Artículo original

Effects of six weeks of moderate-intensity aerobic physical training on metabolic and body parameters of Wistar rats: a pilot study

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Summary

Physical training in animal models promotes changes that can be extrapolated to humans, due to physiological similarities between these species. Thus, the use of rodents submitted to exercise becomes feasible due to the possibility of analyzing variables that would not be admissible in humans. The aim of this study was to evaluate the effects of six weeks of swimming, at moderate intensity, on physical and physiological parameters of Wistar rats. Twelve animals were divided into two groups (trained and non-trained). The trained animals were subjected to six weeks of aerobic training in water with 5% of body weight load. The results between groups were compared by the t test ($p < 0.05$). The variation in body weight was lower in the trained groups. Food and water consumption were higher in the animals submitted to physical training. The relative weight of heart and kidney were higher in trained animals, with no differences in the liver and gastrocnemius relative weight. The gastrocnemius muscle fiber diameter did not differ between groups. The training promoted an increase in the percentage of protein in the carcass and a decrease in body fat percentage, as well as in the diameter of the epididymal region adipocytes. Additionally, the training promoted increased levels of High Density Lipoproteins (HDL-C) and decreased levels of Low Density Lipoproteins (LDL-C) and triacylglycerols (TAG). It was concluded that six weeks of moderate-intensity aerobic training is sufficient to promote improvements in the metabolic profile, weight control, and reduce body fat and increase the protein content in the carcass of rats.

Key words:
Physical activity.
Animal model.
Metabolism.
Metabolic profile.

Efectos de seis semanas de ejercicio físico aeróbico de intensidad moderada sobreparámetros metabólicos y corporales de ratas Wistar: un estudio piloto

Resumen

El entrenamiento físico en un modelo animal promueve cambios que se pueden extrapolar a los seres humanos, debido a las similitudes fisiológicas entre estas especies. El uso de roedores sujetos a ejercicio se vuelve factible debido a la posibilidad de analizar variables que no serían aceptables en los seres humanos. El objetivo de este estudio fue evaluar los efectos de seis semanas de natación a una intensidad moderada en parámetros corporales y fisiológicos de ratas Wistar. Fueron divididos doce animales en dos grupos (entrenados y no entrenados). Las ratas entrenadas fueron sometidas a seis semanas de entrenamiento aeróbico en un medio acuático con una carga relativa a un 5% de su peso corporal. Los resultados entre los grupos se compararon por t-test de student ($p < 0,05$). La variación de la ganancia de peso corporal fue menor en los animales entrenados. El consumo de agua y alimentos fue mayor en los animales delgrupo sometidos a entrenamiento. El peso relativo del corazón y de los riñones fue mayor en los animales entrenados, sin diferencias en el peso relativo del hígado y gastrocnemio. El diámetro de las fibras del músculo no difirió entre los grupos. El entrenamiento provocó un aumento en el porcentaje de proteína en la carcasa y una disminución en el porcentaje de grasa corporal, así como en el diámetro de los adipocitos de la región del epidídimo. Además, el entrenamiento provocó un aumento de los niveles de lipoproteínas de alta densidad (HDL-C) y una disminución de los niveles de lipoproteína de baja densidad (LDL-C) y triacilglicéridos (TAG). Se concluye que seis semanas de entrenamiento aeróbico a una intensidad moderada es suficiente para promover mejoras en el perfil metabólico, control de peso, reducir la grasa corporal y aumentar el contenido de proteínas en la carcasa de las ratas.

Palabras clave:
Actividad física.
Modelo animal.
Metabolismo.
Perfil metabólico.

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Introduction

Regular physical exercise can alter metabolic and body variables that promote health benefits and reduce the risk of non-communicable diseases[1]. Such changes are influenced by the frequency and duration of training, as well as the type and intensity of exercise that is being performed[2]. This occurs, among other factors, due to the fact that the pathways involving energy expenditure during exercise vary according to the activity that is performed[3]. Thus, high-intensity, short-term exercise primarily uses anaerobic glycolytic metabolic pathways while continuing long-term exercises use oxidative pathways. Anaerobic activities tend to promote greater hypertrophic stimuli, whereas aerobic exercise promotes greater energy expenditure and consequently tend to decrease body fat, and improve muscle and cardiovascular function[3].

Several studies in humans have sought to evaluate body and metabolic alterations caused by physical exercise[4-6]. However, some analyzes become unviable and untrustworthy leading to the need to use animal models submitted to physical training[3]. Such models have advantages over studies with humans mainly because they allow greater control of experimental variables and require lower cost[7].

Among the most used animal models for experimentation, mainly that involving physical exercise, the rodents stand out due to physiological similarities to the human species[8,9]. Nevertheless, there is a wide variety of protocols and training periods described in the literature[9], since there is no consensus as to the period necessary to observe significant changes on the metabolic and body parameters of these animals.

