BASIC RESEARCH

The effects of exercise modalities on adiposity in obese rats

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OBJECTIVE: The aim of the present study was to evaluate the effect of both swimming and resistance training on tumor necrosis factor-alpha and interleukin-10 expression, adipocyte area and lipid profiles in rats fed a high-fat diet.

METHODS: The study was conducted over an eight-week period on Wistar adult rats, who were divided into six groups as follows (n = 10 per group): sedentary chow diet, sedentary high-fat diet, swimming plus chow diet, swimming plus high-fat diet, resistance training plus chow diet, and resistance training plus high-fat diet. Rats in the resistance training groups climbed a vertical ladder with weights on their tails once every three days. The swimming groups swam for 60 minutes/day, five days/week.

RESULTS: The high-fat diet groups had higher body weights, a greater amount of adipose tissue, and higher tumor necrosis factor-alpha expression in the visceral adipose tissue. Furthermore, the high-fat diet promoted a negative change in the lipid profile. In the resistance training high-fat group, the tumor necrosis factor-alpha expression was lower than that in the swimming high-fat and sedentary high-fat groups. Moreover, smaller visceral and retroperitoneal adipocyte areas were found in the resistance training high-fat group than in the sedentary high-fat group. In the swimming high-fat group, the tumor necrosis factor-alpha expression was lower and the epididymal and retroperitoneal adipocyte areas were smaller compared with the sedentary high-fat group.

CONCLUSION: The results showed that both exercise modalities improved the lipid profile, adiposity and obesity-associated inflammation in rats, suggesting their use as an alternative to control the deleterious effects of a high-fat diet in humans.

KEYWORDS: Obesity; Adipocytokines; Exercise; Visceral Fat.

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INTRODUCTION

There is a growing concern regarding adipose tissue accumulation, as white adipose tissue is considered the most important fat depot associated with a chronic, low-grade pro-inflammatory metabolic state (1). Studies have demonstrated that metabolic signals from specialized cells (e.g., adipocytes, macrophages, and T-cells) initiate the inflammatory responses that induce alterations in metabolic homeostasis (1) through the secretion of numerous cytokines and adipokines (2,3). Furthermore, there is evidence that adipocyte size may be an important determinant of inflammatory cytokine secretion, including such cytokines as tumor necrosis factor-alpha (TNF-alpha), interleukin-6, and interleukin-1β (2-4).

Additionally, several diseases have been associated with low-grade inflammation, such as insulin resistance, dyslipidemia, type II diabetes, and cardiovascular disease (5-9). TNF-alpha seems to have an important role in the physiopathology of insulin resistance by inducing a diminished expression of glucose transporter 4 (GLUT-4) and decreasing the phosphorylation of insulin receptor substrate-1 (IRS-1) and specific insulin receptors (10,11). Furthermore, Bradley et al. demonstrated that a hyperlipidemic diet (46% fat) increased the expression of TNF-alpha

No potential conflict of interest was reported.

and other pro-inflammatory cytokines in adipose tissue in mice. Moreover, the results demonstrated that low-grade inflammation is associated with higher insulin, leptin and glucose levels (12).

In this sense, there is great interest in studying strategies to prevent or attenuate the effects of adipose tissue accumulation and the low-grade systemic inflammation associated with obesity (1,6,9,11). Several anti-inflammatory cytokines have been studied due to their role in the modulation of pro-inflammatory cytokines (e.g., interleukin-10 and adiponectin). Interleukin-10 (IL-10), produced mainly by macrophages and lymphocytes (11), can significantly inhibit both the expression and synthesis of pro-inflammatory cytokines and adipokines (13,14).

Exercise training is considered an important environmental factor associated with body weight regulation, and this training has been shown to decrease chronic, low-grade systemic inflammation in humans (15-17) and animals (12). Thus, increasing physical activity has become an important aspect of a non-pharmacological strategy to control obesity and weight gain (18-20).

Our research group has already demonstrated that intermittent swimming exercise is more efficient than continuous swimming exercise in decreasing adiposity in rats fed a high-fat diet (18). Bradley et al. (12) showed that voluntary wheel running could decrease visceral white adipose tissue inflammation in high-fat diet- induced obesity in mice. Moreover, the literature has demonstrated that treadmill exercise can minimize inflammation even in non-obese rats (21,22).

Among the exercise interventions investigated, resistance training has been shown to be effective in decreasing the adipose tissue depots in rats (23-25). However, the direct effect of resistance training, with or without a high-fat diet, on adipose tissue inflammation in obesity remains unclear. In addition, there is a lack of studies comparing aerobic exercise and resistance training protocols in obese rats.

Therefore, our hypothesis was that both resistance training and swimming exercise could alter the deleterious effects of a high-fat diet on adipose tissue, parameters of inflammation, and the lipid profile. Due to the significant differences in the volume and intensity of exercise modalities reported in the literature, the role of each one could be different. The aim of the present study was to evaluate the effect of both swimming and resistance training exercise on TNF-alpha and IL-10 expression, adipocyte area and lipid profiles in rats fed a high-fat diet.

