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Aerobic Exercise Sensitizes Brown Adipose Tissue and Mobilizes Fat Oxidation in Obese Rats

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Abstract

Moderate-intensity physical exercise, when performed regularly, induces a challenge to the organism, which leads to the integrated conditioning of several physiological systems. Physical exercise is an important intervention for the treatment and prevention of several diseases, such as obesity. Physical inactivity and obesity are closely linked, in addition, many pathological conditions are generated by fat accumulation. This work explores the molecular mechanism of exercise intervention in brown adipose tissue of obese animals. Rats were submitted to an obesogenic diet accompanied by chronic moderate-intensity physical exercise. Exercise reduced the amount of the rat's body fat, cholesterol and triglycerides levels, and reduced the size of liver lipid droplets. The trained animals had an increase of AMPK phosphorylation in the liver and brown adipose tissue. Besides, exercise induced an increase in mRNA expression of proteins related to lipid metabolism, such as *Pparα, Pgc-1α, Ucp1* and *Ucp* 3, lipoprotein lipase and hormone sensitive lipase in brown adipose tissue. In conclusion, chronic moderate-intensity physical exercise has sensitized the brown adipose tissue (BAT), increasing proteins involved in energetic catabolic metabolism, converting stored fat into heat, maximizing fat oxidation and collaborating as an intervention for the treatment and prevention of obesity.

Keywords: Aerobic Exercise; Obesity; Brown Adipose Tissue; Energetic Metabolism

Introduction

A higher energy consumption than its expenditure results in energy imbalance. Consequently, the calories are stored as lipids in different body regions, and therein lies the problem: The fat accumulation in the vascular system or visceral regions, among others, characterizes obesity, and it can lead to debilitating and deadly diseases [7]. This is a simplistic definition of the first law of thermodynamics [10]. Obesity is a chronic disease with an intricate interplay between biological, physiological, genetic, environmental, social, behavioral and economic factors [1, 10]. For the majority of the obese, reducing a pound of body fat is a real challenge, but its fundamental due to health reasons.

Physical exercise increases the energetic demand of organisms, which includes lipid metabolism. Aerobic exercise of moderate-intensity (range from 45–75% VO_{2max}) presents the ideal conditions for fat oxidation [19]. The stored fat is used as an energy source during and after this type of exercise, and as a result there is a decrease in body fat [21]. In addition to the consumption of stored fat, there are also physiological, biochemical and molecular changes that are linked inseparably from the beneficial effects of physical exercise, which includes stimulus of brown adipose tissue metabolism [8, 16, 31].

There are different types of adipose tissue in organisms, namely White, Beige (which are found interspersed within white) and Brown adipose tissue (BAT) [8]. The last one has this name because it has large amounts of mitochondria that give its brown color. BAT is located in interscapular, subscapular, axillary, perirenal and periaortic regions in rodents [3, 4]. When BAT is stimulated by exercise or cold, the sympathetic nervous system releases norepinephrine targeting the β-adrenergic receptor [3]. Under these conditions, BAT consumes substantial amounts of glucose and fatty acids as fuel for thermogenesis and energy expenditure [24].

Furthermore, studies investigating exercise and BAT have provided conflicting data indicating an increase in BAT activity [6] or a decrease in BAT activity [34, 35]. Therefore, this study aims to provide more data for a better understanding of the mechanisms involved in exercise and brown adipose tissue, as well as an overview of exercise and diet.

Materials and Methods

Animals

Male Sprague Dawley rats 60 days old (250–350 g) were housed in a temperature-controlled room (24 ± 2°C), with a 12-h light, 12-h dark cycle (lights from 08:00 a.m. to 08:00 p.m.). The experiments were carried out in accordance with the International Law on Animal Experimentation and the Ethics Committee has approved the experimental protocols of the University of Santiago de Compostela.

Experimental model

Males were divided into four groups (n=8/group).
Group 1: Diet 10 kcal% Fat – Low Fat Diet (LFD).
Group 2: Diet 10 kcal% Fat + aerobic exercise (AE) - LFD+AE.
Group 3: Diet 45 kcal% Fat - High fat diet (HFD).
Group 4: Diet 45 kcal% Fat + aerobic exercise (AE) – HFD+AE.

Diets and physical exercise were tested concomitantly. Animals had seven days of adaptation to exercise training, 10 min / day. Commercial diets were administered, 10 kcal% Fat, D12450B and 45 kcal% Fat, D12451 (Research Diets, Inc. USA). Exercise training consisted of continuous running on a motor-driven rodent treadmill (TSE-Systems, USA) at 20 m / min, 0º gradient, for 30 min, 5 times a week for 8 weeks.

Rats were euthanized 24 hours after the last exercise training period. Their liver, BAT, gonadal fat, visceral fat, omental fat and retroperitoneal fat were removed, weighed and stored at -80ºC for further analysis.

Weight, Caloric Intake, and Body composition evaluation

Rats were weighed twice a week, and food intake was monitored once a week. The analysis of the body composition was carried out with a nuclear magnetic resonance (NMR) model (EchoMR; Echomedical System Houston, TX). EchoMRI™ analyzes body composition in live animals with high precision and accuracy, measuring whole-body fat and lean body mass. The results are expressed in grams.

