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Research article

Resistance training mitigates hepato-cardiac changes and muscle mitochondrial protein reductions in rats with diet-induced obesity

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ABSTRACT

Aim: To investigate the effect of resistance training (RT) on hepatocardiovascular and muscle mitochondrial parameters in rats that were fed a high-calorie diet for 12 weeks.
Main methods: The animals were divided into four groups: control (C), exercise (E), obese (O), and obese plus exercise (OE). Group E and OE rats performed resistance training by climbing on a vertical ladder with load attached to the end of the tail (1×/day, 3×/week, for 12 weeks). Group O and OE rats were fed a high-calorie diet containing chow and a cafeteria diet for 12 weeks. Under anesthesia, the heart and liver were removed for histopathological analysis, and the gastrocnemius muscle was removed for Western blotting.
Key findings: Group O rats were heavier, with increased fat mass, elevated fasting glycemia, and total triglycerides, and exhibited a significant number of Kupffer cells and diffuse steatosis in the liver. Group O rats also showed increased thickness of the right ventricle, septum, and pulmonary artery. All of these parameters were attenuated by RT. PGC1-α protein levels were increased in both exercise groups. The protein levels of OXPHOS complexes III, IV, and V were reduced in Group O, while RT prevented this alteration.
Significance: RT exerts a protective effect against hepato-cardiac alterations and prevents changes in the muscle mitochondrial protein profile induced by a high-calorie diet.

1. Introduction

Obesity is a complex disease and involves multiple causal factors, such as sex, age, physical inactivity, socioeconomic status and diet [1]. Despite being typical in developed countries [2], a growing incidence of obesity is also observed in developing countries [3, 4, 5], indicating the ubiquitous distribution of this condition worldwide.

Lifestyle modernization may be associated with an increase in obesity and overweight and is directly related to a reduction in daily physical activity [6, 7]. Another important factor is diet consumption. Natural and raw foods have been gradually replaced with ultra-processed foods that are rich in sugar and saturated fats due to their practicality and palatability [8, 9]. Recent studies predict high costs of health services [10, 11] and decreased life expectancy [12] due to the various complications associated with obesity, such as cardiovascular diseases, nonalcoholic fatty liver disease, and type II diabetes [13, 14].

Obesity can induce various hemodynamic alterations, predisposing morphofunctional changes in the heart, which can trigger ventricular dysfunction and insufficiency [15]. In addition, the endocrine function of adipose tissue regarding the synthesis of adipokines is correlated not only with inflammatory conditions and cardiovascular risk but also with hepatic steatosis development [16]. In turn, hepatic steatosis can progress to fibrosis and nonalcoholic liver disease, which are also related to increased cardiovascular risk [17].

The 17-week high-calorie diet model in Wistar rats has already been shown to be effective in establishing obesity and its metabolic consequences, such as weight and fat gain, decreased glucose tolerance [18], oxidative stress, and cardiovascular impairment [19, 20]. Recent studies

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have linked the worsening of obesity to mitochondrial dysfunction in organs involved in energy metabolism, such as skeletal muscle, liver and white adipose tissue [21, 22].

Mitochondria are crucial organelles for the production of ATP, which involves adequate fatty acid oxidation [23]. Mitochondrial disorder or dysfunction can be related to various metabolic diseases [24], such as insulin resistance, diabetes [25, 26] and aging [27].

During contractile activity, the actions of several protein kinases culminate in the activation of the transcription and translation mechanisms of peroxisome proliferator-activated receptor-gamma coactivator 1-alpha (PGC-1α) [28]. PGC-1α is a transcriptional coactivator that is stimulated by exercise and participates in regulating the transcription of genes involved in mitochondrial biogenesis, fusion, and fission, which are essential events for the proper maintenance of organelle activity and function within cells [29]. Among these genes, nuclear respiratory factors 1 (NRF-1) and 2 (NRF-2) are highlighted in the present study [30]. NRF1 can regulate TFAM (mitochondrial transcription factor A) expression by binding to its gene promoter region and can participate in mitochondrial biogenesis and ATP production. TFAM, in turn, can stimulate the replication and transcription of mtDNA and can be involved in the maintenance and repair of some mitochondrial genes [31]. NRF-2 can bind to the antioxidant-responsive element (ARE) in the promoter region in the NRF-1 gene. In addition, there is a feedback mechanism between PGC-1α and NRF-2, in which PGC-1α activates NRF-2, while the latter influences PGC-1α expression. Both factors stimulate mitochondrial biogenesis in different tissues and conditions [32]. Other key proteins, such as mitofusin 2 (MFN2) and mitochondrial fission protein 1 (FIS1) [33], participate in mitochondrial fusion and fission processes, respectively. MFN2 forms an interconnection between mitochondria, and FIS1 leads to the separation of ineffective mitochondrial contents [34, 35]. All of these processes are coupled to increase the number of functional and efficient mitochondria, ensuring improved oxidation.

One study focusing on an obese population reported reductions in the expression of biogenesis and mitochondrial respiratory chain proteins. This phenomenon was not reversed even after six weeks of aerobic exercise [36]. Indeed, improvements in mitochondrial metabolism were detected in animal models of exercise using L-arginine supplementation [37]. On the other hand, resistance training induced positive results for respiratory capacity and increased OXPHOS Complex I without changing PGC-1α, MFN1, or TFAM mRNA levels in the quadriceps skeletal muscle in healthy young men [38]. In ovariectomized rats, resistance training increased PGC-1α and TFAM protein levels in the gastrocnemius skeletal muscle [39].

A consensus about the beneficial effects of exercise training on mitochondrial activity already exists, but these benefits can vary according to exercise training protocol [40, 41, 42, 43]. Additionally, a better exercise training protocol to generate improved benefits for maintaining mitochondrial metabolism in obese or preobese individuals is still not well established [44, 45]. Therefore, we aimed to elucidate whether resistance exercise training can prevent alterations in body weight and blood biochemical parameters, hepatocardiovascular morphological changes, and muscle mitochondrial protein profile impairment induced by high-calorie ingestion.

2. Materials and methods

2.1. Experimental animals

All procedures complied with the ethical principles of animal research and were approved by the Ethical Committee for Animal Research of the School of Sciences and Technology, Sao Paulo State University, Presidente Prudente, Brazil (protocol #01/2017), and complied with ethical principles of animal research.