METHODS

Animals

Sixty male Wistar rats (*Rattus novergicus* var. albinus, Rodentia, Mammalia) (90 days old) from the breeding colony of the Federal University of São Carlos (UFSCar), SP, Brazil, with an initial weight of 300 ± 17.31 g, were used. The animals were kept in individual polypropylene cages (30x20x13 cm), with food and water provided ad libitum, at a controlled temperature and humidity of 22-24℃ and 50-60%, respectively, and a 12-h light/dark cycle. All animal procedures were performed according to the principles of the USA Guide for the Care and Use of Laboratory Animals (26). The study was approved by the Committee of Experimental Animals (protocol no. 031/2009) at the Federal University of São Carlos.

Diets

The experimental groups received the standard rat chow diet (MP-77; Primor, São Paulo, Brazil) in a pellet form, which contains 23 g of protein, 49 g of carbohydrates, 4 g of total fat, and 5 g of fiber per 100 g of diet. The high-fat diet was composed of standard rat chow plus peanuts, milk chocolate, and sweet biscuits in a proportion of 3:2:2:1. This diet contained 20 g of protein, 20 g of total fat, 48 g of carbohydrate, and 4 g of fiber per 100 g of diet. The caloric density of the diets was determined with an adiabatic calorimeter (IKA-C400), and the values were as follows: 5.12 kcal/g for the high-fat diet and 4.07 kcal/g for the chow diet (27). All components of the high-fat diet were ground and blended. All the high-fat diet groups received a high-fat diet for three weeks before the training period and then throughout the training period (28).

Experimental groups

The rats were randomly distributed into the following six experimental groups (ten animals in each group): (I) sedentary chow (Sed-C); (II) sedentary high-fat diet (Sed-HF); (III) swim plus chow (Swim-C); (IV) swim plus high-fat diet (Swim-HF); (V) resistance training plus chow (RT-C); and (VI) resistance training plus high-fat diet (RT-HF). The sedentary groups (Sed-C and Sed-HF) were kept in their cages during the experimental period without any type of exercise. The groups that underwent training (Swim-C, Swim-HF, RT-C, and RT-HF) performed eight weeks of their respective exercise modalities. The training started at the same time for all groups.

Exercise protocols

Swimming. The rats were adapted to the water before the training began. The adaptation consisted of swimming for 30 minutes, once per day for five days, in water at a temperature of 31 ± 1℃. After adaptation, the rats were trained by swimming for 60 minutes/day, five days a week for eight weeks, with a constant overload equivalent to 5% of their body weight (29). The water tanks were 50 cm in height and 30 cm in diameter, and the overload (lead fish sinkers) was attached to the animal's chest using an appropriate vest.

Resistance training

The eight-week resistance training was performed once every three days with one week of familiarization. After this adaptation period, the animals were submitted to the experimental protocol consisting of 8-12 dynamic movements (reps) per climb, as previously described (30) and adapted (25).

The maximum load was determined by the following procedure. Each animal performed an initial climb carrying a load corresponding to 75% of the animal's body weight. Then, additional weight was added in 30-g increments until the rat could not climb the entire length of the ladder. The highest load successfully carried over the entire length of the ladder was considered the rat's maximal carrying capacity for that training session. The next training sessions consisted of four ladder climbs at 50, 75, 90, and 100% of the rat's previous maximum carrying capacity, as determined in the previous session. For the subsequent five ladder climbs, an additional weight was added in 30-g increments until a new maximum carrying capacity was determined. If