After euthanasia, the liver, brown adipose tissue, gonadal fat, visceral fat, retroperitoneal fat and omental fat were weighed and compared between groups.

Quantitative determination of cholesterol and triglycerides

Serum levels of cholesterol and triglycerides were carried out using a commercial kit: Cholesterol - CHOD-POD and Triglycerides - GPO-POD (SPINREACT, Spain). The tests were carried out according to the manufacturers' instructions.

Oil Red O and Harris' Hematoxylin Stain

Livers were sectioned 8 µm thick in a cryostat (HM50E®, Micron cryostat, Thermo Fisher, USA) at -25° C. The sections were mounted on poly-L-lysine coated glass slides from Sigma-Aldrich, USA. The sections were fixed in 10% formalin for one hour. A work solution of Oil Red O was prepared as follows: 0.6 g of Oil Red O (Sigma-Aldrich, USA.) dissolved in 120 mL of isopropanol and 80 mL of distilled water.

The sections were incubated in Oil Red O solution for 30 min. After this time, a washing was carried out in distilled water, followed by 30% isopropanol and distilled water. The sections were stained with Harris' Hematoxylin (Bio Optica, Italy) followed by a washing with distilled water and assembled with mounting and embedding media (Bio Optica, Italy). The images were acquired using a Microscopy EVOS® FL Auto Imaging System (AMAFD1000 - Thermo Fisher Scientific).

Western Blotting

Total liver and BAT protein lysates were submitted to electrophoresis in sodium dodecyl sulfate-polyacrylamide gel, electroblotted onto nitrocellulose membranes and incubated with the indicated antibodies: Phospho-ACC-Ser79 (pACC - #07-303), (Millipore, USA); Phospho-AMPK α -Thr172 (p-AMPK α - #2535) (Cell Signaling, USA); Acetyl-CoA Carboxylase (#3676) and AMPK α (#2532) (Cell Signaling, USA). Protein detection was carried out using IgG peroxidase-linked secondary antibody. The immunoreactivity was detected by enhanced chemiluminescence using Supersignal West Pico Chemiluminescent kit from Thermo Fisher Scientific (USA). Dilutions and incubation time were carried out according to the manufacturers' instructions.

mRNA Isolation and Real-Time PC

Total RNA was isolated with trizol reagent according to the manufacturer's instructions followed by cDNA synthesis with M-MLV Reverse Transcriptase (Invitrogen, USA). Expression of mRNA levels of *Ucp1*, *Ucp3*, *Pparα*, *Pgc1 α*, *Lpl*, *Hsl* in brown adipose tissue were carried out using real-time PCR (TaqMan_; Applied Biosystems, USA) with specific primers and probes.

All reactions were carried out using the cycling parameters: 50ºC for 2 min, 95ºC for 10 min, followed by 40 cycles of 95ºC for 15 seconds and 60ºC for 1 min. For data analysis, the input value of the target gene was standardized to the HPRT value for each sample. Data are expressed in comparison with the average value for the LFD group.

Statistical Analysis

GraphPad Prism version 7 software (GraphPad Software Inc., CA, USA) was used for statistical analysis. Two-way ANOVA, followed by Tukey's multiple comparisons test was carried out. The results were expressed as mean ± standard error (S.E.M.). Differences were considered significant when $p<0.05$. The p values are embedding in figures.

Results

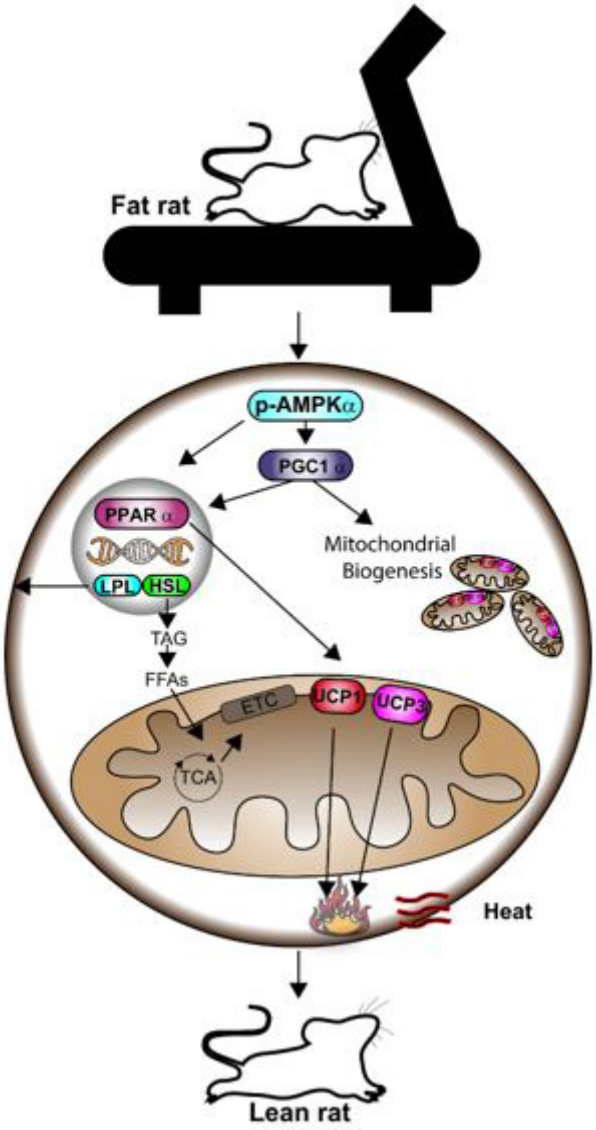
Aerobic exercise decreases body weight, Kcal intake, cholesterol and triglycerides levels

The effectiveness of the diet was proven by weighing the animals. The animals that were fed with HFD presented about 50 grams more than the animals with LFD. The LFD + AE group had the lowest weight. Exercise prevented weight gain induced by HFD (Figure 1A).

