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Physical exercise prevents and mitigates non-alcoholic steatohepatitis-induced liver mitochondrial structural and bioenergetics impairments

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ABSTRACT

Exercise is considered a non-pharmacological tool against several lifestyle disorders in which mitochondrial dysfunction is involved. The present study aimed to analyze the preventive (voluntary physical activity—VPA) and therapeutic (endurance training—ET) role of exercise against non-alcoholic steatohepatitis (NASH)-induced liver mitochondrial dysfunction. Sixty male Sprague–Dawley rats were divided into standard-diet sedentary (SS, n = 20), standard-diet VPA (SVPA, n = 10), high-fat diet sedentary (HS, n = 20) and high-fat diet VPA (HVPA, n = 10). After 9 weeks of diet-treatment, half of SS and HS animals were engaged in an ET program (SET and HET) for 8 weeks, 5 days/week and 60 min/day. Liver mitochondrial oxygen consumption and transmembrane-electric potential ($\Delta\Psi$) were evaluated in the presence of glutamate–malate (G/M), palmitoyl–malate (P/M) and succinate (S/R). Mitochondrial enzymes activity, lipid and protein oxidation, oxidative phosphorylation (OXPHOS) subunits, cytochrome c, adenine nucleotide translocator (ANT) and uncoupling protein-2 (UCP2) content were assessed. HS groups show the histological features of NASH in parallel with decreased of $\Delta\Psi$ and respiratory control (RCR) and ADP/O ratios (G/M and P/M). A state 3 decrease (G/M and S/R), FCCP-induced uncoupling respiration (S/R) and ANT content were also observed. Both exercise types counteracted oxygen consumption (RCR, ADP/O and FCCP-uncoupling state) and $\Delta\Psi$ (lag-phase) impairments. In conclusion, exercise prevented or reverted (VPA and ET, respectively) the bioenergetic impairment induced by NASH, but only ET positively remodeled NASH-induced liver structural damage and abnormal mitochondria. It is possible that alterations in inner membrane integrity and fatty acid oxidation may be related to the observed phenotypes induced by exercise.

1. Introduction

Obesity and related-diseases, such as non-alcoholic steatohepatitis (NASH), is increasing dramatically worldwide among adults (Chiang et al., 2011) and children (Rodriguez et al., 2010). Although the precise prevalence of non-alcoholic fatty liver disease is unclear, it is estimated that 94% of obese adults (Clark, 2006) and 38–53% of obese children are affected (Bellentani and Marino, 2009). The development of diagnostic and therapeutic strategies is dependent on the understanding of the pathogenic mechanisms behind the disease progression. In this field, several authors suggested that oxidative stress and apoptosis are the main mechanisms for NASH development, with mitochondria playing a central role (Begriche et al., 2006; Jiang et al., 2011; Rolo et al., 2012). Therefore, pharmacological (Serviddio et al., 2011) and non-pharmacological strategies have been proposed to prevent mitochondrial degeneration associated with NASH, and hence prevent the progression of the disease (Ascensao et al., 2013; Goncalves et al., 2013; Rector et al., 2011). Among non-pharmacological approaches, physical exercise is a promising strategy to counteract liver mitochondrial damage (Ascensao et al., 2013; Goncalves et al., 2013; Rector et al., 2008a; 2008b, 2011; Thyfault et al., 2009). Rector et al. (2008b) showed that voluntary exercise increased mitochondrial cytochrome c content, enzyme activities and fatty acid oxidation, preventing liver fat accumulation. Later, Thyfault et al. (2009) demonstrated that rats selectively bred for high aerobic capacity had increased hepatic mitochondrial oxidative capacity and consequently, presented attenuated signs of steatosis development and progression. Furthermore, Rector et al. (2011) showed

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that voluntary exercise offers additional health benefits on hepatic mitochondrial function when compared with caloric restriction. In turn, data from our group suggested that the severity of mitochondrial dysfunction was inversely associated with the levels of moderate/vigorous physical activity in a group of NAFLD patients (Gonçalves et al., 2012). All of the previous studies undoubtedly highlight the positive effect of exercise on several mitochondrial components, including the expression and/or activity of different mitochondria enzymes and respiratory complexes. Nevertheless, the protective role of exercise on liver mitochondrial bioenergetics and ultra-structure in a diet model of NASH remain unknown. Our hypothesis is that both voluntary physical activity (VPA) and endurance training (ET) used as preventive and therapeutic strategies, respectively, antagonize ultrastructural and functional mitochondrial alterations induced by a high-fat diet model of NASH. Moreover, oxidative phosphorylation (OXPHOS) machinery components and oxidative damage indices are favorably modulated by exercise, thus contributing to the expected more resistant mitochondrial phenotype.