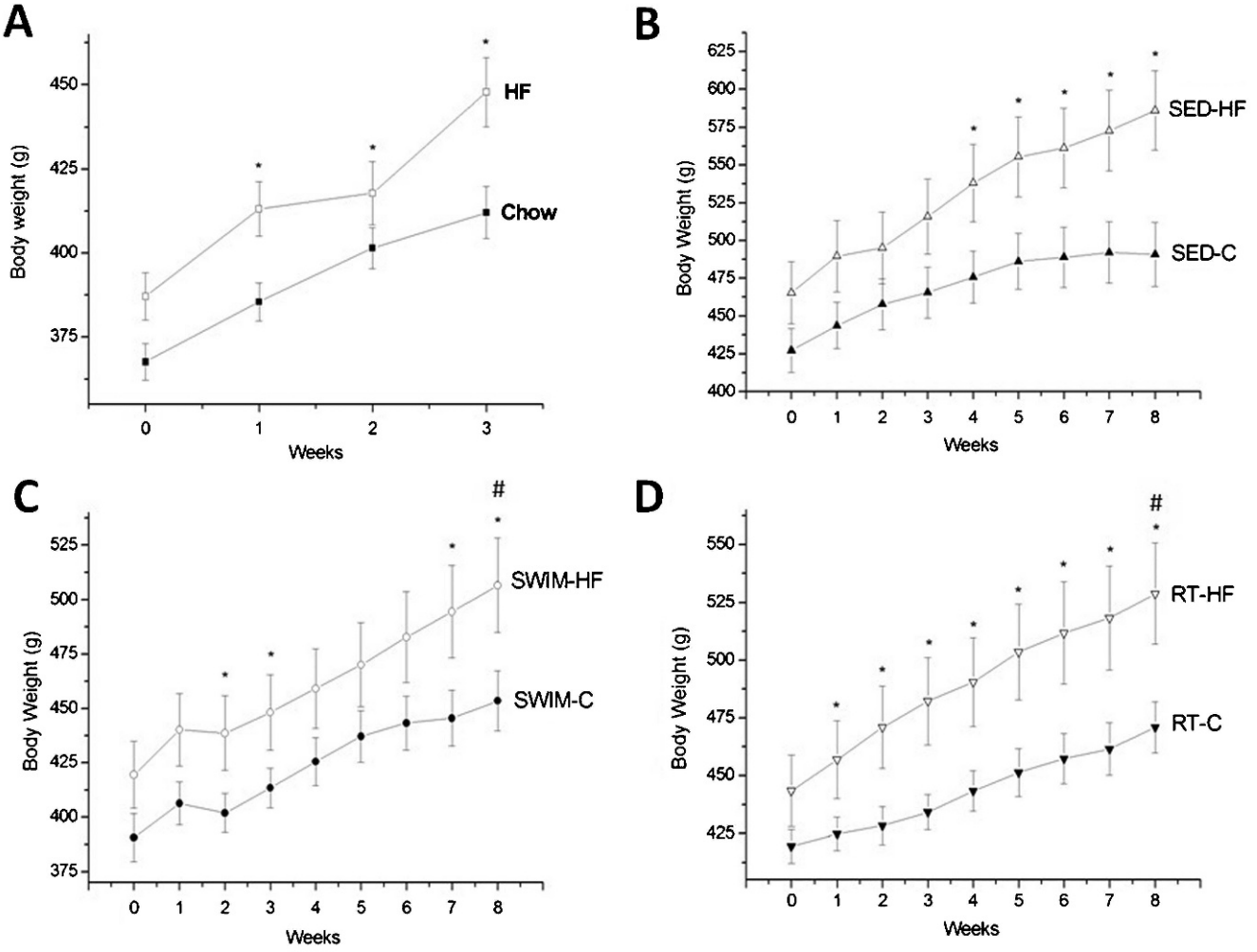

Figure 1 - Body weight evolution. All values are presented as means ± standard error of the mean; *$p \leq 0.05$ comparing before chow *vs.* high-fat diet (HF) 3 weeks before the training period (A) and during training period (B, C and D); #comparing trained groups *vs.* sedentary groups during training period (B, C and D) ($n = 10$ each group). C, chow; HF, high-fat diet; Sed-C, sedentary chow; Sed-HF, sedentary high-fat diet; Swin-C, swimming chow; Swim-HF, swimming high-fat diet; RT-C, resistance training chow; RT-HF, resistance training high-fat diet.

necessary, a stimulus with tweezers was applied to the animal's tail to initiate movement.

Measurements

Body weight and food intake were recorded daily.

Tissue collection

Animals were euthanized by decapitation 48 hours after the last training session. Retroperitoneal (RET), epididymal (EPI), visceral (VISC), and subcutaneous (SUBC) white adipose tissues were immediately removed, weighed and stored at –80℃ for further analysis. The adipose tissue collection was performed according to the descriptions of Cinti (31). Blood was also collected, centrifuged (3000 RPM (1157 g) for 10 min at 4℃), and stored at –80℃.

Analytical methods

The concentrations of total triacylglycerols (TGLs), total cholesterol (TC), and high-density lipoprotein (HDL) were determined enzymatically (Laborlab, São Paulo, Brazil), with sensitivities of 0.7 $mmol \times L^{-1}$, 0.14 $mmol \times L^{-1}$, and 0.5 $mmol \times L^{-1}$, respectively. All assays were performed in duplicate, with a coefficient of variation <0.05.

Adipocyte area determination

Fragments (100 mg) of RET EPI, and VISC adipose tissues were separated and fixed in 0.2 M collidine buffer (pH 7.4) containing 2% osmium tetroxide at 37℃. After 48 hours, the samples were washed with warmed saline and spread on a plate as described previously (32).

The adipocyte area was measured in different cells from the same tissue (EPI, RET, and VISC) approximately 50 times using image analysis software Image-Pro Plus KS-300 (Carl Zeiss, Oberkochen, Germany) and expressed in terms of μm^2. The cells were randomly chosen, and the person analyzing the images was blinded to the group assignments.

Quantitative PCR

Total RNA was extracted from visceral adipose tissues using TRIZOL® (Invitrogen Corporation, Carlsbad, California) according to the manufacturer's instructions. RNA quantification was performed using a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Absorbance of the RNA samples was quantified at 260 and 280 nm, and the 260/280 ratio was calculated. The samples exhibited a 260/280 ratio >1.9, which was selected as

Table 1 - The effects of exercise and diet on food consumption, caloric intake and maximum workload during the experiment period.

