

Resistance training improves body composition and increases matrix metalloproteinase 2 activity in biceps and gastrocnemius muscles of diet-induced obese rats

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OBJECTIVE: We investigated the influence of resistance training on body composition and matrix metalloproteinase 2 activity in skeletal muscles of rats fed a high-fat diet.

METHODS: Thirty-two Wistar rats were divided into four experimental groups (n = 8/each) according to diet and exercise status: Control (standard diet), Obese Control (high-fat diet), Resistance Training (standard diet) and Obese Resistance Training (high-fat diet) groups. Animals were fed a high-fat diet for 12 weeks to promote excessive weight gain. Resistance Training groups performed 12 weeks of training periods after this period in a vertical ladder three times/week. Fat percentage, fat-free mass and fat mass were assessed using dual-energy X-ray absorptiometry, and matrix metalloproteinase 2 activity in biceps and gastrocnemius muscles was analyzed using zymography.

RESULTS: Resistance training significantly reduced body and fat masses and fat percentages in both trained groups ($p < 0.05$). The maximal carrying load between trained groups was not different, but relative force was higher in the Resistance Training group ($p < 0.05$). Of note, increased matrix metalloproteinase 2 activity was noted in the tested muscles of both trained groups ($p < 0.05$).

CONCLUSION: In conclusion, altered body composition and muscle matrix metalloproteinase 2 activity promoted by excessive weight gain were positively modified by resistance training.

KEYWORDS: Resistance Training; Obesity; Matrix Metalloproteinase 2 (MMP-2); Skeletal Muscle and Dual-Energy X-Ray Absorptiometry (DXA).

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INTRODUCTION

Matrix metalloproteinases (MMPs) are important for the integrity of the extracellular matrix (ECM) in skeletal muscles (1). The ECM surrounds muscle fibers and offers structural support and protection, which is essential for maintaining the functional integrity of skeletal muscles (2). Matrix integrity involves the synthesis and degradation of ECM constituents, including collagen, glycoproteins, glycosaminoglycans, and proteoglycans (3). MMPs, primarily MMP-2 and MMP-9, initiate collagen degradation in the

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extracellular environment (3). MMP-2 expression is highly regulated by growth factors, cytokines produced during tissue remodeling (1), biochemical agents, and cell-matrix interactions (4). This enzyme plays an essential role in myofibril proliferation and differentiation, recovery after damage, and local connective tissue homeostasis (5). Adipose tissue influences muscle remodeling by changing collagen biosynthesis organization and altering the homeostasis between MMPs and MMP inhibitors (e.g., tissue inhibitors of metalloproteases [TIMPs]). Exercise training results in developed muscle mass and strength, improved muscle tone, changes in type IV collagen of the basal lamina, and positive effects on retarding obesity-related degenerative changes in skeletal muscle (2).

Resistance training (RT) alters MMP activity. Prestes et al. (6) reported a significant decrease in muscle MMP-2 activity in a model of hypoestrogenism (i.e., ovariectomized rats) and an additional benefit of RT on MMP-2 activity. Our research group showed that muscle (7) and tendon (8,9) remodeling might be impaired in rats with or without loading exercises due to decreased MMP-2 activity. However, the influence of obesity *per se* and RT associated with obesity on MMP-2 activity in skeletal muscles needs further elucidation. Obesity is dependent on interactions between genetic, metabolic, behavioral, and environmental factors (10). Additionally, decreased lean mass may also be present in obesity, which is termed sarcopenic obesity (11). Exercise training may be viewed as a tool to prevent obesity and sarcopenia. Several benefits are observed, such as decreased fat mass and maintained and increased muscle mass, which increase energy expenditure and resting metabolic rate (10). However, few studies have investigated skeletal muscle remodeling and MMP signaling in rat models of diet-induced obesity.

