RESEARCH ARTICLE

Aerobic training induces differential expression of genes involved in lipid metabolism in skeletal muscle and white adipose tissues

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Abstract

Aerobic training induces adaptive responses in skeletal muscles and white adipose tissues, thus facilitating lipid utilization as energy substrates during a physical exercise session. However, the effects of training on cytokines levels and on transcription factors involved in lipid metabolism in muscle and different white adipose depots are still unclear; therefore, these were the aims of the present study. Nineteen adult male Wistar rats were randomly assigned to a trained group or a control, non-trained group. The 10-week training protocol consisted of running on a treadmill, during 1 hour per day, 5 days per week, at 75% of maximum aerobic speed. As expected, trained rats improved their aerobic performance and had augmented citrate synthase activity in the soleus, while the control rats did not. Although body weight was not different between groups, the adiposity index and white adipose depots (ie, epididymal and retroperitoneal) were reduced in trained rats. Training reduced serum concentration of insulin, but failed to change serum concentrations of glucose, triacylglycerol, total cholesterol, and nonesterified fatty acids. Training increased sterol regulatory element-binding protein-1c expression in the gastrocnemius and epididymal adipose tissue, and reduced peroxisome proliferator-activated receptor γ (PPARγ) expression in most of the tissues analyzed. The expression of PPARα and carnitine palmitoyltransferase 1 increased in the gastrocnemius and mesenteric adipose tissue but reduced in epididymal adipose tissue. Triacylglycerol content and tribbles 3 expression reduced in the gastrocnemius of trained rats. Tumor necrosis factor-α and interleukin-6 were increased in all adipose depots evaluated. Collectively, our data indicate that the 10-week aerobic training changed gene expression to improve muscle oxidative metabolism and facilitate lipid degradation in adipose tissues. Our data also highlight the existence of adaptive responses that are

distinct between the skeletal muscle and white adipose tissue and between different adipose depots.

1 | INTRODUCTION

Recent discoveries have changed the understanding about the skeletal muscles, which are now considered as secretory tissues; in this sense, cytokines and other peptides that are produced, expressed and released by muscle fibers exert either autocrine, paracrine or endocrine effects.[1] In addition, the skeletal muscles are plastic organs[2] and develop several adaptations[3,4] in response to physical training regimens. For example, aerobically trained individuals present reduced carbohydrate, but increased fatty acid (FA) oxidation during an exercise session,[5,6] most likely due to increased activity of enzymes involved in the β-oxidation, tricarboxylic acid cycle, and electron transport system.[7,8] Evidence also indicates that aerobic training upregulates membrane-associated FA transport proteins, including the mitochondrial transporter carnitine palmitoyltransferase I (CPT1),[9] thus favoring FA oxidation.

Likewise the skeletal muscles, the white adipose tissues also play endocrine roles, which regulate lipid storage and synthesis, and the secretion of hormones and cytokines.[10,11] Notably, specific regional depots of adipose tissue have differences in structural organization, cellular size, and even biological function.[12] A recent study reported that caloric restriction changed the gene expression of adipokines and M2 macrophage markers in a tissue-specific way in the epididymal, retroperitoneal, and subcutaneous adipose tissues in rats.[13] Indeed, the differential fat distribution rather than the total amount of adipose tissue is associated with disorders like hypertension, diabetes, and cardiovascular disease.[12]

The molecular machinery involved in the triglycerides synthesis and degradation, as well as in FA uptake and oxidation, is regulated by several transcription factors, including the peroxisome proliferator-activated receptors (PPARs)[14] and sterol regulatory element-binding protein (SREBP)-1c.[15] PPAR-α favors FA degradation[16] by promoting the transport of FA across the mitochondrial barrier as a result of increases in the CPT1 and acyl-CoA oxidase expression.[14] Previous data indicate that PPAR-α content in skeletal muscle increases following an aerobic training, thereby suggesting the involvement of this transcription factor on the improvement of lipid oxidation mediated by

training.[17] In contrast, PPAR-γ favors FA deposition in adipose tissue through the activation of genes involved in adipogenesis.[16,18] Interestingly, PPAR-γ also seems to regulate FA uptake and oxidation in skeletal muscle.[19] Rats subjected to a long-term moderate-intensity aerobic training had increased levels of PPAR-γ protein in the muscle, but not in adipose tissue.[19] In the meanwhile, SREBP-1c is a transcription factor that specifically activates genes involved in FA biosynthesis in adipose tissue. SREBP-1c expression was reduced and increased by aerobic training in adipose tissue and skeletal muscle, respectively,[20] suggesting the importance of this transcription factor on lipid metabolism.[15]

Another pathway that regulates lipid metabolism involves the tribbles 3 (TRB3)-mediated degradation of adipose tissue acetyl-CoA carboxylase. Higher expression of TRB3 shifts the FA metabolism from a predominant lipogenesis to a predominant oxidation, allowing tissues to use more energy derived from FA rather than from glucose, as seen during fasting or physical exercise.[21] Earlier studies evidenced that TRB3 in liver and muscle regulates insulin signaling by suppressing AKT in fasted diabetic[22] or obese individuals.[23] Moreover, TRB3 expression was reduced in the liver and muscle after a swimming exercise, thus increasing insulin sensitivity in these animals.[24,25]

Lipolysis in the adipose tissue is also regulated by proinflammatory cytokines.[26,27] In this context, there is evidence that the tumor necrosis factor (TNF)-α and interleukin (IL)-6 stimulate lipolysis by different processes.[26,28] IL-6 seems to modulate the activation of the AMP-activated protein kinase (AMPK) both in the muscle and adipose tissues. Indeed, mice lacking IL-6 present lower activity of AMPK,[28] which regulates cellular energy homeostasis and is involved in mitochondrial biogenesis and function.[29] TNF-α seems to induce the lipolytic process by acting through several intracellular pathways for example, by inhibiting insulin receptor signaling, by counteracting the antilipolytic effect of adenosine on protein G, by reducing Gi-protein content or even by directly inducing the phosphorylation of the protein perilipin and reducing of its expression.[26]

As reviewed above, metabolic adaptations promoted by aerobic training involve very complex and integrated

mechanisms, which are likely tissue-specific. However, little is known whether a given training stimulus may induce specific adaptations, particularly adaptations in lipid metabolism and cytokine levels, according to the adipose tissue depot studied. Therefore, this study investigated the effect of aerobic training on the adaptive responses in muscle and different adipose tissue depots; the adaptive responses investigated herein included the expression of genes related to lipid metabolism and changes in cytokines levels. Moreover, we also determined the effect of aerobic training in promoting TRB3 expression in different adipose tissue depots. By measuring all these parameters mentioned above, the present study advances the knowledge of how physical training induces metabolic adaptation in tissues that are highly responsive to energetic demand, such as the skeletal muscle and adipose tissues. Our hypotheses were that a 10-week aerobic training would: (1) change the expression of transcription factors in muscle and white adipose tissues, facilitating FA oxidation and inhibiting adipogenesis; (2) increase the level of proinflammatory cytokines, facilitating lipolysis in the adipose tissue; (3) reduce TRB3 expression in the tissues evaluated, improving insulin sensitivity.

