

Original article (short paper)

7-week aerobic exercise training reduces adipocyte area and improves insulin sensitivity in Wistar rats fed a highly palatable diet

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Abstract—The aim of this study was to evaluate the effect aerobic exercise training on fat pad mass, adipocyte size, leptin release and insulin sensitivity in rats fed with high fat-palatable diet. Twenty-four male Wistar rats (250-260g) were divided into four groups: sedentary control (CTR/SD), trained control (CTR/TR), obese sedentary (OB/SD) and obese trained (OB/TR). Obese groups were fed with high fat-palatable diet (27% of fat) and control groups fed with AIN-93. Our results showed that aerobic exercise training was effective to reduce body weight and epididymal fat mass in CTR/TR and OB/TR. Insulin and glucose levels were increased in OB/TR compared with OB/SD. Aerobic exercise training reduced the average area of adipocytes in CTR/TR and OB/TR and it was associated with reduced plasma insulin and leptin. In conclusion, 7-week aerobic exercise training reduces adipocyte area and improves insulin sensitivity and leptin levels in high fat-palatable diet-fed Wistar rats.

Keywords: aerobic exercise training, high fat diet, insulin, leptin.

Introduction

The prevalence of obesity is rising around the world and it has been seen as a global health problem. The modern lifestyle, with easy access to food and technological facilities, increases the risk of obesity development. Besides, overfeeding of saturated fat and sucrose are associated with fast white adipose tissue gain (Ma *et al.*, 2011; Sampey *et al.*, 2011). Excessive body fat mass has a negative impact on health that has been related to the development of type 2 diabetes mellitus, insulin resistance, non-alcoholic fatty liver disease and cardiovascular disease (Galic, Oakhill, & Steiberg, 2010).

More than an energy storage, the adipose tissue acts as an endocrine organ releasing different substances that participates in different physiological processes such as tumor necrosis factor alpha (TNF- α), interleukin 6 (IL-6) and leptin (Hotamisligil *et al.*, 1993; Zhang *et al.*, 1994; Weisberg *et al.*, 2003). Leptin is an adipokine that was originally identified as a key molecule in the regulation of food intake and body weight. Circulating leptin levels

are proportional to body fat mass and obese individuals are resistant to leptin action (Ikuni, Lam, Lu, Matarese, & La Cava, 2008).

Studies in adipocyte cell culture have provided strong support for the role of hypoxia, induced by adipocyte hypertrophy, to modulate the overproduction of inflammation-related adipokines as IL-6, IL-1 β and leptin (Wood, De Heredia, Wang & Trayhurn, 2009).

There is an inverse correlation between adipocyte volume and dyslipidemia, insulin resistance and fasting or post-prandial hyperglycemia (Weisberg *et al.*, 2003). Insulin deficiency may also affect the lipid metabolism affecting several other organs such as blood vessels, brain, pancreas, bone mass, and others (Rask-Madsen & Kahn, 2012). Furthermore, it is well established that obesity is associated with a chronic low-grade inflammation of the adipose tissue (Henninger, Eliasson, Jenn Dahl & Hammarstedt, 2014; Vachharajani, & Granger, 2009). Weisberg *et al.* (2003) and Xu *et al.* (2003) demonstrated that macrophage infiltration in adipose tissue is related to obesity and insulin sensitivity, and these conditions are associated to the development of type 2 diabetes mellitus and insulin resistance.

Epidemiological and clinical studies have been shown that physical exercise is an important approach in preventing and for treatment of many cardio metabolic diseases, such as arterial hypertension and type 2 diabetes mellitus. Physical exercise exerts important adaptations in skeletal muscle and adipose tissue metabolism (Ciolac & Guimarães, 2004; Gordecke & Micklesfield, 2014; Guimarães, Duque, De Carvalho, De Moraes & Carmo, 2007, Horská, Kucerova, Suchy & Kotolova, 2014). Thus, considering the negative effect of excessive fat mass on the metabolism, the aim of this study was to evaluate the effect of 7-weeks of aerobic exercise training in fat pad mass, adipocyte size and insulin sensitivity in rats fed with high-palatable diet.