Twenty-four male Wistar rats weighing 200 ± 50 g were maintained in polypropylene cages (3 rats per cage) in a room with controlled temperature (23 ± 2 °C) and a light/dark cycle (12 h/12 h). The cages were cleaned twice per week and filled with environmental enrichment for laboratory rodents. All procedures were carefully performed to ensure the welfare of the animals.

The rats were randomly divided into 4 groups (n = 6/group): sedentary control (C), exercise (E), obesity (O) and obesity plus exercise (OE). Initially, the animals were allowed 7 days to adapt to the new environment and another 7 days to adapt to the vertical ladder used for resistance training. Subsequently, the maximum supported load (MSL) was established before the beginning of resistance training.

2.2. Resistance training (RT)

The training consisted of climbing on a vertical ladder for rodents with 80 degrees of inclination and a 6 mm distance between steps (Insight ltda., Brazil). During the adaptation period, each rat performed three climbs without a load attached to the body in two sessions with a 24-hour interval. Twenty-four hours after this adaptation period, the maximum supported load (MSL) test was performed with an initial load of 50% of the body weight (BW). When the animal completed the climb, an additional 30 g was added for the next trial. When the animal could not climb the ladder or showed tremor in its hindlimbs, the MSL was considered to be the weight of the last climb without alterations. A maximum of eight trials was performed with an interval of one minute between them [46].

After a 72-hour interval, the RT started with 50% of the MSL, with a progressive increase up to 100% of the MSL. The load was added inside a glove attached to the end of the tail, and soft taps were made on the caudal portion of the animal to stimulate climbing. After each climb, the animal was kept on the top of the ladder for 60 s to rest. The training consisted of four repetitions of the climb with 1 min of rest between each repetition, 3 times per week for 12 weeks. The weight load mean in the MSL is in the Supplemental Table 1.

2.3. High-calorie diet

Group C and E rats received standard chow for rodents (PRIMOR™, SP-Brazil) and water. Group O and OE rats received a high-calorie diet (HD) consisting of standard chow, mortadella, cookies, white chocolate, soda, and water (approximately 1,200 kcal per cage) every 2 days or 3 times per week, totalling approximately 200 kcal per animal per day. Body weight (BW) and food intake were recorded weekly. The calculation of Kcal ingested was performed weekly by subtracting the caloric supply from the leftovers. The result was divided by the number of animals per cage, indicating the total calories ingested per animal per week. The diet was offered for twelve weeks according to the RT schedule. Figure 1 shows the timeline of the interventions.

The coefficient of feeding efficiency (CFE) was calculated using the following formula: weight gain (final body weight - initial body weight)/ food consumption divided by total food consumption (grams) × 100. The coefficient of weight gain per caloric consumption (CWGCC) was calculated using the ratio weight gain/total caloric consumption [47].

2.4. Samples

The rats were anesthetized with ketamine hydrochloride (60 mg/kg) plus xylazine (10 mg/kg) after 12 h of fasting and 24 h after the last RT session, and biological samples were collected [48,49]. After the loss of reflexes in the limbs, the animals were placed in a pronated position, and a midline laparotomy was performed. Samples of intraventricular blood were collected, followed by extraction of the gastrocnemius muscle, liver, and periepididymal adipose tissue. The heart, lungs and trachea were collected together without previous dissection after 10% buffered formalin was injected through the trachea to fix the lung parenchyma. Euthanasia occurred after the removal of the heart. All tissues were immediately weighed and stored in liquid nitrogen [50]. The following

ADAPTATION	RESISTANCE TRAINING + HIGH-CALORIE DIET				
14 DAYS: REST WEEK (7 DAYS) + VERTICAL LADDER + MSL (7 DAYS)	WEEKS 1–2 50% MSL	WEEKS 3–4 75% MSL	WEEKS 5–6 85% MSL	WEEKS 7–8 100% MSL	WEEK 9- EUTHANASIA

Figure 1. Timeline of interventions.

formula was used to determine the relative weight of the tissue: (absolute tissue weight/final body weight) × 100.

2.5. Serum analysis

Serum was separated by centrifugation (Eppendorf 5415R centrifuge, Hamburg, Germany) at 200 rcf for 15 min at 4 °C to determine fasting blood glucose and triglyceride levels by commercial kits (BIOCLIN, Minas Gerais - Brazil).

2.6. Histopathological analysis

All procedures were performed according to a standard protocol for histological analysis as previously described [46]. Pieces of the left and right lungs were cut, the heart was sectioned in the coronal direction at the atrial and ventricular levels, and the trachea, liver and gastrocnemius muscle were transversally sectioned. The sections were embedded in paraffin (Dynamics Analytical Reagents, São Paulo, Brazil) and subjected to standard histological processing. Three serial sections with a thickness of 5 μm and a spacing of 15 μm were prepared on all slides.

Briefly, cardiac hypertrophy was assessed by measuring the ventricular wall thickness at the papillary levels in a coronal section fragment that included the atria and ventricles. To measure the thickness of the wall of the right ventricle (RV), the interventricular septum (SI), and the free wall of the left ventricle (LV), two measurements of each wall were taken using the Leica Application Suite LAS 4.2 image analysis system (Leica Microsystems, Switzerland) [45,48] on a slide stained with hematoxylin-eosin (Dolles, São Paulo - Brazil). To verify the analysis of the fractal dimension of the right ventricle (RV) and left ventricle (LV) by quantifying collagen fibers, the slides were stained with Masson's trichrome (Merck KgaA, Darmstadt - Germany) and photographed. Then, the image was submitted to the binarization process for reading and analyzing the fractal dimension using the box-counting method with free ImageJ (NIH) software from the National Institutes of Health (NIH, USA).

To assess the thickness of pulmonary arterioles, the slides were stained with PAS-Alcian blue (Merck, Germany). The thickness was measured in two areas of the pulmonary artery by a Leica Image Analysis System with the Application Suite LAS 4.2.0 (Leica, Microsystems - Switzerland) at 400× magnification.