	Food Consumption (g)	Caloric intake (Kcal)
SED-C	2348.20 ± 74.78	9557.17 ± 304.37
SED-HF	2260 ± 69.70	11571,2 ± 357.30[a]
SWIM-C	2301 ± 63.36	9368.73 ± 257.89
SWIM-HF	2041.77 ± 61.79[a,b]	10453.90 ± 316.38[a,b]
RT-C	2232.40 ± 55.61	9085.86 ± 226.37
RT-HF	1961.22 ± 63.49[a,b,c]	10041.45 ± 325.08[a,b]

	Maximum workload (g)	
	Week 1	**Week 8**
RT-C	600.66 ± 17.75	1451.11 ± 35.14[d]
RT-HF	599.08 ± 19.55	1403.33 ± 37.97[d]

All values are presented as the mean ± standard error of the mean; [a] $p \leq 0.05$ comparing the groups fed a normal chow diet vs. the groups fed a high-fat diet; [b] $p \leq 0.05$ comparing the sedentary vs. training groups; [c] $p \leq 0.05$ comparing the swimming vs. resistance training groups; [d] $p \leq 0.05$ comparing week 1 vs. week 8 (n = 10 in each group). Abbreviations used: Sed-C, sedentary chow diet; Sed-HF, sedentary high-fat diet; Swim-C, swimming chow diet; Swim-HF, swimming high-fat diet; RT-C, resistance training chow diet; RT-HF resistance training high-fat diet.

an indicator of RNA purity. The samples were treated with amplification-grade DNAse (Deoxyribonuclease I) (Invitrogen Corporation, Carlsbad, California). cDNA was synthesized using oligo (dT) primers with the Advantage RT-for-PCR kit (Promega Corporation, Madison, WI USA). PCR amplifications were quantified using SYBR Green PCR (Promega Corporation) using a Rotor-Gene 3000 Real-Time Thermal Cycler (Corbett Research, Sydney, Australia). The primers used in this study were as follows: TNF-alpha (Fwd: 5-GGCTCCCTCTCATCAGTTCCA-3, Rev: 5-CGCTTGGTGG-TTTGCTACGA-3), and IL-10 (Fwd: 5-CAGCTGCGACGCT-GTCATCGA-3, Rev: 5-GCAGTCCAGTAGATGCCGGGTG -3). The results were normalized to the housekeeping gene GAPDH (Fwd: 5-GGTGGAGAGCACCAAGACAGA-3, Rev: 5-GCCGGAGTCGACAATGATG-3). The following primers are available on the NCBI website: TNF-alpha (NM_012675.3), IL-10 (NM_012854.2), and GAPDH (NM_017008.3).

Statistical analysis

All data are presented as the mean ± standard error of the mean (SEM). The statistical analyses were initially performed using the Kolmogorov-Smirnov normality test and the homoscedasticity test (Bartlett criterion). The interaction between the effects of diet and exercise on all variables was analyzed by two-way ANOVA. One-way ANOVA and the independent t-test were also used to compare the sedentary groups with the swimming and resistance training groups, as well as the high-fat diet groups with the chow groups. Fisher's post-hoc test was applied in the event of a significant ($p<0.05$) F ratio. The software package used was Statistica® 6.1 (Tulsa, OK, USA).

RESULTS

Body weight, food consumption and caloric intake

Before starting the diet (week 0), both the normal chow diet (C) and high-fat diet (HF) groups had similar body weights (Figure 1A, $p = 0.06$). However, the high-fat diet groups exhibited higher body weights compared with the normal chow diet groups in weeks 1, 2, and 3. (Figure 1A; $p = 0.003$, $p = 0.032$, and $p = 0.003$, respectively). After the eight-week training period, all groups treated with the high-fat diet (Sed-HF, Swim-HF, and RT-HF) had a higher body weight compared with their respective normal chow diet groups (Sed-C, Swim-C, and RT-C) (Figure 1B, 1C, 1D; $p<0.05$). Nevertheless, the trained HF groups (Swim-HF and RT-HF) had a lower body weight than the Sed-HF group at the end of the training period ($p<0.05$).

There was no statistically significant difference between the sedentary groups (Sed-C and Sed-HF) with regard to food consumption (g) ($p = 0.34$) at the end of the experimental period (a three-week diet and an eight-week training period). The trained high-fat diet groups consumed a lower amount of food compared with the normal chow diet groups ($p<0.05$). Moreover, the Swim-HF group consumed a greater amount of food ($p = 0.002$) than the RT-HF group (Table 1).

The caloric intake (Kcal) was higher in all of the HF groups compared with the C groups (Table 1; $p<0.05$). The Swim-HF and RT-HF groups had lower caloric intakes compared with the Sed-HF group ($p = 0.01$ and $p<0.001$, respectively). There was no statistically significant difference in the interaction between diet and exercise related to either food consumption or caloric intake.

Table 2 - Epididymal, retroperitoneal and visceral adipocyte areas.

	EPI (μm^2)	RET (μm^2)	VISC (μm^2)
SED-C	8921.53 ± 62.45	14805.38 ± 125.16	6832.32 ± 92.77
SED-HF	21935.42 ± 130.23[a]	28945.10 ± 102.23[a]	12578.13 ± 56.90[a]
SWIM-C	8026.83 ± 20.29[b]	10523.88 ± 47.44[b]	6609.82 ± 31.64
SWIM-HF	21147.42 ± 103.34[a,b]	23777.72 ± 327.28[a,b]	12715.79 ± 114.35[a]
RT-C	13298.14 ± 56.30[b,c]	14442.78 ± 80.33[c]	8301.50 ± 45.20[b,c]
RT-HF	18.238.62 ± 76.58[a,b,c]	19408.60 ± 117.48[a,b,c]	11013.45 ± 63.21[a,b,c]

All values are presented as the means ± standard error of the mean; [a] $p \leq 0.05$ comparing the normal chow diet vs. high-fat diet groups; [b] $p \leq 0.05$ comparing the sedentary vs. trained groups; [c] $p \leq 0.05$ comparing the swimming vs. resistance training groups (n = 5 in each group). Abbreviations used: EPI, epididymal; RET, retroperitoneal; VISC, visceral; Sed-C, sedentary chow diet; Sed-HF, sedentary high-fat diet; Swim-C, swimming chow diet; Swim-HF, swimming high-fat diet; RT-C, resistance training chow diet; RT-HF, resistance training high-fat diet.

