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ORIGINAL ARTICLE

Strength training and aerobic exercise alter mitochondrial parameters in brown adipose tissue and equally reduce body adiposity in aged rats

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Abstract

With aging, there is a reduction in mitochondrial activity, and several changes occur in the body composition, including increased adiposity. The dysfunction of mitochondrial activity causes changes and adaptations in tissue catabolic characteristics. Among them, we can mention brown adipose tissue (BAT). BAT's main function is lipid oxidation for heat production, hence playing a role in adaptive thermogenesis induced by environmental factors such as exercise. It is known that exercise causes a series of metabolic changes, including loss body fat; however, there is still no consensus in the academic community about whether both strength and aerobic exercise equally reduces adiposity. Therefore, this study aimed to evaluate the effects of strength training and aerobic exercise regimes on adiposity, proteins regulating mitochondrial activity, and respiratory complexes in BAT of old rats. The rats were divided in two control groups: young control (YC; $N = 5$), and old control (OC; $N = 5$), and two exercise groups: strength training (OST; $N = 5$), and aerobic treadmill training (OAT; $N = 5$). Rats were subjected to an 8-week exercise regime, and their body composition parameters were evaluated (total body weight, adiposity index, and BAT weight). In addition, mitochondrial biogenesis proteins (PGC-1α, SIRT1, and pAMPK) and respiratory chain activity (complexes I, II/III, III, and IV) were evaluated. Results showed that OST and OAT exercise protocols significantly increased the mitochondrial regulatory molecules and respiratory chain activity, while body fat percentage and adiposity index significantly decreased. Taken together, both OST and OAT exercise increased BAT weight, activity of respiratory complexes, and regulatory proteins in BAT and equally reduced body adiposity.

Keywords Aging · Physical exercise · Brown adipose tissue · Adiposity · Metabolism

Abbreviations

AMPK	Adenosine monophosphate-activated protein kinase
BAT	Brown adipose tissue
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetic acid
NADH	Nicotinamide adenine dinucleotide dehydrogenase
NRF-1	Nuclear respiratory factor 1
NRF-2	Nuclear respiratory factor 2
OAT	Aerobic treadmill training
OC	Old control
OST	Strength training
PGC-1α	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha
PMSF	Phenylmethylsulfonyl fluoride
ROS	Reactive oxygen species
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SIRT1	Sirtuin 1
SNS	Sympathetic nervous system

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VEGF Vascular endothelial growth factor
YC Young control

Introduction

Human life expectancy has been steadily increasing worldwide over the years in both developed and developing countries. This phenomenon is associated with a number of factors, such as medical and technological development, greater awareness about health-related aspects, and greater access to health-related information. As aging increases vulnerability to pathologies, the rate of age-related complications increases together with aging [26]. Aging is a hard-wired process in all living beings and is characterized by increased adiposity and declined lifespan [26]. Many interventions, like physical exercise and diet, may reduce aging and increase lifespan. It is known that physical exercise modulates numerous proteins to prevent the aging process. However, studies concerning exercise-induced adaptations at the mitochondrial level in the adipose tissue have long been neglected. Only in recent years, studies have started to focus on the role of mitochondria in adipose tissues [1, 3]. Adipose tissue is a large endocrine, immune, and regenerative organ that can readily adapt to thermal changes and nutrient availability in young and healthy individuals. Several age-related fundamental changes occur at the cellular level in adipose tissue, potentially evolving in adipose tissue dysfunctions, including adipocyte hypertrophy [4]. Physical exercise can influence adipose tissue activity to prevent age-related complications like obesity and insulin resistance, which are associated with shortened lifespan and geriatric disorders.

Mitochondria are crucial in regulating the energy metabolism of many tissues, including the cardiac and skeletal muscle, brain, liver, and adipose tissue. Evidence suggests that mitochondria in the adipose tissues are involved in the whole-body energy homeostasis and cross-talk with striated muscles [1, 3]. Mitochondrial dysfunction in the adipocytes could lead to whole-body pathological consequences [3]. It is not surprising that mitochondrial dysfunctions contribute to metabolism and adipocyte differentiation, as they are involved in the regulation of fatty acid oxidation, oxidative phosphorylation, and reactive oxygen species (ROS) production [3]. However, there are a large number of factors yet to be investigated in relation to mitochondrial and adipose functions. Several research works have pointed out that three important proteins (AMP-activated protein kinase—AMPK, Sirtuin 1—SIRT1, and peroxisome proliferator-activated receptor gamma coactivator 1-alpha—PGC-1α) are involved in regulating the mitochondrial function, but during aging, the functioning of these molecules might diminish [5, 6, 13]. Therefore, a defect occurs in the mitochondrial biogenesis [34]. Regular exercise training has been known to induce these proteins by mimicking their pathway even in the aging [6, 13]. However, the life-extending effect of these molecules by physical exercise in brown adipose tissue (BAT) has not been clearly understood. Therefore, the present study aimed to investigate the effects of both strength and aerobic exercise protocols on body fat compositions and their resulting adaptations on mitochondrial regulatory molecules in BAT.