The inflammatory profile of obesity also influences muscle remodeling (10) due to decreased MMP-2 activity (5) and the inhibition of cells responsible for hypertrophy (satellite cells) (2,12), which contribute to sarcopenia. The latter is strongly correlated with increased functional impairment, physical frailty, and a decreased quality of life (13). Investigations of strategies that prevent and/or attenuate these negative effects of obesity on muscle physiology could provide beneficial effects.

Among the investigated experimental strategies, RT decreased body and fat masses and increased lean mass (14). However, the direct effects of RT on obesity-induced sarcopenia remain unclear. A better understanding of MMP-2 activity could elucidate the physiological responses in skeletal muscle during different stimuli. We investigated the influence of RT on body composition and MMP-2 activity in the skeletal muscles of rats fed a high-fat diet. We hypothesize that RT increases MMP-2 activity, which is negatively influenced by obesity, to favor greater muscle remodeling.

■ MATERIALS AND METHODS

Animals

Thirty-two Wistar rats were used (*Rattus norvegicus var. albinus*, Rodentia, Mammalia) from the breeding colony of the State University of Rio de Janeiro (UERJ) with an initial body mass of approximately 250 ± 30 g. The rats were kept in collective cages (5 rats per cage), and water and chow were given *ad libitum*. The room temperature was

maintained at $22 \pm 2^\circ\text{C}$ and a 12 h light:12 h darkness cycle. Three-month-old virgin female rats were caged with a male rat, and each female was placed in an individual cage with free access to water and food after mating. Excess pups were removed 21 days after birth (day 0) to avoid a litter effect, and only six male pups per dam were maintained to induce higher lactation performance (15). Rat dams received standard laboratory chow (Nuvilab[®], Paraná, Brazil) and water *ad libitum* during the 21 days of lactation.

All animal procedures were conducted in accordance with the guide for the care and use of laboratory animals (National Research Council 1998) and the State University of Rio de Janeiro (UERJ). The Committee of Experimental Animals (CEUA/060/2012) approved the protocol.

Experimental groups

Rats from different litters were randomly divided on postnatal day 21 and distributed into the following four experimental groups (8 animals/group): Control (C; standard diet), Obese Control (C-Ob; high-fat diet), Resistance Training (RT; standard diet) and Obese Resistance Training (RT-Ob; high-fat diet). Animals were kept in their cages for 12 weeks without training until week 13. Animals from the RT groups underwent the 12-week training period as proposed in the protocol.

Diet

All animals from the C and RT groups received a standard rat chow diet (Nuvital, Nuvilab, PR, Brazil) in pellet form containing 23 g of protein, 71 g of carbohydrate, 6 g of total fat, and 5 g of fiber (per %). Animals in the C-Ob and RT-Ob groups received a high-fat diet composed of standard rat chow (Nuvital, Nuvilab, Colombo PR, Brazil) plus shortening (Primor, SC, Brazil) and condensed milk (Nestlé, São Paulo, SP, Brazil). This diet contained 15 g of protein, 30 g of total fat, and 55 g of carbohydrate (15). All components of the high-fat diet were ground and blended. The C-Ob and RT-Ob groups received a high-fat diet during the experimental and RT periods.

Diets were manufactured weekly and stored in pellet form at 4°C in agreement with the American Institute of Nutrition (AIN-93G) recommendations. Animals had free access to water and chow during the experimental period. Food intake (g) was monitored every 3 days during this period, and body weight was evaluated weekly. Our results were consistent with Pinheiro et al. (15) and Carrol et al. (15), who reported that 30% of lipids in the diet induced several abnormalities typical of obesity.

Body mass, food intake, and fat percentage

Food intake (g) was monitored every three days during the experimental period, and body weight was evaluated weekly. Total fat percentage was assessed using dual-energy X-ray absorptiometry (DEXA; Lunar DEXA 200368 GE instrument; Lunar, Wisconsin, USA) and was performed before the last training session after an eight-hour fast while the animals were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg). Analysis was performed using specific software (Encore 2008; Version 12.20; GE Healthcare).

