

Beneficial Effects of Exercise Training (Treadmill) on Body Mass and Skeletal Muscle Capillaries/Myocyte Ratio in C57BL/6 Mice Fed High-Fat Diet

Efectos Benéficos del Ejercicio Físico (Cinta) sobre la Masa Corporal y Relación Capilares/Miocito del Músculo Esquelético en Ratones C57BL/6 Alimentados con una Dieta Alta en Grasas

Victor Faria Motta & Carlos Alberto Mandarim de Lacerda

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SUMMARY: C57BL/6 mice develop signals and symptoms comparable at least in part with the metabolic syndrome in humans. This study aimed to evaluate the beneficial effects of exercise training upon skeletal microcirculation in these mice. Animals were fed one of two diets during an eight week period: standard chow (SC) or very high-fat (HF). Afterwards, the exercise training protocol (treadmill) was established and mice divided into SC and HF sedentary (SC-Sed, HF-Sed) or exercised groups (SC-Ex, HF-Ex), respectively. HF/HF-Sed mice had the greatest body mass (plus 65% than SC-Sed; $P < 0.0001$), and exercise reduced it by 23% ($P < 0.0001$). The plasma insulin was higher in the HF-Sed than in the matched -Ex ($P < 0.001$). The ratio between capillaries/myocytes in HF-Ex group increased by 64% than in HF-Sed group ($P < 0.001$) and increased by 80% in SC-Ex group than in SC-Sed group ($P < 0.001$). In conclusion, exercise improved the lipid profile by reducing body mass gain, insulin resistance, ameliorating the skeletal muscle microcirculation.

KEY WORDS: Aerobic training; Metabolic syndrome; Lipid profile; Skeletal muscle.

INTRODUCTION

The high prevalence of obesity, insulin resistance, dyslipidemia, and hypertension, independently or combined in the metabolic syndrome (MetS) is the main challenge to the modern population and the public health system, considering that it is accompanied by an increased risk for the development of coronary heart disease and other associated diseases (Aguilar-Salinas *et al.*, 2004; Grundy, 2008).

Skeletal muscle is the most abundant human tissue comprising almost 50% of the total body mass, and it is highly adaptable tissue, responding to numerous environmental and physiological challenges (Matsakas & Patel, 2009). It is mainly involved in action and movement, which requires large amounts of glucose, fatty acids, and oxygen. These materials are supplied by blood vessels and incorporated into the muscle fiber through the cell membrane. It is well known that the capillarity of skeletal muscle adapts to various physiological and pathological conditions such as ageing and hypertrophy. Exercise-induced increases the capillarization in skeletal muscle, a phenomenon that happens in healthy humans and animals (Kivelä *et al.*, 2008).

C57BL/6 mice fed a very high-fat diet are an animal model to study MetS because they develop symptoms comparable with the MetS in humans (Fraulob *et al.*, 2010). The aim of this study was to evaluate the beneficial effects of exercise training on skeletal muscle microcirculation in C57BL/6 mice submitted to a very high-fat diet and an exercise training protocol.

MATERIAL AND METHOD

Animals and treatments. Three-month-old male C57BL/6 mice were kept in standard conditions (12h light/dark cycles, $21 \pm 2^\circ\text{C}$, humidity $60 \pm 10\%$) with free access food and water. Animals were fed one of two types of diet during eight weeks: standard chow (SC, 76% of calories from carbohydrates, 10% from fat, and 14% from protein) or high-fat chow (HF, 26% of calories from carbohydrates, 60% from fat, and 14% from protein). Diets were elaborated with purified nutrients by Rhoister (Rhoister, SP, Brazil) in accordance with the American Institute of Nutrition's recommendation - AIN93

(Reeves *et al.*, 1993). After the eighth week of diet, an exercise training protocol was established, and they were randomly divided into four groups: a) SC exercised mice (SC-Ex), b) SC sedentary mice (SC-Sed), c) HF exercised mice (HF-Ex), d) HF sedentary mice (HF-Sed). Food and water were freely allowed and their intakes were monitored daily.

Exercise training protocol. Exercise training was accomplished on a motor treadmill at a moderate-to-low intensity (15 m/min maximal running speed) during 8 weeks, 5 days/week and 1 hour/day, according to the previous description (Marques *et al.*, 2010). The animals were adapted to this procedure for one week before beginning the exercise protocol. Sedentary animals were placed on the stationary treadmill three times a week to provide a similar environment.