Materials and methods

Experimental design

The animals were divided into four groups (young groups were 3 months old and old groups were 18 months old), namely young control group—untrained (YC; $N = 5$), old control group—untrained (OC; $N = 5$), old strength training group (OST; $N = 5$), and old aerobic treadmill running group (OAT; $N = 5$). All the animals were obtained from the Animal Centre of the University of Extremo Sul Catarinense (Criciúma, Brazil). Five animals per cage were housed under a 12-h light/12-h dark cycle/(light from 7 a.m. to 7 p.m.) with free access to food and water ad libitum. The study protocol was reviewed and approved by the local ethics committee according to the Guidelines for Animal Care and Experimentation (number 16/2013).

Exercise protocols

Aerobic treadmill running training

All the rats were accustomed by physical exercise on a nine-channel motor-driven treadmill at a speed of 10 m/min for 10 min/day for 1 week to reduce their stress in response to the new experimental environment. Dynamic aerobic exercise training, prescribed based on the maximal exercise test, was performed at low/moderate intensity (~ 50 to 70% of the maximal running speed during the maximal exercise test) for 13 to 17 min a day, from 7 to 8 p.m., 4 days a week for 8 weeks, with a gradual increase in speed from 0.6 to 1.2 km/h during a total period of 60 days. Each session lasted 50 min and there was a 48-h interval between sessions. Gentle tapping of the tail or hind limb was used to encourage running if the animal stopped.

Resistance training of muscular strength

The strength training entailed that the rats climbed a 1-m ladder with a 2-cm grid inclined at 85° [15], with some modifications according to Scheffer et al. [31]. The rats were familiarized with the given exercise for 1 week. Later, resistance training began. For this protocol, cylinders containing weights were attached with foam tape to the base of the rat's tail.

Briefly, the cylinders were fastened to the tail by wrapping the upper portion of the tail (2–3 cm from the proximal end) with Velcro on top of the foam tape. Then, the initial weights (25% of body weight) were inserted into the cylinders. The rat was then positioned at the base of the climbing apparatus and manually stimulated [35–37]. The weight attached to the tail was gradually increased from 50 to 100% of total weight throughout the 8 weeks of training (weeks 1 and 2, 50%; weeks 3 and 4, 50%; weeks 5 and 6, 75%; weeks 7 and 8, 100%). Three to five sets of 8–12 repetitions, with a 1-min rest between repetitions and a 2-min rest between sets, were performed for 3 or 4 days/week. Each session was 40–50 min in duration, with a 48-h interval between sessions. After reaching the top of the ladder, the rats were allowed to recover in a resting area. This procedure was repeated until the rats finished three sets of training or they failed to climb the entire length of the ladder.

Western blot

After 48 h from the last exercise session, all animals were anesthetized by intraperitoneal administration of ketamine (80 mg/kg) and xylazine (12 mg/kg) and subsequently euthanized. White adipose tissues and BATs were extracted and immediately homogenized in specific buffer containing 1% Triton X-100, 100 mM Tris (pH 7.4), 100 mM sodium pyrophosphate, 100 mM sodium fluoride, 10 mM ethylenediaminetetraacetic acid (EDTA), 10 mM sodium vanadate, 2 mM phenylmethylsulfonyl fluoride (PMSF), and 0.1 mg/mL aprotinin at 4 °C with Polytron MR 2100 (Kinematica, Switzerland). The homogenate was centrifuged at 11,000 rpm for 30 min at 4 °C. The total protein concentration from the supernatant was determined by a colorimetric test using the method by Lowry et al. [22]. The proteins were resuspended and stored in Laemmli buffer containing 100 mmol/L dithiothreitol (DTT) and subsequently assayed for immunoblotting with specific antibodies. To this end, aliquots containing 250 μg of proteins per sample were transferred onto a polyacrylamide gel. Electrophoresis was performed in a Mini-PROTEAN® Tetra electrophoresis system (Bio-Rad, Hercules, CA, USA), with electrophoresis buffer solution. Proteins separated by SDS-PAGE were transferred to the nitrocellulose membrane using a Mini Trans-Blot® Electrophoretic Transfer Cell (Bio-Rad) equipment. Nitrocellulose membranes containing the transferred proteins were incubated in blocking solution for 2 h at room temperature to decrease non-specific protein binding. Membranes were then incubated with specific primary antibodies: anti-PGC1α (Cell Signaling Biotechnology, Beverly, MA, USA); anti-pAMPK, and anti-SIRT1 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) under constant stirring, overnight at 4 °C. Original membranes were re-blotted with β-actin as control protein and then incubated in solution with peroxidase-conjugated secondary antibody for 2 h at room temperature. Then, membranes were incubated for 2 min with the enzymatic substrate and exposed to the RX film in a cassette for development. Intensity and area of the bands were captured using a scanner (HP G2710) and then quantified through the Scion Image program (Scion Corporation, Frederick, MD, USA).