Trained animals ingested fewer Kcal compared to sedentary rats. Even for HFD, which is more palatable, exercise has inhibited appetite (Figure 1B).

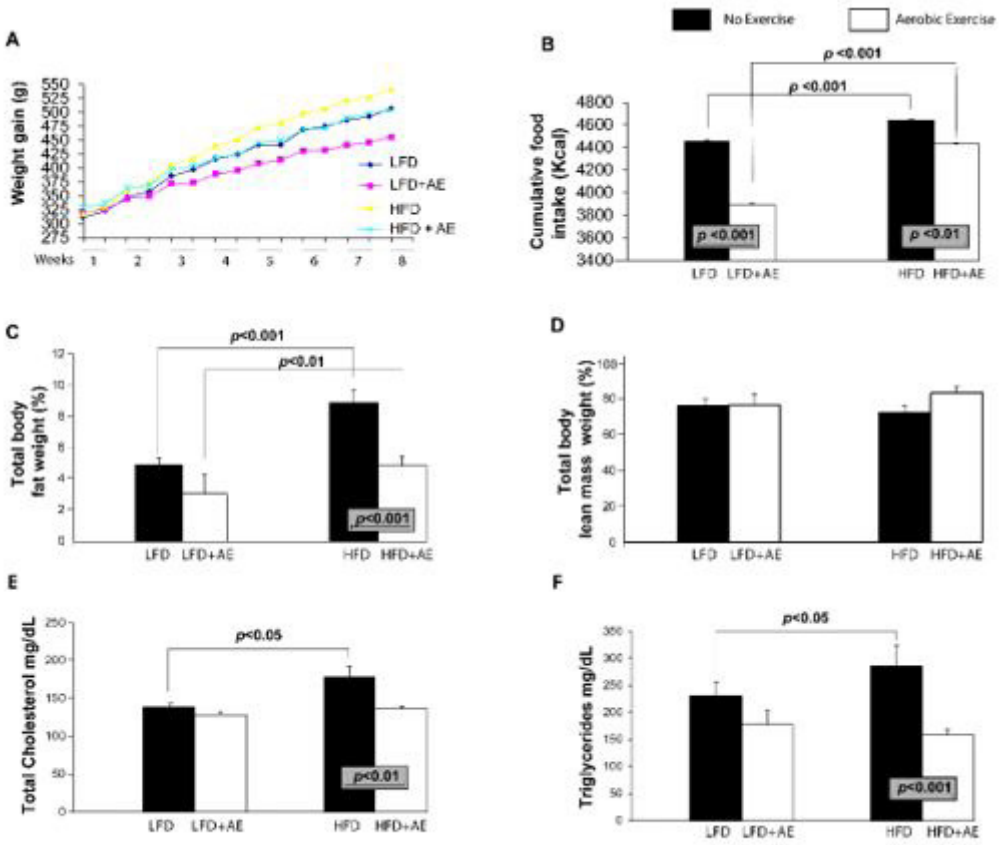
The body composition was investigated by nuclear magnetic resonance. It was observed that HFD induced an increase of 100% in body fat. Whereas the exercise decreased this parameter, trained animals that received HFD showed similar levels the animals that received the LFD diet (Figure 1C). The percentage of lean mass did not change in any group (Figure 1D).

Total cholesterol and triglycerides had a similar profile; HFD significantly increased the serum levels of both and the exercise attenuated increases in these parameters (Figure 1E and F).



AMPK plays a central role in fat oxidation. Exercise sensitizes and induces signaling through this enzyme that triggers transcription factors, such as PGC1 and PPAR. PGC1 signals for PPAR and induces mitochondrial biogenesis. This signaling cascade increases the expression of LPL and HSL enzymes, in addition to mitochondrial uncoupling proteins UCP 1 and UCP 3. The LDL and HSL enzymes catalyze the formation of triacylglycerol fatty acids. The fatty acids are oxidized by β-oxidation with subsequent formation of acetyl-CoA that will be oxidized in the citric acid cycle (TCA). The electrons that pass through the electron transport chain are not used in the formation of ATP, because, in a step prior to ATP synthase, these electrons leak through the mitochondrial uncoupling proteins that dissipate energy into heat. Without ATP accumulation, anabolic metabolism for the formation of fatty acids is inhibited, as also demonstrated in this study by ACC. In summary, exercise sensitizes the proteins involved in fat oxidation in BAT, besides inducing mitochondrial biogenesis, which helps to accelerate the oxidation of stored fat, collaborating as an intervention for the treatment and prevention of obesity.