Resistance training protocol

Climbing sessions were performed three times per week during the 12 weeks of RT. The rats were initially adapted to



the RT protocol by climbing a vertical ladder (1.1×0.18 m, 2 cm grid, 80° incline) with weights attached to their tails. The length of the ladder induced the animals to perform 8-12 movements per climb, as previously described (16). The load apparatus was attached to the animal's tail by wrapping the proximal portion of the tail with a self-adhesive foam strip. A Velcro strap was wrapped around the foam strip and fastened. Each rat was placed at the bottom of the ladder with the load apparatus attached to the tail and was familiarized with climbing. A stimulus was applied with tweezers to the animal's tail to initiate movement when necessary. Rats reached a housing chamber (20×20×20 cm) at the top of the ladder, where they were allowed to rest for 120 s. This procedure was repeated until each rat would voluntarily climb the ladder three consecutive times without any stimulus.

The first training session started two days after the familiarization period and consisted of four to nine ladder climbs while carrying progressively heavier loads. The initial climb consisted of carrying a load that was 75% of the animal's body weight. A series of additional 30-g weights were added until the load hampered the animal's capacity to climb the entire ladder. Failure was established after three non-successful attempts to reach the top of the ladder. The highest load successfully carried over the entire length of the ladder was considered the rat's maximal carrying load (MCL) for that training session.

The training sessions consisted of four ladder climbs with 50%, 75%, 90%, and 100% of the rat's previous MCL, as determined in the previous session. An additional 30-g load was added during subsequent ladder climbs until a new MCL was determined. The RT protocol was adapted from previous studies (17) according to the needs of the present protocol. Furthermore, the relative force for each animal was determined by dividing the body mass by the final MCL obtained at the end of the experimental period.

Tissue preparation and determination of lipid tissue content

Animals from the sedentary and trained groups were decapitated after 24 weeks; the exercised groups were decapitated 48 hours after the last training session. All decapitations were performed during the same period. Entire biceps and gastrocnemius muscles were removed from the anterior and posterior limbs, respectively, frozen in liquid nitrogen, and stored at -84°C for biochemical analysis. Total lipid levels were determined using the sulphophospho-vanillin colorimetric method adapted for tissue (18).

Determination of MMP-2 in muscles

Muscle samples were treated as described previously (7) with minor adaptations. Each group of rats (n=32) was analyzed separately. Frozen tissues (50 mg) were incubated in 1.250 ml of extraction buffer [10 mmol.l⁻¹ cacodylic acid (pH 5.0); 0.15 mmol.l⁻¹ NaCl; 1 mmol.l⁻¹ ZnCl₂; 20 mmol.l⁻¹ CaCl₂; 1.5 mmol.l⁻¹ NaN₃; 0.01% Triton X-100 (v/v)] at 4°C overnight in continuous stirring. The solution was centrifuged for 20 min (13,000 g at 4°C). Aliquots of samples containing 20 µg/µl proteins were subjected to SDS-PAGE with gelatin (1mg/ml). After electrophoresis, gels were washed twice in 2.5% Triton X-100 to remove SDS. Gels were incubated in buffer substrate [50 mmol.l⁻¹ Tris-HCl (pH 8.0); 5 mmol.l⁻¹ CaCl₂; 0.02% NaN₃] at 37°C for 20 h. Gels were stained with Coomassie brilliant blue for 1 h and

destained with acetic acid/methanol/water (1:4:5) for the visualization of activity bands. Samples of 8 animals per group were evaluated to guarantee precision and linearity of analysis, and each sample was normalized for the total amount of protein included. Gels were photographed using a Canon G6 Power Shot 7.1 megapixel camera (Newport News, Virginia, USA), and averages of band intensity were measured using Gene Tools software (Philomath, Oregon, USA). The bands in all groups were 72-62 kDa, which supports MMP-2 activation as previously proposed (19). Active bands were analyzed as described previously (7).

Statistical analyses

Statistical analysis was initially performed using the Shapiro-Wilk Normality test. All variables presented normal distribution and are reported as means ± standard errors of the mean (SEM).