2 | METHOD

2.1 | Animals

The experimental procedures were approved by the local ethics commission for the use of animals (protocol n° 29/2009) and were conducted in accordance with the regulations provided by the National Council for the Control of Animal Experimentation (CONCEA; Brazil). Nineteen adult male Wistar rats (250-300 g), purchased from the CEBIO at the Universidade Federal de Minas Gerais, were housed in controlled facilities (ambient temperature of $23 \pm 2°C$ and lights on from 05:00 until 19:00 hours) and provided with water and chow ad libitum. The experiments were performed in fed rats, between 08:30 and 10:30 hours, and the animals were euthanized 72 hours after the last training session to prevent any acute effect of physical exercise from interfering with our analyses. The skeletal muscles (ie, gastrocnemius and soleus), heart, liver, and white adipose tissues (ie, epididymal, retroperitoneal, and mesenteric) were dissected, removed, weighed and stored at $-20°C$ or $-80°C$ until further analyses.

3 | EXPERIMENTAL DESIGN

3.1 | Familiarization with running on a treadmill

The rats at an age of 12 to 14 weeks were randomly assigned to a trained group (n = 9) or a control, non-trained group (n = 10). The rats were gradually encouraged to exercise on a custom-made treadmill designed for small animals (Gaustec, Nova Lima, MG, Brazil) by light electrical stimulation (0.5 mA). The familiarization consisted of running at a constant speed of 18 m.min^{-1} at a 5% inclination for 5 minutes, during 5 consecutive days,[30] before the first incremental test. The control rats were also familiarized to run on the treadmill.

3.2 | Incremental-speed tests

Each rat performed four incremental tests on the treadmill to determine their maximum aerobic speed (S$_{MAX}$). During these tests, the initial speed corresponded to 6 m.min^{-1} and it was increased by 3 m.min^{-1} every 3 minutes until the rats were fatigued.[31] Fatigue was defined as the point when animals were no longer able to keep pace with the treadmill and exposed themselves to the light electrical stimulation for 10 seconds.[32] The tests performed before (first test) and during the protocol (second and third tests) allowed the adjustment of the intensity of training sessions (75% of the S$_{MAX}$); whereas, the test performed at the end (fourth test) allowed the determination of the overall performance improvement caused by aerobic training.

3.3 | Aerobic training protocol

Two days after the first incremental test, the training protocol was initiated in one of the two groups of rats. Training consisted of one session per day, 5 days a week during a 10-week period. The intensity and duration of training sessions were increased gradually until the rats were able to run at 75% of their S$_{MAX}$ during 60 minutes (Table 1). The control rats were subjected to a slow walk on the treadmill for 5 minutes at a speed of 5 m.min^{-1}, once a day, 5 days a week during the 10-week period. This control protocol was executed to expose all animals to the same stressful conditions (eg, handling and exposure to light electrical stimulation); a recent study using exercise sessions characterized by short-duration, low-intensity treadmill running as a control procedure did not show improvements in aerobic performance.[33]

4 | ANALYTICAL PROCEDURES

4.1 | Serum analysis

The serum concentrations of glucose, total cholesterol, and triacylglycerol were assayed by enzymatic methods using kits produced by Labtest Diagnóstica (Lagoa Santa, MG, Brazil). The serum concentration of insulin was determined

TABLE 1 Ten-week aerobic training protocol

Incremental exercise 1	Aim: to determine the maximum speed (S_{MAX}) attained by the rats to prescribe the intensity of training sessions.
Week 1	Intensity: 28% S_{MAX}
	Duration: 30 min (day 1), 45 min (day 2), 50 min (day 3), 55 min (day 4), and 60 min (day 5)
Week 2	Intensity: 44% S_{MAX} / Duration: 60 min
Weeks 3 and 4	Intensity: 70% S_{MAX} / Duration: 60 min
Incremental exercise 2	Aim: to determine the maximum speed (S_{MAX}) attained by the rats to prescribe the intensity of training sessions.
Weeks 5, 6, and 7	Intensity: 75% S_{MAX} / Duration: 60 min
Incremental exercise 3	Aim: to determine the maximum speed (S_{MAX}) attained by the rats to prescribe the intensity of training sessions.
Weeks 8, 9, and 10	Intensity: 75% S_{MAX} / Duration: 60 min
Incremental exercise 4	Aim: to determine the overall performance improvement caused by aerobic training.

Abbreviation: S_{MAX}, maximum speed attained by the rats.

by an enzyme-linked immunosorbent assay (ELISA; Linco Research, St Charles, MO). The serum concentration of nonesterified fatty acids (NEFA) was determined by the colorimetric method using a kit from RANDOX Laboratories (Ardmore, UK). All these assays were conducted according to the manufacturers' instructions.

4.2 | Muscle analysis

The intramyocellular triacylglycerol content was measured in gastrocnemius muscle (~100 mg) after homogenization in an organic extraction solution consisting of chloroform:methanol (2:1), according to Folch et al.[34] After filtration and centrifugation, the interphase was washed with methanol diluted in water (1:1). After centrifugation and siphoning of the upper phase, the lower chloroform phase containing lipids was evaporated to dryness. The dried samples were reconstituted in phosphate-buffered saline containing 1% Triton X-100,[35] and this preparation was then used in the triacylglycerol assay. The values were normalized to wet tissue weight.