Cross sections of liver pieces were stained with hematoxylin-eosin (HE) (Dolles, São Paulo - Brazil) for morphological analysis. The presence and type of steatosis (0 = absent, 1 = focally present, and 2 = diffusely present) were determined by qualitatively assessing the entire parenchyma. Kupffer cells (macrophages) were counted in 10 high-power fields, which corresponded to approximately 1 mm^2 of each sample [46]. All of these evaluations were blindly performed by a single observer using a standard optical microscope (NIKON, Labophot - Japan).

2.7. Protein expression analysis: Western blotting

The gastrocnemius muscle was homogenized in lysis buffer containing 10 mM EDTA, 100 mM Tris base (pH 7.5), 100 mM sodium pyrophosphate, 100 mM sodium fluoride, 1 mM sodium orthovanadate, 2 mM phenylmethylsulfonyl fluoride (PMSF), and aprotinin 1 mg/mL. All buffer components were purchased from Synth (Synth, Diadema, SP, Brazil) and Sigma (Sigma Aldrich, St. Louis, MO, USA). After the samples were homogenized, 10% Triton X-100 was added and incubated on ice for 30 min. Subsequently, the homogenates were centrifuged at 12,000 g for 20 min at 4 °C. The protein concentration was assessed using the Bradford method (Bio–Rad, Dye Reagent Concentrate, Hercules, CA, USA). Thirty micrograms of protein was loaded on 12% and 15% SDS–PAGE gels for separation [51]. The proteins were transferred onto nitrocellulose membranes (Bio–Rad) and then stained with Ponceau to check that the samples were similarly loaded on the gel. The membrane was washed in a basal solution containing Tris-buffered saline + 0.1% Tween 20. Then, the membrane was incubated in a blocking solution containing Tris-buffered saline + 5% bovine serum albumin for 1 h. After being washed, the membranes were incubated with anti-OXPHOS (1:1, 000, Abcam cat #110413), anti-TFAM (1:1,000, Abcam cat #119684), anti-PGC-1α (1:1,000, Abcam cat #54481) and anti-NRF2 (1:1,000, Abcam cat #89443) antibodies. Subsequently, the membrane was washed and incubated with HRP-conjugated anti-rabbit IgG (Jackson ImmunoResearch Lab) diluted 1:5,000 in blocking solution for 1 h at room temperature. Then, the membrane was washed, and bands were detected by chemiluminescence with Clarity™ Western ECL Substrate solution (Bio–Rad, CA, USA). The membranes were exposed to a photodocumenter (Amersham Imager 600 - GE Healthcare, USA). The abundance of proteins was estimated by densitometric analysis of the bands using free ImageJ (NIH) software. Protein loading was normalized by Ponceau-stained membranes.

2.8. Statistical analysis

The data are presented as the mean ± standard error of the mean. Comparisons among the groups were made using two-way ANOVA followed by Tukey's multiple comparison test. Kruskal–Wallis nonparametric tests for qualitative results (hepatic steatosis) were performed with GraphPad Prism (GraphPad Software, Inc., San Diego, USA), and $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. A high-calorie induced enlarged fat mass, increased body weight and hyperglycemia, while RT mitigates these changes

Table 1 shows the characteristics of the animals. The O group had increased body mass, which was accompanied by enlarged fat mass compared to those in the other groups. On the other hand, the OE group showed a 40% reduction in fat mass compared to the O group, but these animals were still enlarged compared to Group C and E rats. Group E rats showed relatively increased gastrocnemius muscle weight compared to rats in the O and OE groups. Calorie consumption was elevated in the O and OE groups compared to the C and E groups. The CFE was increased in the O group compared to the other groups, and the CWGCC was also elevated in the O group compared to the E and OE groups. Fasting glycemia and triglyceridemia were elevated in Group O rats compared to rats in the other groups, while the E group showed reduced fasting blood glucose compared to the C group.

Table 1. Characteristics of the animals.

	C	O	E	OE
Body Weight (g)	423.0 ± 9.5	526.3 ± 12.5*	405.8 ± 13.1**	448.4 ± 9.2
Weight Gain (g)	113.4 ± 14.8	182.2 ± 16.8*	114.4 ± 10.1	141.8 ± 18.4
AW Adipose Tissue	5.9 ± 0.2	16.1 ± 1.0*	5.6 ± 0.7	9.7 ± 0.4**
RW Adipose Tissue	1.4 ± 0.07	3.0 ± 0.1*	1.3 ± 0.1	2.2 ± 0.08**
AW Muscle	2.63 ± 0.07	2.80 ± 0.13	2.58 ± 0.13	2.48 ± 0.08
RW Muscle	0.619 ± 0.01	0.516 ± 0.02	0.638 ± 0.02*	0.557 ± 0.01
Kcal/week/animal	618.6 ± 12.8	812.1 ± 8.9*	686.3 ± 12.5**	750.5 ± 7.2$^{\#}$
CWGCC	0.22 ± 0.01	0.28 ± 0.02*	0.19 ± 0.01	0.18 ± 0.02
CFE	2.48 ± 0.05	3.71 ± 0.1*	2.22 ± 0.1	2.27 ± 0.2
Fasting Glycemia (mg/dL)	157.8 ± 3.2	217.0 ± 2.3*	122.1 ± 4.7**	143.8 ± 6.2
Triglyceridemia (mg/dL)	50.8 ± 6.7	123.9 ± 17.4*	60.0 ± 5.4	77.9 ± 2.0

Values are expressed as the mean ± SEM of control (C), obese (O), exercise (E) and obese plus exercise (OE) rats. Body weight (g): *$P < 0.0001$ *vs.* C, E and OE, **$P = 0.04$ *vs.* OE; Weight gain (g): *$P = 0.02$ *vs.* C and E; Absolute weight (AW) of periepididymal adipose tissue: *$P < 0.0001$ *vs.* C, E and OE, **$P < 0.05$ *vs.* C and E; Relative Weight (RW) of periepididymal adipose tissue: *$P < 0.0001$ *vs.* C and E, **$P < 0.05$ *vs.* C, E and O. Absolute weight (AW) of the gastrocnemius muscle: No significance; Relative weight (RW) of the gastrocnemius muscle: *$P = 0.0002$ *vs.* O and OE; Calorie consumption (Kcal/week/animal): *$P < 0.0001$ *vs.* C, E and OE, **$P = 0.0003$ *vs.* C and OE, $^{\#}P < 0.0001$ *vs.* C; CWGCC: *$P = 0.002$ *vs.* E and OE; CFE: *$P < 0.0001$ *vs.* C, E and OE; Fasting glycemia: *$P < 0.001$ *vs.* C, E and OE, **$P = 0.01$ *vs.* C and OE; Fasting triglyceridemia: *$P < 0.0001$ *vs.* C, E and OE. N = 06 animals per group.