Graphical Abstract: The effects of physical exercise on brown adipose tissue

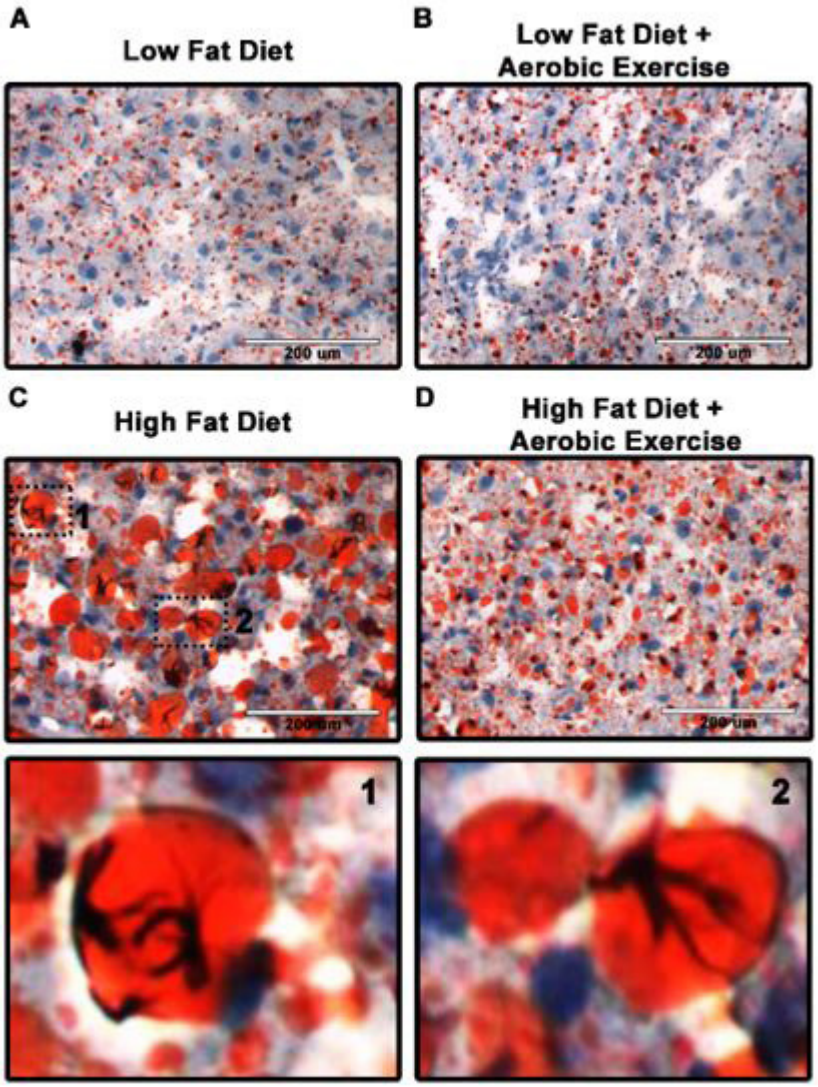


A) Kinetics of weight gain over eight weeks, measurements were taken twice a week. B) Cumulative food intake (Kcal). C) Body fat weight. D) Body lean mass weight. E) Cholesterol. F) Triglycerides. The results were expressed as a mean ± standard error (S.E.M.). Two-way ANOVA, followed by Tukey's multiple comparisons test, was carried out. *p* values are embedding in figures. *n*=8 per group. Black columns - sedentary animals. White columns - trained animals. Low Fat Diet (LFD), High Fat Diet (HFD, Aerobic exercise (AE).

Figure 1: Aerobic exercise decreases the parameters associated with obesity

The effect of physical exercise and HFD on hepatic fat deposits

Histological analysis were carried out in animal livers. The hepatic steatosis was investigated. Lipid droplets hypertrophy was observed in HFD (Figure 2C). The exercised animals from HFD had lower lipid droplets volume compared to those that did not have exercise training (Figure 2D).