For the citrate synthase assay, the soleus muscle was dissected, cut into small pieces, and extracted in a medium consisting of 50 mM Tris-HCl, 1 mM EDTA at a pH 7.4. Citrate synthase activity was assayed in medium containing 1% Triton, 1 mM acetyl-CoA and 0.5 mM oxaloacetate to which the homogenated was added. The kinetics of the reaction was measured by following the change in E_{340} in a recorder spectrophotometer at 25°C.[36]

4.3 | Real-time quantitative polymerase chain reaction

Total RNA from epididymal (EAT), retroperitoneal (RAT), and mesenteric (MAT) and gastrocnemius muscle were extracted using Tri-Phasis (BioAgency, Sao Paulo, SP)

according to the manufacturer's instructions. After quantification, the samples were treated with DNAse and the reverse transcription was carried out using an oligo (dT) primer and Moloney murine leukemia virus. The purity of RNA was checked spectrophotometrically, and the evaluation of messenger RNA (mRNA) levels was done by reverse transcription polymerase chain reaction (PCR). Complementary DNA was amplified using specific primers for PPAR-α (forward: 5'- TACCACTATGGAGTCCACGCATGT-3'; reverse: 5'- TTGCAGCTTCGATCACACTTGTCG-3'), CPT1 (forward: 5'- ACGTGAGTGACTGGTGGGAAGAAT-3'; reverse: 5'- TCTCCATGGCGTAGTAGTTGCTGT-3'), TRB3 (forward: 5'-TGCGTCGCTTTGTCTTCAGCAACT-3', reverse: 5'-ATCCAGTCATCACGCAGGCATCTT-3'), PPAR-γ (forward: 5'-AGATCATCTACACCATGCTGGCCT-3'; reverse: 5-aggaactccctggtcatgaatcct-3'), SREBP-1c (forward: 5'-cgccacaatgccattgaga-3'; reverse: 5'-gcagatttattcagctttgcttca-3'), S26 (forward: 5'cgattcctgacaaccttgcta-3'; reverse: 5'cgtgcttcccaagctctatgt-3') and SYBR® green PCR buffer in an ABI Prism 7000 platform (Applied Biosystems). S26 was co-amplified as an endogenous normalizing gene. The specific primers sequences were designed from sequences available in geneBank (BLAST, NCBI). The following amplification parameters were set at different stages: stage 1 - one cycle at 50°C for 2 minutes, stage 2 - one cycle at 95°C for 10 minutes, stage 3 - 40 cycles at 95°C for 0.15 minutes and at 60°C for 1 minutes. To analyze the target genes expression, we used the ΔΔCT semi-quantitative method.[37] The expression levels were represented as the ratio of the signal intensity for each target mRNA relative to S26 mRNA.

4.4 | Assay of cytokines in tissues

IL-6 and TNF-α concentrations in adipose tissues (ie, EAT and RAT) and in skeletal muscle (ie, gastrocnemius) were determined in duplicate using commercially available rat

sensitive and ultrasensitive ELISA kits (R&D systems, Minneapolis), respectively. The assays were carried out according to the manufacturers' instructions. All data are expressed as pg of cytokine per mg of total tissue weight.

5 | CALCULATED VARIABLE

5.1 | Adiposity index

The EAT, RAT, and MAT adipose tissues were collected and weighed separately; these weights were then used to calculate the adiposity index as follows: (EAT + RAT + MAT) ÷ body weight × 100.[38]

5.2 | Statistical analysis

All data are expressed as the means ± SEM. The data regarding aerobic performance (ie, time to fatigue during the incremental tests) were analyzed using a mixed-design two-way analysis of variance (ANOVA), with the effect of group (trained vs control rats) representing a between-rats analysis and with the effect of moment (fourth vs third vs second vs first test) representing a within-rats analysis. This ANOVA was followed by the post-hoc Student-Newman-Keuls test. The remaining data, which were collected as single-point in time, were compared between groups using unpaired the Student t tests. The significance level was set at $\alpha < .05$.

6 | RESULTS

The training protocol improved aerobic performance, as evidenced by a 42% longer time to fatigue during the fourth incremental test in trained than in control rats (Figure 1). In contrast, time to fatigue was not changed in control rats when comparing the four incremental tests. During the 10-week period, the body weight gain and the weights of MAT, heart, liver, and gastrocnemius, and soleus muscles were not different between the two groups (Table 2). However, trained rats showed reduced weights of EAT and RAT, which were, respectively, decreased by 27% and 39% relative to control rats (Table 2). Thus, trained rats showed a lower adiposity index than control rats, despite the lack of intergroup differences in body weight (Table 2).

Aerobic training decreased the triacylglycerol content in gastrocnemius muscle by 20% (Table 3) and increased the activity of citrate synthase, a marker of oxidative burst, in soleus muscle by approximately 18% (Table 3). Regarding the metabolic parameters measured in serum, aerobic training did not change the concentrations of NEFA, triacylglycerol, total cholesterol and glucose, but
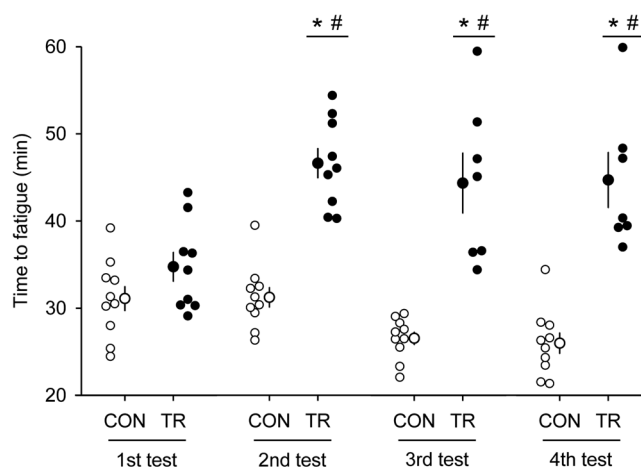

FIGURE 1 Effects of aerobic training on the time to fatigue (min) attained in the incremental tests at the beginning (first test), during (second and third tests) and at the end of the 10-week period (fourth test). The black symbols represent the trained group (TR; n = 9), whereas the white symbols represent the control group (CON; n = 10). Values are expressed as the means ± SEM; individual data are presented next to the mean group values. * Significantly different when compared to the control group in the same test ($P < .05$). #Significantly different when compared to the first incremental test in the trained group ($P < .05$)

decreased by 27% the concentration of insulin (Table 4). Regarding the tissue content of proinflammatory cytokines, IL-6, and TNF-α were increased by aerobic training in EAT and RAT, but not in gastrocnemius muscle (Figure 2A,B).