Thus, the aim of this study was to evaluate the effect of six weeks of moderate-intensity aerobic training on body, metabolic and food and water consumption parameters in Wistar rats.

Material and methods

Animals

The present study was approved by the ethics commission on the use of animals - CEUA (Ethics Committee on Animal Use of Federal University of Lavras) of the Federal University of Lavras. Twelve adult male rats *(Rattus norvegicus albinus)* of the Wistar lineage, obtained from the Central Bioterium of the Federal University of Lavras (UFLA) were used. Animals, with six weeks of age, in a healthy state were selected and submitted to a period of seven days of acclimatization in metabolic cages. During the entire experimental period the rodents remained under ideal temperature (22±2 °C), humidity (45±15%), and lighting (light/dark cycle of 12/12 hours) conditions. Commercial ration and water were provided ad libitum during the entire experimental period.

At the end of this acclimatization period, the animals were randomly divided into two groups containing six animals each. One group was composed of non-trained animals, while the other group had animals which would be submitted to physical training in water under moderate intensity. The animals of non-trained group were not submitted to any procedure in the aquatic environment.

Physical Training

After the above-mentioned period of adaptation, acclimatization in the aquatic medium was carried out. At this stage, the animals of the group submitted to physical training spent two hours daily, for seven days, in a polyethylene tank with a total capacity of 300 liters, containing water to a depth of five centimeters at a temperature of approximately 32±2 °C. The purpose of this acclimatization was to reduce the animals' stress in the aquatic environment, without causing adaptation to the physical training[10].

In the following week, the animals were submitted to swimming sessions with progressive increase in duration. This stage consisted of swimming without load, in a depth of 50 cm (in order to avoid the animal coming into contact with the bottom of the tank), where the animals swam for 10 minutes on the first day, with daily 10-minute increases until, at the end of six days, each animal would be swimming uninterruptedly for 60 minutes, without load[11,12].

During the subsequent six weeks, the animals swam for 60 minutes daily, at a frequency of five times per week, with relative overload of 5% of their body weight. This load was used with the intention of causing an increase in the endurance capacity of the animals, characterizing aerobic exercise of moderate intensity[10-12].

After each training session, the animals were dried with absorbent towels, and when they were completely dry, they were placed in their respective cages, as proposed by the American Physiological Society[13].

Throughout the entire experimental period, the ration and water consumption and and urine excretion were measured daily, and the animals were weighed every week. At the end of the experimental period the animals were induced to a fast of eight hours and were euthanized by cardiac puncture under anesthesia (Sodium thiopental 50 mg/kg i.p.). Blood samples were collected in a syringe containing EDTA anticoagulant, and the plasma was used for analysis of the biochemical parameters such as glucose, total cholesterol, HDL-C and triacylglycerides (TAG), utilizing specific colorimetric kits (Gold Analisa Diagnósticos®, Belo Horizonte, Brazil) as performed by Amr &Abeer[14]. The LDL-C and VLDL-C levels of each animal were obtained by using the Friedewald formula[15]: LDL-C= Total cholesterol –HDL-C – (TG/5).

Collection of Biological Material and Histological Analysis

After cardiac puncture, the animals were submitted to ample opening of the abdominal cavity until the internal organs were exposed. The heart, liver, right kidney and right gastrocnemius muscle were then collected and weighed. The values of the weight of these organs and carcass were obtained individually[16], and then the relative mass of each organ was calculated in relation to the weight of the clean carcass (weight of organ/weight of clean carcass).

For the histological analyses, fragments of epididymal adipose tissue and gastrocnemius muscle were collected. These tissues were fixed in 10% buffered formaldehyde and routinely processed for preparing the histological slides, which were hematoxylin-eosin stained[17]. The slides were analyzed by optical microscopy.

After weighing, the gastrocnemius muscle was sectioned in the medial region and both portions were stored for 48 hours in 10% buffered formaldehyde. Subsequently, the tissues were routinely processed and 5μm sections were used for histological slide preparation and stained with hematoxylin-eosin. Five different muscle sections of each animal were photographed (at 40X) and in each, ten fibers were randomly selected, for a total of 50 fibers analyzed in each muscle. Muscle fiber diameter was then determined by calculating the mean of the shortest distance of each fiber (μm) as described by Mierzejewska-Krzyzowska *et al.*[18].

Histomorphometric analyzes were obtained using a system for capturing and analyzing images, consisting of binocular microscope Olympus CX31 (Olympus Optical do Brazil Ltda, São Paulo, SP) with attached camera (SC30 CMOS Color Camera for Light Microscopy, Olympus Optical's Brazil Ltda, São Paulo, SP). The measurements were made using version 6.0 of the Image-Pro® Express software (Media Cybernetics, Rockville, MD, USA).

