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Raman spectral characteristics of neck and head of femur in low-density lipoprotein receptor gene knockout mice submitted to treadmill aerobic training

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ABSTRACT

Hypercholesterolemia is associated with deterioration of bone tissue. Trough Raman spectroscopy, the present study aimed to assess the molecular changes in terms of mineral and organic bone-tissue remodeling in low-density lipoprotein receptor gene knockout mice (LDLr −/−) submitted to treadmill aerobic training. In order to evaluate alterations in trabecular bone and cartilage surface, neck and head femoral were assessed into four groups: (CON, $n = 7$), the 1) animals did not perform any physical activity and had hypercholesterolemia (CON, $n = 7$); The trained groups where animals underwent a protocols of low, moderate and high training for 8 weeks and 4 times per week on a treadmill with progressive intensities: running speed (8–13 m/min) and time of exercise (20-60 min). These progressive intensities were needed to adjust the working load for the physical training to 2) 30–49% (LOW, $n = 7$), 3) 50–79% (MOD, $n = 7$), and 4) 80–100% (HIGH, $n = 7$) of the maximum running speed. Each group was examined for molecular changes in both bone phases (mineral and organic) using principal components analysis (PCA). All exercised groups showed a significant increase ($p < 0.05$) of bone remodeling in trabecular area and a higher content of proteins (organic content) in cartilage independently of physical activity intensity. Thus, this work demonstrated that physical training could act as a bone-protector factor in hyperlipidemic animal model as well as Raman technique associated with PCA analysis may be feasible methodology for assessment or monitoring of bone at the molecular level.

1. Introduction

Bone is a metabolically active and extremely organized tissue that undergoes continuous remodeling throughout life in order to maintain the correct balance between bone resorption by osteoclasts and bone deposition by osteoblasts [1]. The phenomenon of bone turnover reflects bone cell activity that integrates a rigid framework (extracellular matrix) composed of collagen fibrils (organic phase) and hydroxyapatite crystals (mineralized phase) [2]. Basically, bone remodeling serves to adjust bone architecture to meet changing mechanical needs and helps to repair microdamage in the bone matrix, thus preventing the accumulation of old bone [3]. The regulation of bone remodeling is both systemic and local and may be influenced by several factors, such as diet and physical activity [4].

In morphological terms, there are two major types of bone: cortical, which has a mechanical function and is protective, and trabecular, which provides strength and, more importantly, the majority of the metabolic function (major site of bone remodeling) [5]. It is possible to identify different types of bone in a single bone segment. For instance, the long bone femur which is subject to most of the load during daily activities has different regions including the femoral neck (FN), femoral head (FH) and femoral diaphysis (FD). While FN is mainly composed of trabecular bone and FD is composed of cortical bone, FH has an articular surface which is composed of cartilaginous tissue [6].

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It has been suggested that high levels of circulating lipids in blood cause alterations in bone tissue. For instance, Yamaguchi et al. [7] showed that both low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol levels were correlated with low bone mass in postmenopausal women. Luegmary et al. [8] showed that hyperlipidemia may lead to an imbalance in the bone remodeling process, reducing bone mass by increasing the activity and differentiation of osteoclasts in vitro. Using hyperlipidemic rats, Krieger et al. [9] found alterations in bone remodeling, as demonstrated by the increase in bone resorption and number of osteoclasts and the decrease in osteogenesis. Recently, Soares et al. [10,11] demonstrated that hyperlipidemic LDLr −/− mice had bones with reduced resistance that were more susceptible to fractures.

On the other hand, the biomedical literature has extensively reported that the mechanical loading generated by physical activity plays an important role in bone development [12,13]. Although it has been reported that aerobic exercises could be considered a strategy to reduce the risk of chronic diseases such as hypercholesterolemia [14] and favor the increase of HDL, which decreases the risk for cardiovascular complications [15], studies investigating the effects of physical activity on the bone quality of subjects or animals with hyperlipidemia are scarce. Given that hyperlipidemia can be deleterious for bone quality and that physical activity could counterbalance this outcome, it is reasonable to hypothesize a beneficial interaction among these variables.

In recent years, Raman spectroscopy has become an important tool in the assessment of bone quality, because it can be used quickly and nondestructively with fresh tissue, highlighting both mineral and organic contents [16]. Raman spectra provides information about the chemical structure of the biological tissue by the inelastic scattering of the incident light during the polarization of the electron cloud [17]. Bone quality as well as bone biochemical composition may be investigated by calculation of integrated Raman bands [18–20] and using multivariate analysis such as principal components (PCA) [21,22]. Unlike bone densitometry (DXA), which is the gold standard for bone mineral assessment, Raman spectroscopy may be useful for obtaining information about both bone mineral and organic phases [19].