Maximum workload

The maximum workload capacity of the resistance training groups (RT-C and RT-HF) increased throughout the training period (Table 1). The workloads were higher in weeks four and eight compared with week one ($p<0.001$) in both resistance training groups ($p<0.001$).

Relative white adipose tissue weight and adipocyte area

The mean areas of adipocytes in the epididymal, retroperitoneal and visceral adipose tissues of the Sed-HF group were larger ($p<0.001$) than those of the Sed-C group (Table 2). Similar results were observed between the trained high-fat diet groups (Swim-HF and RT-HF) and the trained normal chow groups (Swim-C and RT-C) ($p<0.005$). Moreover, regardless of the diet, all adipocyte areas were smaller in the swimming groups compared with the sedentary groups ($p<0.001$), with the exception of the visceral adipocyte area in both the swimming high-fat diet

group ($p = 0.14$) and the swimming chow group ($p = 0.55$). The resistance training chow diet group (RT-C) had larger epididymal and visceral adipocyte areas than the sedentary (Sed-C) and swimming (Swim-C) groups ($p<0.001$). In addition, the retroperitoneal adipocyte area was larger in the RT-C group than in the Swim-C group ($p<0.001$). All adipocyte areas were smaller in the resistance training high-fat diet group (RT-HF) compared with the sedentary high-fat diet group (Sed-HF) and the swimming high-fat diet group (Swim-HF) ($p<0.001$).

All high-fat diet groups exhibited significantly higher relative epididymal, retroperitoneal, visceral, and subcutaneous adipose tissue weights compared with the normal chow diet groups (Figures 2A and 2B; $p<0.05$). The relative weight of the epididymal tissue was lower in the Swim-HF ($p = 0.03$) and RT-HF ($p = 0.04$) groups compared with the Sed-HF group. Additionally, the Swim-C group had a lower relative epididymal weight compared with the Sed-C group ($p = 0.03$). Nevertheless, the relative visceral weights were higher in the Swim-HF group than in the sedentary ($p = 0.009$) and resistance training ($p<0.001$) high-fat diet groups.

Gene expression

There was an interaction between diet and exercise with regard to TNF-alpha ($p<0.001$), but not IL-10 ($p = 0.53$), expression. The swimming exercise training seemed to be more effective in increasing anti-inflammatory adipokine (IL-10) expression than the resistance training. The swimming groups (Swim-C and Swim-HF) exhibited higher expression levels of IL-10 than the Sed-C, Sed-HF, RT-C, and RT-HF groups ($p<0.05$), except the Swim-HF group, compared with the Sed-HF group (Figure 3A). Additionally, the Swim-C group exhibited significantly higher levels than the Swim-HF group ($p = 0.001$). However, the resistance training groups exhibited a lower expression level of the pro-inflammatory adipokine TNF-alpha. TNF-alpha expression levels were lower in the trained high-fat groups (Swim-HF, $p = 0.01$, and RT-HF, $p<0.001$) compared with the Sed-HF groups. Nevertheless, the Swim-HF groups exhibited significantly higher expression than the RT-HF group (Figure 3B; $p<0.001$). The Swim-HF and Sed-HF groups

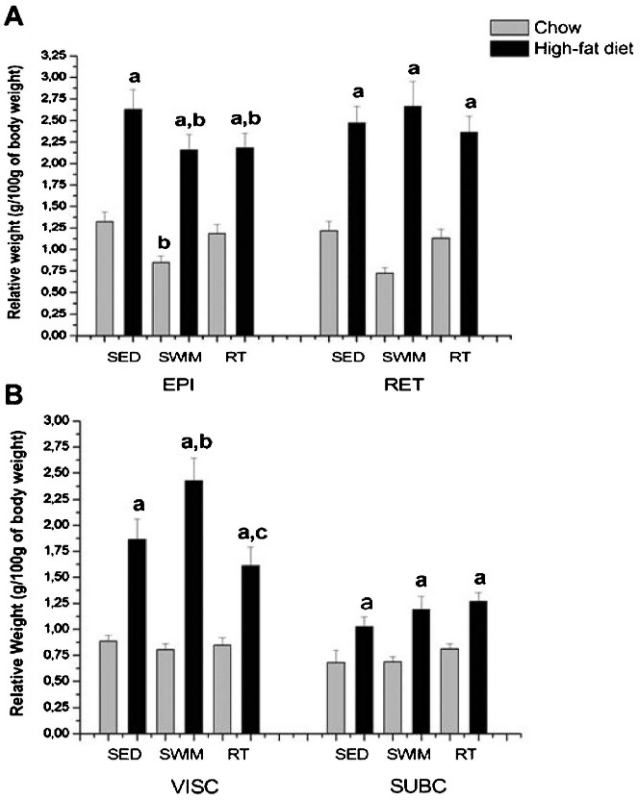

Figure 2 - Relative weight of adipose tissue. All values are presented as means ± standard error of the mean; [a]$p \leq 0.05$ comparing normal chow diet *vs* high-fat diet groups; [b]$p \leq 0.05$ comparing sedentary *vs* trained groups; [c]$p \leq 0.05$ comparing swimming *vs* resistance training groups ($n = 10$ each group). A, EPI, epididymal; RET, retroperitoneal; B, VISC, visceral; SUBC, subcutaneous; SED, sedentary; SWIM, swimming; RT, resistance training.