Determination of total body weight

After euthanasia, epididymal, retroperitoneal, and mesenteric adipose tissues and brown adipose tissue were extracted and weighed using an analytical scale looking for differences between groups. To collect different fat types, we sprayed ethanol around 1–2 cm below the neck and cut with scissors. At this point, a pad of white fat (WAT) was observable. We then removed the butterfly-shaped brown fat. Without removing the WAT, we picked with forceps the extreme lower of it, pulled it up, and isolated all the area without leaving any fat. Then, we placed all the tissue on a clean surface and used a blade to separate the WAT. The brown fat was recognizable due to its dark brown color. After weighing the BAT with the precision scale, the body BAT percentage was calculated by dividing the total body weight for the BAT weight in each rat and then converted into percentage.

Activities of electron transport chain enzymes

The samples were frozen and defrosted thrice in hypotonic assay buffer to fully expose the enzymes to substrates and achieve maximal activities. NADH dehydrogenase (complex I) activity was analyzed by measuring the rate of NADH-dependent ferricyanide reduction as absorbance at 420 nm [7]. Complex II/III activities were measured via cytochrome c reduction by succinate [11]. The activity of cytochrome c oxidase (complex IV) was instead assayed as a decrease in absorbance at 550 nm due to oxidation of previously reduced cytochrome c [29].

Statistical analysis

Data on biochemical and body measurements are expressed as means ± SEM. Blot analysis data are expressed as means ± SEM, of densitometric units (molecule protein levels/β-actin protein levels ratio). Differences between groups were evaluated using one-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test. *P* value of less than 0.05 was considered significant. Statistical Package for the Social Sciences (SPSS) version 16.0 for Windows was used for data analysis.

Results

The present study evaluated the body composition in old rats that were exposed to aerobic exercise and strength training by using a climbing apparatus (Fig. 1). Old rats had significantly greater body weight than their younger counterparts, and two strength training and aerobic exercise protocols promote no alteration to this parameter. The body fat was significantly reduced in trained rats as compared to that in the untrained ones. Both epididymal and mesenteric fat percentages, and adiposity index, were significantly higher in the OC group than in the YC group, whereas BAT percentage was significantly decreased in the OC when compared to the YC, OAT, and OST groups (Table 1). Conversely, in the OST and OAT groups, there was a significant decrease in such parameters (epididymal, retroperitoneal and mesenteric fats, and adiposity index), although without any statistical distinction between them. However, these trained groups were not able to revert the adipose tissue level in comparison to untrained old rats (Table 1).

The effect of two exercise protocols in promoting mitochondrial regulatory proteins in old rats was also evaluated. As shown in Fig. 2, PGC-1α (Fig. 2a), pAMPK (Fig. 2c), and SIRT1 (Fig. 2c) levels were increased significantly in OST and OAT groups in comparison with YC and OC groups.

Fig. 1 The rat was positioned at the base of the climbing apparatus and motivated manually to climb the ladder. Resistance training began by using cylinders containing weights that were attached with foam tape to the base of the tail of the rat. Briefly, the cylinders were fastened to the tail by wrapping the upper portion of the tail (2–3 cm from the proximal end) with Velcro on top of the foam tape

The physical exercise intervention on mitochondrial activity was analyzed in BAT of old rats. Activity of electron transport complexes (I, II/III, III, and IV) in the OC group showed a significant decrease as compared to the YC one, whereas OAT and OST groups showed a significant increase in the activity of respiratory chain complexes (Fig. 3a–d). The OAT group showed a greater increase in the activity of complex I, II/III, and IV, whereas OST and OAT groups both showed an increase in the complex III activity without statistical significance in comparison with YC and OC groups.

Discussion

This study was designed to demonstrate the effects of two different exercise protocols (OST and OAT) in aged rats. We observed that aged rats showed an increase in epididymal, retroperitoneal, and mesenteric fat, as well as adiposity index and increased total body weight, whereas young rats had lower percentages. Furthermore, both aerobic and strength exercise protocols decreased body weight and fat index percentage of the old rats. However, aerobic training was shown to be effective in reducing the percentage of the epididymal, and mesenteric fats and adiposity index. Kohrt et al. [18] reported that regular exercise lowered the increased body fat due to aging. Similar results were observed by other researchers [17, 39]. Increased storage of fat in the body is a major factor in age-related complications. Regular training increased the metabolic activity in aged rats and prevented body fat storage. The present study shows that both the exercise protocols decreased body weight and fat percentage in aged rats, which proves the involvement of exercise in the energy expenditure to reduce age-related complications.