Euthanasia. At 16th week, animals were not fed, but had free access to water for 6 hours and then were deeply anesthetized (sodium pentobarbital, ip, 150 mg/kg) and blood samples were obtained by cardiac puncture for biochemistry analyzes and centrifuged at 120 g for 15 min at room temperature and plasma was stored -80°C until assay. The right soleus muscle was removed and fixed at room temperature in newly prepared fixative (4% w/w formaldehyde in phosphate buffer pH 7.2, 0.1M).

Metabolic measurements. Total cholesterol (TC), triglycerides (TG), LDL-Cholesterol (LDL-C) and HDL-Cholesterol (HDL-C) were measured by a colorimetric assay (Bioclin, Belo Horizonte, MG, Brazil). The plasma insulin concentrations were measured using RIA insulin kit cat. 07-260. 121 (ImmuChem Coated Tube, Orangeburg, NY, USA). All samples were analyzed in a double test, for which the coefficient of variation was 1.4%. Insulin resistance was estimated by homeostasis model assessment index as HOMA-IR= [insulin x glucose]/ 22.5 (Matthews *et al.*, 1985).

Stereology of skeletal muscle . Muscle fragments were embedded in Paraplast plus (Sigma-Aldrich Brazil, Sao Paulo, SP, Brazil), sectioned at 5µm thick and stained with hematoxylin and eosin. Digital images were kept (JPEG, 24-bit color, 1280x1024 pixels, LC Evolution camera and Olympus BX51 microscope) and analyzed (five fields per muscle) with the help of the Image-Pro Plus software (version 7.02, Media Cybernetics, Silver Spring, MD, USA). A test-system containing 36 points (PT) was applied to a screen in order to acquire data. The volume density was estimated for myocytes and for intramuscular capillary by point counting ($V_v[\text{structure}] = P_p[\text{structure}] / PT$, where P_p is the number of points hitting the structure (myocytes or intramuscular capillary). Afterwards, the ratio between $V_v[\text{capillaries}]$ -to- $V_v[\text{cardiomyocytes}]$ was studied (Mandarim-de-Lacerda *et al.*, 2010).

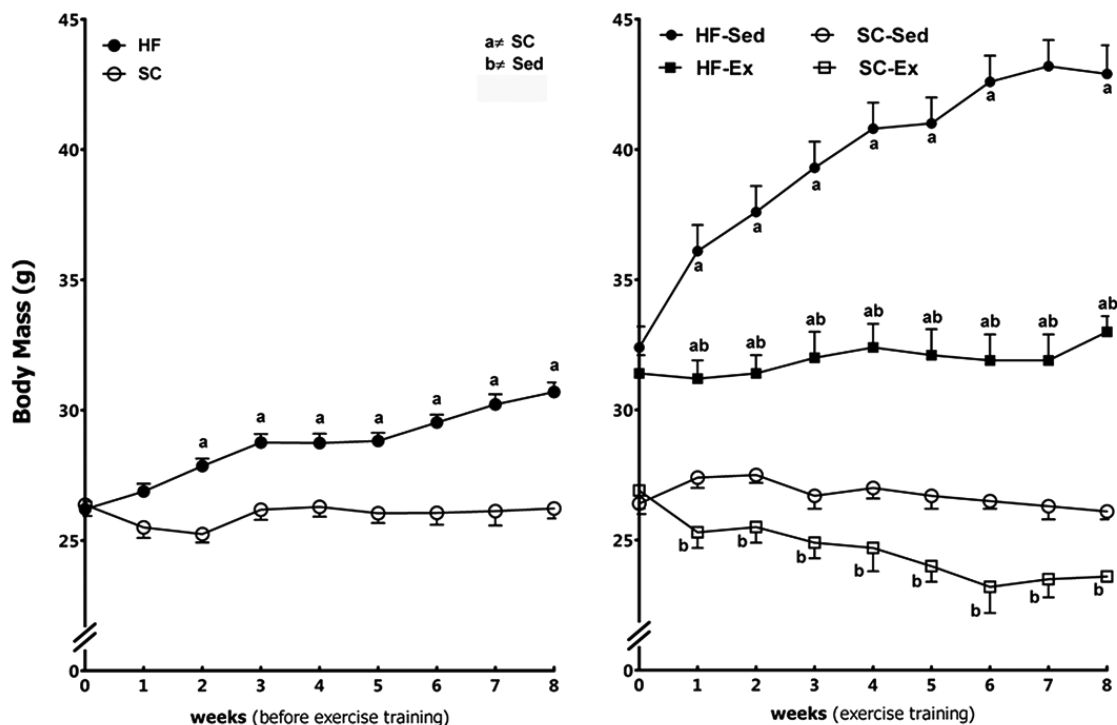


Figure 1. Body mass evolution before the exercise training (left side) and during the exercise training (right side). Abbreviations: SC, standard chow; HF, high-fat diet; Ex, exercise; Sed, sedentary. In signaled cases $P < 0.05$ when [a] is different from SC counterpart, [b] is different from Sedentary counterpart.

