary data obtained in our laboratory indicated a significant decrease in circulating VitD in rats bearing the AH-130 hepatoma compared to controls. On this line, the aim of the present study was to investigate the involvement of VitD deficiency in the pathogenesis of muscle wasting in AH-130 bearing rats. Rats were divided into four experimental groups: control, AH-130, VitD-treated and AH-130 VitD-treated. VitD (calcitriol, 1,25OH VitD) was administered daily *per os* (5μg/kg initial body weight, dissolved in corn oil). Untreated groups received vehicle alone. Treatment started the day of tumor transplantation and the animals were sacrificed after 7 days.
Both VitD-treated groups (controls and tumor hosts) showed reduced body weight and decreased GSN and tibialis mass in comparison to the respective untreated groups. Although preliminary, these results apparently suggest that VitD administration does not prevent tumor-induced muscle wasting. Such observation might be consistent with results obtained on C2C12 myocyte cultures, where the presence of calcitriol in the culture medium impairs myoblast complete differentiation to myotubes, suggesting that VitD-activated signals might inhibit myogenesis.

THERAPEUTIC PARACETAMOL TREATMENT IN OLDER PERSONS INDUCES DIETARY AND METABOLIC MODIFICATIONS RELATED TO SULFUR AMINO ACIDS

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Sulfur amino acids are determinant for the detoxification of paracetamol (N-acetyl-p-aminophenol) through sulfate and glutathione conjugations. Long term paracetamol treatment is common in elderly, despite a potential cysteine/glutathione deficiency. Detoxification could occur at the expense of anti-oxidative defenses and whole body protein stores in elderly. We tested how older persons satisfy the extra demand in sulfur amino acids induced by long term paracetamol treatment, focusing on metabolic and nutritional aspects.
Effects of 3 g/d paracetamol for 14 days on fasting blood glutathione, plasma amino acids and sulfate, urinary paracetamol metabolites, and urinary metabolomic were studied in independently living older persons (five women, five men, mean (± SEM) age 74 ± 1 y). Dietary intakes were recorded before and at the end of the treatment and ingested sulfur amino acids were evaluated.
Fasting blood glutathione, plasma amino acids and sulfate were unchanged. Urinary nitrogen excretion supported a preservation of whole body proteins, but large scale urinary metabolomic analysis revealed an oxidation of some sulfur-containing compounds. Dietary protein intake was 13% higher at the end than before paracetamol treatment. Final sulfur amino acid intake reached 37 mg/kg/d. The quantity of sulfur excreted in urinary paracetamol conjugates corresponded to 20% of the dietary intake in sulfur amino acids. Older persons accommodated to long term paracetamol treatment by increasing dietary protein intake without any mobilization of body proteins, but with increased oxidative damages. The extra demand in sulfur amino acids led to a consumption far above the corresponding population-safe recommendation.

A LEUCINE SUPPLEMENTATION AFTER AN IMMOBILIZATION-INDUCED ATROPHY IN OLD RATS ENHANCED PROTEIN ANABOLISM BUT FAILED IN MUSCLE MASS RECOVERY

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Background: By contrast to adult, we have shown (Magne et al. 2011) that immobilization during ageing generated muscle atrophy which is not recovered. This is not due to a sustained muscle proteolysis since it was rapidly normalized. This study was undertaken to assess if an alteration of muscle protein synthesis during the immobilization and the recovery periods was involved and if a leucine supplementation may improve muscle mass following immobilization.

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ethods: 23-month-old rats were immobilized for 8 days (I8) and allowed to recover during 40 days. Half of the rats received a control diet whereas the other half were fed the same diet supplemented with 4.5% leucine after cast removal. Muscle proteasome-dependent proteolytic activities, FOXO3a-phosphorylation were assessed in *gastrocnemius*. Protein synthesis and protein S6 phosphorylation were measured at both the postabsorptive (PA) and post prandial (PP) states

Results: At I8, immobilized-muscles were atrophied by 21% that was explained by an increased proteasome activities (+20 to +80%) but also by a large decreased in protein synthesis at PP (-30%). During recovery, proteolysis and protein synthesis were normalized. With the leucine-diet, proteolysis was normalized earlier and protein synthesis was 30% higher in PP than in controls. These observations were correlated with an increased phospho-FOXO3a/FOXO3a ratio (+80%) and a sustained increase of phospho-S6 amount (+30%) However, despite this improvement of muscle anabolism, leucine failed to improve muscle mass recovery.

Conclusion: After immobilization, leucine supplementation, despite its beneficial effect on muscle protein anabolism failed in muscle mass recovery. This discrepancy needs to be further studied.

DIFFERENTIAL INSULIN SIGNALING REGULATION IN SKELETAL MUSCLE AND ADIPOSE TISSUE FROM OLD RATS FED A LONG-TERM LEUCINE EXCESS

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Leucine acts as a signal nutrient in promoting protein synthesis in skeletal muscle and adipose tissue via mTOR/S6K1 pathway activation, and may be of interest to prevent age-related sarcopenia. However, hyper-activation of mTOR/S6K1 has been suggested to inhibit the first steps of insulin signaling and finally promote insulin resistance.