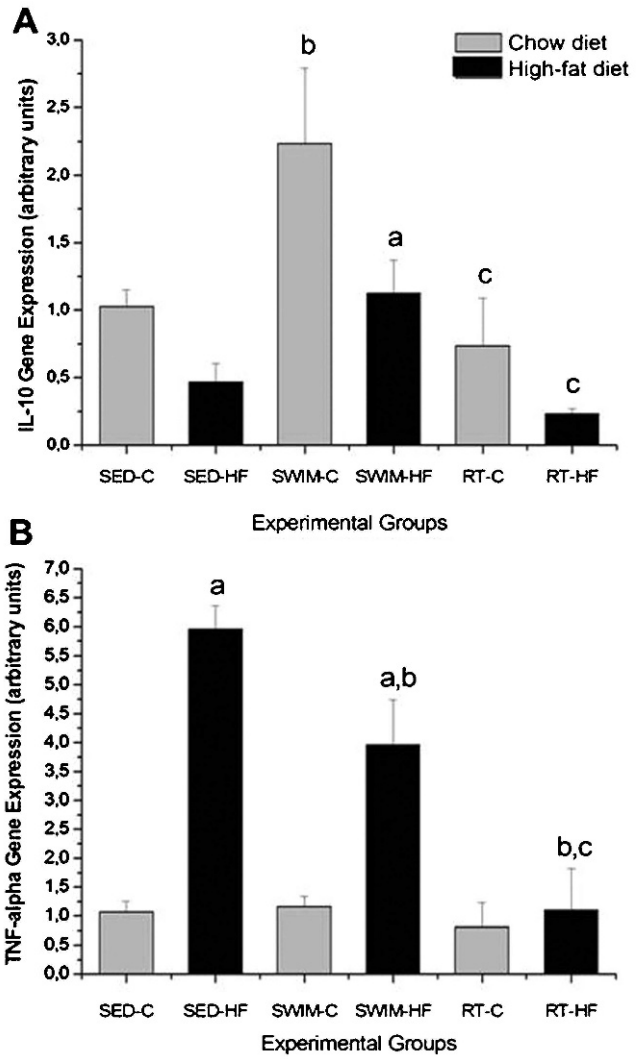

Figure 3 - IL-10 (A) and TNF-alpha expression (B). All values are presented as means ± standard error of the mean; [a]$p \leq 0.05$ comparing normal chow diet *vs* high-fat diet groups; [b]$p \leq 0.05$ comparing sedentary *vs* trained groups; [c]$p \leq 0.05$ comparing swimming *vs* resistance training groups ($n = 5$ each group). IL-10, interleukin-10; TNF-alpha, tumor necrose factor-alpha; Sed-C, sedentary chow diet; Sed-HF, sedentary high-fat diet; Swim-C, swimming chow diet; Swim-HF, swimming high-fat diet; RT-C, resistance training chow diet; RT-HF resistance training high-fat diet.

exhibited higher levels ($p<0.001$) of TNF-alpha expression compared with the respective normal chow diet groups (Swim-C and Sed-C). However, there was no significant difference between the RT-HF and RT-C groups with regard to TNF-alpha expression ($p = 0.69$).

Lipid parameters

All exercised groups exhibited lower levels of total cholesterol and triacylglycerols (TGLs) and higher values of HDL than the sedentary groups ($p<0.05$), with the exception of the Swim-HF group, which exhibited TGL values similar to the Sed-HF group (Table 3). Moreover, the TGL levels in the Swim-HF group were higher than those in the RT-HF group ($p = 0.001$), and they were also higher in the Sed-HF group than in the Sed-C group ($p = 0.01$). Furthermore, higher values ($p = 0.02$) of total cholesterol were found in the Swim-HF group compared with the respective normal chow diet group (Swim-C). When the trained groups were compared, the Swim-C group was found to have lower values ($p = 0.02$) than the RT-C group. The HDL values were higher in the Swim-HF group compared with the Swim-C ($p = 0.04$) and RT-HF ($p<0.001$) groups.

DISCUSSION

Our study showed that both swimming and resistance training can alter the deleterious effects of a high-fat diet in rats. However, this study adds some new findings related to the training modalities. The exercise protocols have significant differences both in intensity and volume. In this sense, low-volume/high-intensity resistance training was effective in controlling the expression of TNF-alpha in the visceral adipose tissue, controlling body weight, minimizing the increase on the retroperitoneal and visceral adipocyte areas, and producing significant changes in the lipid profile. On the other hand, high-volume/moderate-intensity swimming was effective in increasing the expression of IL-10 in the visceral adipose tissue, controlling body weight, reducing the epididymal and retroperitoneal adipocyte areas, and inducing positive effects on the lipid profile.