Body mass, total fat percentage, and MMP-2 activity were analyzed using two-way analysis of variance (ANOVA; diet [normal chow *vs.* high-fat diet] X training [sedentary and RT groups]) followed by Tukey's post hoc test (significant Main Effects) for multiple comparisons.

The final MCL and relative force for trained groups were analyzed using Student's *t* test. Data were analyzed using Statistics 7.0 software package (Stat. Soft. Tusa Inc., OK, USA) with an α level of 0.05.

■ RESULTS

Table 1 depicts the following results. There were no statistically significant interactions between groups for body mass. The C-Ob and RT-Ob groups had higher final body masses compared to the C and RT groups ($p<0.05$). The RT group tended toward a lower body mass than the C group (431.52 ± 16.38 *vs.* 453.97 ± 14.06, $p=0.8055$), but the difference was not statistically significant. Of note, RT-Ob had a lower final body mass than the C-Ob group ($p=0.003$). Bicep mass was greater in the C-Ob group compared to the RT group ($p=0.010$). The same result was noted for gastrocnemius mass between the C-Ob and RT groups ($p=0.0042$) and the C-Ob and C groups ($p=0.030$).

Some effects of high-fat diet consumption were observed in the C-Ob group, including higher fat and lower lean masses compared to the other groups ($p<0.05$, Figures 1b and c). In contrast, the RT group had a decreased fat percentage ($p<0.05$, Figure 1a) compared to the C ($p=0.001$), C-Ob ($p<0.001$), and RT-Ob ($p<0.001$) groups, and lower fat and higher lean masses were also noted ($p<0.05$; Figure 1).

We confirmed our hypothesis by observing higher MMP-2 activity in the biceps of rats in the RT group compared to the C group ($p=0.034$). However, no difference was observed between the C-Ob and RT-Ob groups (Figure 2). Consistent with our hypothesis, higher MMP-2 activity in the gastrocnemius muscle was also noted in the RT-Ob compared to the C ($p=0.007$) and C-Ob groups ($p=0.001$). However, no significant difference was noted between the RT-Ob and RT groups. Finally, it should be mentioned that no interaction was observed between groups for total MMP-2 activity in biceps and gastrocnemius muscles. Pro-, intermediate, and active MMP-2 proteins were also tested, and similar results were obtained. MMP-2 expression was the same as total MMP-2 activity (data not shown).



Table 1 - Body mass and body composition of experimental groups.

	C	C-Ob	RT	RT-Ob
Final body mass	453.97 ± 14.06	566.42 ± 22.16 ^a	431.52 ± 16.83 ^b	500.32 ± 16.36 ^{a,b,c}
Gastrocnemius mass (g)	2.49 ± 0.10	2.57 ± 0.05 ^a	2.47 ± 0.12 ^b	2.57 ± 0.07
Gastrocnemius lipid content (mg.100 mg tissue ⁻¹)	1.31 ± 0.16	1.41 ± 0.10	1.19 ± 0.07	1.29 ± 0.09
Gastrocnemius mass/body mass	0.00548 ± 0.00014	0.00460 ± 0.00023	0.00572 ± 0.00014	0.00051 ± 0.00028
Biceps mass (g)	0.203 ± 0.007	0.226 ± 0.007	0.213 ± 0.011 ^b	0.216 ± 0.007
Biceps lipid content (mg.100 mg tissue ⁻¹)	1.60 ± 0.18	2.18 ± 0.28	1.55 ± 0.13	1.81 ± 0.28
Biceps mass/Body mass	0.00044 ± 0.000015	0.00040 ± 0.000017	0.00049 ± 0.000016	0.00043 ± 0.00002
Energy intake (kcal/day)	101.5 ± 3.79	93.41 ± 2.62	97.41 ± 3.22	90.25 ± 2.75

Values are presented as means ± SEM; P<0.05 (n=8 per group). C=Control group; C-Ob=Obese Control group; RT=Resistance Training group; RT-Ob=Obese Resistance Training group. Statistically significant differences compared to: ^aC group; ^bC-Ob group and ^cRT group.

