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 del grupo 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 lipoproteínas 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.


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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. Such changes are influenced by the frequency and duration of training, as well as the type and intensity of exercise that is being performed. 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. 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.

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

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. Nevertheless, there is a wide variety of protocols and training periods described in the literature, 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 experiment. Periodic weighing of the animals was performed every week. At the end of the experimental period the animals 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 Analis Diagnósticos®, Belo Horizonte, Brazil) as proposed by the American Physiological Society.

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 Analis Diagnósticos®, Belo Horizonte, Brazil) as proposed by the American Physiological Society.

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. The slides were analyzed by optical microscopy.

**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.

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 uninterrupted for 60 minutes, without load.

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.

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.

**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, 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. 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 Mierzejevska-Krzyzowska et al.14.

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 do 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 cell15.

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 Soxhlet20 equipment. The percentage of protein in the carcass was obtained using the Kjeldahl method21.

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 program21.

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 studies22,23. 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 intake24. Furthermore, the maintenance of body weight occurred probably because the diet was provided ad libitum to the animals25, 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 exercise27. 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 exercise28.

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-Marcondes29. 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 fibers30, which may explain the results observed in the gastrocnemius muscle.

In addition, the increased synthesis of mitochondrial proteins favors lipid oxidation31, 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 animals33,34 as observed in this study.

The physiological mechanisms of exercise-induced lipolysis first involves hydrolyzing intramuscular TAGs and then adipose tissue35.
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. This same mechanism may explain the lower TAG and LDL-C circulating levels, concomitant to the HDL-C increase observed in the present study. 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.

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. Thus the results of this study confirm the benefits of exercise to combat this risk.

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).*

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**References**

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