3.2. A high-calorie diet provoked steatosis and increased local inflammation in the liver while RT prevented these abnormalities

A significant number of Kupffer cells (Figure 2F) and robust type 2 diffuse steatosis (Figure 2E, $P < 0.05$) were detected by histopathological analysis in the liver in the O group (Figure 2B), and this effect was not detected in the C or E group (Figures 2A, C and E). The OE group showed a discrete focus of steatosis (Figures 2D, E).

3.3. A high-calorie diet contributed with factors for pulmonary hypertension development while RT prevented this condition

Cardiovascular histopathological analysis (Figure 3) showed increased thickness in the pulmonary artery in the O group compared to the other groups (Figure 3A). This finding was associated with increased thickness in the interventricular septum and an enlarged wall in the right ventricle (Figures 3B and D). Additionally, the OE group also showed

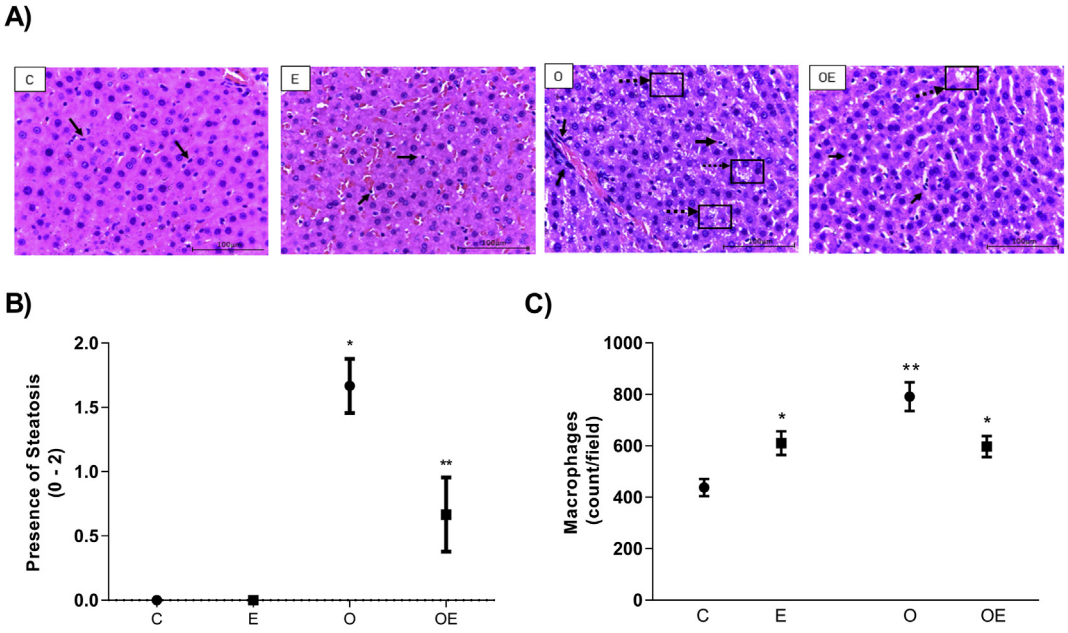

Figure 2. Histopathological assessment of the liver. Hematoxylin-eosin-stained images at 400× magnification. In A) C Group: unchanged liver parenchyma, with a mean number of Kupffer cells = 438. O group: Diffuse microvesicular steatosis along the parenchyma, with a mean number of Kupffer cells = 758. E group: unchanged liver parenchyma, with a mean number of Kupffer cells = 649. OE Group: Parenchyma with discrete steatotic focus (square), with a mean number of Kupffer cells = 637. Kupffer cells are indicated by continuous arrows. Dotted frames and arrows indicate steatosis. B) Presence of hepatic steatosis. *$P < 0.05$ *vs.* C, E and OE; **$P < 0.05$ *vs.* C, E and O. C) Quantification of macrophages. *$P < 0.05$ *vs.* C; **$P < 0.05$ *vs.* C, E and OE.