The impact of long-term dietary leucine supplementation on insulin signaling and sensitivity was investigated in old rats (18-month old) fed a 15% protein diet supplemented (LEU group) or not (C group) with 4.5% leucine for 6 months. The resulting effects on muscle and fat were examined.

mTOR/S6K1 signaling pathway was not significantly altered in muscle from old rats subjected to long-term dietary leucine excess whereas it was increased in adipose tissue. Phosphorylation of IRS1 on serine 635/636 was increased in adipose tissue but not in muscle. Overall glucose tolerance was not changed but insulin-stimulated glucose transport was improved in muscles from leucine-supplemented rats related to improvement in Akt expression and phosphorylation in response to food intake. No change in skeletal muscle mass was observed whereas perirenal adipose tissue mass accumulated (+ 45%) in leucine-supplemented rats.

A prolonged leucine supplementation in old rats differently modulates IR/IRS/Akt and mTOR/S6K pathways in muscle and adipose tissue. It does not increase muscle mass but seems to promote hypertrophy and hyperplasia of adipose tissue that did not result in insulin resistance.

HYPERCYST(E)INEMIA IN *CDO*$^{-/-}$ MOUSE MODEL IS ASSOCIATED WITH AN ACCUMULATION OF TISSUE ACID-LABILE SULFIDE AND LOW CIRCULATING HOMOCYSTEINE LEVELS

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Cysteine (Cys) is considered a semi-essential amino acid in mammals as it is synthesized via transsulfuration of homocysteine (Hcy) by two enzymes, cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE). Cysteine can be oxidized by cysteine dioxygenase (CDO) to taurine and sulfate. Non-oxidative desulfuration reactions mediated by CBS and CSE can transform cysteine to reduced sulfur, generating endogenous H_2S. Cysteine is also used for the synthesis of glutathione (GSH) a reduced tripeptide that also contains glutamate and glycine. To gain more insight into the physiological control of cysteine metabolism, functions of CDO and the consequences of a loss of CDO activity, we generated *CDO*$^{-/-}$ and *CDO*$^{+/+}$ mice. Because of the disruption of flux through CDO-dependent pathways, *CDO*$^{-/-}$ mice exhi... an increase in serum cyst(e)ine levels compared to *CDO*$^{+/+}$ mice. Surprisingly, serum Hcy levels markedly decreased in *CDO*$^{-/-}$ compared to *CDO*$^{+/+}$ mice. Serum Cys levels were positively associated with tissue acid-labile sulfide levels. The association of Cys with GSH was slightly positive in *CDO*$^{+/+}$ mice whereas no association was found in *CDO*$^{-/-}$ mice. Treatment of *CDO*$^{-/-}$ mice with a taurine-supplemented diet decreased tissue acid-labile sulfide and GSH levels compared to non-taurine treated *CDO*$^{-/-}$ mice. On the other hand, serum Hcy levels increased in taurine treated *CDO*$^{-/-}$ mice when compared to non-taurine treated *CDO*$^{-/-}$ mice. Taken all together these results suggested that in the absence of CDO cysteine is preferentially metabolized via desulfuration pathways with only a small increase in net flux to glutathione. The increase in tissue acid-labile sulfide levels and the decrease of the serum Hcy level in *CDO*$^{-/-}$ mice suggest the idea that serum hypercyst(e)inemia could lead to increased flux of cysteine through the reactions catalyzed by CBS and CSE (enzymes involved in both desulfuration and transsulfuration), resulting in an increase of H_2S accumulation in tissues and a reduction in circulating Hcy levels.

COMBINED APPROACH TO COUNTERACT EXPERIMENTAL CANCER CACHEXIA: EICOSAPENTENOIC ACID AND TRAINING EXERCISE

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Cancer cachexia is a syndrome characterized by loss of skeletal muscle protein, depletion of lipid stores, anorexia, weakness, and perturbations of the hormonal homeostasis. Despite several therapeutic approaches were described in the past, effective interventions countering cancer cachexia are still lacking.

The aim of the present work was to verify the ability of eicosapentaenoic acid (EPA) to prevent the muscle depletion in Lewis lung carcinoma-bearing mice and to test the ability of exercise training to increase the EPA effect. EPA alone did not prevent the muscle loss induced by tumor growth, while the combination with the exercise induced a partial rescue of muscle strength and mass. Moreover, the association of EPA and exercise reduced the dramatic PAX-7 accumulation and stimulated the increase of PCG-1 protein.

Overall, the present data suggest the exercise as an effective tool that should be added on combined therapeutic approaches against cancer cachexia.

HYPERINSULINEMIA AND INSULIN RESISTANCE, EARLY CARDIOVASCULAR RISK FACTORS IN CHILDREN WITH CHRONIC KIDNEY DISEASE

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Background/aims: Pediatric chronic kidney disease (CKD) is associated with increased risk of cardiovascular disease. Still, hyperinsulinemia and insulin resistance, common cardiovascular risk factors, are not extensively investigated in children with CKD. We hypothesize that insulin abnormalities are present also in pediatric mild to moderate CKD, and associated with inflammation and malnutrition.

Methods: We enrolled 26 children with CKD, and 34 healthy controls for analyses of blood samples and body composition. Insulin resistance was assessed using the homeostasis model assessment for insulin resistance (HOMA-IR).

Results: The patients had higher insulin levels and HOMA-IR compared to the controls ($p < 0.01$ and $p < 0.005$), and they correlated inversely with estimated glomerular filtration rate (rho $= -0.52$, $p < 0.01$; rho $= -0.37$, $p = 0.08$). No association was found with inflammation or malnutrition.

Conclusion: High insulin levels and HOMA-IR appear to be common in pediatric CKD patients, already in mild to moderate renal failure. We