For the epididymal adipose tissue, a diameter was evaluated, from measurement of the shortest distance between the two extremities of the cell[19].

All the histological measurements were taken by a single trained evaluator.

Chemical Composition of the Body

After removal of internal organs, skin, head, feet and tail, the clean carcasses were weighed and stored at -20°C. Subsequently, water, protein and fat percentages in the carcass were measured. To measure the water percentage, carcasses were processed and oven-dried at 105 ºC for 24 hours. The percentage of fat present in the carcass was evaluated by extraction with ethyl ether using Soxhlet[20] equipment. The percentage of protein in the carcass was obtained using the Kjeldahl method[20].

The data were analyzed by one way ANOVA, and the means were compared by the t test at 5%. The analysis were performed using the BioEstat 3.0 statistical software program[21].

Results

The change in body weight was lower in the animals that underwent physical training ($p < 0.05$), while the food intake of these animals was higher compared to non-trained rats ($p < 0.05$). The water consumption and relative kidney weight of trained animals was higher than in the non-trained ($p <0.05$), with no difference in urine output (Figure 1). Likewise, there was no significant difference in the relative liver weights (Figure 2).

The fiber diameter, as well as the relative weight of the gastrocnemius was not altered by the six weeks of training (Figure 2). The relative weight of the heart was higher in trained animals. Body composition analysis showed that the training caused a decrease in fat content ($p <0.05$) and increased the protein percentage in the carcass ($p <0 05$). The percentage of this water in the carcass was not affected by physical training (Figure 2). The diameter of adipocytes from the epididymal region was lower in the trained animal groups ($p <0.05$).

With regards to blood biochemical parameters, it was observed that physical training promoted an increase in serum HDL-C and decrease in LDL-C and TAG ($p <0.05$). There were no significant differences in blood glucose levels as well as total cholesterol and VLDL-C in animals submitted, or not, to physical training (Figure 3).

Discussion

The main results obtained in present study show those six weeks of physical training were sufficient to increase protein and decrease fat content in rats' carcass. Furthermore, this training period was sufficient to promote metabolic improvements as observed in HDL-C, LDL-C and TAG levels. The increase in food intake associated with less variation in weight gain during physical training observed in this study corroborates previous studies[22,23]. Such results may be explained by higher energy expenditure induced by exercise, which promotes an increase in basal metabolic rate and increases the need for caloric intake[24]. Furthermore, the maintenance of body weight occurred probably because the diet was provided *ad libitum* to the animals[24], promoting a balance between energy intake and expenditure, so that the animals did not gain or lose weight.