Therefore, the present study aimed to assess the molecular changes in FN (trabecular bone) and FH (articular cartilage region) in low-density lipoprotein receptor gene knockout mice (LDLr −/−) by the unique biochemical information provided by the Raman spectroscopy. In order to elucidate the effects of treadmill aerobic training on Raman spectral features, PCA was applied in two different femoral regions: trabecular and articular. In addition, different intensities of physical exercise (low, moderate and high) were employed during 8 weeks of testing. Correlations between triglycerides and total cholesterol serum were assessed via PCA in an attempt to establish a causal relationship between these variables.

2. Materials and Methods

2.1. Experimental Model

Experimental procedures were conducted according to the international guidelines for animal handling and were approved by the Research Ethics Committee for animal care of the Federal Institute of Education, Science and Technology of South of Minas Gerais (IFSULDEMINAS) (protocol no. 5A/2015). Twenty-seven genetically modified male mice with a knockout of the low density lipoprotein receptor (LDLr −/− mice) were obtained from the bioterium of IFSULDEMINAS (Muzambinho, MG, Brazil). These experimental animals show elevated plasma levels of cholesterol and triglycerides, irrespective of the diet consumed [10]. Mice were kept in ventilated racks with controlled temperature (22–24 °C) under a 12/12 h light/dark cycle, fed with standard chow and water “ad libitum”.

At 3 months of age (at skeletal maturation), the hypercholesterolemic animals were randomly divided into four experimental groups as follows: control group (CON, $n = 7$) where the animals did not perform any physical activity; low intensity group (LOW, $n = 7$), where the trained animals were submitted to the treadmill protocol with an intensity equal to 30–49% of the maximum load; moderate intensity group (MOD, $n = 7$), where the trained animals were submitted to the treadmill protocol with an intensity equal to 50–79% of the maximum load; high intensity group (HIGH, $n = 7$), where trained animals were submitted to the treadmill protocol with an intensity equal to 80–100% of the maximum load.

2.2. Treadmill Training Protocol

The animals were adapted to a treadmill for 10 min for 4 days before starting training. The trained groups underwent a protocol of low, moderate and high training four times per week for 8 weeks on a treadmill (EP 131, Insight Equipment, Ribeirão Preto, SP, Brazil) with progressively increasing intensity: running speed (8–13 m/min) and duration of exercise (20–60 min). These progressive intensities were needed to adjust the working load for the physical training to 30–49% (LOW), 50–79% (MOD) and 80–100% (HIGH) of the maximum running speed as described by Ferreira et al. [23].

2.3. Bone Samples

All mice were anesthetized with an intramuscular administration of 40 mg·kg^{-1} xylazine HCl (Xilazin 2%, 50 mL; Syntec do Brazil Ltda., São Paulo, Brazil) and 50 mg.kg^{-1} ketamine HCl (Cetamin 10%, 50 mL; Syntec do Brazil Ltda.) and euthanized with an intracardiac injection of KCl solution (potassium chloride 10%; Laboratório Ariston Ltda., São Paulo, Brazil). The right femur was harvested and all visible soft tissues were removed. The femurs were then stored in plastic and sterilized bottles and frozen in a freezer (− 23 °C) until spectral analysis since chemical fixation is not advisable for Raman studies. In order to evaluate alterations in the trabecular bone and articular surface (cartilage area), the femoral neck (FN) and the femoral head (FH) were assessed, respectively (Fig. 1). Prior to Raman analysis, the bone samples were gradually warmed to room temperature and their surfaces were adequately positioned in the Raman spectrometer without any chemical fixation or treatment.

2.4. Dispersive Raman Spectroscopy

Raman spectra were obtained using a dispersive Raman spectro-

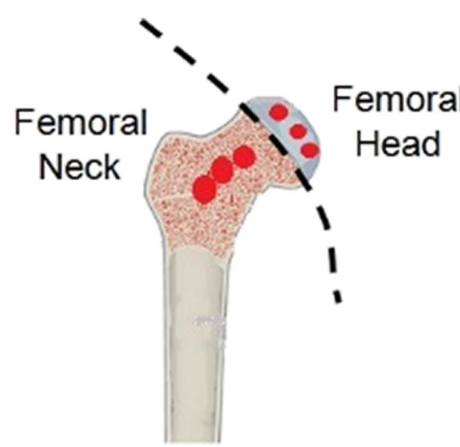

Fig. 1. Indication of the sites (red circles) on the bone where the Raman measurements were taken: triplicates on head of femur with a thick layer of cartilage and neck of femur composed mainly of trabecular bone. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)