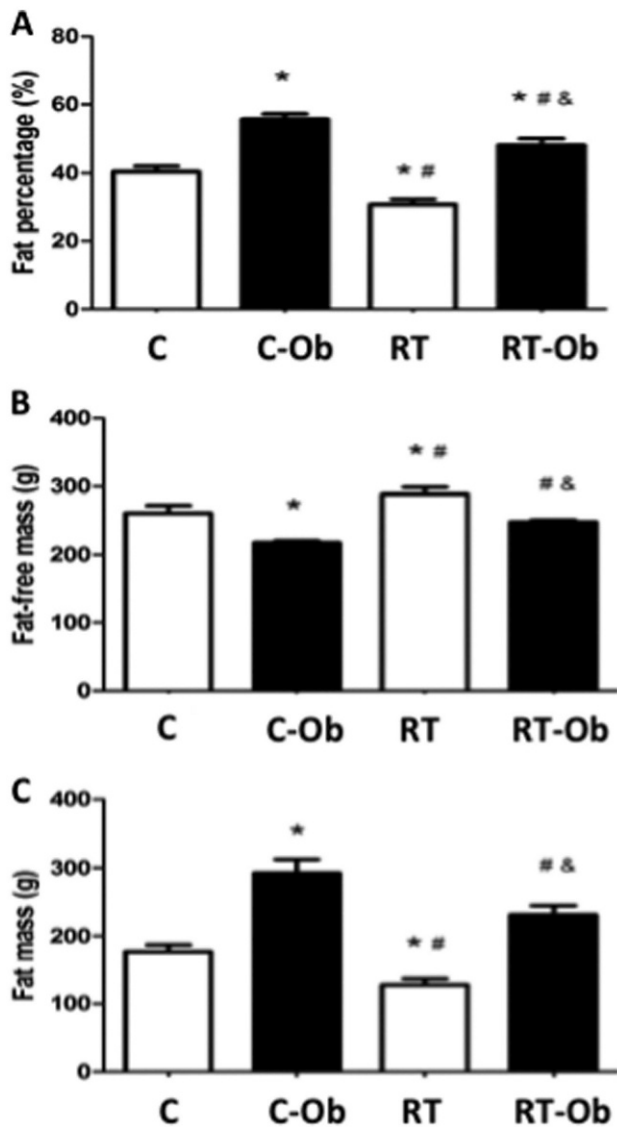


Figure 1 - Effects of resistance training and high-fat diet on fat percentage (a), fat-free mass (b), and fat mass (c). Statistically significant differences compared to: *C group, #C-Ob group, and &RT group.

MCL was not significantly different between the trained groups at the beginning [RT (331.30 ± 14.03 g) vs. RT-OB (383.82 ± 14.36 g); NS] and end of the training period [RT (914.32 ± 8.07 g) vs. RT-Ob (842.10 ± 27.86 g); NS]. However, the relative force was significantly higher in the RT group compared to the RT-Ob group (2.14 ± 0.09 vs. 1.69 ± 0.08; p<0.001).

DISCUSSION

We investigated the influence of RT on body composition and MMP-2 activity in the skeletal muscles of rats fed a high-fat diet. Interestingly, RT increased MMP-2 activity, which may contribute to tissue remodeling. Confirming our hypothesis, MMP-2 activity in the trained groups was greater compared to all other groups. Similar results were observed in previous studies (6,9), which demonstrated the interaction between RT and increased MMP-2 activity in gastrocnemius and soleus muscles in ovariectomized rats. Some factors may have contributed to this interaction, including the increase in training load (body mass + training load), the migration of satellite cells to the point of injury, and the release of pro-inflammatory cytokines (20).

