Resistance Training Suppresses Intra-abdominal Fatty Acid Synthesis in Ovariectomized Rats

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Key words
- exercise training
- lipid metabolism
- visceral adipose tissue
- ovarian hormone deficiency

Abstract

Ovarian hormone loss is associated with a shift in fat distribution to intra-abdomin al adipose tissue (intra-AAT) depots and with lipid metabolism disorders, which predisposes individuals to developing insulin resistance. Resistance training (RT) prevents increases in intra-AAT after ovarian hormone loss. However, the molecular mechanisms underlying these changes remain unclear. We investigated the effects of ovariectomy and RT on gene expression related to lipogenesis and fat oxidation in the intra-AAT of ovariectomized rats. Sprague-Dawley rats (n=6/group) were divided into the groups: sham-sedentary, ovariectomized-sedentary, sham-RT and ovariectomized-RT. RT groups performed a 10-week climbing program on a ladder with progressive overload. Intra-AAT was subjected to morphometric and mRNA analysis. Ovariectomized-sedentary group had larger adipocytes and higher expression of peroxisome proliferator-activated receptor-γ (PPAR-γ), sterol regulatory element-binding protein-1c (SREBP-1c), stearoyl-CoA desaturase-1 (SCD-1), acetyl-CoA carboxylase (ACC), hormone-sensitive lipase (HSL) and lower expression of the oxidative carnitinepalmitoyltransferase-I (CPT-1). RT counteracted OVX-induced increases in PPAR-γ and SCD-1 and decreased SREBP-1c. ACC and HSL were downregulated in ovariectomized-RT compared with the ovariectomized-sedentary group. Ovariectomized-RT group had the highest CPT-1 gene expression. Adipocyte size decreased in ovariectomized-RT group. Results suggest that RT reduces intra-AAT adipocyte size in ovariectomized rats by suppressing intra-AAT fatty acid synthesis and enhancing fatty acid β-oxidation.

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Introduction

Menopause is the result of ovarian hormone loss, including estrogen deficiency [34]. Evidence suggests that estrogen regulates fat distribution [24]. Menopause or ovariectomy in animal models is associated with increased body fat mass and a redistribution of fat from peripheral to central depots [1,48]. Intra-abdominal adipose tissue (intra-AAT) is a major risk factor for developing insulin resistance (IR), type 2 diabetes (T2D), cardiovascular diseases and hypertension [1,32,43]. Therefore, the personal and public health impacts of menopause-associated diseases are significant because women may now expect to spend nearly a third of their lives in the postmenopausal state [32].

During conditions of ovarian hormone loss, the increased intra-AAT appears to be triggered by changes in the expression of genes related to lipolysis and lipogenesis. Lipolysis is a tightly regulated process, which consists of the activation of key lipases, such as hormone-sensitive lipase (HSL) [8]. Increased HSL activity increases the release of fatty acids (FA) from adipocytes to the plasma, which may contribute to the development of IR and to ectopic fat deposition [16].

Estrogen also appears to have a strong influence on the lipogenic process of fat storage. After estrogen supplementation, both postmenopausal women and ovariectomized (OVX) rodents show lipogenesis inhibition via the downregulation of peroxisome proliferator-activated receptor-γ (PPAR-γ), sterol regulatory element-binding protein-1c (SREBP-1c), stearoyl-CoA desaturase-1 (SCD-1) and acetyl-CoA carboxylase (ACC) [9,23]. SREBP-1c and PPAR-γ are key transcription factors in the control of both lipogenesis [3] and adipogenesis [27]. PPAR-γ increases the number of small and new adipocytes. Some anti-diabetogenic drugs stimulate PPAR-γ expression. However, these drugs act mainly in subcutaneous compartments [39].

Stimulating PPAR-γ in intra-AAT is harmful because PPAR-γ also triggers the expression of lipogenic genes, such as SREBP-1c and its downstream genes ACC and SCD-1 [7,8,43], each of these genes causing severe obesity over the long term [43].

In addition to its effect on the lipogenic process, it has recently been shown that the AAT of both humans and rodents perform β-oxidation [11]. Although adipocytes prefer to use glucose as an energy source, adipocytes turn to FA oxidation under certain conditions, such as after body mass loss/maintenance [4]. β-oxidation requires the entry of free FA into mitochondria. Carnitine palmitoyltransferase-1 (CPT-1) provides the rate-limiting step in promoting the entry of FA into mitochondria [47].

In postmenopausal women, hormone replacement therapy has been shown to favorably modulate body fat distribution, IR, T2D and cardiometabolic risk. However, this therapy can cause serious side effects. Lifestyle interventions leading to body mass loss generally induce preferential mobilization of visceral fat [5,39]. The increase in body fat mass caused by the decreased levels of circulating ovarian hormones is not directly associated with hyperphagia but is mostly associated with decreased energy expenditure [9,32]. As a key component of energy expenditure [15], exercise is an effective tool for preventing increases in body mass and intra-AAT obesity after ovarian hormone loss.