Methods

Animals and experimental conditions

Twenty-four male Wistar rats (weighting 250-260g) were obtained from Central Animal Facility (University of São Paulo, campus of Ribeirão Preto) and divided into four groups: sedentary control (CTR/SD), trained control (CTR/TR), obese sedentary (OB/SD) and obese trained (OB/TR).

Animals were housed in polypropylene cages (41×34×30 cm) containing three animals in each, with *ad libitum* access to water and food, kept on a 12 h light/dark cycle. Obese groups were fed with high-palatable diet (carbohydrate: 53%, protein: 20% and fat: 27%) and Control groups were fed with AIN-93 (carbohydrate: 64%, protein: 20% and fat: 16%). High-palatable diet and AIN-93 are isocaloric (16.8 kJ/ g). The experiment lasted 11 weeks. Animals were fed for 4 weeks prior to exercise training that was carried out for a further 7 weeks. Trained rats rested during 48 hours prior to euthanasia. Animals were euthanized by decapitation without previous anesthetic procedure after 12 hours fasting overnight.

All procedures were reviewed and approved by the Ethics Committee on Animal Use in Research (CEUA/PUSP-RP protocol number 10.1.1290.53.5) in compliance with the US National Institutes of Health Guide Care and Use of Laboratory Animals.

Exercise Training Protocol

Before starting exercise training, all rats underwent an adaptation period attempting to minimize the potential stress from equipment and effort (for details see Table 1).

Table 1. Adaptation scheme to treadmill exercise.

| Exercise session | 1st | 2nd | 3rd | 4th | 5th |
|------------------|-----|-----|-----|-----|---------|
| Speed (m/ min) | 5 | 5 | 10 | 10 | maximum |
| Duration (min) | 30 | 60 | 30 | 60 | test |

Training speed was determined after a maximum incremental exercise test (adapted from Hohl *et al.*, 2009), which began at 11.6 m/min and increased by 1.6 m/min every 2 minutes until 20 m/min. Subsequently, the speed was increased by 3.2 m/min and rats ran until exhaustion (determined when the animal touched the bottom of the bay five times within one minute). The maximal speed (Smax) was considered to be the speed at which exhaustion occurred.

Training intensity progressively increased from 40%_{ms} on the first week, 50-55%_{ms} from the 2nd to 4th week and 60%_{ms} from the 5th to 7th week. Exercise sessions last 60 minutes, 5 days a week, for 7 weeks. Rats were trained on a treadmill with individual lanes designed for small animals (Gesam, São Paulo, Brazil).

Body Weight, Food Intake and Epididymal Fat

Body weight gain was measured weekly and food intake was controlled daily. At the end, epididymal fat was removed; weighed and small fragments were prepared for histological analyses.

Adipose Tissue Histology

Fragments of adipose tissue were maintained for 24 hours in paraformaldehyde (4%), processed for dehydration (alcohol) diafanization (xylene), infiltration (paraffin) and included in paraffin. Histological sections of 4µM were stained with hematoxylin-eosin (HE) for morphological evaluation. Images (20x zoom) were obtained using a camera (Olympus QColor 3, Olympus America, USA) connected to the microscope (Olympus BX51, Olympus, Japan). ImageJ software (National Institute of Health, USA) was used to determine adipocyte surface area.

Biochemical Parameters

Blood samples were collected and centrifuged for 15 minutes at 3000 rpm. Serum was used to determine glucose concentration using colorimetric method (Labtest commercial kit, Brazil). Leptin and insulin concentration were determined in plasma using Luminex® technology (panel Milliplex RADPK-81K, Millipore, USA).

Statistical analysis

Data are expressed as mean ± standard error mean (SEM) for n experiments. Analysis of variance (*two-way* ANOVA) followed by Bonferroni post-hoc test were done using GraphPad Prism software. Significance was considered at 5% ($P < 0.05$).