Brown adipose tissue (BAT) is crucial in energy expenditure of the body to counteract obesity-related morbidity. The effect of physical exercise on BAT is contradictory, and this could be due to different exercise protocols, duration, and type of exercise, including running and swimming [30]. The present study shows that OST and OAT increased the levels of BAT in old rats. BAT activity is sustained by the sympathetic nervous system (SNS). Studies have found that physical exercise activates the SNS on BAT recruitment and thermogenic activity [5, 30]. The present data support that both the exercises could increase the delivery of oxygen due to SNS activation. This is further confirmed by the increased activation of PGC-1α by both OST and OAT, which might facilitate the vascularization by regulating the VEGF expression. Bostrom et al. [2] found that the overexpression of PGC-1α induced the BAT gene program. Moreover, they found that VEGF was upregulated after endurance exercise by PGC-1α. Our study corroborated their findings that exercise increases BAT. However, young rats had a higher percentage of BAT than

Table 1 Effects of physical exercise protocols on physiological parameters of old rats. Total body weight, epididymal, retroperitoneal, mesenteric, and brown adipose fat pad and adiposity index are express as percentage of body weight (% of b.w.)

Parameters	YC	OC	OST	OAT
Body weight (g)	278.8 ± 5.28	497.8 ± 19.93*	467.2 ± 24.81*	459.2 ± 10.50*
Epididymal fat (%)	2.47 ± 0.18	10.76 ± 0.56*	7.37 ± 1.13#	6.41 ± 0.45#
Retroperitoneal fat (%)	1.60 ± 0.04	11.22 ± 1.73*	6.55 ± 1.30#	6.32 ± 0.80#
Mesenteric fat pad (%)	1.96 ± 0.14	7.29 ± 0.80*	4.38 ± 0.56#	4.02 ± 0.23#
Adiposity index (%)	2.55 ± 0.11	6.90 ± 0.09*	4.85 ± 0.55#	4.17 ± 0.31#
BAT (%)	0.11 ± 0.005	0.06 ± 0.004*	0.09 ± 0.005#	0.09 ± 0.005#

OST, old strength training; *OAT*, old aerobic training

*$p < 0.05$ compared to the young control group

#$p < 0.05$ compared to the old control group

old ones that underwent exercise. This could be associated with the body weight and adiposity ratio.

The proper activity of adipose tissue requires well-functioning mitochondria. Adipose tissue performs a number of functions such as lipolysis, fatty acid synthesis, and fatty acids beta-oxidation, and hence, adipose tissue needs more ATP to maintain its normal activity. Adipose tissue dysfunction occurs with aging due to reduced production of mitochondrial regulatory proteins, and thus this study aimed to investigate the effect of physical exercise on mitochondrial regulatory proteins (PGC-1α, SIRT1, and AMPK) in adipose tissue. Early studies reported that overexpression of PGC-1α increased the mitochondrial production and respiratory rates [20, 40]. In order to perform such functions, it co-activates multiple transcription factors such as glucocorticoid receptors, nuclear respiratory factors (NRF-1 and NRF-2), and estrogen-related receptors. In this study, we observed the increased activity of PGC-1α in both the exercise protocols, while aging rats without exercise had decreased PGC-1α content. As we discussed earlier, due to aging, the production of PGC-1α declines, but any stimuli like exercise can induce PGC-1α activation through de-acetylation. However, prolonged exercise alters mitochondrial dynamics by affecting the