Aerobic or resistance training could reduce body mass and intra-AAT obesity [2,15]. However, aerobic training does not prevent the sarcopenia that is associated with menopause [25]. Resistance training can promote skeletal muscle hypertrophy in postmenopausal women [20,28]. Therefore, resistance training may be a better choice during this period. Although long-term resistance training reduces intra-AAT in both postmenopausal women [2] and ovariectomized rats [19], the expression of genes related to lipogenesis (SREBP-1c, ACC and SCD-1), fat oxidation (CPT-1) and lipolysis (HSL) in the intra-AAT of OVX rats remains uncharacterized. Due to the difficulty in obtaining intra-AAT from systematically trained humans, rodents are an ideal animal model [45].

Thus, the aim of this study was to investigate the effects of RT in OVX rats on the expression of genes related to lipogenesis and fat oxidation in intra-AAT. To our knowledge, these mechanisms remain uncertain. Our initial hypothesis was that RT could at least partly prevent the deleterious effects of ovariectomy on intra-AAT lipid metabolism.

Materials and Methods

Animals

In this study, 24 seven-week-old female Sprague-Dawley rats from the breeding colony from the State University of Sao Paulo (UNESP, Araraquara, Brazil) were used. The animals were housed under a constant temperature of 22 ± 2 °C in collective polypropylene cages (3 rats per cage), were fed "ad libitum" with water and a standard rodent chow, and had a 12-h light/12-h dark cycle. Food intake and body mass were monitored daily over the entire experimental period. All animal procedures were conducted in accordance with the "Ethical standards in sport and exercise science research: 2014 update" [12] and were approved by the Committee of Experimental Animals from the Federal University of São Carlos (protocol no. 008/2010). When the rats reached 10 weeks of age (body mass: 243–263 g), they were subjected to ovariectomy or sham surgery, according to a previously described technique [17], using a mixture of ketamine-xylazine (61.5–7.6 mg/kg, i.p.) as an anaesthetic.

Experimental groups

The rats were randomly distributed into the following 4 experimental groups (6 animals per group): sham-sedentary (SHAM-SED) or ovariectomized-sedentary (OVX-SED), which were kept in their cages over the whole experimental period without any type of exercise; and SHAM-resistance trained (SHAM-RT) and ovariectomized- resistance trained (OVX-RT) groups, which performed a 10-week course of resistance training. The training started 3 weeks after the surgery.

Resistance exercise training

The resistance training protocol was adapted from Hornberger and Farrar [14] and was performed in the dark phase, which is the active period of rats. This protocol required the animals to climb a vertical ladder (1.1 × 0.18 m, 2 cm grid, 80° incline) with weights attached to their tails. The size of the ladder induced the animals to perform 8–12 movements per climb. The load apparatus was attached to the tail by wrapping the proximal portion of the tail with a self-adhesive foam strip.

Three weeks after the surgery, the trained groups started a 10-week resistance training course of climbing sessions that were performed 3 times per week. Before beginning training, the rats were adapted to the resistance training protocol for one week. With the load apparatus attached to the tail, each rat was placed at the bottom of the ladder and familiarized with climbing. At the top of the ladder, the rats reached a housing chamber (20 × 20 × 20 cm), where they were allowed to rest for 120 s. This procedure was repeated until the rats would voluntarily climb the ladder 3 consecutive times without stimulus. The familiarization week consisted of 4–8 ladder climbs carrying progressively heavier loads. The initial climb consisted of carrying a load with 75% of the animals' body mass, and on each additional series 30 g weights were added until the load did not allow the animals to successfully climb to the housing chamber. The highest load successfully carried up the entire ladder was considered to be the maximal carrying capacity for that training session. The training sessions consisted of 4 ladder climbs with 65, 85, 95 and 100% of the rat's previous maximal carrying capacity, followed by climbs with 30 g loads added until a new maximal carrying capacity was reached.

Feeding efficiency

Feeding efficiency was calculated as grams of the body mass gained per gram of food intake, per week.

Tissue sampling

All animals were fasted overnight prior to being euthanized by decapitation 92 days post-surgery and 48 h after the last training session for the purpose of sampling in a state of accumulated training effect but beyond the acute effects of the last exercise session. Subcutaneous (gluteal)-AT, intra-AAT (mesenteric and omental) and uteri were rapidly removed and weighed. Approximately 100 mg of intra-AAT and subcutaneous-AT was placed in a fixative 0.2 M collidine buffer containing 1% osmium tetroxide and placed in an oven at 37 °C for 24 h for morphometric analysis. A portion of the intra-AAT was immediately frozen in liquid nitrogen and stored at −80 °C for further qPCR analysis.

Morphometric analysis

Fragments of subcutaneous and intra-AAT (100 mg) were separated and fixed in 0.2 M collidine buffer (pH 7.4) containing 2% osmium tetroxide at 37 °C. After 48 h, the cells were resuspended in warm saline and immediately dispersed on glass slides for fat cell area measurement per the method described by Hirsch and Gallian [13].