Results

Body weight, epididymal fat and food intake

Body weight was significantly reduced in CTR/TR group (about 33%) as compared with CTR/SD group and similar result

was found in OB/TR compared with OB/SD. As expected, the OB/SD showed an increase (about 32%) in epididymal fat compared with the CTR/SD group. On the other hand, trained groups had 40% less epididymal fat mass than sedentary groups. A lower food intake was observed in both trained groups compared with sedentary groups (CTR/TR: -12% and OB/TR: -26%) as well as in OB/SD (-28%) compared with the CTR/SD group. Data are represented in Figure 1.

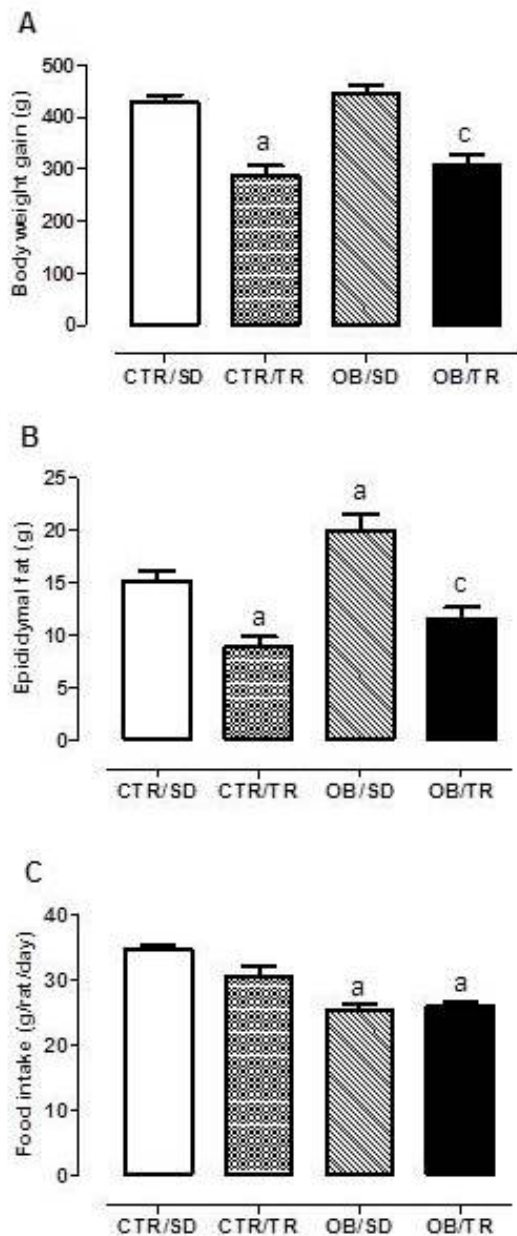


Figure 1. Body weight gain (panel A), epididymal fat pad (panel B) and daily food intake (panel C) control sedentary (CTR/SD), control trained (CTR/TR), obese sedentary (OB/SD) and obese trained (OB/TR). Data are mean±SEM. ANOVA two-way, Bonferroni post test ($P<0.05$). ^adifferent from CTR/SD, ^cdifferent from OB/SD.

Fasting glucose, Insulin and Leptin

The high palatable diet intake lead to higher levels of blood glucose (about 15%), insulin (about 54%) and leptin (103%) in

the OB/SD group compared with CTR/SD. Exercise training prevented blood glucose increase in the OB/TR group that showed glucose concentration lower than the OB/TR group. Interestingly, we found that CTR/TR group had lower insulin (about 45%) and leptin (57%) compared with CTR/SD, similar result was seen in OB/TR that had 52% less insulin and 81% less leptin than the OB/SD group (Figure 2).