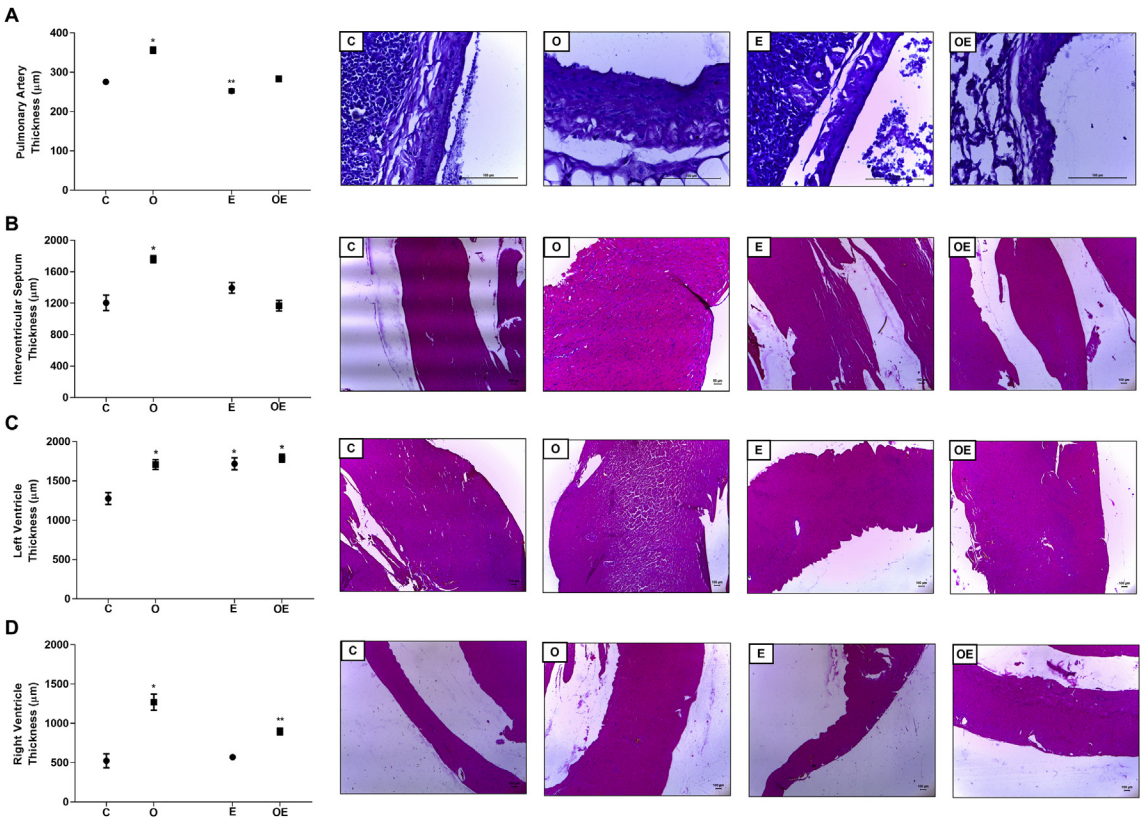

Figure 3. Cardiovascular parameters. Values are expressed as the mean ± SEM of the control (C), obese (O), exercise (E) and obese plus exercise (OE) groups. A) Pulmonary artery thickness: $^{*}P < 0.0001$ *vs.* C, E and OE; $^{**}P < 0.05$ *vs.* C and OE; B) Interventricular septum thickness: $^{*}P < 0.0001$ *vs.* C, E and OE; C) Left ventricle thickness: $^{*}P < 0.05$ *vs.* C; D) Right ventricle thickness: $^{*}P < 0.05$ *vs.* C, E and OE; $^{**}P < 0.05$ *vs.* C and E; E) Fractal dimension the left ventricle; F) Fractal dimension the right ventricle: $^{*}P < 0.05$ *vs.* C, E and OE. N = 6.

increased thickness of the right ventricle wall compared to the C and E groups, but no change in the thickness of the pulmonary artery was observed. The O, E, and OE groups showed increased left ventricular thickness compared to the C group (Figure 3C). Fractal dimension analysis of the left ventricle showed no changes in the different groups (Figure 3E). The collagen fiber density of the right ventricle in the O group was increased compared to that in the other groups (Figure 3F).

3.4. Twelve-week RT improved PGC1-α protein levels

PGC1-α protein levels were increased in the E and OE groups compared to the O group (Figure 4A). TFAM and NRF2 protein levels were unchanged among the groups (Figure 4B–C).

3.5. A high-calorie diet impaired OXPHOS protein complexes (CIII, CIV and CV) which were prevented by RT

The expression of the protein complexes involved in the mitochondrial OXPHOS (oxidative phosphorylation supercomplexes chain III, IV and V) was reduced in the O rats compared to the E rats (Figure 5C–E).

3.6. RT improved Nrf1, Tfam, Cycs and Ppargc1a, reduced MnSod mRNA levels in the gastrocnemius muscle

Additional analysis (Supplemental Figure 3) involving the gene expression of some proteins related to biogenesis and mitochondrial function (Supplemental Figure 3A–D) were obtained, and we demonstrated the efficacy of RT, as indicated by the elevated mRNA levels of *Nrf1*, *Tfam*, *Cycs* and *Ppargc1a* in the gastrocnemius muscle. Supplemental Table 2 shows the sequence of oligonucleotides. Similarly, RT also seemed to improve local oxidative stress, and the exercised animals showed reduced mRNA levels of *MnSod* (Supplemental Figure 3H).

3.7. A high-calorie diet increased fission and reduced fusion in the mitochondria of the skeletal muscle, while RT promoted opposite effects

A high-calorie diet increased *Fis1* gene expression, while RT reduced *Fis1* gene expression (Supplemental Figure 3E). On the other hand, HD feeding reduced *Mfn2* mRNA expression, which was increased by RT (Supplemental Figure 3F). Additionally, *Nos3* mRNA levels were increased in the exercise groups (Supplemental Figure 3G).

4. Discussion

A high-calorie diet (HD) has become a common feature due to increased ingestion of processed foods, inducing a series of metabolic changes and culminating in the development of obesity worldwide [52, 53]. In the present study, we investigated the effects of resistance training on the prevention of obesity and possible alterations in the heart, liver, and/or expression of mitochondrial proteins in muscle in Wistar rats fed a HD. We found that eight weeks of RT could prevent various negative effects of HD feeding. RT reduced fat mass and, consequently, body weight and prevented the increases in glycemia and triglyceridemia. Additionally, RT prevents steatosis in the liver and maintains proper pulmonary artery wall morphology, preventing pulmonary hypertension development. Diet-induced obesity impairs the expression of some mitochondrial proteins in skeletal muscle, while RT prevents these alterations.

The groups that were fed a high-calorie diet showed notable increases in body mass, likely due to the increased gain in fat mass. Twelve weeks of resistance training mitigated this enlargement, indicating the efficacy