The effects of aerobic training on transcription factors related to lipid metabolism in different tissues were also investigated. Trained rats showed increased mRNA

TABLE 2 Body weight and weight of tissues in control and trained rats

	Control	Trained	P value
Initial body weight (g)	193 ± 3	190 ± 7	.61
Final body weight (g)	414 ± 15	407 ± 8	.73
Change in body weight (g)	221 ± 14	218 ± 11	.87
EAT (g)	4.9 ± 0.4	3.6 ± 0.2*	.01
MAT (g)	1.9 ± 0.3	1.5 ± 0.2	.21
RAT (g)	3.8 ± 0.5	2.3 ± 0.1*	.03
Adiposity index	2.5 ± 0.2	1.8 ± 0.1*	<.01
Cardiac muscle (g)	1.35 ± 0.02	1.36 ± 0.01	.94
Gastrocnemius muscle (g)	3.11 ± 0.05	2.97 ± 0.04	.48
Soleus muscle (g)	0.60 ± 0.03	0.59 ± 0.03	.72
Liver (g)	13.0 ± 0.2	13.9 ± 0.2	.33

Note: The data are represented as the means ± SEM of 8 to10 rats.
Abbreviations: EAT, epididymal adipose tissue; MAT, Mesenteric adipose tissue; RAT, retroperitoneal adipose tissue.
*$P < .05$ vs the control group.

TABLE 3 Citrate synthase activity in the soleus and triacylglycerol content in the gastrocnemius of control and trained rats

	Control	Trained	P value
Citrate synthase activity ($\mu mol \cdot mg^{-1} \cdot protein^{-1} \cdot min^{-1}$)	48.5 ± 1.8	58.7 ± 4.3*	.03
Triacylglycerol ($mg \cdot mL^{-1} \cdot g^{-1}$)	7.8 ± 0.5	6.2 ± 0.5*	.04

The data are represented as the means $\pm$ SEM of 8-10 rats.
*$P < .05$ vs the control group.

expression of SREBP-1c, a lipogenic transcription factor, in gastrocnemius muscle (103%), EAT (142%), and RAT (161%), but not in MAT (Figure 3A). In contrast, aerobic training decreased the expression of PPAR-γ in gastrocnemius muscle (50%), EAT (42%), and MAT (55%), but not in RAT (Figure 3B). Trained rats showed a higher expression of PPAR-α and CPT1 in gastrocnemius muscle (173% and 126%, respectively) and MAT (54% and 168%), but a lower expression in EAT (73% and 80%) and no changes in RAT (Figure 4A,B). At last, aerobic training decreased TRB3 expression by 60% in gastrocnemius muscle but failed to change the expression in EAT, RAT, and MAT (Figure 5).

7 | DISCUSSION

Our findings indicate that a 10-week aerobic training reduced the body adiposity, serum concentration of insulin and muscle content of triacylglycerol. Moreover, the trained rats exhibited higher concentrations of proinflammatory cytokines in adipose tissues, as well as several molecular adaptations in skeletal muscle and adipose tissue depots. Overall, the training-induced adaptations occurred in the direction of a facilitated FA oxidation, together with improved insulin sensitivity. Notably, the different metabolic tissues investigated, including the three adipose tissue depots (ie, epididymal, mesenteric, and retroperitoneal), exhibited distinct molecular adaptations in response to aerobic training, which may indicate that these tissues have particular metabolic

TABLE 4 Serum concentrations of glucose, insulin, triacylglycerol, nonesterified fatty acids, and total cholesterol in control and trained rats

	Control	Trained	P value
Glucose ($mg.dL^{-1}$)	132.8 ± 1.8	127.9 ± 3.3	.18
Insulin ($ng.mL^{-1}$)	2.2 ± 0.3	1.5 ± 0.2*	.05
Triacylglycerol ($mg.dL^{-1}$)	159.9 ± 19.1	159.6 ± 21.1	.99
Nonesterified fatty acids ($mmol.L^{-1}$)	0.7 ± 0.1	0.6 ± 0.1	.18
Total cholesterol ($mg.dL^{-1}$)	96.7 ± 4.4	93.1 ± 7.1	.66

The data are represented as the means $\pm$ SEM of 8-10 rats.
*$P < .05$ vs the control group.

machineries that underlie adaptation induced by repeated exposure to exercise. For instance, training increased the expression of transcription factors involved in FA oxidation in skeletal muscle and MAT but decreased expression in EAT.