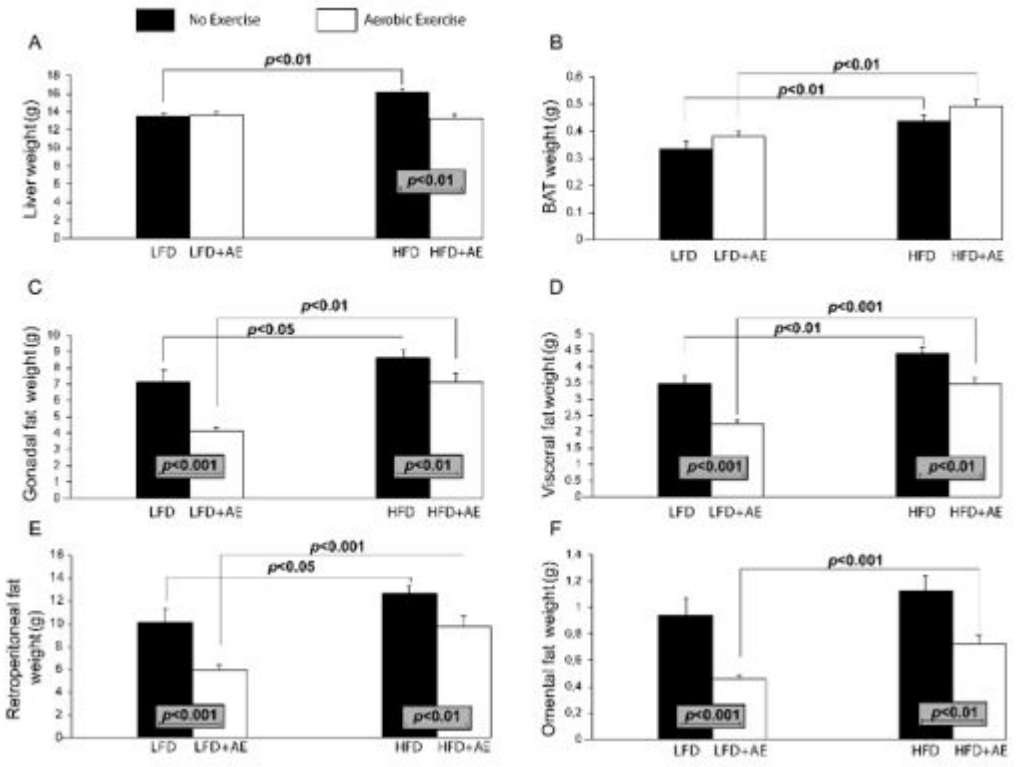


Liver histological analyses stained with Oil Red O – stained lipids (red color) and Harris' Hematoxylin - stained acidic structures in the cell, including nuclei (blue-black color). A) Low Fat Diet. B) Low Fat Diet + Aerobic Exercise. C) High Fat Diet, the numbers 1 and 2 represent detailed lipid droplts of this group. D) High Fat Diet + Aerobic Exercise.

Figure 2: Physical Exercise Reduces Liver Lipid Droplets

Aerobic exercise modulates the weight of the liver and of body fat deposits

Liver, BAT, gonadal, visceral, retroperitoneal and omental fat showed significant increases in their weight, that was induced by HFD (Figure 3). Liver mass increases with HFD and exercise modulated this effect (Figure 3A). Exercise also decreased different body fat deposition weight in LFD and HFD groups (Figure 3C-F). On the other hand, BAT was an exception, once the diet itself increases it weight, and exercise versus LFD or exercise versus HFD had no difference (Figure 3B).



A) Liver weight. B) Brown adipose tissue (BAT) weight. C) Gonadal fat weight. D) Visceral fat weight. E) Retroperitoneal fat weight. Omental fat weight. The results were expressed as a mean ± standard error (S.E.M.). Two-way ANOVA, followed by Tukey's multiple comparisons test, was carried out. *p* values are embedding in figures. *n*=8 per group. Black columns - sedentary animals. White columns - trained animals.

Figure 3: Aerobic exercise prevents weight gain in the liver and different fat stores

Aerobic exercise increase AMPK signaling and inhibited fat acid synthesis

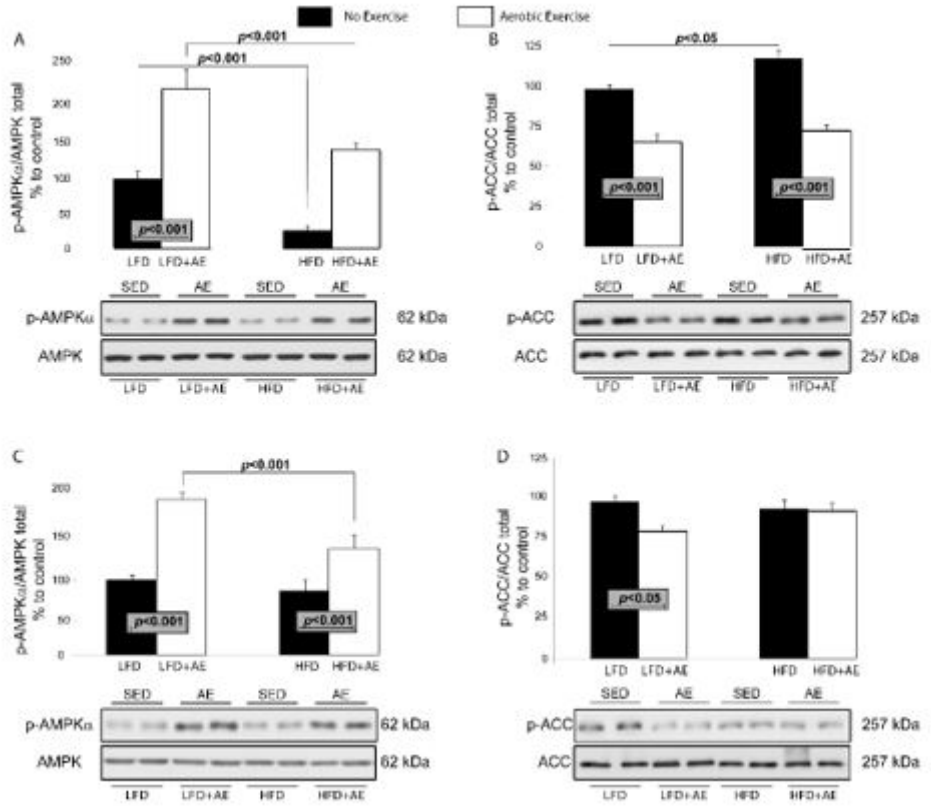
Western blotting was carried out to investigate the protein content of critical enzymes of lipid metabolism, AMPKα and p-ACC. Whereas, exercise increased p-AMPK in the liver by 100%, HFD decreased it. Already in trained animals feed with HFD, the AMPK phosphorylation increased (Figure 4A). Exercise prevented the effect induced by HFD.