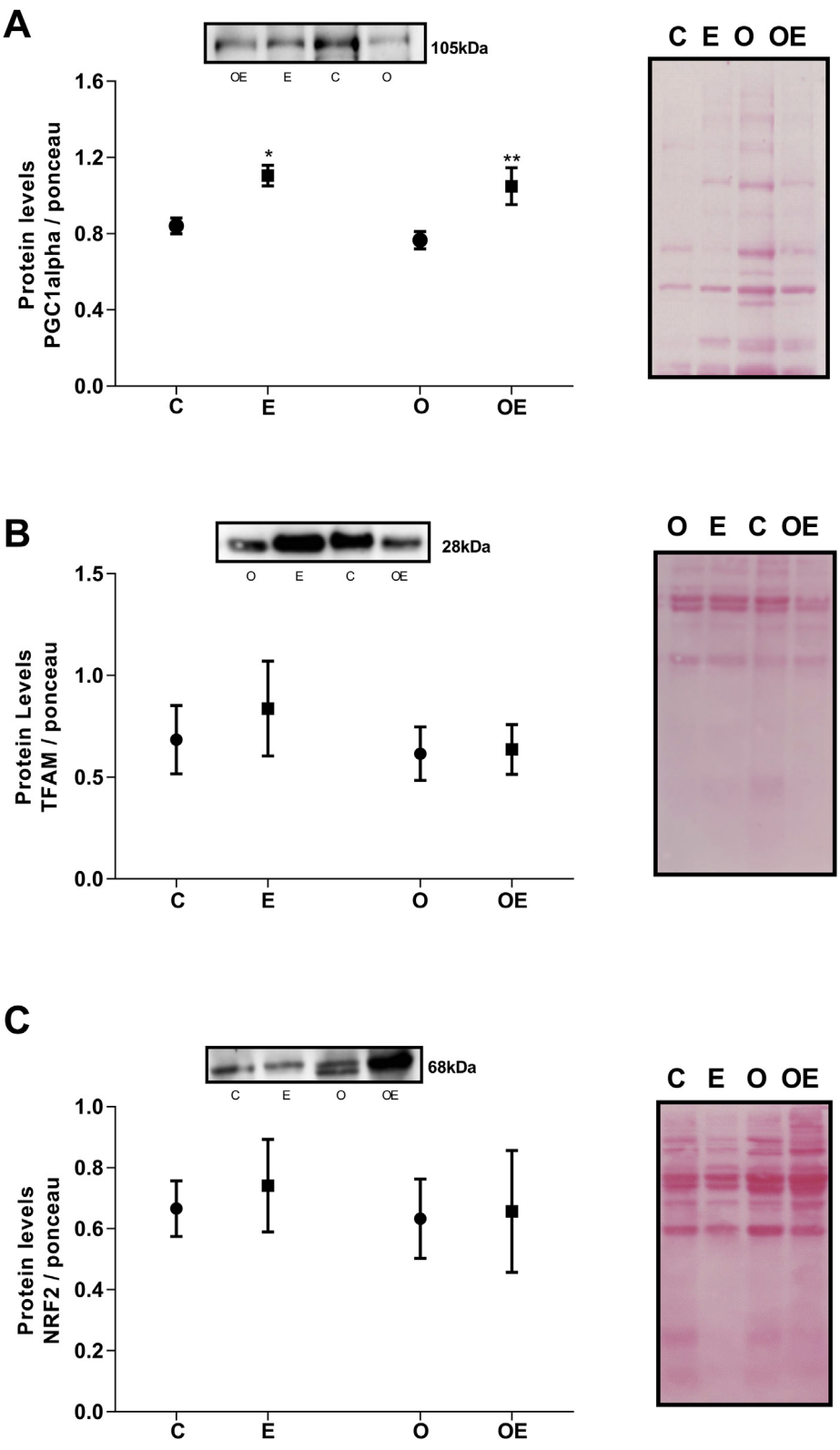

Figure 4. Protein levels of mitochondrial biogenesis markers. Values are expressed as the mean ± SEM of the control (C), obese (O), exercised(E) and obese plus exercise (OE) groups. A) PGC-1α protein levels: $*P < 0.05$ *vs.* C and O, $**P < 0.05$ *vs.* O. B) TFAM protein levels: No significance was found. C) NRF2 protein levels: No significance was detected. N = 6 animals per group. Respective Ponceau-stained membranes are shown beside each protein analysis. Supplemental Figure 1 is showing non-adjusted images of the blots and Ponceau-stained membrane for each protein of the Western blotting assay. Supplemental Figure 2 is showing two different experiments of Western blotting for each protein.

of the training protocol on the regulation of fat mass gain, suggesting that this is an accessible tool to avoid obesity development. The coefficient of food efficiency is an interdependent variable associated with body weight, suggesting the fundamental role of the diet in this condition [47]. The higher the CFE and CWCCG coefficients are, the more efficient the diet will be, which is related to greater incorporation of calories into the body mass. This effect was observed in the O group after they ingested the high-calorie diet for 12 weeks. On the other hand, consistent with the literature [54], the OE group did not show an increase in these coefficients, although this group also ingested a HD and had similar calories as the O group during the 12 weeks of training. Thus, even though both groups consumed the same diet and similar calories, an obesogenic effect was observed in the O group only, which occurred due to the higher incorporation of fat mass. RT abrogated the effect of the diet, avoiding