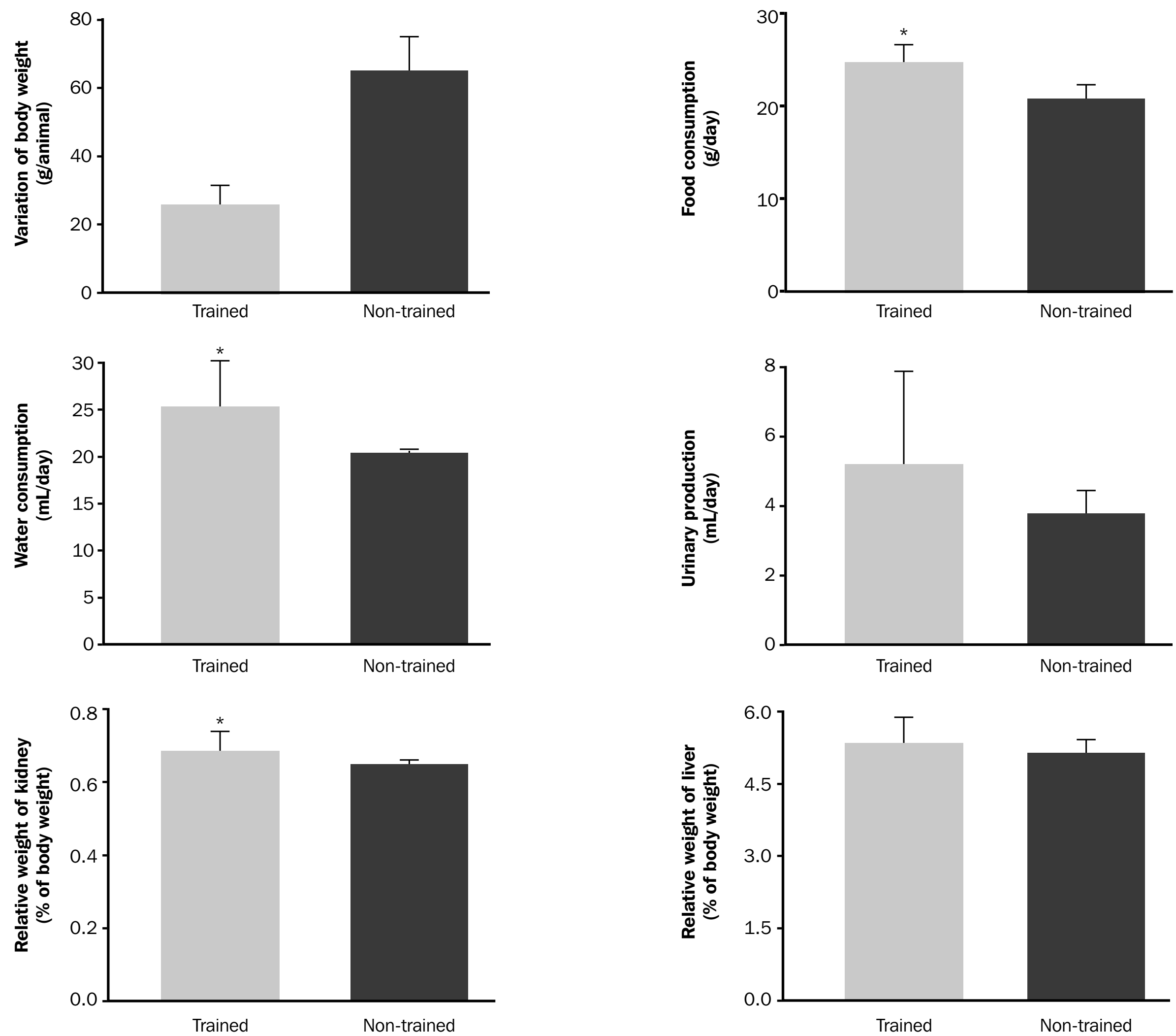
Furthermore, the higher water consumption during training, as observed in the present study, confirms the results of Michna *et al.*[25], who observed an increase of over 20% in water consumption in animals subjected to voluntary exercise. This effect was also observed by Droste *et al.*[26], who associated this result to the increased evaporation rate that occurs due to increased body temperature and respiratory rates during exercise. Furthermore, the increase in the relative weight of the kidney is an adaptation to exercise, being related to higher filtration volume due to the water consumption increase, as well as the changes in homeostatic mechanisms related to the high ejection volume in the heart and increased blood pressure during exercise[27]. In line with this, the increase in relative heart weight observed in animals subjected to swimming is also a physiological adaptation arising from the increased workload of this organ during exercise[28].

Another change related to the adaptation to training observed in this study was greater protein deposition in the carcass of trained animals, as observed by Salomão and Gomes-Marcondes[29]. Thus, even though the relative weight and fiber diameter of the gastrocnemius muscle did not differ between trained and non-trained animals, the higher percentage of protein in the carcass demonstrates an increase in protein synthesis throughout the body. Additionally, endurance exercise mainly increases the synthesis of mitochondrial proteins that respond less to hypertrophy than glycolytic fibers[30], which may explain the results observed in the gastrocnemius muscle.

In addition, the increased synthesis of mitochondrial proteins favors lipid oxidation[31], justifying a decrease in the fat percentage in the carcass of the trained animals. Such results were also observed by Aoki *et al.*[32] where trained animals had 28% less fat in the carcass compared to non-trained animals. Furthermore, the effects of physical training to decrease body fat were not only observed in the parameters of the carcass, since the diameter of adipocytes from the epididymal region was also lower in trained animals. As accumulation of fat in this region is preferred in rats, it may be suggested that the same results could be extrapolated to abdominal fat in humans. These results may be explained by the increase in lipolysis and energy expenditure induced by aerobic exercise, also resulting in less weight gain in the trained animals[33,34] as observed in this study.

The physiological mechanisms of exercise-induced lipolysis first involves hydrolyzing intramuscular TAGs and then adipose tissue[34].

Figure 1. Parameters related to the variation in body mass, food intake, water intake, urine output and relative weight of kidney and liver of rats, trained or non-trained, for six weeks.



*Highest mean between groups ($p < 0.05$ by t test).