The p-ACC had the opposite effect of the previous enzyme, the diet induced an increase in the phosphorylation of this enzyme, while exercise inhibited it. Interestingly, the levels of p-ACC in trained animals (LFD + AE and HFD + AE) remained the same (Figure 4B).

The p-AMPK levels in BAT had a profile similar to the liver of trained rats, including the percentage. However, HFD did not induce alterations in AMPK-α phosphorylation in this tissue. Although exercise induced an increase in p–AMPK levels, HFD + AE did not reach the levels seen in the LFD + AE groups. HFD appears to have inhibited part of the exercise's effect (Figure 4C).

Exercise has induced a decrease in p-ACC levels in LFD + AE group in BAT. In HFD animals, it did not have the same effect (Figure 4D).

There was no difference between the total protein content, which indicates that the effects occurred exclusively by enzymatic phosphorylation and not by increasing the amount of enzyme.



A) Liver immunocontent of p-AMPK. B) Liver immunocontent of p-ACC. C) BAT immunocontent of p-AMPK. C) BAT immunocontent of p-ACC. Upper panel representative western blotting of phosphorylated proteins and the lower panel represents a total protein. The results were expressed as a mean ± standard error (S.E.M.). Two-way ANOVA, followed by Tukey's multiple comparisons test, was carried out. *p* values are embedding in figures. *n*=8 per group. Black columns - sedentary animals. White columns - trained animals. Low Fat Diet (LFD), High fat Diet (HFD, Aerobic exercise (AE).

Figure 4: Physical exercise increases AMPK immunocontent in the liver and BAT

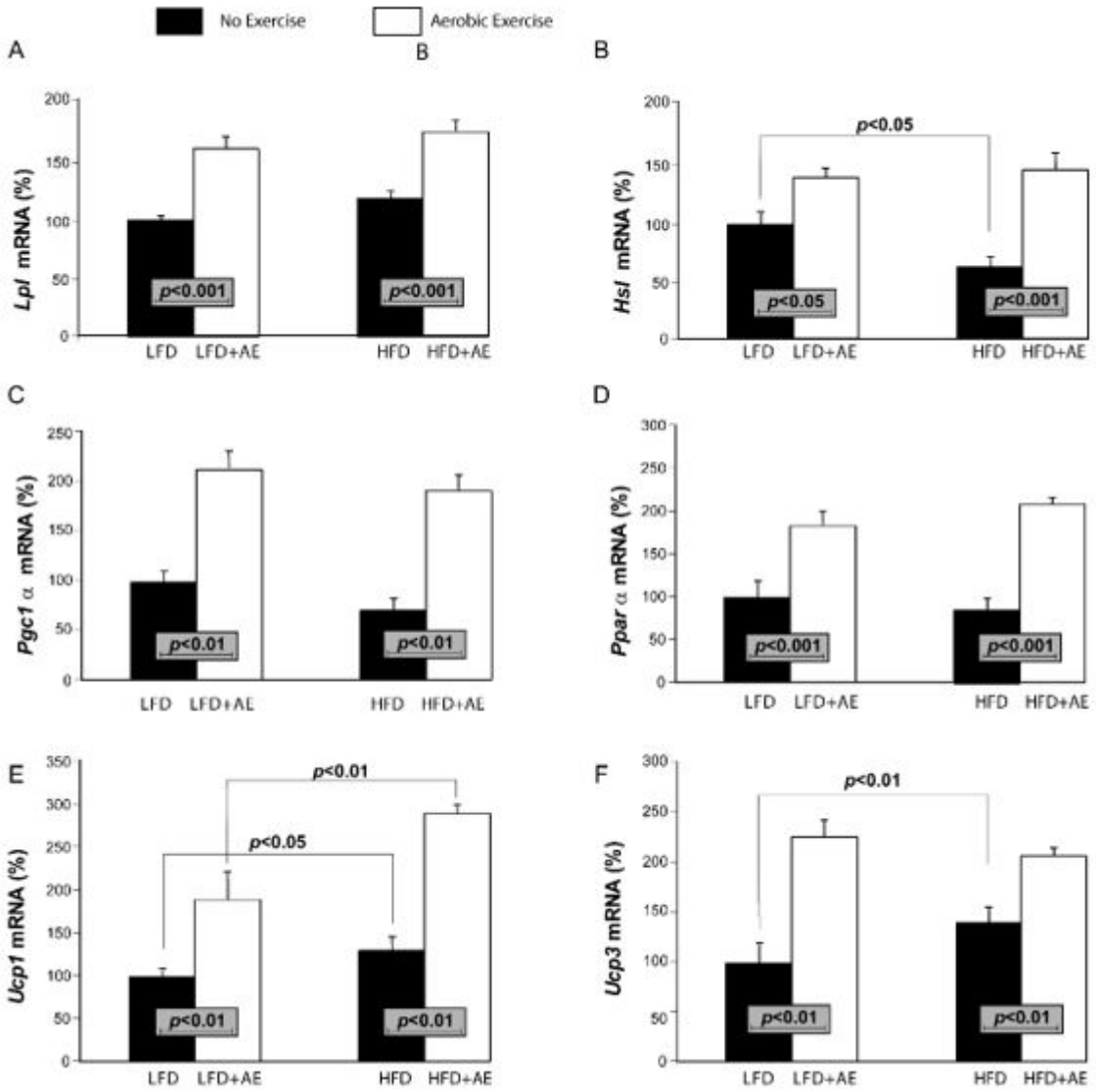
Aerobic exercise increases the mRNA expression of lipid metabolism enzymes, mitochondrial uncoupling proteins and transcription factors