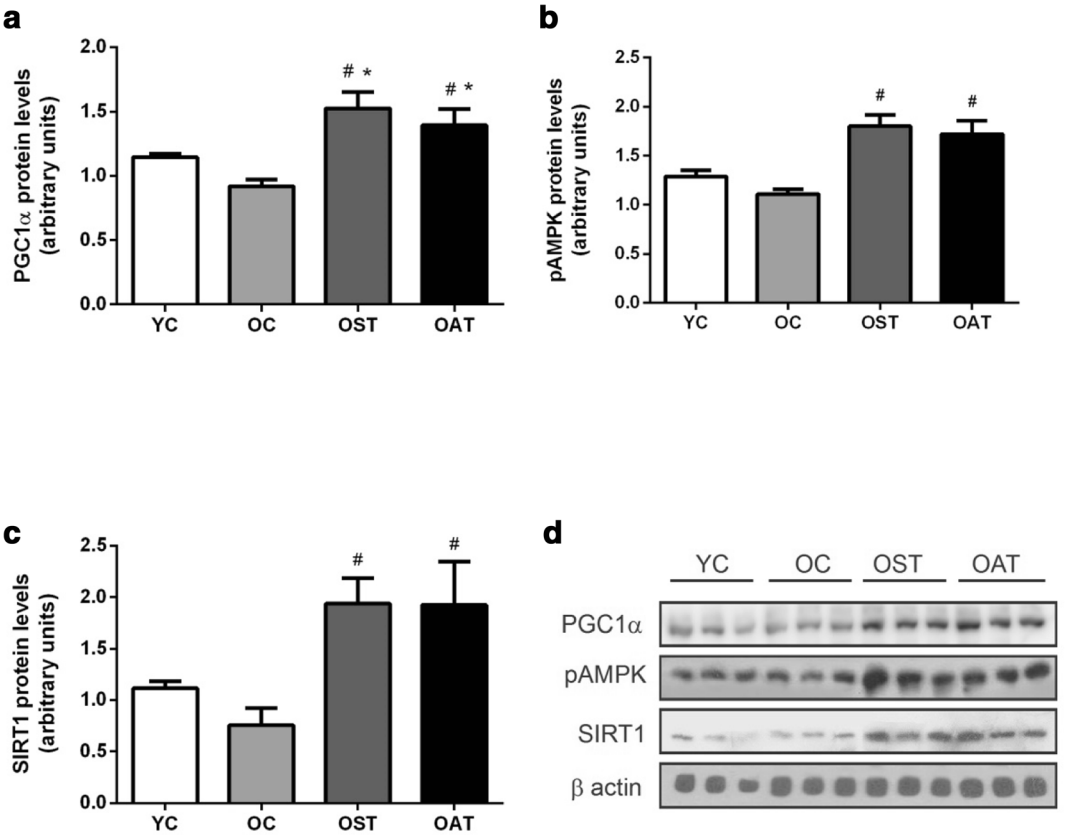

Fig. 2 Effects of different physical exercise protocols on mitochondrial regulatory molecules in brown adipose tissue of old rats. PGC1-α (**a**). pAMPK (**b**). SIRT1 (**c**). OST, old strength training; OAT, old aerobic training. *$p < 0.05$ compared to the young control group; #$p < 0.05$ compared to the old control group

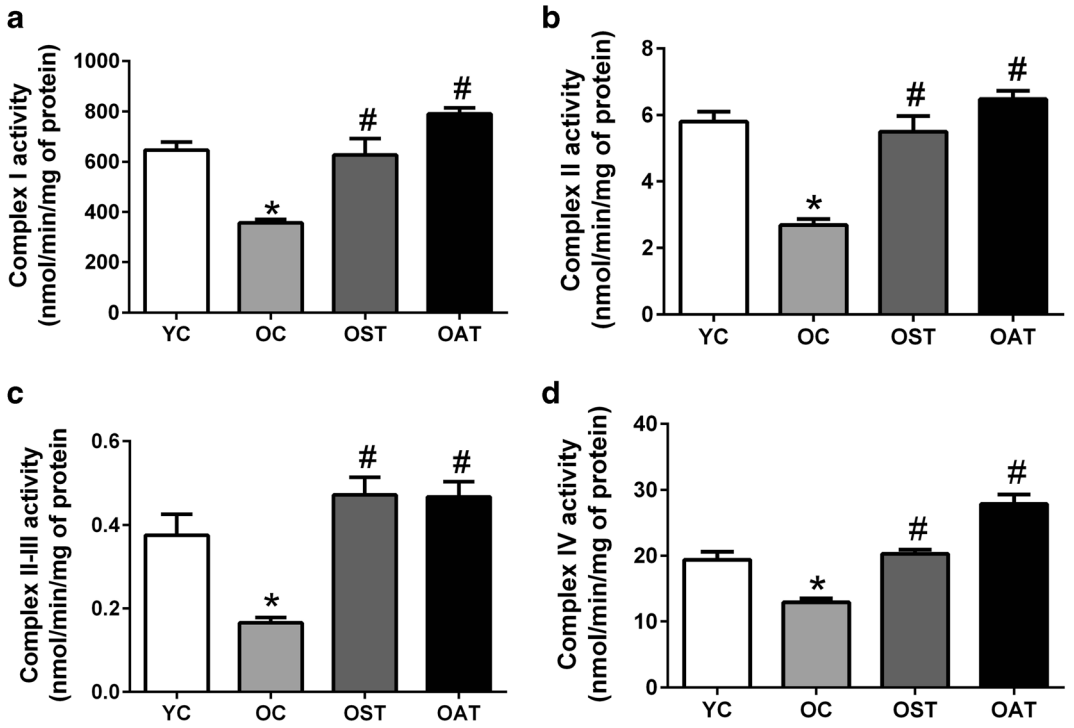

Fig. 3 Effects of exercise protocols on respiratory chain activities of old rats. Activity of the complex I (**a**). Activity of complex II (**b**). Activity of complex II/III (**c**). Activity of complex IV (D). OST, old strength training; OAT, old aerobic training. $N = 5$ rats per group. $^{*}p < 0.05$ compared to the young control group; $^{\#}p < 0.05$ compared to the old control group