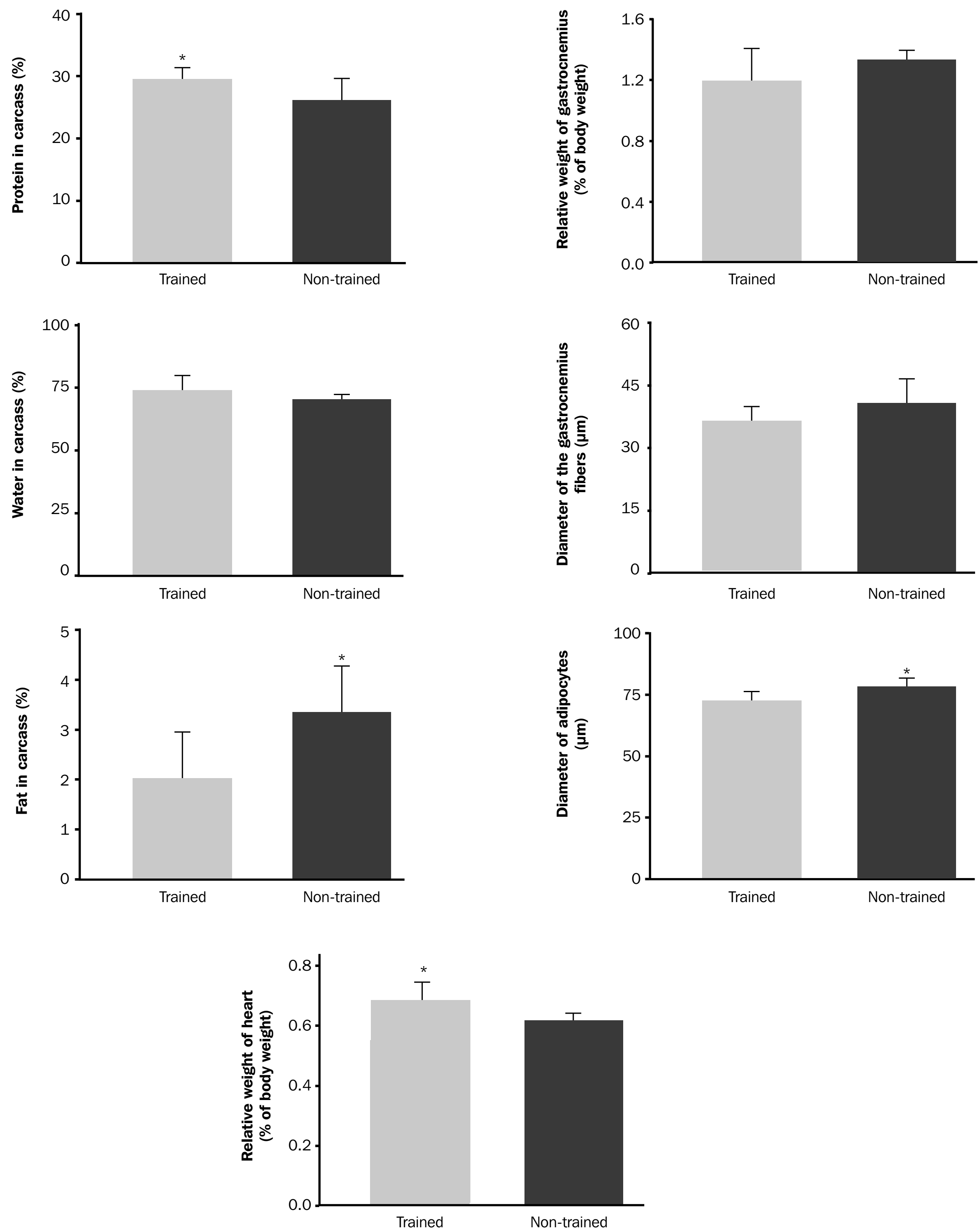
This process causes the release of TAGs from adipocytes, mediated by hormone-sensitive lipase (HSL), there being a formation of free fatty acids and glycerol in the plasma, which are fuels for energy supply to skeletal muscle, kidneys, liver and myocardium[35]. This same mechanism may explain the lower TAG and LDL-C circulating levels, concomitant to the HDL-C increase observed in the present study[36]. Regular aerobic exercise influences the activity of enzymes such as lipoprotein lipase (LPL), hepatic lipase (HL) and lecithin-cholesterol acyltransferase (LCAT), promoting such improvements in metabolic profile[36].

Thus, even if the physical training had not alter the total cholesterol and VLDL-C levels of the animals, the HDL-C increase concomitant with decreased LDL-C demonstrates improved metabolic profile during the exercise. The higher LDL-C associated with decreased HDL-C is related to the increased risk of atherosclerosis and other cardiovascular diseases[37]. Thus the results of this study confirm the benefits of exercise to combat this risk[38].