An optical microscopy system and the software Image-Pro Plus 3.2 were used to measure the areas of triacylglycerol depots of adipocytes. At least 100 cells per animal were measured, for a total of at least 500 adipocyte areas (size) per group. The mean values and standard errors were expressed in μm^2. The cells were randomly chosen, and the research analyzing the images was blinded to the group assignments.

Total RNA extraction and reverse transcription

Total RNA was extracted with TRIZOL (Invitrogen, Carlsbad, California) according to the manufacturer's protocol. Briefly, approximately 100 mg of intra-AAT was placed in 1 mL of Trizol and homogenized with a Power Gen 1000 homogenizer (Fisher Scientific). The RNA pellet was dissolved in nuclease-free water and stored at −80 °C. The RNA concentration and purity were determined by optical density readings at 260 nm and the 260/280 nm and 260/230 nm ratios, respectively, and the RNA integrity was checked through denaturing formaldehyde agarose gel electrophoresis.

To remove genomic DNA, total RNA (1 µg) from each sample was treated with deoxyribonuclease I (DNase I; Invitrogen Corporation, California, USA) following the manufacturer's protocol. Treated RNA was reverse-transcribed into cDNA using M-MLV reverse transcriptase and oligo (dT) (Promega Corporation, Madison, WI, USA).

Quantitative (q) PCR

The qPCR reaction for each gene was performed in duplicate in a CFX 96 real-time PCR detection system (Bio-Rad, San Francisco, USA). Each reaction contained 20 ng of cDNA, 0.4 µM of each primer and 10 µL of SsoFast™ EvaGreen® Supermix (Bio-Rad, San Francisco, USA) in a total reaction volume of 20 µL. The gene-specific primers were purchased from Invitrogen Life Technologies, Inc., and are listed in **Table 1**. The thermal cycling program was 95 °C for 30 min, followed by 40 cycles of 95 °C for 15 min, 57–61 °C for 30 min, and 72 °C for 30 min. The specificity of the amplification products was confirmed by analysis of their melting curves and by size estimation after electrophoresis in 2% agarose gel. RPLOP (Large Ribosomal Protein) was used as an endogenous control rather than GAPDH (glyceraldehyde-3-phosphate dehydrogenase) or β-actin, because it was the only one of the 3 that was stable within and between groups. The relative expression of the quantitative RT-PCR products was determined by the ΔΔCt method, where the relative expression is calculated using the following equation: fold induction = $2^{-\Delta\Delta CT}$ [21].

Fasting glucose

This parameter was assessed following euthanasia with Accu-Chek Active blood glucose meter (Roche Diagnostics Laboratory, Germany). The results are expressed in mg/dL.

Statistical analysis

Normality (Shapiro-Wilk) and the homoscedasticity (Bartlett criterion) tests were performed. Because all variables showed normal distribution and homoscedasticity, they were compared using the two-way ANOVA (taking into consideration the intervening variables OVX vs. RT). When the group means were different ($p < 0.05$), post-hoc analysis (Tukey's test) for multiple comparisons was performed. Statistical analyses were performed using Instat 3.0 (GraphPad, San Diego, CA, USA, 1998). Data are presented as mean ± SEM.

Results

Maximal overload capacity

The maximal overload capacity in the resistance-trained animals increased progressively over the 10-week training period. However, there was no difference between the SHAM and OVX groups during the same period (**Fig. 1**).

Uterine mass and oestrous cycle phases

The lower uterine mass (**Table 2**) and the absence of an estrous cycle (data not shown) in the OVX groups confirmed the success of the ovariectomy.

Body mass gain, food intake (Table 2) and feeding efficiency (Fig. 2)

Table 2 shows that 3 weeks after the ovariectomy and before the training period, the OVX rats had a body mass gain that was approximately 2 times greater than that of the SHAM rats. At the end of experiment, the OVX-SED group gained more body mass than all the others. Resistance training decreased the body mass gain in both the SHAM-RT and OVX-RT groups compared with the SHAM-SED and OVX-SED groups, respectively. The OVX-RT group had a higher body mass than the SHAM-RT group.

The food intake was highest in the OVX-SED group. The RT groups had lower food intake compared with their respective sedentary groups. The SHAM-RT group had the lowest food intake.

The feeding efficiency was highest in the OVX-SED group. The feeding efficiency of the SHAM-RT and OVX-RT groups was

Table 1 Oligonucleotide primers used for quantitative RT-PCR.

