Rational: Obesity is a worldwide epidemic with collateral diseases, including liver damage. Data report that obesity and liver dysfunction are associated with increased levels of oxidative damage, inflammation and apoptosis, involving mitochondria in the process. Our group with the support of FCT (PTDC /DES/113580/2009) recently studied this topic [1-4]. However, new data highlighted that endoplasmic reticulum (ER) stress is also a central regulator in nonalcoholic fatty liver disease, including in nonalcoholic steatohepatitis (NASH). The imbalance between cellular protein folding needs and the capacity of the ER to deal with it, induces ER stress and an unfolded protein response (UPR) [5]. Recent data suggest that signaling of the NASH-related UPR is linked to oxidative stress and inflammation by various mechanisms [6] and exacerbated by visceral fat-derived cytokines secretion [7]. Moreover, a cooperative link between ER and mitochondria in the establishment and regulation of calcium homeostasis, oxidative stress and apoptosis in several physiopathological conditions, including liver dysfunction through mitochondria-associated endoplasmic reticulum membranes (MAMs) has also been described [8]. Many strategies have been explored against NASH-related cellular disturbances, including exercise. In fact, the effects of exercise in modifying key proteins of the hepatic metabolism in obesity should not be taken lightly [9, 10]. However, if the exercise benefits in the liver in the context of obesity-related NASH may also be related with the modulation of ER function remains scarcely studied. To analyze the effects of voluntary physical activity (VPA) and endurance training (ET) against ER stress in obesity-induced NASH, and to better comprehend the cross-talk between mitochondria and ER in this obesity-related liver disease. As exercise is an effective preventive and therapeutic strategy against deleterious conditions in several tissues, VPA on free wheel and ET in treadmill will be considered in this project. High fat diets (HFD) have been successfully used to induce NASH in rats [11] and to study obesity-related liver dysfunction [1-4, 9]. This “ecological” dietary model mimics the Western-style diet, and will be used in this study. An important contribution to elucidate the ER mechanisms associated with the possible role of exercise in the prevention of ER stress in obesity-induced NASH will be provided. Our hypothesis is that, in an obesity setting, an active life-style decreases liver ER stress, oxidative stress and subsequent, inflammation and apoptosis, resulting in decreased hepatic damage. Moreover, it is our belief that a positive cross-talk between mitochondria and the ER will be involved in this protective phenotype. Therefore, physical activity should likely be advised in patients with NASH.

Sets of wild-type rats will be assigned into the following six groups: sedentary, sedentary diseased (rats fed with a HFD), active (32 wks VPA or 12 wks ET) and active diseased (fed with a HFD and submitted to VPA or ET). NASH-related liver morphological alterations will be evaluated by light and electron microscopy. Using polymerase chain reaction and western blotting (WB), the expression of genes and the content of some proteins from the three branches of the UPR – inositol requiring enzyme (IRE-1α), activating transcription factor 6 (ATF6), and PKR-like ER-associated protein kinase (PERK) will be assayed. Proteins involved in the inflammatory response, as well as adiponectin and leptin will be assayed in serum by enzymelinked immune sorbent assay (ELISA). Moreover, IL-1β, IL-6, IL-10 and TNF-α will also be assessed in hepatic and visceral adipose tissues by WB. Proteins related with the activation of the apoptotic signaling and some proteins of the Bcl-family will be assessed by spectrophotometry and WB, respectively. Moreover, the structure and molecular composition of MAMs will be characterized by electron microscopy and WB. This project will improve the links between CIAFEL and FMUP Porto, Faculty of Biology Barcelona and CNC Coimbra. FMUP have quality works on the effects of liver disease on ER and the CNC is a gold-standard group concerning mitochondrial function. Moreover, based on a recent collaboration with the Nencki Institute of Biology, Poland, the International Consultant of this project is a very recognized expert in the field that can contribute for the success of this project.