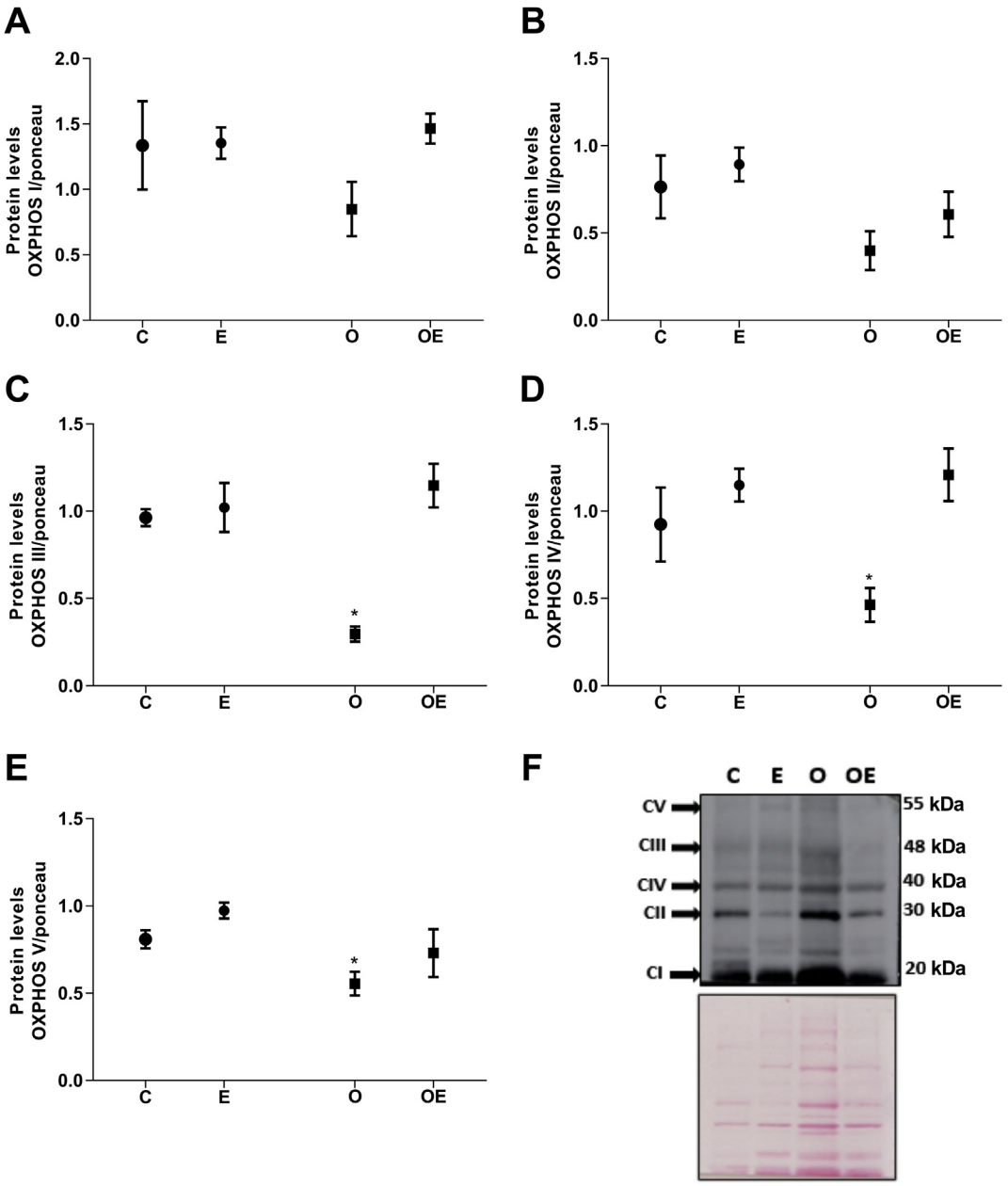

Figure 5. Protein expression of mitochondrial oxidative phosphorylation protein complexes. Values are expressed as the mean ± SEM of the control (C), obese (O), exercise (E) and obese plus exercise (OE) groups. A) OXPHOS I protein levels: no significance; B) OXPHOS II protein levels: no significance; C) OXPHOS III protein levels: $^{*}P < 0.001$ *vs.* C, E and OE; D) OXPHOS IV protein levels: $^{*}P = 0.01$ *vs.* E and OE; E) OXPHOS V protein levels: $^{*}P = 0.002$ *vs.* E; F) Above: Image showing the target proteins in the Western blot. Below: Ponceau-stained membrane is shown. N = 5 animals per group. Supplemental Figure 1 is showing non-adjusted images of the blots and Ponceau-stained membrane for OXPHOS protein complexes of the Western blotting assay. Supplemental Figure 2 is showing two different experiments of Western blotting for each protein.

the excessive gain of body mass during the 12 weeks of HD ingestion, maintaining lower levels of CWCCG, and reducing the gain in fat mass. Elevated values of serum triglycerides and fasting blood glucose were detected in the O group, emphasizing the negative effect of HD feeding. According to the literature, the consumption of a diet rich in calories, lipids and sugar triggers dyslipidemia and increases body fat and other comorbidities, such as insulin resistance and dyslipidemia [55]. In contrast, RT prevented the increases in these parameters in the group fed a hypercaloric diet. In addition, RT prevented increases in glycemia and triglyceridemia in the group fed standard chow, showing the positive effect of training on serum parameters [56].

We believe the increased visceral adiposity in Group O contributed to the robust accumulation of macrophages in the liver and the development of type 2 steatosis [57]. On the other hand, RT prevented weight gain in rats fed a HD and promoted a reduction in the number of resident macrophages in the liver. In addition, the OE group had mild liver steatosis, confirming the protective effect of resistance training on this organ. A previous study [58] using a similar animal model of diet-induced obesity showed an increase in fat depots and an increase in the expression of FAS (fatty acid synthase) in the liver in nonexercised animals, which is known to be implicated in the development of nonalcoholic fatty liver disease and impaired fatty acid oxidation. However, strength training reduced the proinflammatory condition in the liver, with a reduction in local fat deposits, a reduction in FAS expression, and an improvement in lipid regulation in this organ. Studies using aerobic exercise have not been able to abrogate the side effects of increased

caloric intake [59, 60], which motivated us to investigate the efficacy of resistance training in controlling body composition.

It is likely that the overload imposed by body mass in the sedentary obese group had an effect on cardiovascular tissue, such as the increases the wall thickness in the right ventricle, the intraventricular septum, and the pulmonary artery wall that were observed in Group O. These results are closely related since the increase in blood pressure caused by the increase in the thickness of the pulmonary artery can generate adaptive hypertrophy in the right ventricle, which should perform more vigorous contractions to circulate the blood to reach the lungs [61]. This right ventricular compensation can lead to insufficient diastolic function and possible long-term ventricular death [62]. Although blood pressure was not assessed in our animals, we believe that of pulmonary hypertension may have been developing in Group O rats, since at least two of the three main characteristics of pulmonary hypertension [46] were present. Although RT maintained the increased right ventricle wall thickness, it promoted a reduction in the pulmonary artery and interventricular septum thickness, possibly preventing pulmonary hypertension development. It has been demonstrated that the liver and heart can influence each other, with liver diseases affecting the heart and heart diseases affecting the liver, and diseases can affect the heart and liver simultaneously [63], which are known as hepato-cardiac diseases [64]. Thus, we suggest that the inflammatory condition in the liver, as indicated by an increase in the local macrophages and the development of steatosis, can also influence the cardiovascular changes observed in this experimental model due to changes induced by adiposity and inflammation [65], with possible cardiac involvement in the long term. Again, RT improved the morphology of the liver in the high-calorie diet group and, consequently, contributed to the prevention of some possible alterations in the heart.