2. Methods

2.1. Animal treatment

Sixty male Sprague–Dawley rats (aged 5–6 weeks and weighting 125–150 g) purchased from Charles River (L'Arbresle, France) were randomly assigned into six groups (n = 10): standard-diet sedentary (SS), standard-diet voluntary physical activity (SVPA), standard-diet endurance-trained (SET), high-fat diet sedentary (HS), high-fat diet voluntary physical activity (HVPA) and high-fat diet endurance-trained (HET). Throughout the experimental protocol all the animals were housed in individual cages in a normal environment (21–22 °C; 50–60% humidity) in 12 h light/dark cycles, receiving food and water *ad libitium*. The study was approved by local Institutional Ethics Committee and followed the Guidelines for Care and Use of Laboratory Animals in research advised by the Federation of European Laboratory Animal Science Associations (FELASA). Several authors are accredited by FELASA to perform animal experimentation.

2.2. Diet and exercise protocol

The animals were fed with a standard (Lieber–DeCarli diet #710027) or with a high fat (Lieber–DeCarli diet #712031) liquid diet purchased from Dyets Inc. (Bethlehem, USA) as previously described to induce NASH (Lieber et al., 2004). The feeding protocol included 1 week of adaptation to the liquid diet, in which the standard diet was given to all animals, followed by 17 weeks of standard or high fat diet according to the experimental groups (Fig. 1). Although diets have the same amount of calories (1 kcal/ml) differences exist in the content of each one. In the standard diet, 35%, 18% and 47% of energy is derived from fat, proteins and carbohydrates, respectively, while 71%, 18% and 11% of energy is derived from fat, proteins and carbohydrate in the high-fat diet group. The quantification of food consumption was daily obtained from graduated feeding tubes (Dyets Inc.) and calculated in accordance with manufactures instructions (1 ml = 1 Kcal). After the diet adaptation period, animals from the voluntary physical active groups (SVPA and HVPA) were housed in cages equipped with running wheels until the end of the experimental protocol. The running distance was daily obtained from a digital counter between 08.00 and 10.00 h. After 8 weeks of diet consumption, half of the SS and HS animals continued sedentary while the other half was adapted to the treadmill (SET and HET) for 5 days at 15 m/min and 0% grade until 30 min running was achieved. Habituation was followed by 8 weeks of endurance exercise during 5 days/week, 60 min/day at a starting speed of 15 m/min that was gradually increased over the training program until 25 m/min was reached. Sedentary animals (SS and HS) were placed on a non-moving treadmill 5 days/week for 60 min in order to expose the animals to the same environmental conditions without promoting any physical training adaptation.

2.3. Blood and tissue sampling

All animals were anaesthetized (Ketamine 90 mg/kg and Xylazine 10 mg/kg) 48 h after the last exercise bout and after 12 h of overnight fasting. Thereafter, the abdominal and chest cavity were opened and blood was rapidly drawn from the heart, centrifuged (3000 rpm, for 10 min at 4 °C) and stored at −80 °C. The organs were then perfused with NaCl 0.9% and the liver, *soleus*, mesenteric, perirenal and

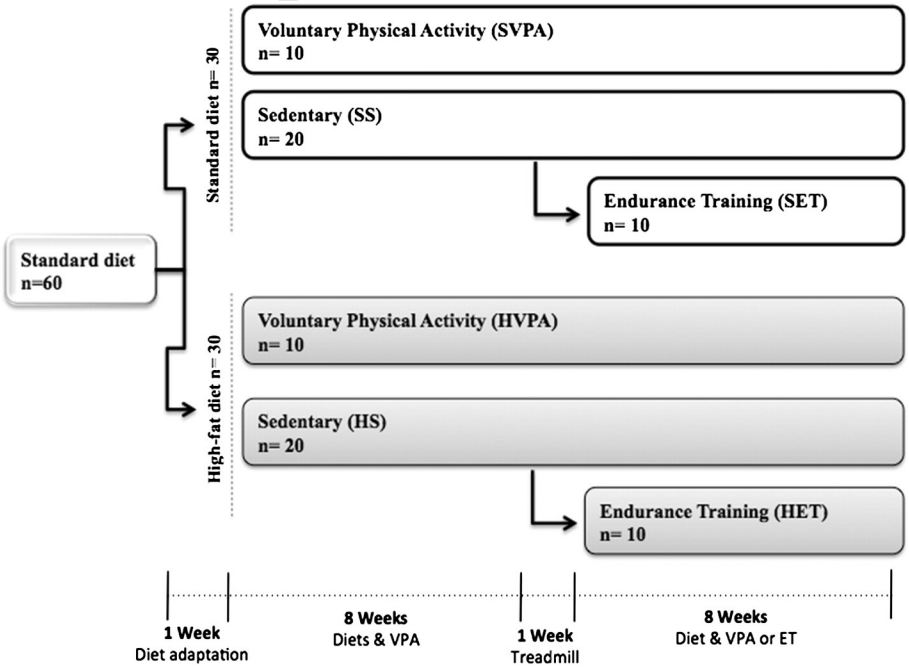

Fig. 1. Flow chart of diet and exercise protocol design.