Abstract:

Type II diabetes is a worldwide epidemics resulting from poor lifestyle choices, including dietary habits and lack of exercise. Evidence exists showing that life in the womb predisposes the offspring for metabolic diseases in the adult life. Mitochondria are mediators of hyperglycemia-induced fetal developmental complications. Mitochondria are present in most eukaryotic cells and vary in number from hundreds to thousands, depending on metabolic activity of the tissue. Mitochondrial-formed ATP can be used by the cell for many functions including the maintenance of transmembrane ion gradients, protein synthesis, vesicular transport, metabolite secretion (as insulin in pancreatic beta-cells) as well as muscle activity. Mitochondria have also an important role in calcium homeostasis, intermediate metabolism, cell death regulation and generation of oxygen free radicals. Diabetes during pregnancy can affect fetal cellular signaling pathways, including mitochondrial bioenergetics, leading to the development of several metabolic diseases in the adulthood. Although pharmacological approaches exist to control diabetic-induced complications, a voluntary change in lifestyle is often more effective, also sparing mother and fetus from being exposed to chemical agents. Voluntary physical activity (VPA) during pregnancy is a powerful approach, which can be benefic for mother, fetus and later child. By using a rodent model of gestational diabetes, we intend to demonstrate for the first time that hyperglycemic pregnant mothers subjected to VPA have offspring with a more robust mitochondrial function and demonstrating better cognitive functions when compared with offspring from sedentary pregnant mothers. For the present work, we will use the Goto-Kakizaki (GK) animal model. The GK rat has been generated as an animal model of non-insulin-dependent diabetes mellitus. This animal is a spontaneously diabetic rat, produced by selective inbreeding of Wistar rats with the highest glucose values during oral glucose tolerance tests. We expect to confirm the protective effect from VPA even when both mother and father were hyperglycemic. If protection resulting from VPA is obtained, this relevant work will show that VPA contributes to protect the future offspring from the development of further metabolic disease and will decrease the incidence of organ degeneration motivated by in utero hyperglycemic insult. From a human point of view, the impact is very high. The results can be translated into a motivational will to perform moderate exercise during pregnancy if a diabetic condition is present. We believe that this change in paradigm will lead many health-related professionals to recommend physical activity to the diabetic mother, in case no other counter-indications exist.

The results obtained in this project will be disseminated as full length publications in high impact peer-reviewed scientific journals (e.g. Diabetes, Clinical Sciences, PNAS or others), and presented under the form of posters or short oral talks in different national and international meetings). The present project is a fortunate combination of six recognized scientists with very strong CVs and different backgrounds (the Principal Investigator, António Moreno, Paulo J. Oliveira, Carlos Palmeira, Anabela Rolo, António Ascensão and José Magalhães), and originated in two different excellent institutions, the Center for Neuroscience and Cell Biology (CNBC/UC), and the Research Centre in Physical Activity, Health and Leisure (CIAFEL/UP). The team has extensive experience in animal models for hyperglycemia and physical activity, with more than 40 papers published so far when combined. This project, which shows a high impact to the society, will also contribute to the formation of human resources, and may result in several Master and PhD theses. We also intend to organize and teach graduate courses on subjects related with the research proposed, as well as to organize science awareness activities focused on showing that VPA can be a solution for many metabolic diseases in humans.
Abstract:
Obesity is a major public health challenge as the number of severely obese patients increases in several countries. Surgical treatment (bariatric surgery) is the most effective option for long-term weight reduction in severely obese patients. Despite beneficial metabolic outcomes, bariatric surgery (BS) negatively influences bone metabolism. BS patients show a rise in biochemical markers of bone turnover (BTM), with bone resorption exceeding bone formation. Bone mineral density (BMD) decreases following BS, with several patients developing osteopenia or osteoporosis, while others develop more severe metabolic bone disorders. These patients also seem to be more prone to fall, further aggravating the risk of fractures. Indeed, recent evidences suggest that the obese patients risk of fracture increases following BS. Despite this, there is controversy regarding the real magnitude of the effects of BS on bone metabolism. Since dual-energy X-ray absorptiometry (DXA) derived measurements of BMD are the gold standard for diagnosing osteopenia and osteoporosis, it is also used to assay metabolic bone disease in BS patients. However, the extreme variations in weight, body size and body composition that follow BS negatively affect the specificity and sensibility of DXA, hindering its ability to monitor the effects of BS on bone. The etiological mechanisms of BS induced bone loss are also largely unknown. Initially, bone losses were attributed to calcium and vitamin D malabsorption, however more recent studies suggest that other mechanisms are involved. BS induces profound changes in the energy regulation metabolism and in gastrointestinal physiology. As several of these hormones also affect bone metabolism, it is thought that they are also involved in BS induced bone loss. Since the etiology of BS induced bone loss is largely unknown, treatment is also essentially symptomatic. As clinicians lack adequate strategies to manage metabolic bone disease in these patients, treatment relies mostly on calcium and vitamin D supplementation, which is not effective. Several evidences show that exercise is an effective therapeutic strategy to prevent bone mass losses in several health conditions. However, no study so far has examined the therapeutic effects of an exercise-training program in the prevention of BS induced bone loss. Our main objective in this research project is therefore to investigate the effects on bone metabolism and fracture risk of an exercise-training program specifically tailored to improve bone health and balance of patients that underwent BS. To accomplish our goals we will perform a randomized controlled trial on volunteer obese patients (n=80; BMI>40 Kg.m\(^{-2}\)) elected to perform BS that meet our inclusion and exclusion criteria. Patients will be followed for about 13 months and will be randomly assigned into 2 groups i) a group receiving standard follow-up and medical care, or ii) a group that will undergo a 11 months duration Exercise Training Intervention program designed to improve bone health and reduce fall risk in addition to the standard follow-up and medical care. All patients will be assessed i) before the surgery, ii) one month, iii) 6 months, and iv) 12 months after the surgery. A broad set of parameters will be assayed in each patient, namely: BTM, BMD, bone tissue biomechanical properties, hormones involved in the regulation of energy, gastrointestinal and bone metabolism, body composition, BMI, nutritional intake, balance, muscle strength, cardiorespiratory fitness and daily physical activity. This broad set of variables will allow us to understand in a much more comprehensive way the effects of an exercise-training program on bone metabolism of BS patients, contributing also to further elucidate the mechanisms underling BS induced bone loss and fracture risk increase. To accomplish the main goal we will use established methods in the literature as well as novel procedures, which will enable us to overcome some of the limitations of previous studies. At the end of the study we expect to have collected consistent data about whether an exercise-training program is or is not able to effectively prevent BS induced bone losses and fracture risk increases. We believe that this data will help clinicians to make evidence based decisions regarding the best treatment options for the management of metabolic bone disease in their patients who are submitted to BS. The growing number of bariatric surgeries performed worldwide together with the complete lack of studies on the therapeutic effects of exercise in the prevention of bone mass losses in these patients supports the need for research in this area. Our past experience in bone biology research as well as in the conduction of exercise intervention clinical trials, together with the expertise of our partners in this research project supports our chances of successfully achieving the objectives that we are proposing.