RT-PCR was used to investigate the expression in mRNA levels of proteins involved in lipid metabolism in BAT. Aerobic exercise increased mRNA expression of lipoprotein lipase (LPL) (Fig. 5A). The diets did not interfere in the expression of mRNA LPL. Hormone-sensitive lipase (HSL), which is another enzyme involved in lipid catabolism, has increased due to physical exercise. HFD decreased the expression of HSL mRNA to LFD in BAT (Figure 5B).

mRNA expression of Peroxisome proliferator-activated receptor gamma coactivator alpha (PGC1 α) (Figure 5C) and Peroxisome proliferator-activated receptor alpha (PPARα) (Figure 5D) increased due to exercise in BAT. HFD did not induce a difference in mRNA expression of both mRNA.

Two mitochondrial uncoupling proteins were investigated, namely uncoupling protein 1 and isoform 3 (UCP-1 and UCP-3). The HFD showed an increase in both UCP1 and UCP3 mRNA (Figure 5E and F). Exercise triggered a 100% increase in UCP1 mRNA levels in sedentary LFD animals. While in HFD+AE it was 150% higher compared to HFD animals (Figure 4A).

The exercise group (LFD + AE) induced an increase of 125% in UCP3 mRNA expression compared to LFD (Figure 5F), whereas HFD increased it. Besides, the association between exercise and HFD increased in 63% the UCP3 mRNA concerning to HFD.



A) Lipoprotein lipase (*Lpl*) mRNA. B) Hormone-sensitive lipase (*Hsl*) mRNA. C) Peroxisome proliferator-activated receptor-gamma coactivator alpha (*Pgc1* α) mRNA. D) Peroxisome proliferator-activated receptor alpha (*Ppar α*) mRNA. E) Uncoupling proteins 1 (*Ucp1*) mRNA. F) Uncoupling proteins 3 (*Ucp3*) mRNA. The results were expressed as a mean ± standard error (S.E.M.). Two-way ANOVA, followed by Tukey's multiple comparisons test, was carried out. *p* values are embedding in figures. *n*=8 per group. Black columns - sedentary animals. White columns - trained animals. Low Fat Diet (LFD), High fat Diet (HFD, Aerobic exercise (AE).

Figure 5: Physical exercise modulates mRNA expression of proteins related to lipid metabolism in BAT

Discussion

To improve obesity knowledge, an experimental model was used to investigate how exercise optimizes / accelerates the biochemical and molecular mechanisms in fat oxidation through BAT sensitization in obese rats. The effectiveness of this obesity model was validated by standard parameters that changed in obese people, such as exponential weight gain, percentage of total body fat weight, total cholesterol and triglycerides. Most of them increased in our animal model by HFD.

In addition, immunohistochemistry of the liver was also carried out. This tissue acts as a tube to metabolically connect various tissues, including skeletal muscle and adipose tissue [29]. Obese animals showed hypertrophy in cytplasmatic lipidic droplets of hepatocytes, and exercise was partly efficient in decreasing the size of fat molecules. Lipids from the liver are an energy reservoir during and post-exercise. Triacylglycerols in these cells are hydrolyzed to release fatty acids that are subsequently released into the

circulation, providing fuel for the working muscles [23]. Therefore, an exercise-induced decrease in the size of lipidic droplets in hepatocytes was expected.

Physical exercise is used as an intervention for obesity, accelerating the oxidation of body fat and using it as energy to carry out an activity. Nevertheless, the effects of exercise goes far beyond that, as exercise stimulates several metabolic pathways, accelerating biochemical processes even hours after the last training session [26]. In fact, physical exercise was able to prevent weight gain triggered by HDFin this study. Furthermore, trained animals ingest fewer calories than sedentary animals feed with LFD or HFD. The hunger and food intake induced by exercise depend on its intensity and duration [33]. Part of this effect could be explained by the relation between acylated ghrelin and leptin [33]. However, there are multiple variations in appetite-related hormone, and further studies are needed to summarize how gender and habitual physical activity induce changes in those hormones [9].

The assessment of fat deposits revealed that sedentarism and HFD intake led to greater deposition of white fat in different body regions. The adipocytes present in white fat cells, in addition to providing energy, play a critical role in secreting hormones such as leptin, influencing processes such as food intake and insulin resistance [5].