mitochondrial biogenesis. In addition, longer endurance exercise promotes the biogenesis, fusion, fission, and mitophagy which may result in increased *ex-novo* mitochondria formation and possibly helps to remove damaged or dysfunctional mitochondria, thus improving metabolic activity. Our study is in agreement with the study of Suwa et al. [33] who reported that exercise increased PGC-1α activation due to de-acetylation process. The activity of PGC-1α, determined not only by its expression, but also by a number of post-translational modifications, including phosphorylation and acetylation [28]. However, a number of studies reported that a decrease in post-translational modifications is associated with aging [8]. The present data suggest that the redundant activity of PGC-1α due to aging is possibly reversed by the exercise-induced de-acetylation process. Moreover, dysfunctional mitochondria due to aging which may cause poor metabolic activity can be reprogrammed by an exercise stimulus. Canto and Auwerx [6] described that physical activity intervenes the metabolic sensors like AMPK and SIRT1, thereby affecting the activity of PGC-1α through phosphorylation and de-acetylation, respectively.

It has been considered that physical exercise is associated with the metabolic activity of adipose tissue to regulate the energy deficit. AMPK and SIRT1 are the important regulators of energy status. The link between exercise and these two proteins in energy homeostasis is a crucial step in adipocyte differentiation [12, 14, 27]. Therefore, this study aimed to observe the linking mechanism of AMPK and SIRT1 in BAT of old rats. Studies have shown that physical exercise activated AMPK and SIRT1, but this activation may directly phosphorylate the PGC-1α by AMPK, or increased AMPK by physical exercise inducing the activation of SIRT1 for further de-acetylation of PGC-1α to regulate the energy expenditure for increasing mitochondrial biogenesis [12, 13, 24]. Both the exercise protocols performed in this study augmented AMPK and SIRT1 levels in order to increase the PGC-1α. As a consequence, mitochondrial activity increased in old rats, and this was further confirmed by increased respiratory complex activities. Taken together, exercise is a converging factor of AMPK and SIRT1 on PGC-1α to increase the mitochondrial biogenesis.

Aging is associated with lower mitochondrial protein synthesis rates, disturbances in mitochondrial enzyme activities, and lower oxidative capacity [16]. In this study, we observed that the OC group had significantly low complex I (Fig. 3a), complex II (Fig. 3b), complex III (Fig. 3c), and complex IV (Fig. 3d) levels as compared to YC group. Studies have shown that old rats have reduced mitochondrial complex activities, whereas other studies have reported that there is no age-related decrease in ETC activities [4, 9, 10]. The results of the present study showed that both OST and OAT promoted a significant

increase in respiratory chain activity in all complexes as compared to the OC group, and OAT proved to be more effective in complexes I, II/III, and IV. In complexes II/III (Fig. 3b) and III (Fig. 3c), both types of exercise promoted significant increases in respiratory chain activity as compared to old rats, however, with no significant difference between them in complex III activity. Several studies suggested that the decline in the function of mitochondria due to aging is partially normalized by exercise [19, 38]. Moreover, the poor performance of mitochondria due to aging in adipose tissue induces prematurely aged BAT cells. BAT cells are highly vascularized tissues involved in maintaining homeostasis. The increased amount of BAT cells is related to lower body weight. BAT cells are present in a considerable amount in adults, and aging decreases the number of BAT cells and increases the body weight. Importantly, the activity of BAT is not only decreased during aging but also in obesity and other aging-related problems [25]. BAT cell mitochondria are functionalized by uncoupling protein 1 (UCP1) which allows the translocation of protons to dissipate the energy [21], which are negatively correlated with aging and obesity. Studies have shown that regular exercise increased the energy expenditure by regulating BAT in order to control the obesity-related problems in aging [21, 33]. In the context of aging, mitochondrial enzyme expression is reduced in old mice of BAT [23]. Moreover, BAT specific dysfunctions impact the whole-body energy homeostasis in relation to aging. Also, BAT physiology affect molecular pathways that regulate lifespan. For example, SIRT1 levels decline with aging in several tissues, including adipose tissue, and this reduction lead to accelerate the age induced obesity. Mitochondria is the core functional unit in metabolic control of many cells, including BAT. BAT is the origin and target of many extra and intra cellular signals and it relies on mitochondrial function for maintaining intracellular metabolism. Since SIRT1, AMPK, and PGC-1α highly influence the mitochondrial content and functionality, exercise could revert their activities partly or fully in order to postpone the aging, particularly in BAT cells [32]. In this study, both OST and OAT increased the levels of SIRT1, AMPK, and PGC-1α, suggesting a regulatory effect of exercise on mitochondrial enzymes. In addition, we speculated whether the given anesthesia in adipose tissue could influence our results. However, the time between anesthesia application and tissue removal is very short (approximately 2 min) and cannot be sufficient to alter the results in the BAT cells induced by exercises.