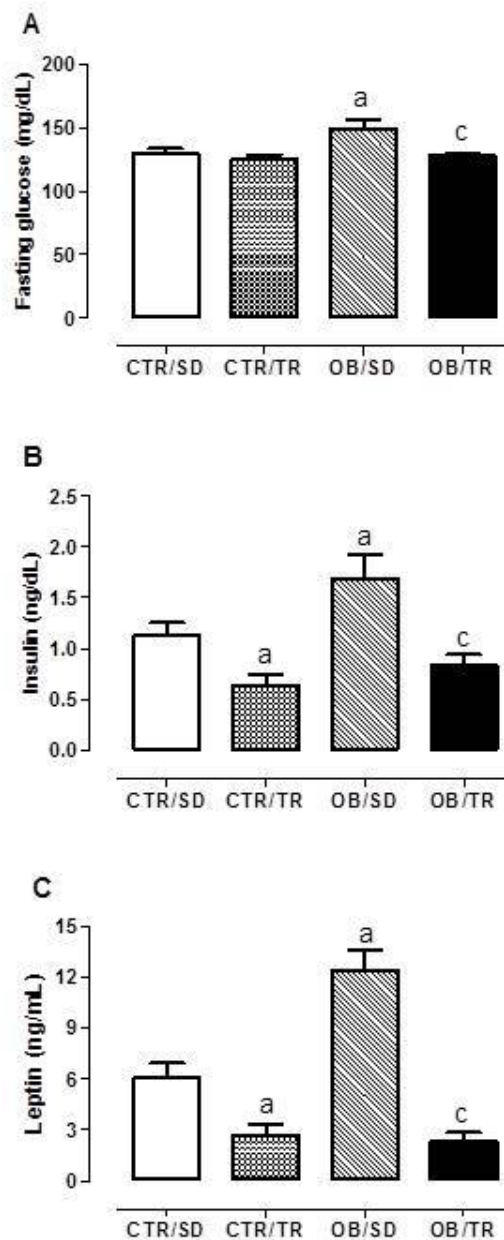


Figure 2. Fasting glucose (panel A), insulin (panel B) and leptin (panel C) from control sedentary (CTR/SD), control trained (CTR/TR), obese sedentary (OB/SD) and obese trained (OB/TR). Data are mean±SEM. ANOVA two-way, Bonferroni post test ($P<0.05$). ^adifferent from CTR/SD, ^cdifferent from OB/SD.

Adipocyte area

Exercised groups had reduced adipocyte area compared with their sedentary counterparts (CTR/TR: - 49%, OB/TR: 41%). Representative and quantitative analyses are show in Figure 3.

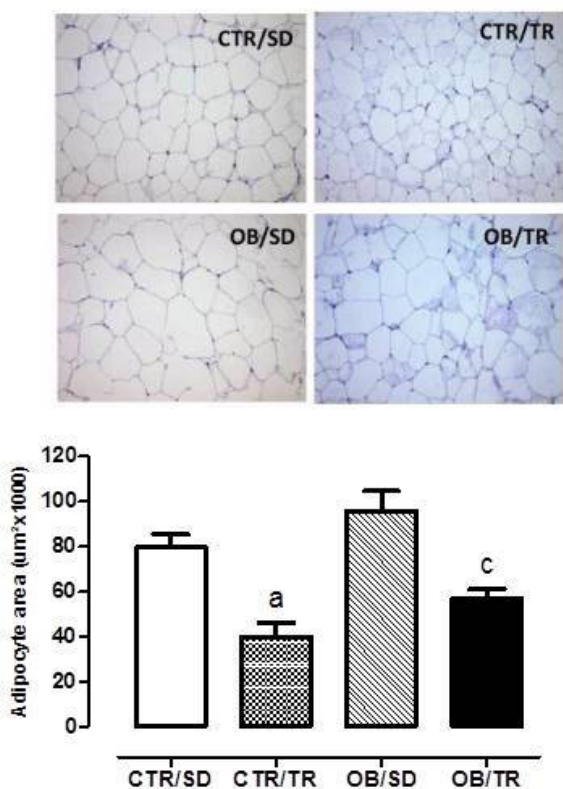


Figure 3. Representative/ quantitative analysis for adipocyte size from control sedentary (CTR/SD), control trained (CTR/TR), obese sedentary (OB/SD) and obese trained (OB/TR). Data are mean±SEM. ANOVA two-way, Bonferroni post test ($P < 0.05$). ^adifferent from CTR/SD, ^cdifferent from OB/SD.