BAT has been investigated as a therapeutic target in obesity (32). This tissue has many respiratory chain uncoupling proteins producing heat (thermogenesis), which has been implicated as an essential site of facultative energy expenditure (15). About 15% of the daily energy expenditure is used by thermogenesis (30), about 20% of the oxygen consumption (28). For example, transgenic mice with deficiency in brown adipose tissue, develop obesity and morbid complications (20). Depending on the type, intensity and duration of exercises, it is possible to stimulate energy expenditure through mitochondrial biogenesis and BAT proteins activity (20). In this study, HFD and HFD + AE increased the weight of BAT. Exercise alone has not changed this tissue weight. However, trained animals has increased immunocontent of an enzyme that plays an essential role in cellular energy homeostasis, adenosine monophosphate (AMP)-activated protein kinase (AMPK).

AMPK is a cellular energy sensor that regulates energy homeostasis pathways in any kind of cell (11). Once activated by ADP, and mainly by high levels of AMP, it leads to an inhibition of anabolic pathways, also upregulating the catabolic metabolism. Thus, this improves the formation of ATP through increasing the expression of nuclear genes that encode factors involved in mitochondrial biogenesis and functions, such as PGC-1α and uncoupling proteins (22).

Besides, AMPK modulates proteins from glucose and fatty acid pathways to increase the formation of ATP (11). In this study, trained animals had an increase in the content of AMPK in the liver and BAT.

To complement this result, the immunocontent of Acetyl-CoA carboxylase (ACC) was investigated. ACC catalyzes the first step committed in the biosynthesis of long-chain fatty acids (14). Trained animals had lower phosphorylation of p-ACC, while in sedentary animals it was increased. There was no difference between diets neither due to exercise in HFD feed animals. The maintenance of p-ACC levels shows that synthesis of fatty acid remains activated, what we believe that is regulating BAT content.

In this study, trained animals had an increase in *Lpl* and *Hsl* mRNAs, whereas, HFD decreased only *Hsl* mRNA levels. We find different results in the literature, since most articles demonstrate a reduction in this enzyme and a consequent reduction in the uptake of FAT. However, the exercise protocol was not the same (16, 27). These enzymes are necessary for recruiting adipose tissue as an energy source. As the energy demand of BAT cells presents changes in most of the fatty acid oxidation pathways investigated in this study, the increased expression of *Hsl* and *Lpl* mRNA can be interpreted as a compensating mechanism for the energy demand performed by physical exercise in BAT cells.

In this study, physical exercise induced increases in mRNA Pgc1α. The PGC-1α is a co-activator protein that increases the probability of gene expression when interacting with transcription factors (18). Once interacting with PPARs, PGC-1α plays a crucial role in regulating of BAT function (18, 25).

PPARα is a transcription factor that controls the expression of genes related to lipid metabolism, such as mitochondrial biogenesis, β-oxidation, peroxisomal β-oxidation, fatty acid uptake, binding, assembly and transport of lipoprotein (18, 25). PPARα deficiency causes disturbance in fatty acid metabolism accompanied by a significant decrease in serum's short-chain and an increase in long-chain acyl carnitines (17). PPARα is activated in conditions of energy deprivation such as prolonged fasting, promoting fatty acid catabolism (13). Our results show that trained rats had an increase in PPARα mRNA. It is possible to consider that the type of exercise carried out for this study resulted in adaptations of PPARα, similar to those found in fasting (17).

PPARα mediates the transcriptional regulation of UCP 1 and UCP 3 genes of mitochondrial uncoupling proteins in BAT (12). UCP1 represents approximately 10% of the mitochondrial protein content and plays a thermogenic role through the catalysis of proton-leak. The overexpression of UCP1 has been implicated in preventing the development of obesity (2). In this experimental model, trained animals had high levels of *Ucp1* and *Ucp3* mRNA in BAT, and this result points to one of the multiple mechanisms that physical exercise sensitizes / accelerates the oxidation of body fat and combats obesity.

Conclusion

The present study demonstrated the effects of chronic aerobic exercise on obesity. According to our results, brown adipose tissue plays a fundamental role in the nutritional homeostasis, supporting energy balance and, consequently, accelerating catabolic metabolism, using fatty acids as an energy source, which generates heat. Moreover, it contributes to the reduction of fat in the liver and in the deposits of white fat in rodents. In summary, aerobic exercise stimulates / sensitizes the BAT and increases energy metabolism, maximizing fat oxidation.

In addition to the limitations of this study to investigate only lipid metabolism without considering glucose and amino acids metabolism, for future studies, it is necessary to investigate the activation of the formation of AMPK and ATP in BAT and WAT to establish and compare changes in energy metabolism, and clarify the increase in the expression of *Lpl* mRNA.

Conflict of Interest

The authors declare no conflict of interest.

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Author Contributions

JG– Planning, management, data analysis/interpretation and writing the manuscript; KDBM – Mouse colony management and experiments; MLSO-Review & Editing, data curation and funding acquisition; NS data interpretation and writing the manuscript, CES – Investigation, methodology, conceptualization; CD – Supervision, and funding acquisition.

Compliance with Ethical Standards

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