Data analysis. The differences among the groups were tested by one-way analysis of variance (ANOVA) and post hoc test of Tukey (in the first eight weeks of the study) or Student t-test (comparing sedentary and exercised groups).

RESULTS

Biometry. Figure 1 shows the body mass evolution in all the groups. The administration of high fat diet is directly

related to significant weight gain since a second week after starting the experimental protocol ($P < 0.05$). At the end of eight weeks, the increase in body mass reached 17% more in the HF group when compared with SC group ($P < 0.0001$, one-way ANOVA). At the end of the experiment, the HF-Sed obtained the highest body mass (more than 65% compared with SC-Sed, $P < 0.0001$, one-way ANOVA). Even with the maintenance of the HF diet, the exercise training influenced the body mass of the group HF-Ex, which remained relatively stable throughout the exercise protocol. The HF-Sed group continued increasing the body mass, reaching a difference of about 70% when compared to SC-Sed ($P < 0.0001$, one-way ANOVA).

Table I. Biochemical data. Abbreviations: SC, standard chow; HF, high-fat diet; Ex, exercise; Sed, sedentary. In signaled cases $P < 0.05$ when [a] is different from SC counterpart, [b] is different from Sedentary counterpart (ANOVA and Tukey post hoc test).

| Data | SC-Sed | SC-Ex | HF-Sed | HF-Ex |
|---------------------------|-----------|-----------------------|------------------------|------------------------|
| Total Cholesterol (mg/dl) | 121.3±1.5 | 121.0±3.6 | 190.6±3.6 ^a | 169.3±7.7 ^b |
| LDL-c (mg/dl) | 10.9±1.1 | 7.9±1.4 | 60.8±4.1 ^a | 22.6±2.5 ^b |
| HDL-c (mg/dl) | 100.3±1.6 | 110.0±4.6 | 105.0±2.1 | 133.8±5.8 |
| Triglycerides (mg/dl) | 49.3±1.4 | 43.4±2.6 | 109.8±0.3 ^a | 64.8±3.9 ^{ab} |
| Insulin (mg/dl) | 17.7±0.2 | 11.0±0.2 ^b | 20.4±0.9 | 12.2±1.0 ^b |
| HOMA-IR (mmol/uU/ml) | 6.2±2.5 | 3.7±1.9 ^b | 9.1±3.7 ^a | 4.7±1.9 ^b |

Biochemistry. The data are show in Table 1. The high fat diet could rise significantly cholesterol and triglycerides levels ($P < 0.001$, one-way ANOVA). The exercise reduced significantly all metabolic values analyzed

($P < 0.001$, one-way ANOVA), with a greater emphasis on the HF-Ex group, which reduced the HOMA index values in 50% ($P < 0.001$, one-way ANOVA).

Serum HDL cholesterol was significant between HF-Ex and -Sed groups ($P < 0.001$, one-way ANOVA); however, plasma concentrations of LDL-cholesterol, triglycerides and total cholesterol were higher in HF-Sed and -Ex groups when compared to SC-Sed and -Ex. These values were higher in the HF-Sed compared to the HF-Ex group ($P < 0.001$, one-way ANOVA). The plasma insulin concentrations was higher in HF-Sed compared to the corresponding exercised group ($P < 0.001$, one-way ANOVA).

Stereology of skeletal muscle. The Figure 2 shows the cross-section views of the soleus muscle in all groups where the capillaries were observed and counted. Figure 3 shows a bar graph of the ratio between $V_v[\text{capillaries}] / V_v[\text{myocytes}]$. This ratio in HF-Ex group increased by 64% than in HF-Sed group ($P < 0.001$, t-test) and increased by 80% in SC-Ex group than in SC-Sed group ($P < 0.001$, t-test).