Conclusions

In conclusion, both OST and OAT exercises promoted greater weight BAT and increased mitochondrial complex activities and mitochondrial regulatory proteins (PGC-1α, SIRT1, and pAMPK) in BAT, and equally reduced body adiposity. These findings may be one of the mechanisms through which exercise regulates the proper activity of adipose tissue and maintains the normal functioning of mitochondria in order to reduce aging-related body adiposity.

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Compliance with ethical standards

The study protocol was reviewed and approved by the local ethics committee according to the Guidelines for Animal Care and Experimentation (number 16/2013).

Conflict of interest The authors declare that they have no conflicts of interest.

References

1. Bernlohr DA (2014) Exercise and mitochondrial function in adipose biology: all roads lead to NO. Diabetes 63(8):2606–2608
2. Bostrom P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, Rasbach KA, Boström EA, Choi JH, Long JZ et al (2012) A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature 481:463–468
3. Boudina S, Graham TE (2014) Mitochondrial function/dysfunction in white adipose tissue. Exp Physiol 99(9):1168–1178
4. Bratic A, Larsson NG (2013) The role of mitochondria in ageing. J Clin Invest 123(3):951–957
5. Cannon B, Nedergaard J (2004) Brown adipose tissue: function and physiological significance. Physiol Rev 84(1):277–359
6. Canto and Auwerx (2009) PGC-1alpha, SIRT1 and AMPK, an energy sensing network that controls energy expenditure. Currr Opin Lipidol 20(2):98–105
7. Cassina A, Radi R (1996) Differential inhibitory action of nitric oxide and peroxynitrite on mitochondrial electron transport. Arch Biochem Biophys 328(2):309–316
8. Cloos PA, Christgau S (2004) Post-translational modification of proteins: implications for aging antigen recognition, and autoimmunity. Biogerontology 5(3):139–158
9. Cocco T, Sgobbo P, Clemente M, Lopriore B, Grattagliano I, Di Paola M, Villani G (2005) Tissue specific changes of mitochondrial functions in aged rats: effect of a long-term dietary treatment with N-acetylcysteine. Free Radic Biol Med 38:796–805
10. Davies SM, Poljak A, Duncan MW, Smythe GA, Murphy MP (2001) Measurements of protein carbonyls, ortho and meta-tyrosine and oxidative phosphorylation complex activity in mitochondria from young and old rats. Free Radic Biol Med 31:181–190
11. Fischer JC, Ruitenbeek W, Stadhouders AM, Trijbels JMF, Sengers RCA, Janssen AJM, Veerkamp JH (1985) Investigation of mitochondrial metabolism small human skeletal muscle biopsy specimens. Clin Chim Acta 145:89–100