The 10-week training, with sessions performed at 75% of the S_{MAX}, improved the performance (ie, time to fatigue) of trained rats relative to controls. In addition, the higher activity of citrate synthase observed in trained rats suggests improved FA oxidation in skeletal muscle in
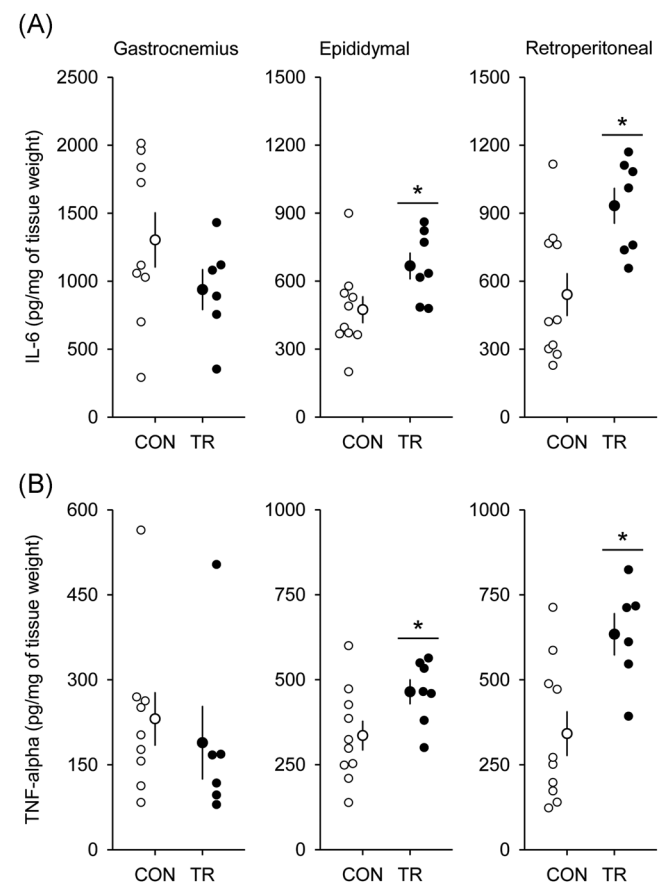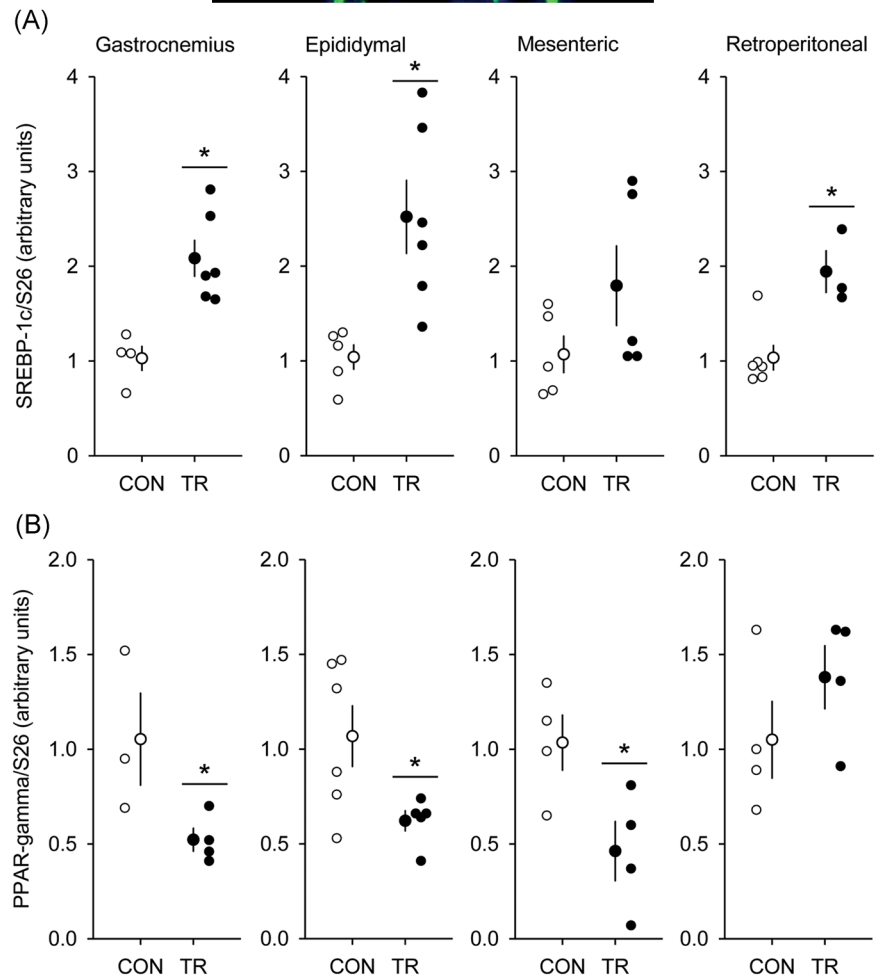

FIGURE 2 Effects of aerobic training on the concentrations of IL-6 (panel A) and TNF-α (panel B) in the gastrocnemius muscle, and in the epididymal and retroperitoneal adipose tissues. The black symbols represent the trained group (TR; n = 6-7), whereas the white symbols represent the control group (CON; n = 8-10). Values are expressed as the means $\pm$ SEM; individual data are presented next to the mean group values. *Significant different when compared to the control group ($P < 0.05$). IL-6, interleukin-6; TNF-α, tumor necrosis factor-α

response to training.[39] Indeed, aerobic training protocols performed during similar periods (8 weeks) and at similar intensities (~60%-85% of maximum speed) increased the speed at which the maximum rate of oxygen consumption was attained and reduced the gross oxygen cost of running[33]; altogether, these adaptations may explain the greater tolerance to aerobic exercise observed in our trained rats.

Despite not influencing body weight gain, the 10-week training markedly reduced the weight of two adipose tissues evaluated (ie, EAT and RAT), thereby decreasing body adiposity. Importantly, no changes in the weight of the heart, liver, and gastrocnemius, and soleus muscles were observed. Therefore, our data suggest that aerobic training preserved or even augmented lean body mass, although we did not measure this parameter. Reductions in adipose tissue weight and/or body adiposity are commonly reported following aerobic training consisting of a treadmill run.[40,41] For example, 6 weeks of training have been shown to reduce by 50% the weight of periuterine adipose tissue in female rats,[41] whereas an 8-week training lowered body adiposity, although it did not change the body weight of trained mice.[40]

The present findings revealed that training did not change the serum concentrations of triacylglycerol, NEFA, and total cholesterol. Additionally, although glucose concentration was not affected by training, insulin concentration was lowered.

A mechanism underlying aerobic training-induced amelioration of insulin sensitivity is associated with the expression of TRB3, a protein that promotes inhibition of insulin signaling pathway by binding to AKT. Previous studies showed a reduction in TRB3 gene expression in liver[24,42] and muscle[25] in animals after a physical exercise session. In this context, it is suggestive that inhibition of this protein plays a role in improving insulin sensitivity induced by regular exercise.[43] Accordingly, our trained rats showed a reduced TRB3 gene expression in gastrocnemius, a response that likely promoted a better muscle insulin sensitivity.[43]