Genes	Sense primer (5′–3′)	Antisense primer (3′–5′)	Accession no
PPAR-γ	AAGGGGCCTGGACCTCTGCTG	ATAAGGCGGGGACGCAGGCT	NM_001145366.1
SREBP-1c	TGCCCTAAGGGTCAAAACCA	TGGCGGGCACTACTTAGGAA	L16995.1
ACC	ACAGAGATGGTGGCTGATGTC	GATCCCCATGGCAATCTG	NM_022193.1
SCD-1	TGCTGATGTGCTTCATCCTG	GGGAAACCAGGATATTCTCC	NM_009127.4
CPT-1	ACGTTGGACGAATCGGAGC	AAAGCATCTTCCATGCAGCAG	NM_031559.2
HSL	GCGCCTATTCAGGGACAGA	CCAGGAAGGAGTTGAGCCAT	NM_012859.1
RPLPO	AGGGTCCTGGCTTTGTCTGTGG	AGCTGCAGGAGCAGCAGTGG	*NM_022402.2*

SREBP-1c = sterol regulatory element-binding protein-1c, ACC = acetyl-CoA carboxylase, SCD-1 = stearoyl CoA desaturase-1, CPT-1 = carnitinepalmitoyltransferase 1, PPAR-γ = Peroxisome proliferator-*activated*receptor gamma; HSL = hormone-sensitive lipase, RPLPO = large ribosomal protein

lower than that of the SHAM-SED and OVX-SED groups, respectively. The feeding efficiency was similar between the SHAM-SED and OVX-RT groups (**Fig. 2**).

Intra-abdominal and subcutaneous adipocyte size

The intra-AAT and subcutaneous adipocyte size was largest in the OVX-SED group. The SHAM-RT and OVX-RT groups had smaller intra-AAT and subcutaneous adipocytes than did the SHAM-SED and OVX-SED groups, respectively. The intra-AAT adipocyte size was larger in OVX-RT than in SHAM-SED group (**Fig. 3a, b**).

Fasting glucose

There were no differences in the fasting glucose levels of the SHAM-SED, OVX-SED and OVX-RT groups. The SHAM-RT group had a lower fasting glucose level than the OVX groups (**Table 2**).

Expression of lipid metabolism genes

The ovarian hormone loss induced increases in the gene expression of the lipogenic transcription factors PPAR-γ and SREBP-1c as well as their downstream targets, SCD-1 and ACC. RT prevented the OVX-induced increases in PPAR-γ and SCD-1 gene expression and the decrease in SREBP-1c gene expression. Only the ACC expression was higher in OVX-RT than in the SHAM-SED group. All these genes were downregulated in OVX-RT compared with the expression in the OVX-SED group. In the non-OVX groups, PPAR-γ, SREBP-1c and ACC were downregulated, but SCD-1 was upregulated in the SHAM-RT group (**Fig. 4a–d**).

The gene involved in the mitochondrial β-oxidation process, CPT-1, was downregulated in both the OVX-SED and SHAM-RT groups compared with the SHAM-SED group. CPT-1 expression was highest in the OVX-RT group (**Fig. 5**). The expression of the gene HSL was higher in the OVX groups. RT decreased the HSL expression in the OVX animals (**Fig. 6**).

Discussion

The main finding of the study was that RT counteracts disturbances in the intra-AAT of OVX rats. Ovarian hormone loss induced higher expression of the lipogenic genes PPAR-γ, SREBP-1c, SCD-1 and ACC and the lipolytic gene HSL and lower expression of the oxidative gene CPT-1 during fasting. The OVX-SED group had the largest adipocytes. However, RT counteracted both the OVX-induced increases in the expression of PPAR-γ and SCD-1 and also decreased the expression of SREBP-1c. ACC and HSL were lower in OVX-RT than in the OVX-SED group, and CPT-1 was highest in the OVX-RT group. These regulations of the lipid metabolism of the OVX-RT group were coincident with

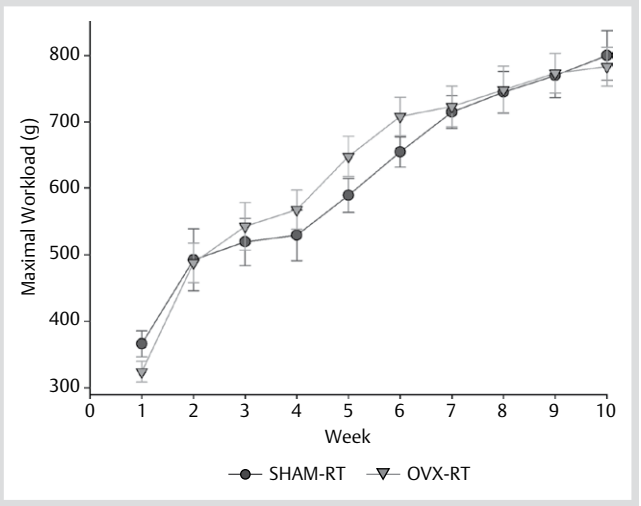

Fig. 1 Incremental maximal overload capacity of SHAM-RT and OVX-RT rats during the training period (n = 6/group). SHAM-RT = sham-resistance training and OVX-RT = ovariectomized-resistance training. Data are the means ± SEM.