12. Fulco M, Sartorelli V (2008) Comparing and contrasting the roles of AMPK and SIRT1 in metabolic tissues. Cell Cycle 7(23):3669–3679
13. Gerhart-Hines Z, Rodgers JT, Bare O, Lerin C, Kim SH, Mostoslavsky R, Alt FW, Wu Z, Puigserver P (2007) Metabolic control of muscle mitochondrial function and fatty acid oxidation through SIRT1/PGC-1 alpha. EMBO J 26(7):1913–1923
14. Habinowski SA, Witters LA (2001) The effects of AICAR on adipocyte differentiation of 3T3-L1 cells. Biochem Biophys Res Commun 286(5):852–856
15. Hornberger TA Jr, Farrar RP (2004) Physiological hypertrophy of the FHL muscle following 8 weeks of progressive resistance exercise in the rat. Can J Appl Physiol 29(1):16–31
16. Igbal S, Ostojic O, Singh K, Joseph AM, Hood DA (2013) Expression of mitochondrial fission and fusion regulatory proteins in skeletal muscle during chronic use and disuse. Muscle Nerve 48: 963–970
17. Kim HS, Kim DG (2013) Effect of long-term resistance exercise on body composition, blood lipid factors, and vascular compliance in the hypertensive elderly men. J Exerc Rehabil 9(2):271–277
18. Kohrt WM, Malley MT, Dalsky GP, Holloszy JO (1992) Body composition of healthy sedentary and trained, young and older men and women. Med Sci Sports Exerc 24(7):832–837
19. Konopka AR, Miranda Suer K, Christopher Wolff A, Matthew Harber P (2014) Markers of human skeletal muscle mitochondrial biogenesis and quality control: effect of age and aerobic exercise training. J Gerontol A Biol Sci Med Sci 69(4):371–378
20. Lagouge M, Argmann C, Gerhart-Hines Z, Meziane H, Lerin C, Daussin F, Messadeq N, Milne J, Lambert P, Elliott P, Geny B, Laakso M, Puigserver P, Auwerx J (2006) Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1 alpha. Cell 127(6):1109–1122
21. Lo KA, Sun L (2013) Turning WAT into BAT: a review on regulators controlling the browning of white adipocytes. Bio Sci Rep 33(5):e00065
22. Lowry O, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193(1): 265–275
23. Mennes E, Dungan CM, Frendo-Cumbo S, Williamson DL, Wright DC (2014) Aging-associated reductions in lipolytic and mitochondrial proteins in mouse adipose tissue are not rescued by metformin treatment. J Gerontol A Biol Sci Med Sci 69:1060–1068
24. Niederberger E, King TS, Russe OQ, Geisslinger G (2015) Activation of AMPK and its impact on exercise capacity. Sports Med 45(11):1497–1509
25. Peng XR, Gennemark P, O'Mahony G, Bartesaghi S (2015) Unlock the thermogenic potential of adipose tissue: pharmacological modulation and implication for treatment of diabetes and obesity. Front Endocrinol 6:174
26. Picard F, Guarente L (2005) Molecular links between aging and adipose tissue. Int J Obes 29:S36–S39
27. Picard F, Kurtev M, Chung N, Topark-Ngarm A, Senawong T, Machado De Oliveira R, Leid M, McBurney MW, Guarente L (2004) Sirt1 promotes fat mobilization in white adipocytes by repressing PPAR-gamma. Nature 429(6993):771–776
28. Puigserver P, Rhee J, Lin J, Wu Z, Yoon JC, Zhang CY, Krauss S, Mootha VK, Lowell BB, Spiegelman BM (2001) Cytokine stimulation of energy expenditure through p38 MAP kinase activation of PPAR gamma coactivator-1. Mol Cell 8:971–982
29. Rustin P, Chretien D, Gerard B, Bourgeron T, Rotig A, Saudubray JM, Munnich A (1994) Biochemical and molecular investigations in respiratory chain deficiencies. Clin Chim Acta 228:35–51
30. Sanchez-Delgado G, Martinez-Tellez B, Olza J, Aguilera CM, Gil A, Ruiz JR (2015) Role of exercise in the activation of brown adipose tissue. Ann Nutr Metab 67(1):21–32
31. Scheffer DL, Silva LA, Tromm CB, da Rosa GL, Silveiira PC, de Souza CT, Latini A, Pinho RA (2012) Impact of different resistance training protocols on muscular oxidative stress parameters. Appl Physiol Nutr Metab 37(6):1239–1246
32. Stanford KI, Middelbeek RJ, Goodyear LJ (2015) Exercise effects on white adipose tissue: beiging and metabolic adaptations. Diabetes 64(7):2361–2368
33. Suwa M, Nakano H, Kumagai S (2003) Effects of chronic AICAR treatment on fiber composition, enzyme activity, UCP3, and PGC-1 in rat muscles. J Appl Physiol 95:960–968
34. Thirupathi A, de Souza CT (2017) Multi-regulatory network of ROS: the interconnection of ROS, PGC-1 alpha, and AMPK-SIRT1 during exercise. J Physiol Biochem 73:487–494
35. Thirupathi A, Pinho R (2018) Effects of reactive oxygen species and interplay of antioxidants during physical exercise in skeletal muscles. J Physiol Biochem 74:359–367
36. Vilela TC, Muller AP, Damiani AP, Macan TP, da Silva S, Canteiro PB, de Sena Casagrande A, Pedroso GDS, Nesi RT, de Andrade VM, de Pinho RA (2017) Strength and aerobic exercises improve spatial memory in aging rats through stimulating distinct neuroplasticity mechanisms. Mol Neurobiol 54:7928–7937
37. Vilela TC, Effting PS, Dos Santos Pedroso G, Farias H, Paganini L, Rebelo Sorato H, Nesi RT, de Andrade VM, de Pinho RA (2018) Aerobic and strength training induce changes in oxidative stress parameters and elicit modifications of various cellular components in skeletal muscle of aged rats. Exp Gerontol 106:21–27
38. White Z, Terrill J, White RB, McMahon C, Sheard P, Grounds MD, Shavlakadze T (2016) Voluntary resistance wheel exercise from mid-life prevents sarcopenia and increases markers of mitochondrial function and autophagy in muscles of old male and female C57BL/6J mice. Skelet Muscle 6(1):45
39. Woods JA, Wilund KR, Martin SA, Kistler BM (2012) Exercise, inflammation and aging. Aging Dis 3(1):130–140
40. Wright DC, Han DH, Garcia-Roves PM, Geiger PC, Jones TE, Holloszy JO (2007) Exercise induced mitochondrial biogenesis begins before the increase in muscle PGC-1 alpha expression. J Biol Chem 282(1):194–199