The fractal dimension is a mathematical analysis that characterizes nonsymmetrical structures [66]. A recent study linked the increase in the fractal dimension in the right ventricle to hypertrophy and insufficiency resulting from induced pulmonary hypertension [67]. In the present study, the fractal dimension was used to assess tissue density and possible tissue stiffness based on collagen fiber analysis. In this context, abnormal collagen expansion has already been associated with fibrosis and myocardial tissue stiffness [68]. We hypothesized that an increase in the fractal dimension in the RV in the O group correlated with the other results associated with cardiovascular thickness due to the involvement of obesity, forcing these animals to have more rigid and pathologically hypertrophied ventricles. We supposed that the thickness of the left ventricular wall was increased in the O group because of body weight (constant overload), and the factors triggered by adiposity already correlated with ventricular remodeling [69]. On the other hand, the thickness of the left ventricular wall was also increased in the exercise groups, probably due to resistance training, which is an inducer of cardiac hypertrophy [70].

Mitochondria are organelles that act directly on homeostasis because their role in the production of ATP is essential for the whole organism. ATP production involves five protein complexes (named CI - CV) in the electron transport chain [71, 72]. Thus, damage to the production, maintenance, and/or activity of mitochondria directly affects energy supply and activities of cells. PGC1-α is a transcriptional cofactor induced by exercise and is the main regulator of metabolic adaptations for the use of substrates, stimulator of mitochondrial biogenesis, and modulator of exercise-induced muscular phenotypic adaptations through interacting and coactivating several transcription factors. However, the great majority of these effects have been described for aerobic/endurance training [73]. In the present study, RT improved PGC1-α protein expression in the gastrocnemius muscle of exercised obese animals, reinforcing the role of RT in mitochondrial homeostasis. In addition, the damaging association between obesity and sedentarism not only affects mitochondrial biogenesis proteins but also affected the protein levels of electron transport chain complexes in skeletal muscle. Reduced protein levels of complexes III, IV, and V were detected in high-fat diet-fed animals. These findings corroborate the literature, which indicated that obesity was a limiting factor for the oxidative capacity of OXPHOS complexes and indicated mitochondrial dysfunction [74, 75, 76]. RT prevented these alterations in obese rats, maintaining OXPHOS complexes with similar expression levels as those in the control and exercised groups, suggesting improvements in mitochondrial function. In the literature, it has already been demonstrated that RT increases the protein expression of all five OXPHOS complexes in mitochondria in the vastus lateralis muscle in older adults [77]. However, this is the first study to demonstrate a relevant preventive role of RT in rats with diet-induced obesity.

Recent studies note the induction of mitochondrial dysfunction due to obesity onset, and oxidative stress is one of the main determinants of this change in mitochondria [78, 79]. During oxygen consumption via cellular respiration, several reactive oxygen species (ROS) are formed [80]. In the electron transport chain, complexes I and III are the main producers of ROS [81], which, when released, can be converted into hydrogen peroxide (H_2O_2) by the enzyme MnSod, whose function is to protect mtDNA from apoptotic processes and oxidative stress. Although we did not evaluate ROS production, our findings suggest that excessive ROS production might occur, which could eventually generate worse cellular damage, considering the reduction in *Mnsod* mRNA levels found in the obese groups. Therefore, a limiting factor in this study was the lack of assessment of oxidative stress in muscle tissue. Future studies should be conducted to investigate oxidative stress in skeletal muscle. Li et al. [82] reported a decrease in oxidative function and an increase in superoxide production in obese animals fed a high-fat diet for 12 weeks. However, the same author noted that aerobic exercise was a mitigating factor in these effects, protecting tissues from oxidative stress; we found similar effects in the present study with RT.

Considering all the processes involving the quality control of mitochondria, we highlighted mitochondrial dynamics, which include fusion, fission, and mitophagy [83]. The main purpose of fusion is to combine mitochondrial contents to maintain a highly active interconnected network, and fission aims to separate mitochondria, eliminate ineffective contents and maintain organelles with high membrane potential, while damaged organelles are sent for mitophagy [84]. During these processes, several proteins participate in proper mitochondrial function, including FIS1 and MFN2, two key proteins involved in mitochondrial fission and fusion, respectively. Changes in *Fis1* and *Mfn2* gene expression suggest an imbalance in these mechanisms. While the fusion process seems to be stimulated by RT, fission seems to prevail in response to diet-induced obesity and is reduced by RT. In fact, it is likely that the energy demanded induced by RT stimulates mitochondrial fusion to produce higher levels of energy, but RT seems to prevent the exacerbated production of dysfunctional organelles reducing fission. The ingestion of a HD increased the expression of the fission gene because the inefficient production of energy and excess ROS generation stimulate this process. Therefore, we believe that the mitochondria of obese rats did not perform efficient fusion, losing the transmission of mtDNA and exhibiting an increase in fission due to the increase in dysfunctional organelles [85]. On the other hand, in rats that exercised and were fed a HD, fission and fusion gene levels were maintained, suggesting a balance in these processes due to resistance training. PGC1-α affects fusion and fission, and therefore, a decrease in PGC1-α expression can impair the activity of other mechanisms [86]. A limiting factor of the study is that the levels of proteins related to fusion and fission processes were not evaluated. Although the results presented here are coherent, they may not represent what occurs at the protein level regarding these mitochondrial proteins. In addition, we evaluated only two genes involved in fission and fusion. Future investigation will be performed to elucidate these mechanisms. Taking all these results into account, we believe that an overload of function, oxidative stress, and a possible increase in fission may be related to the low levels of mitochondria, in addition to the low efficiency of organelles in sedentary obese rats. Other studies in the literature that evaluated the effect of obesity on mitochondrial fusion and fission also showed decreases in

fusion markers, increases in fission markers and direct effects on mitochondrial dysfunction [82, 87].

5. Conclusion

In summary, we concluded that performing resistance exercise training during a period of high-calorie diet ingestion prevented some metabolic alterations induced by the diet. Resistance exercise training shows a protective effects against the negative effects of a high-calorie diet on homeostasis, minimizing obesity development, reducing hepato-cardiac morphological changes, avoiding pulmonary hypertension development, improving the profile of mitochondrial proteins in muscle, and favoring better local metabolic performance.

Declaration

Author contribution statement

Patricia Monteiro Seraphim: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Caroline Pancera Laurindo, Karen Cristina Rego Gregorio, Ana Caroline Rippi Moreno: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Julia Maia Viudes Agostinho, Evelyn Carvalho Campos: Performed the experiments.

Gisele Alborghetti Nai: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Maria Tereza Nunes: Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

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