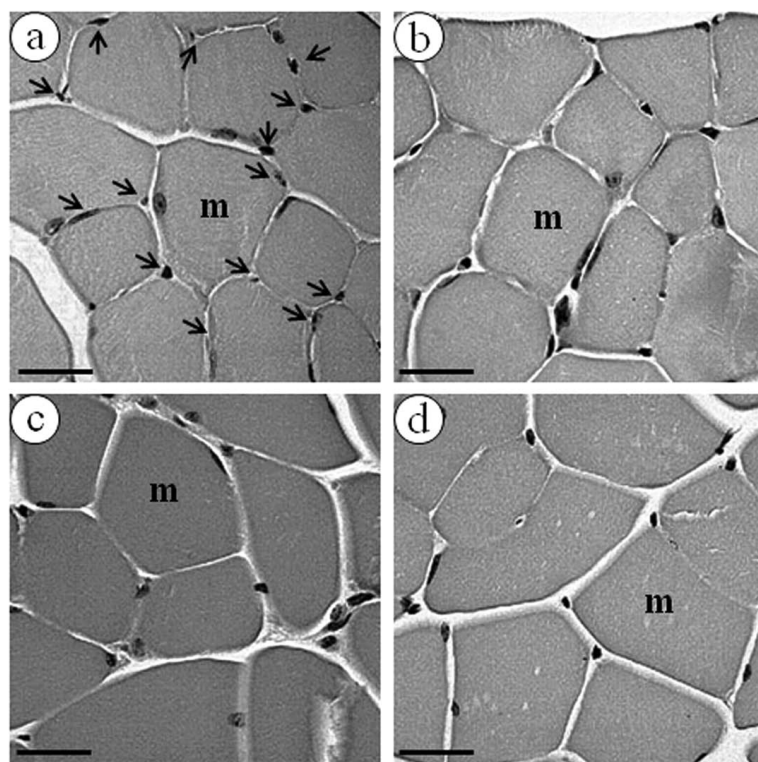


Fig. 2. Photomicrographs of the soleus muscle in cross-section stained by hematoxylin and eosin. Same magnification in all pictures (bar = 120 μm). The myocytes are signaled with m, and capillaries are marked with arrows. The groups are: (a) SC-Ex, (b) SC-Sed, (c) HF-Ex and (d) HF-Sed. Abbreviations: SC, standard chow; HF, high-fat diet; Ex, exercise; Sed, sedentary.

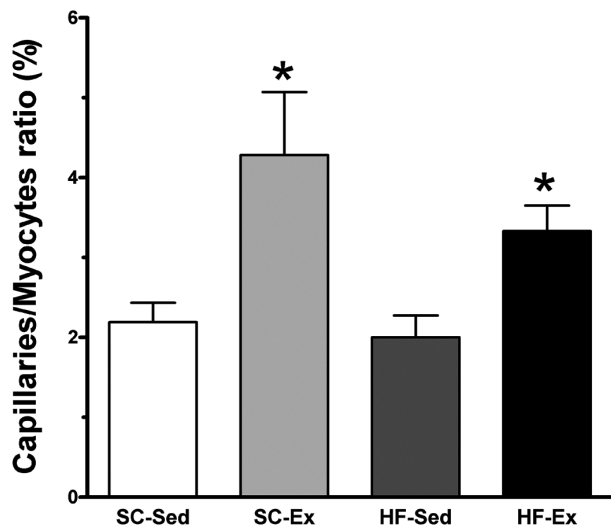


Fig. 3. Capillaries/Myocytes ratio in the right soleus muscle of mice estimated by stereology. Abbreviations: SC, standard chow; HF, high-fat diet; Ex, exercise; Sed, sedentary. In signaled cases * $P < 0.05$ different from Sedentary counterpart.

DISCUSSION

In mice, the administration of the HF diet resulted in body mass gain accompanied by abnormal lipid profile, alterations in the ratio between $V_v[\text{capillaries}]/V_v[\text{myocytes}]$ in the skeletal muscle and in the carbohydrate metabolism.

The effects of the consumption of HF diet on visceral adiposity, and their relationship with the development of chronic diseases and obesity are issues of great importance in public health (Hida *et al.*, 2005). The framework of HF diet induces insulin resistance, which is associated with impairment in the ability of fatty acid oxidation and increased fat accumulation in target tissues, impairing insulin action (Hegarty *et al.*, 2003).

It is known that muscle contraction is effective in causing improvement in fat oxidation and improving insulin sensitivity (Matsuzawa-Nagata *et al.*, 2008). It is necessary to consider the intensity, frequency and duration of exercise as well as the type of diet, which may lead to different metabolic adaptations (Horowitz, 2003). The fact that all models of exercise have the same beneficial effects on health is questionable. The moderate exercise is considered promoting a reduction in body mass and adiposity, improving the lipid profile, as reported in previous studies of our laboratory (Marques *et al.*; Schultz *et al.*, 2012).