Muscle cell basal lamina plays an important role in structural support and has a pivotal function in maintaining the physiological integrity of myofibrils (2). This basal lamina also serves as an electrolytic barrier and repairs muscle fibers after injury due to exercise-imposed overload (2). However, MMPs and TIMPs in skeletal muscle play fundamental physiological functions in ECM maintenance (21). MMPs are at the heart of a wide range of muscle functional, pathological, and developmental processes. These enzymes regulate muscle growth by promoting the release of growth factors, which act in local tissue repair after injury (1). Finally, these enzymes also play central roles in the maintenance and remodeling of the ECM by stimulating quiescent satellite cells. Once activated, these cells migrate to sites of injury, where they fuse to each other or to damaged fibrils, which allows tissue regeneration (22). Previous studies of the influence of physical training on pre-mRNA pathways in quadriceps and gastrocnemius muscles of old mice using ultrastructural cytochemistry showed that the activation of satellite cells during endurance exercises depends on the intensity, rather than the duration, of the exercise session; this activation also occurs during high-intensity resistance exercises (23,24).

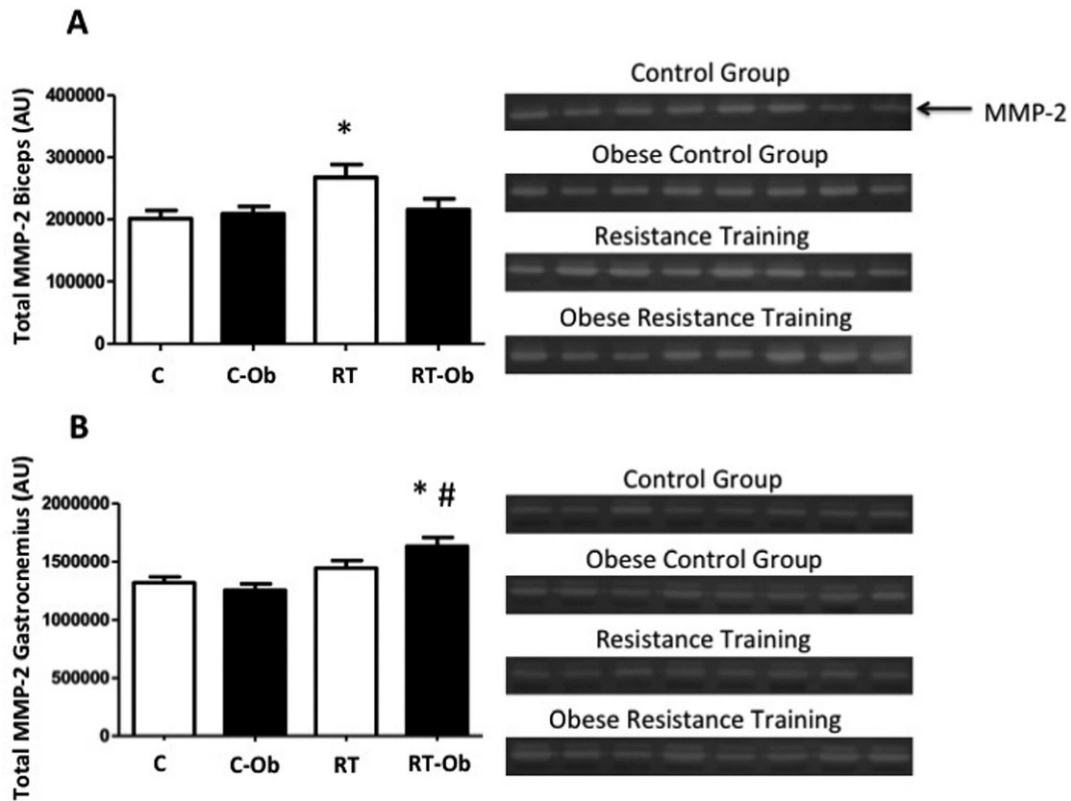


Figure 2 - Effects of resistance training and high-fat diet on total MMP-2 activity in biceps (A) and gastrocnemius (B) muscles. Total MMP-2 activity was determined by adding the integrated optical densities obtained (expressed in arbitrary units) for the three bands (pro-, intermediate, and active MMP-2 forms). Data are reported as means \pm SEM ($p < 0.05$). *Statistically significant difference compared to C; #statistically significant difference compared to C-Ob.