Our trained rats also had lower triacylglycerol content in gastrocnemius muscle, which may have resulted from greater mitochondrial lipid oxidation. This adaptive response is likely a consequence of a facilitated transport of long-chain FAs into the mitochondria and a higher oxidative phosphorylation
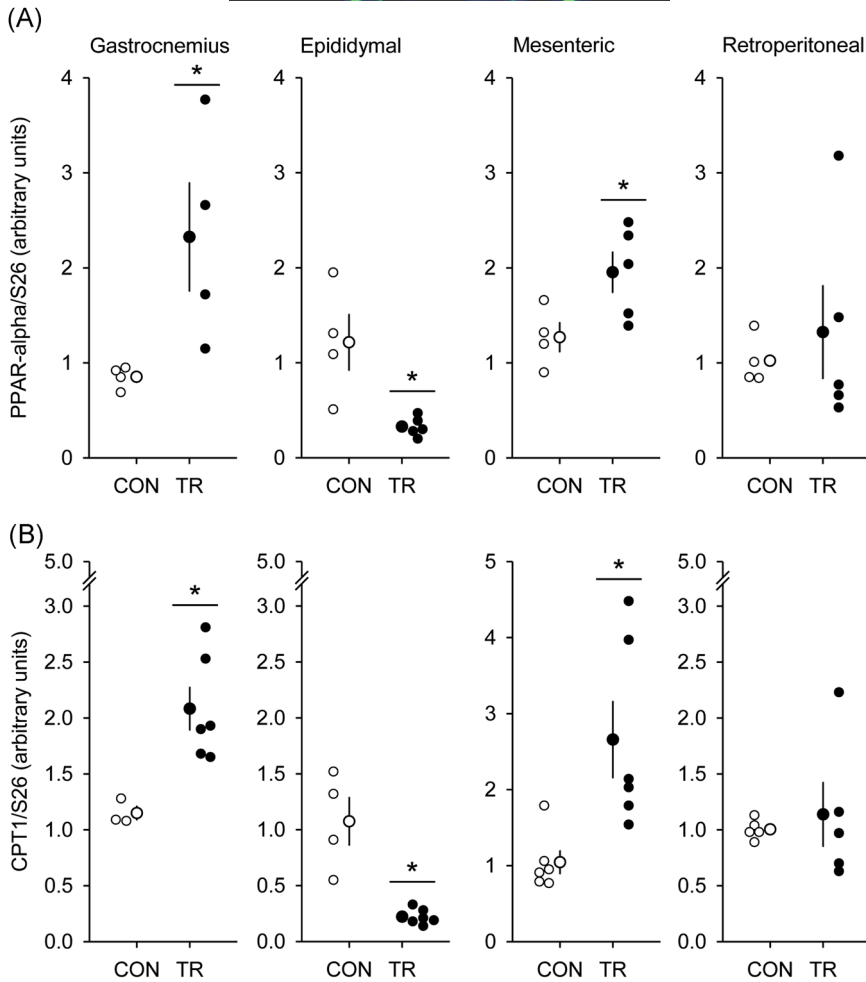
FIGURE 4 Effects of aerobic training on mRNA expression of PPARα (panel A) and CPT1 (panel B) in the gastrocnemius muscle, and in the epididymal, mesenteric, and retroperitoneal adipose tissues. The black symbols represent the trained group (TR; n = 4–6), whereas the white symbols represent the control group (CON; n = 3–6). Values are expressed as the means ± SEM; individual data are presented next to the mean group values. *Significant different when compared to the control group ($P < .05$). CPT1, carnitine palmitoyltransferase 1; mRNA, messenger RNA; PPARγ, peroxisome proliferator-activated receptor γ

dependent on the functioning of the tricarboxylic acid cycle, as evidenced, respectively, by greater expression of CPT1 and citrate synthase activity in the gastrocnemius of trained rats. There is evidence that higher muscle triacylglycerol content is associated with diminished insulin sensitivity.[44] As training reduced muscle triacylglycerol content, improved insulin sensitivity would be expected in the muscle of trained rats;
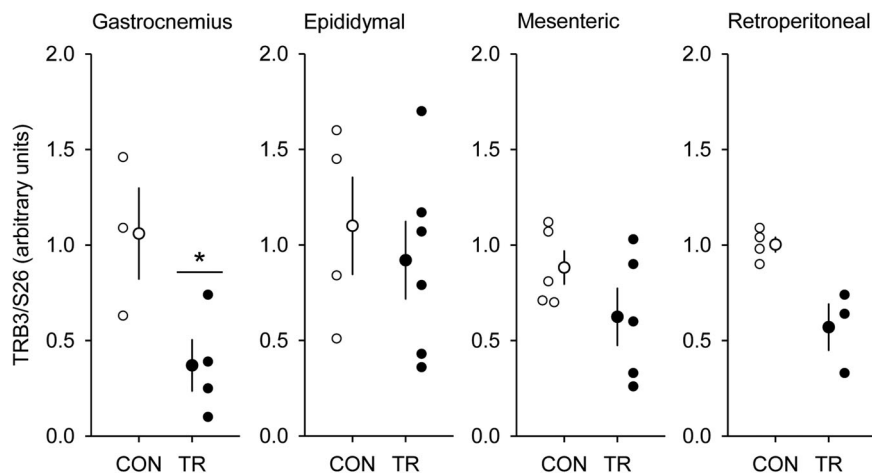

FIGURE 5 Effect of aerobic training on mRNA expression of TRB3 in the gastrocnemius muscle, and in the epididymal, mesenteric, and retroperitoneal adipose tissues. The black symbols represent the trained group (TR; n = 3–6), whereas the white columns represent the control group (CON; n = 3–5). Values are expressed as the means ± SEM; individual data are presented next to the mean group values. *Significant different when compared to the control group ($P < .05$). mRNA, messenger RNA; TRB3, tribbles 3

indeed, the lower serum insulin concentration may indirectly indicate increased tissue sensitivity.

The effects of aerobic training on transcription factors are not completely elucidated. In the present study, an increased SREBP-1c gene expression was observed in the adipose tissue (ie, EAT and RAT) and in gastrocnemius after the 10 weeks of training. This finding is similar to those reported by a study that subjected mice to a 2-week swimming training.[15] In contrast, PPARγ gene expression was reduced in all tissues that were analyzed in trained rats; this result agrees with data collected in the vastus lateralis of individuals subjected to a 9-day training on a cycle ergometer.[9] Overall, the changes in the expression of PPARγ and SREBP-1c observed in response to an aerobic training seems to allow muscles and adipose tissues to increase the storage of lipids-which is regulated by SREBP-1c[15]-without increasing adipogenic pathways-- which are regulated by PPARγ.[18]

Training-induced greater expression of PPARα and CPT1 in gastrocnemius and in one adipose tissue evaluated (ie, MAT). This finding, which is in agreement with findings reported for trained humans,[9] highlights the role of PPARα in regulating its target gene, CPT1. Interestingly, we observed lower expression of PPARα and CPT1 in EAT of trained rats. A possible explanation to this outcome is the marked reduction in EAT weight (27%) after the training protocol, which may have induced an adaptive response to reduce the activation of oxidative pathways, thereby avoiding exaggerated, non-physiological adipose weight loss in nonobese rats.