Abstract:
Resistant hypertension (RH) is a major problem for patients, healthcare system and professionals. Presently, it is a medical problem without a solution, as the available treatment options to lower blood pressure in these patients, namely antihypertensive drugs and renal denervation, have reduced success.\(^1,\(^2\) Despite promising, the evidence about the impact of exercise training in the control of blood pressure in RH is fragile. Only three studies exist\(^3\)-\(^10\); two\(^13\),\(^14\) used a water-based exercise intervention, which is difficult to implement due to staff and infrastructures requirements. The only land-based study\(^12\) has several limitations, as it fails to provide crucial information, namely frequency, intensity, time, and type of exercise, as well as exercise adherence and the timing of the blood pressure measurements, which is a critical aspect as the evaluation should avoid the postexercise hypotension and the detraining effects. Additionally, the 3 studies did not control modifications in diet, physical activity, and body fat, hence not accounting their contribution to the changes in blood pressure, hence not observing the independent effect of exercise. Moreover, none of these studies provided any insight about the mechanisms of blood pressure reduction or did a follow-up evaluation to clarify whether the effects of exercise could be maintained. Therefore, due to lack of success of the available treatments, the methodological limitations of the available evidence and the high burden of disease attributable to high blood pressure, we aim to test the hypothesis that aerobic exercise is an effective antihypertensive therapy in patients with RH; and, that the positive changes induced in the blood pressure control are mediated by exercise-induced increase in nitric oxide bioavailability. In this sense, the main objective is to assess in a randomized controlled trial whether exercise training reduces ambulatory blood pressure in patients with RH. As secondary objectives we aim to evaluate the effects of the exercise intervention on: the use of blood pressure lowering agents; risk factors, body composition, daily physical activity, dietary intake, quality of life, cardiorespiratory fitness; inflammatory biomarkers, autonomic function, arterial stiffness; endothelial function, damage and repair/regeneration; nitric oxide bioavailability, endothelial Nitric Oxide Synthase (eNOS) and oxidative stress. Third, to evaluate the effect of exercise on blood pressure and the former parameters three months post-intervention, to clarify whether the effects of exercise could be maintained after the end of the training period. To accomplish these goals 60 patients with RH will be recruited and randomized into exercise training or control groups and followed up for 6 months. The patients in the exercise group will participate in a 3-month outpatient program. The control group will receive usual medical care. At baseline, after the intervention and 3 months after the end of the intervention both groups will undergo the following evaluations: body composition, casual and ambulatory blood pressure, cardiorespiratory fitness and hemodynamic at peak exercise, quality of life, daily physical activity, dietary intake, salt intake, arterial stiffness, autonomic function, endothelial damage, endothelial repair/regeneration, and determination of inflammatory biomarkers, nitric oxide, eNOS, and oxidative stress levels. With the present study we expect to promote, develop and expand the knowledge in this field by assessing the impact of exercise not only on blood pressure, but also on a pool of markers that provide a wide picture of the mechanisms underlying the effects of exercise. Our laboratories have the skills and the majority of the equipment to accomplish our goals. We are incorporating young investigators and enrolling different institutions in an attempt to gather a team with expertise covering all the project area. The consultant will provide additional expertise in some key aspects. In summary, the methods and techniques in use in this project could provide a new view in the setting of exercise and RH. Ultimately, we expect to reinforce the scientific evidence regarding the role of exercise training as a cost-effective tool in the treatment of patients with RH.