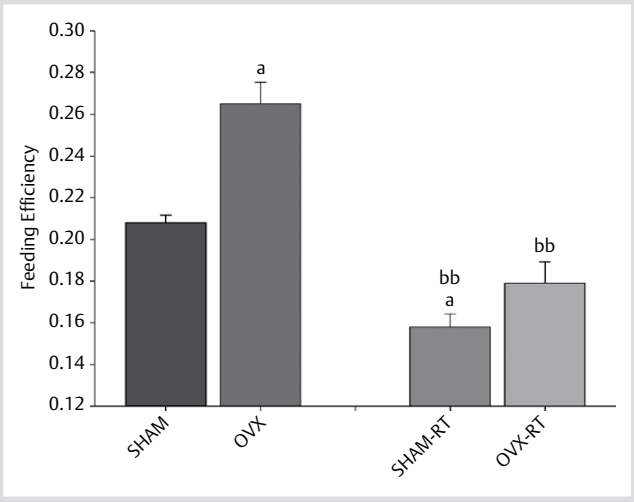

Fig. 2 Feeding efficiency of SHAM-SED, OVX-SED, SHAM-RT and OVX-RT rats. (n = 6/group). SHAM-SED = sham sedentary, OVX-SED = ovariectomized sedentary, SHAM-RT = sham-resistance training and OVX-RT = ovariectomized-resistance training. Data are the means ± SEM. [a] $p < 0.05$ vs. SHAM-SED, [bb] $p < 0.001$ vs. OVX-SED.

Table 2 Uterine, initial, pre-RT and final body mass; food intake; and glucose.

	SHAM-SED	OVX-SED	SHAM-RT	OVX-RT
uterus mass (g/100 g body mass)	0.203 ± 0.01	0.054 ± 0.08 [aa]	0.195 ± 0.03 [bb]	0.044 ± 0.02 [aa,cc]
initial body mass (g)	252.7 ± 7	244.8 ± 12	244.3 ± 7	250.3 ± 7
body mass (g) at the beginning of the training	291.7 ± 4	319.5 ± 4 [aa]	278.3 ± 7 [bb]	318.7 ± 2 [aa,cc]
final body mass (g)	336.3 ± 7	392.7 ± 7 [aa]	301.3 ± 5 [aa,bb]	367.1 ± 4 [a,b,cc]
food intake (g/day)	20.89 ± 0.3	23,32 ± 0.2 [aa]	19.54 ± 0.2 [aa,bb]	20.99 ± 0.2 [bb,cc]
glucose (mg/dL)	155.2 ± 3	163.6 ± 2	147.2 ± 4 [bb]	161.8 ± 1 [c]

Data are presented as means ± SEM; n = 6 for each group

[a] $p < 0.05$, [aa] $p < 0.001$ vs. SHAM-SED

[b] $p < 0.05$, [bb] $p < 0.001$ vs. OVX-SED

[c] $p < 0.05$, [cc] $p < 0.001$ vs. SHAM–RT

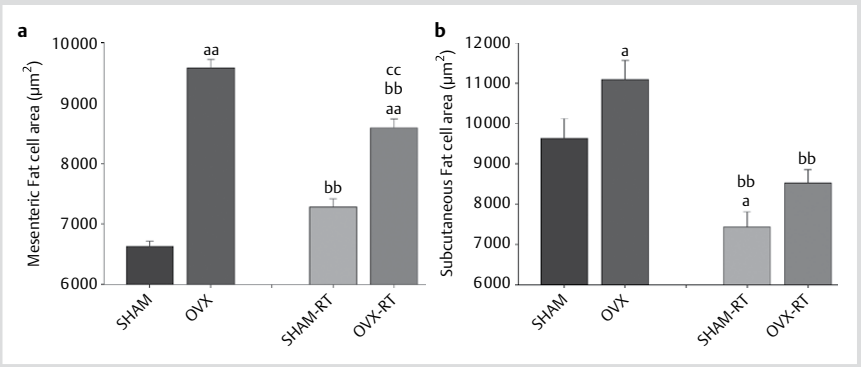

Fig. 3 **a** Mesenteric and **b** subcutaneous adipocyte size of SHAM-SED, OVX-SED, SHAM-RT and OVX-RT rats. (n = 6/group). SHAM-SED = sham sedentary, OVX-SED = ovariectomized sedentary, SHAM-RT = sham-resistance training and OVX-RT = ovariectomized-resistance training. Data are the means ± SEM. $^{a}p < 0.05$, $^{aa}p < 0.001$ vs. SHAM-SED, $^{bb}p < 0.001$ vs. OVX-SED, $^{cc}p < 0.001$ vs. SHAM-RT.