In the literature, different high-fat diets are found to induce a positive energy balance and obesity in rats and mice (12,33-35). Specifically, these diets have been shown to impair glucose metabolism, stimulate abnormal glucose production, and promote both insulin resistance and a chronic, low-grade pro-inflammatory metabolic state (12,27). In the present study, we used the diet previously described by Estadella et al. (27). Our research group has demonstrated that this particular diet can induce weight gain and promote deleterious effects on adiposity, fatty liver development, and the lipid profile in rats (18,28). Moreover, our study demonstrated that a high-fat diet can induce a chronic, low-grade pro-inflammatory metabolic state in rats.

Table 3 - Total serum cholesterol, HDL and triacylglycerol levels.

	Total Cholesterol (mg/dl)	HDL (mg/dl)	TGL (mg/dl)
SED-C	51.05 ± 3.06	13.85 ± 0.68	73.66 ± 4.22
SED-HF	57.83 ± 2.34	13.77 ± 0.66	88.94 ± 6.71^{a}
SWIM-C	$39.61 \pm 1,36^{b}$	15.85 ± 1.21^{b}	62.72 ± 4.30^{b}
SWIM-HF	$46.94 \pm 4.22^{a,b}$	$19.85 \pm 0.85^{a,b}$	87.83 ± 6.44
RT-C	$48.22 \pm 1.62^{b,c}$	18.69 ± 0.62^{b}	65.27 ± 4.32^{b}
RT-HF	47.22 ± 2.41^{b}	$17.87 \pm 1.01^{b,c}$	$66.94 \pm 5.15^{b,c}$

All values are presented as the mean $\pm$ standard error of the mean. [a] $p \leq 0.05$ comparing the normal chow diet vs. high-fat diet groups; [b] $p \leq 0.05$ comparing the sedentary vs. trained groups; [c] $p \leq 0.05$ comparing the swimming vs. resistance training groups (n = 10 in each group). Abbreviations used: HDL, high-density lipoprotein; TGL, triacylglycerol; Sed-C, sedentary chow diet; Sed-HF, sedentary high-fat diet; Swim-C, swimming chow diet; Swim-HF, swimming high-fat diet; RT-C, resistance training chow diet; RT-HF resistance training high-fat diet.

Our results showed that the high-fat diet exerted a strong influence on body weight at three weeks. Previous studies have demonstrated that a few weeks of a high-fat diet are sufficient to induce body weight gain, fat accumulation and lipid profile alterations in rats (12,18,27,36). Additionally, the body weights of all the high-fat diet groups, both sedentary and trained, were significantly increased until the end of the experiment compared with the body weights of the chow diet groups. However, the trained groups had a lower body weight than the sedentary groups. Our results are in agreement with the other studies in the literature reporting that exercise alone does not promote a significant loss of body weight in humans and animals (12,15). Therefore, changes in diet are more related to body weight changes than exercise (15).

Lower food consumption was associated with an increased caloric intake in the HF groups. These results also suggest that rats are sensitive to both energy and the high-fat contents of diets with regard to the controlling of their food intake by satiety (37,38). Moreover, our data showed that both the swimming and resistance training groups had a lower food consumption and caloric intake compared with the sedentary groups. Previous studies showed similar results (18,35). Furthermore, Ropelle et al. (34) demonstrated that exercise increases the expression of IL-6 and IL-10 in the hypothalamus, which increases insulin and leptin sensitivity through the reduction of IkappaB kinase (IKKb) and inhibition of endoplasmic reticulum (ER) stress, culminating in a decrease in food intake. Thus, the increase in energy expenditure through exercise training and the lower caloric intake could have contributed to the lower body weight gain found in both of the trained HF groups (Swim-HF and RT-HF).

In the current study, a high-fat diet also promoted higher relative weights of all adipose tissues and larger adipocyte areas of the epididymal, retroperitoneal, and visceral adipose tissues. Other studies have demonstrated the same results (18,27,39,40). In the trained groups fed a high-fat diet, the relative weight of the epididymal adipose tissue was lower compared with that of the sedentary group fed the same diet. Moreover, high-volume/moderate-intensity swimming was not able to reduce the visceral adipocyte area. Our findings are consistent with other studies that did not observe a significant decrease in either visceral tissue relative weight or adipocyte area with continuous exercise in rats fed a high-fat diet (18,36). These results suggest a higher free fatty acid (FFA) mobilization from other adipose tissues compared with the visceral adipose tissue during exercise.

These results could be associated with the load that was used in the swimming exercise in our study (5% of the rat's body weight). This intensity of exercise is considered to correspond to the transition between aerobic and anaerobic

metabolism (lactate threshold) in rats, as previously described by Gobatto et al. (29). The corresponding intensity of exercise promotes a substantial increase in catecholamine levels (41); visceral adipose tissue is very sensitive to catecholamines due to a higher number of alpha and beta adrenergic receptors (42). In light of these facts, we speculate that the load used by the obese rats during swimming was not enough to promote a significant increase in catecholamine levels, resulting in a smaller mobilization of visceral adipose tissue during the swimming exercise. Recently, Araujo et al. (33) demonstrated that the load corresponding to the maximum steady-state lactate level (the gold standard for the identification of the anaerobic threshold) in obese rats is 6% of the rats' body weights. Therefore, it seems that the load that we used for obese rats is a limitation to our study.