Increased adiposity may exert some negative influences on tissue remodeling, and some pro-inflammatory cytokines stimulate MMP-2 synthesis and activation, which contributes to collagen degradation (3,25). Our results demonstrated that obesity induced by a high-fat diet promoted unfavorable fat accumulation, altered body composition, and decreased muscle mass (*i.e.*, favored sarcopenia). Recently, some adipocytokines, such as interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF)- α , were correlated with decreased muscle mass and increased adipose tissue (20). The imbalance between muscle mass and obesity, *i.e.*, sarcopenic obesity, favors functional decline and increases obesity-related cardiovascular morbidity-mortality (20).

We noted that the RT-Ob group had lower body and fat masses and higher lean mass compared to the C-Ob group. Therefore, the proposed training sessions effectively improved and/or prevented obesity-related deleterious effects on body composition and mass and on skeletal muscle tissue. Although not tested, we could infer that improvements in body composition occurred as a consequence of higher energy expenditure during and after the training session due to increases in lean body mass and the subsequent increases in basal metabolic rate, energy expenditure, and fat oxidation (25).

It is noteworthy that the strength levels of the trained groups, as analyzed by MCL, were similar. Our findings are consistent with previous reports on obesity after 8 weeks (14) and on ovariectomized rats over 12 weeks (8,26). Benton et al. (13) proposed resistance sessions as a strategy for the

treatment and prevention of sarcopenic obesity in humans due to the increase in basal metabolic rate and the resultant negative energy balance. Additionally, changes promoted by resistance sessions induce muscle fiber alterations favoring muscle hypertrophy, as observed by increased cross-sectional areas of both type I and II fibers, which are essential for muscular endurance and strength.

Although we did not notice a difference in MLC between groups, one of our important findings was the observed benefit in the animals' capacity to respond to the stimulus of force imposed by the training. The analysis of relative force among animals not subjected to a high-fat diet (RT group) revealed greater MLC than among animals fed a high-fat diet, which suggests that changes in energy consumption induced by a high-fat diet would decrease physical capacity and impair muscle remodeling, which affects the contractile force.

A model of metabolic syndrome in Zucker rats showed decreased proliferation and satellite cell differentiation that was related to the increased expression of pro-inflammatory cytokines (27). The results indicated an imbalance in protein metabolism favoring catabolism, decrements in muscle tissue mass, and a reduced capacity to generate force. In this sense, we consider the lack of the analysis of inflammatory cytokines, gene and protein expression levels, and muscle cross-sectional area as limitations of the present study. These interactions should be investigated to clarify the involved mechanisms.

During aging, the lack of an anabolic environment due to physical inactivity and/or inadequate intake of nutrients,



associated with a catabolic trend, are key factors that may compromise lean mass (13). Finally, energetic and protein control in diets could aid in the prevention of fat accumulation and could promote lean mass gains. Increases in muscle mass have been inversely related to the negative effects of a high-fat diet. Therefore, RT could be viewed as a strategy to minimize the deleterious effects of the consumption of these diets.

In conclusion, the alterations of body composition and MMP-2 activity in skeletal muscles that are promoted by this experimental model of obesity could be positively modified by RT. Therefore, the RT proposed in this study can serve as a tool against some deleterious effects induced by the consumption of high-fat diets. However, further studies are necessary to clarify the exact mechanisms involved in these findings.

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AUTHOR CONTRIBUTIONS

Souza MV, Leite RD, Lino AD, and Marqueti RC elaborated the research project and performed experimental interventions. Bernardes CF participated in the research project and performed experimental interventions. Araújo HS participated in the research project and co-supervised the experimental interventions. Bouskella E, Shiguemoto GE, Perez SE and Kraemer-Aguiar LG supervised the research project.

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