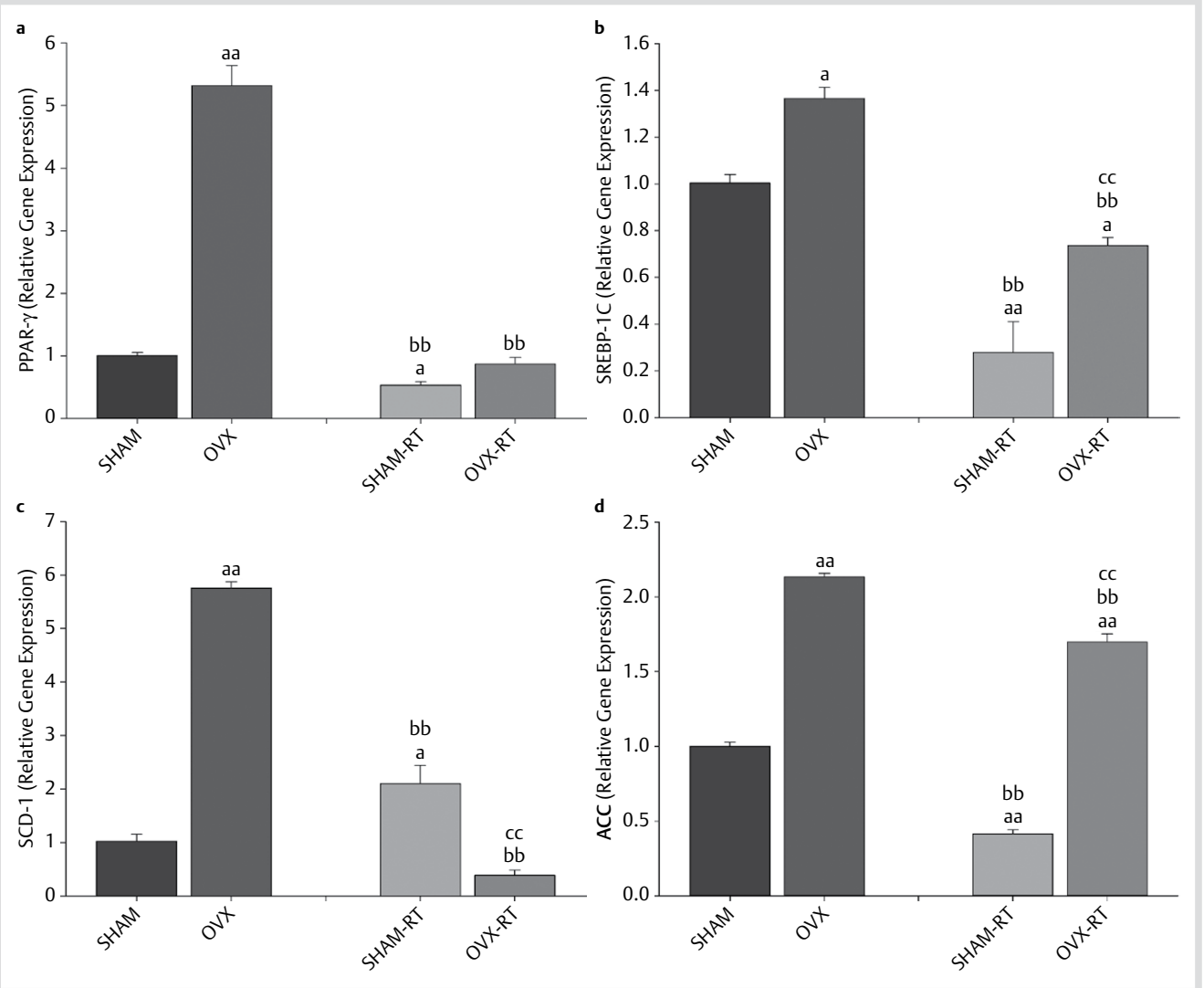

Fig. 4 Gene expression of the genes involved in lipogenesis in the intra-AAT adipose tissue of SHAM-SED, OVX-SED, SHAM-RT and OVX-RT rats (n = 6/group). **a** PPAR-gamma, **b** SREBP-1c, **c** SCD-1 and **d** ACC. SHAM-SED = sham sedentary, OVX-SED = ovariectomized sedentary, SHAM-RT = sham-resistance training and OVX-RT = ovariectomized-resistance training. Data are the means ± SEM. $^{a}p < 0.05$, $^{aa}p < 0.001$ vs. SHAM-SED, $^{bb}p < 0.001$ vs. OVX-SED, $^{cc}p < 0.001$ vs. SHAM-RT.

decreases in adipocyte size. Thus, our initial hypothesis was confirmed.

Consistent with previous studies, both body mass [34] and food intake [6] increased in the OVX rats compared with the SHAM rats. During the 3 weeks following the surgery, the OVX rats gained more body mass than the SHAM rats. Resistance training prevented the increases in both food intake and, partly, body mass, resulting in a similar change in body mass between the OVX-RT and SHAM-SED groups during training period. The higher body mass of the OVX-RT rats compared with that of the SHAM-SED rats appears to be caused by the delay (3 weeks) in starting resistance training after ovariectomy. It has been shown that beginning exercise early after the loss of ovarian hormones prevents the body mass gain induced by an ovariectomy [35].