In the present study, mice that underwent a protocol of exercise had a reduction in body mass and serum levels of TG

and TC, and improved HDL-cholesterol. In addition, exercise improves insulin action in liver, probably due to the fact that during exercise, after glycogen depletion, the fatty acid becomes the main fuel for exercise on muscle metabolism. During the recovery period, glycerol is necessary to replenish the glycogen.

Hyperinsulinemia is a compensatory response to insulin resistance that causes adipogenesis, and this compensatory effect eventually leads to diabetes (Ferrannini, 2006). Mice fed a HF diet have free fatty acids and excessive circulating glucose, which increases insulin resistance, lipolysis and insulin secretion (Karasawa *et al.*, 2009). Physical inactivity in humans also contributes to the development of insulin resistance (Bassuk & Manson, 2005). In contrast, insulin sensitivity may increase with exercise, independent of body mass loss and changes in body composition (O'Donovan *et al.*, 2005), which was confirmed in the present study, in which all exercised groups had increased insulin sensitivity.

It is well known that the microcirculation is impaired by insulin resistance/diabetes (Gomes *et al.*, 2004; Lioupis, 2005). Otherwise, insulin is beneficial, restoring microvascular reactivity to inflammatory mediators in type 2 diabetes (Rastelli *et al.*, 2005). The main effect of exercise on insulin resistance is the increased expression of intracellular insulin signaling, particularly in GLUT4 skeletal muscle (Teran-Garcia *et al.*, 2005). The uptake by skeletal muscle represents the largest component of glucose disposal (Okura *et al.*, 2007). Most of the uptake of glucose is used in the synthesis of glycogen. GLUT4 plays an essential role in cellular signaling mechanism of insulin and its expression is also decreased by the intake of HF diet. In addition, as measured in the present study, capillaries are recruited in skeletal muscle by exercise training, probably by opening microcirculation and improving the capillaries/myocyte ratio.

In conclusion, exercise training improves the lipid profile and reduces not only the body mass gain, but the insulin resistance. All these effects are beneficial and help controlling obesity and other co-morbidities. One of the beneficial effects observed in this animal model is the improvement of the capillaries/myocytes ratio. These findings demonstrate the beneficial effects of exercise to mitigate the adverse effects of HF diet. Thus, understanding these data from experiments with animals can help the ongoing studies related to clinical research.

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MOTTA, V. F. & MANDARIM-DE-LACERDA, C. A. Efectos beneficiosos del ejercicio físico (cinta) sobre la masa corporal y relación capilares/miocytes del músculo esquelético en ratones C57BL/6 alimentados con una dieta alta en grasas. *Int. J. Morphol.*, 30(1):205-210, 2012.

RESUMEN: Los ratones C57BL/6 desarrollan señales y síntomas similares al menos en parte con el síndrome metabólico en los seres humanos. Este estudio tuvo como objetivo evaluar los efectos beneficiosos del ejercicio físico sobre la microcirculación ósea en estos ratones. Los animales fueron alimentados con una de dos dietas durante un período de ocho semanas: comida estándar (CE) o muy alta en grasas (AG). Posteriormente, fue establecido un protocolo de entrenamiento físico (cinta) y los ratones fueron divididos en grupos CE y AG sedentarios (CE-Sed, AG-Sed) o grupos de ejercicios (CE-Ej, AG-Ej), respectivamente. Los ratones AG/AG-SED tuvieron una masa corporal mayor (más del 65% de CE-Sed, $p < 0,0001$), y el ejercicio se redujo en un 23% ($p < 0,0001$). La insulina en el plasma fue mayor en el AG-Sed que en el pareado-Ej ($p < 0,001$). La relación entre capilares/miocytes en el grupo AG-Ej aumentó en un 64% más que en el grupo AG-Sed ($p < 0,001$) y aumentó en un 80% más en el grupo CE-Ej que en el grupo CE-Sed ($p < 0,001$). En conclusión, el ejercicio mejora el perfil lipídico mediante la reducción de la ganancia de masa corporal, resistencia a la insulina, mejorando la microcirculación del músculo esquelético.

PALABRAS CLAVE: Entrenamiento aeróbico; Síndrome metabólico; Perfil lipídico; Músculo esquelético.

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Correspondence to:

Carlos Alberto Mandarim de Lacerda

Departamento de Anatomia

Laboratório de Morfometria, Metabolismo e Doença Cardiovascular

Universidade do Estado do Rio de Janeiro (UERJ)

Av. 28 de setembro 87 (fds), 20551-030

Rio de Janeiro, RJ.

BRAZIL

URL: www.lmmc.uerj.br.

Phone: (+55.21) 2868-8316

Fax: 2868-8033.

Email: mandarim@uerj.br

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