Conclusions

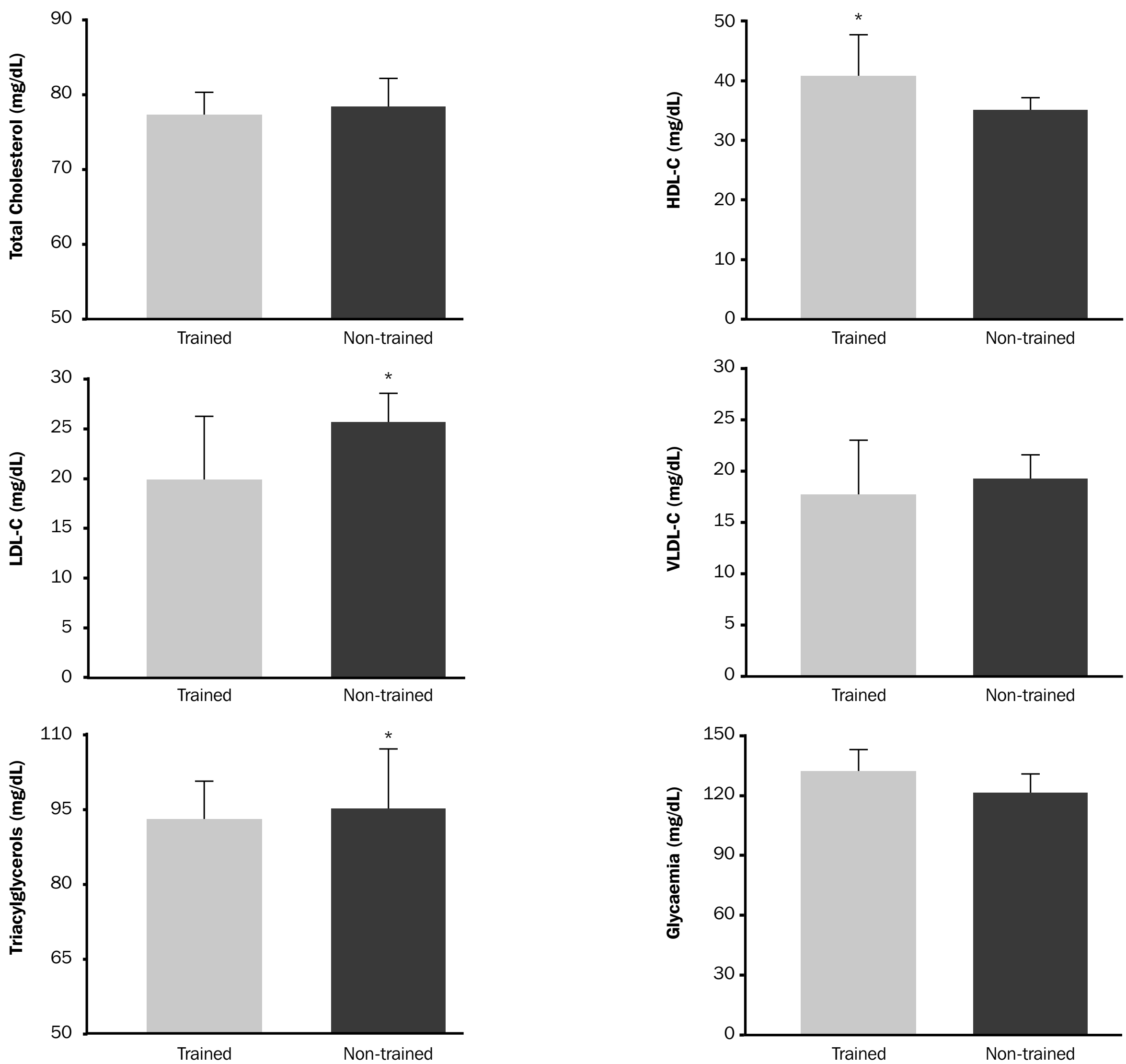
Aerobic training, at moderate intensity, promoted improvement of the metabolic profile of the animals, in which decreased body fat and weight maintenance was also observed during the trial period. Additionally, the increase in the protein content of the carcasses of trained animals shows that six weeks of training were sufficient to cause alterations in that parameter.

Figure 2. Body chemical composition parameters, relative weight of the gastrocnemius muscle, adipocyte and muscle fiber morphology of rats, trained or non-trained, for six weeks.



*Highest mean between groups ($p < 0.05$ by t test).

Figure 3. Blood biochemical parameters of rats, trained or non-trained, for six weeks.



*Highest mean between groups ($p < 0.05$ by t test).