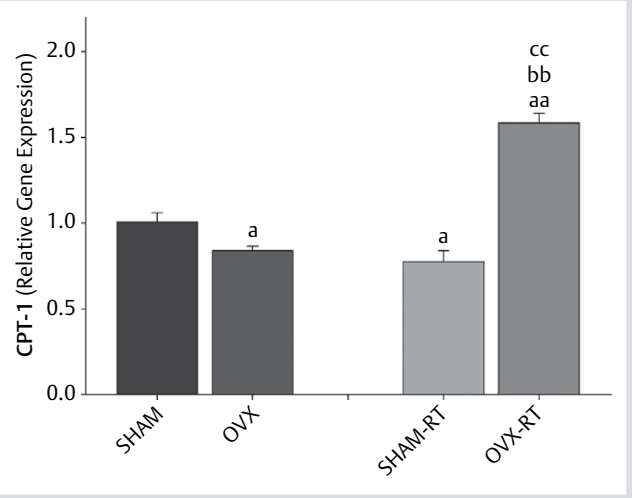

Fig. 5 Gene expression of CPT-1 in the intra-AAT adipose tissue of SHAM-SED, OVX-SED, SHAM-RT and OVX-RT rats (n = 6/group). SHAM-SED = sham sedentary, OVX-SED = ovariectomized sedentary, SHAM-RT = sham-resistance training and OVX-RT = ovariectomized-resistance training. Data are the means ± SEM. [a] $p < 0.05$, [aa] $p < 0.001$ vs. SHAM-SED, [bb] $p < 0.001$ vs. OVX-SED, [cc] $p < 0.001$ vs. SHAM-RT.

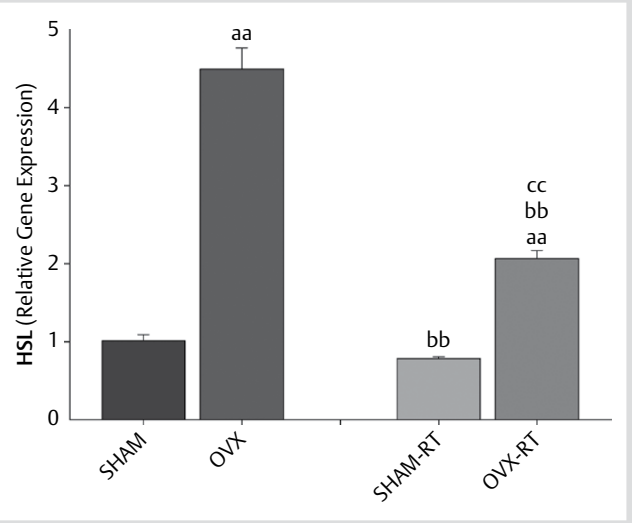

Fig. 6 Gene expression of lipolytic HSL in the intra-AAT adipose tissue of SHAM-SED, OVX-SED, SHAM-RT and OVX-RT rats (n = 6/group). SHAM-SED = sham sedentary, OVX-SED = ovariectomized sedentary, SHAM-RT = sham-resistance training and OVX-RT = ovariectomized-resistance training. Data are the means ± SEM. [a] $p < 0.05$, [aa] $p < 0.001$ vs. SHAM-SED, [bb] $p < 0.001$ vs. OVX-SED, [cc] $p < 0.001$ vs. SHAM-RT.

These data indicate the importance of beginning exercise training soon after the loss of ovarian hormones to prevent obesity in both rats and menopausal women.

Central obesity in the postmenopausal state increases the risk of developing IR and high blood pressure. These clinical conditions predispose the development of many chronic diseases, such as T2D, cardiovascular disease and metabolic syndrome [24,33]. Obesity is associated with adipocyte expansion [39]. The OVX-SED group had larger subcutaneous and intra-AAT adipocytes compared with the other groups. The increase was greater in the intra-AAT (45% vs. 15% in subcutaneous adipocytes). It is well known that ovarian hormone loss promotes a shift of FA from subcutaneous to intra-AAT depots in both humans [22,39] and rodents [32,40]. The lower subcutaneous expansion capacity is related to ectopic fat deposition because the subcutaneous fat expansion is needed to serve as a buffer to absorb lipids in the postprandial period [46]. In addition, large adipocytes, mainly in intra-AAT, are generally less sensitive to insulin and secrete more pro-inflammatory cytokines, such as TNF-α and leptin [39].

The findings on the body mass and adipocyte size are supported by the concept of feeding efficiency. When feeding efficiency is increased, greater body mass gains occur even for the same amount of food consumption. Ovariectomized mice became obese even without increasing food intake after the ovariectomy [5,40], indicating the higher capacity for energy conversion and storage in the OVX mice. Thus, the higher feeding efficiency of the OVX-SED group accounted for this greater body mass gain.

The increased feeding efficiency of ovariectomized rodents has been suggested to be caused, at least partly, by a decrease in body energy expenditure, which occurs not only because of the reduced spontaneous physical activity during dark hours but also because of a still unknown independent mechanism during light hours [5,32]. In addition to decreased exercise levels, decreases in both the total and sleeping energy expenditure of postmenopausal woman have also been demonstrated [22].

Our results demonstrated that resistance training prevented OVX-induced increases in feeding efficiency. The key benefit of resistance training is increased muscle mass, which is the main determinant of energy expenditure [37] and also affects feeding efficiency. Resistance exercise on a ladder has been shown to effectively increase the lean mass in OVX rats [14,31]. Thus, it is tempting to speculate that the lower feeding efficiency in the trained animals compared with the corresponding sedentary animals is due to the increased muscle mass, which is also associated with lower body mass and adipocyte size in these animals.