The main purpose of the current study was to analyze the inflammatory response of both high-volume/moderate-intensity swimming exercise and low-volume/high-intensity resistance training in rats fed a high-fat diet. In this sense, our results suggest an important role of both types of exercise in regulating the low-grade inflammatory status in obesity. The high-fat diet induced a higher (six-fold) TNF-alpha expression level in visceral adipose tissue. Both high-fat diet trained groups (Swim-HF and RT-HF) exhibited a lower expression of this pro-inflammatory adipokine compared with the Sed-HF group. These findings are in accordance with the literature (1,12,43), where studies have shown a decrease in both TNF-alpha expression in adipose tissue and serum TNF-alpha levels after aerobic training. Bradley et al. (12) demonstrated that voluntary exercise could decrease TNF-alpha expression and improve insulin sensitivity in obese mice through a high-fat/high-sucrose diet. Even with a lower training volume, the high-intensity resistance training seemed to be more effective in controlling the TNF-alpha expression compared with the swimming exercise.

According to Gregor and Hotamisligil (1), TNF-alpha is more highly expressed in hypertrophied adipocytes than in normal size adipocytes, and this finding could support the results in the current study showing that the resistance training promoted smaller visceral adipocyte areas. TNF-alpha plays a key role in the development of insulin resistance, type II diabetes, atherosclerosis and metabolic syndrome, mainly by activating the c-jun N-terminal kinase (JNK) and inhibiting the κ kinase and the protein kinase R (PKR) (1). Thus, our results suggest that low-volume/high-intensity resistance training could be an important intervention strategy for both the prevention and treatment of the inflammatory state of obesity and associated chronic diseases.

On the other hand, high-volume/moderate-intensity swimming exercises promoted higher IL-10 expression levels in rats fed the chow diet and the high-fat diet, while resistance training was not able to induce the same adaptations. Our results are in agreement with previous studies (34,44) that observed an increase in IL-10 expression in obese and lean rats through aerobic exercises. IL-10 is an important immunoregulatory cytokine with multiple biological effects. In the cytoplasm, it has been shown that IL-10 blocks nuclear factor kappa B (NF-kB) at two levels, namely by suppressing the inhibitor of κ kinase (IKK) activity and NF-kB DNA binding activity (45). Therefore, low levels of IL-10 have been observed in obesity, metabolic syndrome and type II diabetes (11). Moreover, Ropelle et al. (34) observed that the intra-hypothalamic infusion of recombinant IL-10 blocked IKK/NF-kB signaling and endoplasmic reticulum (ER) stress while restoring serine-threonine kinase (Akt) and the signal transducer and activator of transcription-3 (STAT3) phosphorylation, promoting a re-balance in the energy intake in obese animals. According to Waters et al. (46), the increase in IL-10 expression seems to be an important protective factor against insulin resistance, atherosclerotic plaque instability and acute coronary ischemia.

Our results also confirmed the evidence that a high-fat diet promotes changes in the lipid profile (18,27). Furthermore, the high-fat diet modified the serum lipid profile by increasing triacylglycerol (TGL) and total cholesterol (TC) levels. Studies in the literature have shown that the lipid profile alteration is strongly associated with metabolic syndrome (47), insulin resistance and nonalcoholic fatty liver disease (48). The current results showed that both the swimming and resistance training groups had higher HDL levels compared with sedentary groups, regardless of the diet administered. Furthermore, the resistance training yielded lower TGL levels. In contrast, several studies have reported a decrease in TGL levels after aerobic exercise training (18,47,48). These effects could be due to the higher levels of TNF-alpha expression in the swimming groups compared with the resistance training groups after eight weeks of exercise in the current study. The effect of TNF-alpha in reducing fatty acid oxidation in hepatocytes and skeletal muscle through the induction of protein phosphatase 2C and the suppression of AMP-activated protein kinase (AMPK) has been previously demonstrated (49). Thus, the reduced rates of fatty acid oxidation are accompanied by an increased accumulation of bioactive lipids, such as TGLs (49).

In conclusion, the effect of different exercise modalities on low-grade inflammation and obesity has attracted much interest. In the present study, both resistance training and swimming positively affected the inflammation parameters, body weight, adipocyte area and lipid profile in obese rats. These data support the finding that both exercise modalities seem to be effective in controlling the effects of the consumption of a high-fat diet. However, the influence of each modality on the analyzed parameters seems to be different. Therefore, we can suggest that concurrent training, i.e., swimming exercise at a high volume/moderate intensity along with resistance training at a low volume/high intensity, could be a great choice for the prevention and/or treatment of obesity. Furthermore, despite the fact that exercise has been shown to alter various pathways associated with obesity, many questions still remain unanswered. Thus, more studies are needed in this field to evaluate the exercise-induced changes associated with obesity and chronic low-grade inflammation.

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AUTHOR CONTRIBUTIONS

Speretta GF and Rosante MC conceived and designed the study, drafted the manuscript, analyzed and interpreted the data, critically revised the manuscript for intellectual content, and approved the final version of the

manuscript. Duarte FO, Leite, RD, Lino AD, Andre RA, Silvestre JG, Selistre de Araujo HS, and Duarte AC drafted the manuscript, interpreted the data and critically revised the manuscript for intellectual content.

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