References

1. Slentz CA, Houmard JA, Kraus WE. Modest Exercise Prevents the Progressive Disease Associated with Physical Inactivity. *Exerc Sport Sci Rev.* 2007;35(1):18-23.
2. Malagoni AM, Mandini S, Boari B, Felisatti M, Zamboni P, Manfredini R. Sport Therapy for Hypertension: Why, How, and How Much? *Angiology.* 2009;60(2):207-16.
3. Ghosh S, Golbidi S, Werner I, Verchere BC, Laher I. Selecting exercise regimens and strains to modify obesity and diabetes in rodents: an overview. *Clin Sci (Lond).* 2010;119:57-74.
4. Lewis GD, Farrell L, Wood MJ, Martinovic M, Rowe GC, Souza A, *et al.* Metabolic Signatures of Exercise in Human Plasma. *Sci Transl Med.* 2010;2(33):32-7.
5. Nybacka Å, Carlström K, Ståhle A, Nyrén S, Hellström PM, Hirschberg AL. Randomized comparison of the influence of dietary management and/or physical exercise on ovarian function and metabolic parameters in overweight women with polycystic ovary syndrome. *Fertil Steril.* 2011;96(6):1508-13.
6. Mazini-Filho ML, Matos DG de, Rodrigues BM, Aidar FJ, Gabriela, Venturini R de O, *et al.* The effects of 16 weeks of exercise on metabolic parameters, blood pressure, body mass index and functional autonomy in elderly women. *Int Sport J.* 2013;14(2):86-93.
7. Voss MW, Vivar C, Kramer AF, van Praag H. Bridging animal and human models of exercise-induced brain plasticity. *Trends Cogn Sci.* 2013;10:525-44.
8. Garland T, Schutz H, Chappell MA, Keeney BK, Meek TH, Copes LE, *et al.* The biological control of voluntary exercise, spontaneous physical activity and daily energy expenditure in relation to obesity: human and rodent perspectives. *J Exp Biol.* 2011;214:206-229.
9. Seo DY, Lee SR, Kim N, Ko KS, Rhee BD, Han J. Humanized animal exercise model for clinical implication. *Pflugers Arch Eur J Phys.* 2014; [Ahead to print] Available from: http://www.ncbi.nlm.nih.gov/pubmed/24647666

10. Rambo LM, Ribeiro LR, Oliveira MS, Furian AF, Lima FD, Souza MA, *et al.* Additive anticonvulsant effects of creatine supplementation and physical exercise against pentylenetetrazol-induced seizures. *Neurochem Int*. 2009;5:333-40.

11. Gobatto CA, de Mello MA, Sibuya CY, de Azevedo JR, dos Santos LA, Kokubun E. Maximal lactate steady state in rats submitted to swimming exercise. *Comp Biochem Physiol* A *MolIntegr Physiol.* 2001;1:21-7.

12. Lima FD, Oliveira MS, Furian AF, Souza MA, Rambo LM, Ribeiro LR, *et al.* Adaptation to oxidative challenge induced by chronic physical exercise prevents Na+,K+-ATPase activity inhibition after traumatic brain injury. *Brain Res.* 2009;1279:147-55.

13. American Physiological Society (APS). Exercise protocols using rats and mice. Resource Book for the Design of Animal Exercise Protocols [Internet]. 1st ed. Washington: National Academy Press; 2006. p. 152. Available from: http://www.the-aps.org/mm/SciencePolicy/AnimalResearch/Publications/Animal-Exercise-Protocols/book14824.pdf

14. Amr AR, Abeer EE-K. Hypolipideimic and Hypocholestermic Effect of Pine Nuts in Rats Fed High Fat , Cholesterol-Diet. *World Appl Sci J.* 2011;15:1667-77.

15. Friedewald WT, Levy RI, Fredrickson DS.Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem*. 1972;18:499-502.

16. Page KA, Hartzell DL, Li C, Westby AL, Della-Fera MA, Azain MJ, *et al.* β-Adrenergic receptor agonists increase apoptosis of adipose tissue in mice. *Domest Anim Endocrinol.* 2004;26:23-31.

17. Messora MR, Oliveira LFF, Foureaux RC, Taba M, Zangerônimo MG, Furlaneto FAC, *et al.* Probiotic therapy reduces periodontal tissue destruction and improves the intestinal morphology in rats with ligature-induced periodontitis. *J Periodontol.* 2013;84:1818-26.

18. Mierzejewska-Krzyżowska B, Drzymała-Celichowska H, Celichowski J. Gender differences in the morphometric properties of muscle fibres and the innervation ratio of motor units in rat medial gastrocnemius muscle. *Anat Histol Embryol.* 2011;40:249-55.

19. Velasco M V, Tano CT, Machado-Santelli GM, Consiglieri VO, Kaneko TM, Baby AR. Effects of caffeine and siloxanetriol alginate caffeine, as anticellulite agents, on fatty tissue: histological evaluation. *J Cosmet Dermatol.* 2008;7:23-9.