Adipocyte size has been shown to be closely related to IR [39], the central disorder in the pathogenesis of T2D. Adipocyte size is also a defining feature of metabolic syndrome [24]. Estrogen and/or exercise may indirectly protect against insulin resistance by decreasing adipose tissue inflammation and modulating lipid metabolism [38,41]. It is possible that the synergy between these factors was responsible for the lower fasting glucose level found in the SHAM-RT group relative to the OVX groups. Although there was no difference between the trained and sedentary OVX groups, it is tempting to speculate that the trained group is protected against the onset of IR, which occurs approximately 26 weeks following ovariectomy [40], given that the OVX-RT group had smaller adipocytes. Smaller adipocytes are more sensitive to insulin and secrete a lower amount of proinflammatory adipokines, which may prevent the onset of IR [38,39,46].

There is a strong link between the morphologic features of intra-AAT and the gene expression patterns [29]. Growing evidence shows that abnormal fat metabolism may have a major role in triggering IR and T2D. Diabetic humans and IR mice showed a persistently higher expression of lipogenic factors during fasting [26,44]. The gene expression of the lipogenic PPAR-γ, SREBP-1c and ACC should be downregulated during fasting [26,30]. We found a persistently higher gene expression of the lipogenic genes for PPAR-γ, SREBP-1c, ACC and SCD-1 during fasting in the intra-AAT of the OVX-SED rats, which may suggest a possible link between the abnormal lipogenesis of intra-AAT and the onset of IR after ovarian hormone loss.

White adipocytes, despite having a relatively lower mitochondrial density, coordinate β-oxidation either to oxidize incoming FA and carbohydrate fuels through the carboxylic acid cycle and the respiratory chain or to store these fuels in the form of TG [18,30]. Mesenteric AT has higher capacity for mitochondrial FA oxidation than other intra-AT depots. CPT-1 encodes the enzyme that catalyzes the rate-limiting step of mitochondrial β-oxidation, the major pathway by which FA are oxidized to generate energy [29]. The lower CPT-1 gene expression in the OVX-SED group suggests a lower oxidative capacity in this group compared with others.

In addition to reduced CPT-1, the HSL gene was upregulated in the ovariectomized rats, this gene being associated with lipolytic activity. These results suggest a higher capacity to mobilize stored TG (HSL) and a lower capacity of mitochondrial fatty acid oxidation (CPT-1), indicating that the FA mobilized from TG in the condition of ovarian hormone loss are mainly released into blood circulation. It may explain why both postmenopausal women and rodents have higher systemic levels of free FA [9,19,38]. This condition may lead to the onset of liver steatosis and IR previously shown to occur in the absence of ovarian hormones [38]. Higher FA turnover may be harmful because of its association with IR, dyslipidemia and impaired liver metabolism [30].

The main contribution and original finding of our study is that resistance training counteracted the intra-AAT molecular disturbances that are promoted by ovariectomy by counteracting the transcription of molecules related to FA synthesis, such as PPAR-γ, SREBP-1c and SCD-1, in ovariectomized rats. These molecular responses strongly suggest that resistance training depresses lipogenesis in the intra-AAT of ovariectomized rats and can be a mechanism that contributes to lower adipocyte size, which helps to decrease the risk of developing IR [39]. Furthermore, reduced SCD-1 is closely related to obesity resistance through increases in fat oxidation [7].

The increased expression of HSL in TA in low- and moderate-intensity, long-duration exercise stimulated by chemical or mechanical signals may have beneficial effects, since this type of exercise predominantly uses FA as energy source [10,42]. On the other hand, increases in HSL activity were not observed in short-duration and high-intensity exercises, probably due to AMPK inhibition [36,42]. High lipolytic activity in intense exercise is not necessarily needed, considering that carbohydrates are the main source of energy in this type of exercise. Consistent with these notions, the trained groups showed that our exercise protocol does not stimulate HSL. However, it was effective in decreasing HSL expression in OVX animals, which can be beneficial in terms of decreasing ectopic fat deposition.

Indeed, we found higher expression of CPT-1, a key enzyme in the mitochondrial oxidation of FA [30], in trained OVX rats, suggesting that a relatively higher amount of FA can be oxidized in intra-AAT through mitochondrial β-oxidation. The mitochondrial β-oxidation process promotes energy expenditure, which may have also aided in the reduction of adipocyte size and body mass in the OVX-RT rats [4,11,18]. The higher expression of genes related to fat oxidation (upregulated CPT-1) and the lower related to FA mobilization (downregulated HSL) could explain the lower systemic levels of free FA previously reported in a similar OVX-RT rat model [19].

Overall, to our knowledge, this study is the first to demonstrate that resistance training prevents ovariectomy-induced molecular disturbances in intra-AAT lipid metabolism. The results clearly indicate that resistance training may be an effective treatment for controlling the effects of menopause and ovariectomy on the expression of genes that encode enzymes involved in intra-AAT lipid metabolism. Therefore, we conclude that the ovarian hormone loss associated with ovariectomy changes the expression of genes that favor the development of intra-AAT obesity, which can be prevented through resistance training.

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