Our results showed that training increased the concentrations of TNF-α and IL-6 in the adipose tissue (ie, EAT and RAT), without concomitant changes in the gastrocnemius. Despite the fact that increased concentrations of some inflammatory cytokines, such as TNF-α and IL-6, have been traditionally related to a dysfunctional adipose tissue, recent studies have highlighted that these cytokines are also implicated in the physiological control of the intermediary metabolism.[45,46] These studies have shown that adipose tissue inflammation is required for a proper tissue remodeling (which includes quantitative and qualitative modifications in adipose tissue-resident cells) and, consequently, for a proper control of fat tissue expansion.[45-47] In addition, a lipolytic role for TNF-α in adipocytes[27] and for IL-6 in exercising muscle has also been demonstrated.[48] Thus, we suggest that the increase in proinflammatory cytokines in adipose tissue induced by training can be determinant for tissue remodeling. Nevertheless, additional studies are required to better explain the involvement of these cytokines on lipid metabolism, especially in adipose depots of trained rats.

The present study enhances the understanding of how chronic physical activity or physical training promotes several health benefits.[11,49] The adaptations occurring in muscle and white adipose tissues[40] justify, for example, the use of aerobic training as a nonpharmacological intervention to prevent the development of metabolic diseases.[11] During physical exercise sessions, the enhanced sympathetic outflow disrupts lipid homeostasis to attend the increased muscle demand for substrates.[50] Thus, repeated the physical exercise (or activity) impacts lipid homeostasis through the induction of the lipolytic pathway and inhibition of FA synthesis.[41] Indeed, our results are in line with the latter statement, but they also revealed site-specific adaptive responses (in the skeletal muscle and adipose tissues). The physiological significance of these site-specific adaptions should be addressed in further studies.

In summary, our data demonstrate that a 10-week aerobic training was effective in promoting adaptive responses aimed at improving muscle oxidative metabolism to attend the increased metabolic demand during physical exertion. Alongside the improved oxidative metabolism, training promoted adaptations favoring better muscle insulin sensitivity. Of note, the latter response was observed in nonobese and nondiabetic rats. Adaptive responses pointing to an inhibited synthesis and a facilitated degradation of FA were also observed in adipose tissues. These adaptations were distinct between specific adipose depots, suggesting a differential role of adipose tissues in metabolic changes induced by aerobic training.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests.

AUTHOR CONTRIBUTIONS

REFERENCES

1. Pedersen BK, Febbraio MA. Muscles, exercise and obesity: skeletal muscle as a secretory organ. *Nat Rev Endocrinol.* 2012;8:457-65.

2. Hoppeler H, Baum O, Lurman G, Mueller M. Molecular mechanisms of muscle plasticity with exercise. *Compr Physiol.* 2011;1:1383-412.

3. Starritt EC, Angus D, Hargreaves M. Effect of short-term training on mitochondrial ATP production rate in human skeletal muscle. *J Appl Physiol (1985).* 1999;86:450-454.

4. Wisdom KM, Delp SL, Kuhl E. Use it or lose it: multiscale skeletal muscle adaptation to mechanical stimuli. *Biomech Model Mechanobiol.* 2015;14:195-215.

5. Hurley BF, Nemeth PM, Martin WH, 3rd, Hagberg JM, Dalsky GP, Holloszy JO. Muscle triglyceride utilization during exercise: effect of training. *J Appl Physiol (1985).* 1986;60:562-567.

6. Jansson E, Kaijser L. Substrate utilization and enzymes in skeletal muscle of extremely endurance-trained men. *J Appl Physiol (1985).* 1987;62:999-1005.

7. Holloszy JO. Adaptation of skeletal muscle to endurance exercise. *Med Sci Sports.* 1975;7:155-164.

8. Kiens B, Essen-Gustavsson B, Christensen NJ, Saltin B. Skeletal muscle substrate utilization during submaximal exercise in man: effect of endurance training. *J Physiol.* 1993;469:459-78.

9. Tunstall RJ, Mehan KA, Wadley GD, et al. Exercise training increases lipid metabolism gene expression in human skeletal muscle. *Am J Physiol Endocrinol Metab.* 2002;283:E66-E72.

10. Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. *J Clin Endocrinol Metab.* 2004;89:2548-56.

11. Stanford KI, Middelbeek RJ, Goodyear LJ. Exercise effects on white adipose tissue: beiging and metabolic adaptations. *Diabetes.* 2015;64:2361-2368.

12. Bjorndal B, Burri L, Staalesen V, Skorve J, Berge RK. Different adipose depots: their role in the development of metabolic syndrome and mitochondrial response to hypolipidemic agents. *J Obes.* 2011;2011:490650.

13. Narita T, Kobayashi M, Itakura K, et al. Differential response to caloric restriction of retroperitoneal, epididymal, and subcutaneous adipose tissue depots in rats. *Exp Gerontol.* 2018;104:127-137.

14. Mandard S, Muller M, Kersten S. Peroxisome proliferator-activated receptor α target genes. *Cell Mol Life Sci.* 2004;61:393-416.

15. Ikeda S, Miyazaki H, Nakatani T, et al. Up-regulation of SREBP-1c and lipogenic genes in skeletal muscles after exercise training. *Biochem Biophys Res Commun.* 2002;296:395-400.

16. Tyagi S, Gupta P, Saini AS, Kaushal C, Sharma S. The peroxisome proliferator-activated receptor: a family of nuclear receptors role in various diseases. *J Adv Pharm Technol Res.* 2011;2:236-240.

17. Horowitz JF, Leone TC, Feng W, Kelly DP, Klein S. Effect of endurance training on lipid metabolism in women: a potential role for PPARα in the metabolic response to training. *Am J Physiol Endocrinol Metab.* 2000;279:E348-E355.

18. Farmer SR. Regulation of PPARγ activity during adipogenesis. *Int J Obes.* 2005;29(Suppl 1):S13-S16.

19. Kawamura T, Yoshida K, Sugawara A, et al. Regulation of skeletal muscle peroxisome proliferator-activated receptor γ expression by exercise and angiotensin-converting enzyme inhibition in fructose-fed hypertensive rats. *Hypertens Res.* 2004;27:61-70.

20. Ebrahimi M, Fathi R, Ansari Pirsaraei Z, Talebi-Garakani E, Najafi M. Expression of the key metabolic regulators in the white adipose tissue of rats; the role of high-fat diet and aerobic training. *Comparative Exercise Physiology.* 2018;14:6-277.