Discussion

Our results showed that 7-week aerobic exercise training was effective in reducing body weight, by approximately 30%, and epididymal fat mass, by approximately 40%, in rats from both exercised groups. The effects of exercise training to prevent body weight gain have also been shown by Levin e Dunn-Meynell (2004) using 6 weeks of wheel running with 8 weeks high fat and sucrose fed rats. Higa, Spinola, Fonseca-Alaniz e Evangelista, (2014) had showed a reduction in epididymal fat in obese mice trained for 8 weeks of sessions of 60 minutes at 60% of maximal speed conducted five days per week. A greater use of energy substrates by skeletal muscle increases the demand for supply this energy production (Baker, Mc Cormick, & Robergs, 2010; Scomparin *et al.*, 2006). Then, these results observed in our study could be explained by the activation of the central nervous system induced by exercise training, which activates sympathetic nervous system and increases the release of catecholamines, which in turn, promotes lipolysis to maintain energy supply in trained animals.

It is well established that exercise training is an important approach to control the content of adipose tissue, especially on visceral fat, which is more susceptible to lipolysis in experimental models (Arner, 1995; Gomes *et al.*, 2010; Moreno-Eutimio, & Acosta-Altamirano, 2014; Goyal, Nimmakayala &

Zonszein, 2014). In agreement with previous studies, our results demonstrated that aerobic exercise training during 7 weeks was efficient in reducing the average area of adipocytes from epididymal fat pad in both trained groups, even when animals were fed with palatable high fat diet. Indeed, previous studies have shown a lower diameter of adipocyte in animals fed with high fat diet or high cholesterol diet and were exercised with moderate continuous exercise either swimming or running wheels for 4 to 8 weeks (Gollisch *et al.*, 2009; Guerra *et al.*, 2007).

In addition to the increase of insulin levels in OB/SD group, it was observed an increase in fasting glucose, showing that the high palatable diet turns metabolism to an insulin-resistance state. Possibly, insulin signaling and glucose uptake were improved by exercise training in the OB/TR group. Mechanisms that triggered this result could be related to the increase in glucose transporter (GLUT -4) expression and/or translocation as shown in different studies (Richter & Hargreaves, 2013; Torres-Leal, De Capitani, Tirapegui, 2009). Gomes and colleagues (2012) demonstrated that moderate exercise on a treadmill prevented insulin resistance in obese animal. Additionally they also demonstrated that the same exercise program was efficient to reduce fat pads and normalize glucose tolerance.

Different studies have shown a hypothalamic leptin resistance in obese animals (Koh, Park & Quon, 2008; Yang, & Barouch, 2007). Our results demonstrate that the OB/SD group had higher levels of leptin compared with the control group. This result was expected in view of the fact that the circulating leptin is proportional to the adipose tissue mass (Farooqi *et al.*, 2002; Ravussin *et al.*, 2014), mainly to visceral adipose tissue since it produces significant amounts of leptin (Coelho, Oliveira & Fernandes, 2013). In addition, insulin stimulates the production of leptin (Barr *et al.*, 1997; Tsai, Asakawa, Amitani & Inui, 2012) thus, hyperleptinemia observed in OB/SD rats could be related not only to adipocyte size, but also to hyperinsulinemia.

Trained groups had similar levels of leptin, both lower than sedentary counterparts. This result is in accordance with those demonstrated by Estadella and colleagues (2004) in obese rats fed with cafeteria diet and submitted to swimming training for 7 weeks. However, a recent study showed no change in plasma leptin levels after consumption of high fat diet and moderate continuous treadmill training, 5 days a week for 8 weeks (Haghshenas *et al.*, 2014). Exercise training could modulate leptin concentration through different mechanisms. Zhao *et al.* (2011) demonstrated improvements in hypothalamic leptin signaling in rats submitted to chronic exercise. In the same line of thought, Yi *et al.* (2013) showed an increase in leptin receptors on liver and vascular smooth muscle. Other studies proposed mechanisms related with reduction on fat pad mass (Estadella *et al.*, 2004; Park *et al.*, 2012).

Conclusion

The exercise training protocol proposed was effective to prevent adipocyte enlargement and the rise of leptin levels in high palatable diet fed rats. In addition, the insulin level was lower in trained rats demonstrating an improvement of insulin sensitivity.

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