20. AOAC (Association of Official Analytical Chemistry). Official Methods of Analysis.17th ed. Washington D. C.; 2000.

21. Ayres M, AYRES-Jr M, Ayres DL, Santos AS. Bioestat 3.0 -Aplicações estatísticas nasáreas das ciências biológicas e médicas. Belém P. A.: Sociedade Civil Mamirauá; 2003. p. 151-205.

22. Melzer K, Kayser B, Saris HW, Pichard C. Effects of physical activity on food intake. *Clin Nutr.* 2005;24:885-95.

23. Vandersluis AD, Venier NA, Colquhoun AJ, Sugar L, Pollak M, Kiss A, *et al.* Exercise Does Not Counteract the Effects of a "Westernized" Diet on Prostate Cancer Xenografts. *Prostate.* 2013;73:1223-32.

24. Meyer JS, Itoh Y, Okamoto S, Welch KM, Mathew NT, Ott EO, *et al.* Circulatory and metabolic effects of glycerol infusion in patients with recent cerebral infarction. *Circulation*. 1975;51:701-12.

25. Michna L, Wagner GC, Lou Y, Xie J, Peng Q, Lin Y, *et al.* Inhibitory effects of voluntary running wheel exercise on UVB-induced skin carcinogenesis in SKH-1 mice. *Carcinogenesis.* 2006;27:2108-15.

26. Droste SK, Gesing A, Ulbricht S, Mu MB, Linthorst ACE, Reul JMHM. Effects of Long-Term Voluntary Exercise on the Mouse Hypothalamic-Pituitary-Adrenocortical Axis. *Endocrinology.* 2014;144:3012-23.

27. Nagaraja HS, Jeganathan PS. Forced swimming stress-induced changes in the physiological and biochemical parameters in albino rats. *Indian J Physiol Pharmacol.* 1999;43:53-9.

28. Zou Y, Li J, Ma H, Jiang H, Yuan J, Gong H, *et al.* Heat shock transcription factor 1 protects heart after pressure overload through promoting myocardial angiogenesis in male mice. *J Mol Cell Cardiol.* 2011;51:821-9.

29. Salomão EM, Gomes-Marcondes MCC. Light aerobic physical exercise in combination with leucine and/or glutamine-rich diet can improve the body composition and muscle protein metabolism in young tumor-bearing rats. *J Physiol Biochem.* 2012;68:493-501.

30. Araki S, Izumiya Y, Hanatani S, Rokutanda T, Usuku H, Akasaki Y, *et al.* Akt1-mediated skeletal muscle growth attenuates cardiac dysfunction and remodeling after experimental myocardial infarction. *Circ Heart Fail.* 2012;5:116-25.

31. Donato J, Pedrosa RG, Cruzat VF, Pires ISDO, Tirapegui J. Effects of leucine supplementation on the body composition and protein status of rats submitted to food restriction. *Nutrition*. 2006;22:520-7.

32. Aoki MS, Almeida ALRA, Navarro F, Costa-Rosa LFBP, Bacurau RFP. Carnitine supplementation fails to maximize fat mass loss induced by endurance training in rats. *Ann Nutr Metab.* 2004;48:90-4.

33. Botezelli JD, Cambri LT, Ghezzi AC, Dalia RA, Scariot PPM, Ribeiro C, *et al*. Different exercise protocols improve metabolic syndrome markers , tissue triglycerides content and antioxidant status in rats. *Diabetol Metab Syndr.* 2011;3:1-8.

34. Higa TS, Bergamo FC, Mazzucatto F, Fonseca-Alaniz MH, Evangelista FS. Physical training prevents body weight gain but does not modify adipose tissue gene expression. *Brazilian J Med Biol Res.* 2012;45:988-94.

35. Dallas C, Gerbi A, Tenca G, Juchaux F, Bernard F-X. Lipolytic effect of a polyphenolic citrus dry extract of red orange, grapefruit, orange (SINETROL) in human body fat adipocytes. Mechanism of action by inhibition of cAMP-phosphodiesterase (PDE). *Phytomedicine.* 2008;15:783-92.

36. Ghorani, V, Rashidlamir, A, Farzanegi, P. Effects of 6 Weeks of Aerobic Training and Fenugreek Extract Administration on Plasma Apo A-I of Female Patients with Type 2 Diabetes. *Iranian Journal of Health and Physical activity.* 2014; 5:29-34.

37. Lavekar AS, Salkar HR. Treadmill Test to Detect Stress Induced Ischemic Heart Disease in Type 2 Diabetes Mellitus Patients Asymptomatic for CAD: A Hospital Based Cross-sectional Study in Rural Population of Central India. *J Diabetes Metab.* 2013;4:1-5.

38. Szostak J, Laurant P. The forgotten face of regular physical exercise: a "natural" anti-atherogenic activity. *Clin Sci.* 2011;121:91-106.