21. Neels JG, Olefsky JM. Cell signaling. A new way to burn fat. *Science.* 2006;312:1756-1758.

22. Du K, Herzig S, Kulkarni RN, Montminy M. TRB3: a tribbles homolog that inhibits Akt/PKB activation by insulin in liver. *Science.* 2003;300:1574-1577.

23. Koh HJ, Toyoda T, Didesch MM, et al. Tribbles 3 mediates endoplasmic reticulum stress-induced insulin resistance in skeletal muscle. *Nat Commun.* 2013;4:1871.

24. Lima AF, Ropelle ER, Pauli JR, et al. Acute exercise reduces insulin resistance-induced TRB3 expression and amelioration of the hepatic production of glucose in the liver of diabetic mice. *J Cell Physiol.* 2009;221:92-97.

25. Matos A, Ropelle ER, Pauli JR, Frederico MJ, dePinho RA, Velloso LA, DeSouza CT, et al. Acute exercise reverses TRB3 expression in the skeletal muscle and ameliorates whole body insulin sensitivity in diabetic mice. *Acta Physiol.* 2010;198:61-69.

26. Langin D, Arner P. Importance of TNFα and neutral lipases in human adipose tissue lipolysis. *Trends Endocrinol Metab.* 2006;17:314-20.

27. Botion LM, Brasier AR, Tian B, Udupi V, Green A. Inhibition of proteasome activity blocks the ability of TNFα to down-regulate G(i) proteins and stimulate lipolysis. *Endocrinology.* 2001;142:5069-75.

28. Kelly M, Keller C, Avilucea PR, et al. AMPK activity is diminished in tissues of IL-6 knockout mice: the effect of exercise. *Biochem Biophys Res Commun.* 2004;320:449-54.

29. Marin TL, Gongol B, Zhang F, et al. AMPK promotes mitochondrial biogenesis and function by phosphorylating the epigenetic factors DNMT1, RBBP7, and HAT1. *Sci Signal.* 2017;10:10.

30. Wanner SP, Guimaraes JB, Rodrigues LO, Marubayashi U, Coimbra CC, Lima NR. Muscarinic cholinoceptors in the ventromedial hypothalamic nucleus facilitate tail heat loss during physical exercise. *Brain Res Bull.* 2007;73:28-33.

31. Zanchi NE, Bechara LRG, Tanka LY, Debbas V, Bartholomeu T, Ramires PR. Efeitos do treinamento físico aeróbio sobe a bioatividade do óxido nítrico e a vasodilatação aórtica. *Rev Bras Educ Fís Esp.* 2006;20:239-247.

32. Soares DD, Lima NR, Coimbra CC, Marubayashi U. Evidence that tryptophan reduces mechanical efficiency and running performance in rats. *Pharmacol Biochem Behav.* 2003;74:357-62.

33. Teixeira-Coelho F, Fonseca CG, Barbosa NHS, et al. Effects of manipulating the duration and intensity of aerobic training sessions on the physical performance of rats. *PLOS One.* 2017;12:e0183763.

34. Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem.* 1957;226:497-509.

35. Crosson SM, Khan A, Printen J, Pessin JE, Saltiel AR. PTG gene deletion causes impaired glycogen synthesis and developmental insulin resistance. *J Clin Invest.* 2003;111:1423-32.

36. Alp PR, Newsholme EA, Zammit VA. Activities of citrate synthase and NAD + -linked and NADP + -linked isocitrate dehydrogenase in muscle from vertebrates and invertebrates. *Biochem J.* 1976;154:689-700.

37. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C (T)) Method. *Methods.* 2001;25:402-408.

38. Lacerda DR, Moraes MM, Nunes-Silva A, et al. Aerobic training reduces immune cell recruitment and cytokine levels in adipose tissue in obese mice. *Appl Physiol Nutr Metab.* 2018;44:512-520.

39. Vigelso A, Andersen NB, Dela F. The relationship between skeletal muscle mitochondrial citrate synthase activity and whole body oxygen uptake adaptations in response to exercise training. *Int J Physiol Pathophysiol Pharmacol.* 2014;6:84-101.

40. Higa TS, Spinola AV, Fonseca-Alaniz MH, Evangelista FS. Remodeling of white adipose tissue metabolism by physical training prevents insulin resistance. *Life Sci.* 2014;103:41-48.

41. Koh HJ, Hirshman MF, He H, et al. Adrenaline is a critical mediator of acute exercise-induced AMP-activated protein kinase activation in adipocytes. *Biochem J.* 2007;403:473-81.

42. Marinho R, Ropelle ER, Cintra DE, et al. Endurance exercise training increases APPL1 expression and improves insulin signaling in the hepatic tissue of diet-induced obese mice, independently of weight loss. *J Cell Physiol.* 2012;227:2917-2926.

43. Marinho R, Mekary RA, Munoz VR, Gomes RJ, Pauli JR, deMoura LP. Regulation of hepatic TRB3/Akt interaction induced by physical exercise and its effect on the hepatic glucose production in an insulin resistance state. *Diabetol Metab Syndr.* 2015;7:67.

44. Timmermans RJ, Saris WH, vanLoon LJ. [Insulin resistance: the role of intramuscular triglyceride and the importance of physical activity]. *Ned Tijdschr Geneeskd.* 2006;150:122-127.

45. Menezes-Garcia Z, Oliveira MC, Lima RL, et al. Lack of platelet-activating factor receptor protects mice against diet-induced adipose inflammation and insulin-resistance despite fat pad expansion. *Obesity (Silver Spring).* 2014;22:663-72.

46. Wernstedt Asterholm I, Tao C, Morley TS, et al. Adipocyte inflammation is essential for healthy adipose tissue expansion and remodeling. *Cell Metab.* 2014;20:103-18.

47. Choe SS, Huh JY, Hwang IJ, Kim JI, Kim JB. Adipose tissue remodeling: its role in energy metabolism and metabolic disorders. *Front Endocrinol.* 2016;7:30.

48. Ostrowski K, Rohde T, Zacho M, Asp S, Pedersen BK. Evidence that interleukin-6 is produced in human skeletal muscle during prolonged running. *J Physiol.* 1998;508(Pt 3):949-53.

49. Gollisch KS, Brandauer J, Jessen N, et al. Effects of exercise training on subcutaneous and visceral adipose tissue in normal- and high-fat diet-fed rats. *Am J Physiol Endocrinol Metab.* 2009;297:E495-E504.

50. Bosma M. Lipid homeostasis in exercise. *Drug Discov Today.* 2014;19:1019-23.