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Aerobic training reduces immune cell recruitment and cytokine levels in adipose tissue in obese mice

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Abstract

Obesity is associated with an energy imbalance that results from excessive energy intake, low diet quality and a sedentary lifestyle. In this regard, the increased consumption of a high-refined carbohydrate diet (HC) is strongly related to higher adiposity and low-grade inflammation. Aerobic training is a well-known non-pharmacological intervention to treat obesity and metabolic disturbances. However, the mechanisms through which aerobic training ameliorates the low-grade inflammation induced by the HC diet need to be further investigated. Herein, our hypothesis was that aerobic training would decrease the recruitment of leukocytes in the adipose tissue thereby reducing the levels of cytokines and improving metabolism in mice fed the HC diet. Male Balb/c were assigned to the following groups: control non-trained (C-NT), control trained (C-T), HC-NT and HC-T. Mice were submitted to moderate-intensity training sessions that consisted of running 60 min/day for 8 weeks. The intravital microscopy technique was performed *in vivo* in anesthetized mice to visualize the microvasculature of the adipose tissue. The HC diet induced obesity and increased the influx of immune cells into the adipose tissue. In contrast, HC-T mice presented a lower adiposity and adipocyte area. Furthermore, HC-T mice showed an increased resting energy expenditure, a decreased recruitment of immune cells in the adipose tissue, reduced cytokine levels, and ameliorated hyperglycemia and fatty liver deposition relative to HC-NT mice. Collectively, our data enhance the understanding about the anti-inflammatory effect of aerobic training and shed light on the adipose tissue-mediated mechanisms by which training promotes a healthier metabolic profile.

Key-words: Aerobic training; inflammation; high-refined carbohydrate diet; obesity; fat mass; cytokines.

1 Introduction

Excessive energy intake, low quality diet and physical inactivity favor the development of obesity and metabolic dysfunction (Aller et al. 2011; Oliveira et al. 2013; Pedersen and Saltin 2015). Chronic low-grade inflammation is closely linked to metabolic dysfunction induced by obesity and is characterized by the immune cell infiltration and higher cytokine levels in adipose tissue (Lumeng et al. 2007; Sekimoto et al. 2015; Pedersen and Saltin 2015; Guzik et al. 2017). Thus, the recruitment of immune cells into the adipose tissue plays an essential role in the perpetuation of adipose tissue inflammation that, in turn, is related to insulin resistance and hepatic steatosis (Lumeng and Saltiel 2011; Oliveira et al. 2015).

Sugar is consumed in significant amounts in Western diets, and this high consumption has been positively associated with long-term weight gain and development of type 2 diabetes and related metabolic conditions (Malik and Hu, 2012). Noteworthy, not only the amount of carbohydrates ingested, but also their quality has been associated with metabolic dysfunction. The consumption of high-refined carbohydrate (HC) diets, particularly those containing high amounts of sucrose, induces obesity, fatty liver deposition and adipose tissue inflammation (Oliveira et al. 2013; Menezes-Garcia et al. 2014; Lacerda et al. 2015; Oliveira et al. 2015; Martins et al. 2017). Moreover, the HC diet-induced rapid fat tissue expansion is associated with leukocyte influx into adipose tissue vessels and increased levels of proinflammatory cytokines (Nishimura et al. 2008; Oliveira et al. 2013).

Sedentary behavior is another key risk factor for metabolic disease. As physical inactivity is accompanied by fat accumulation and adipose tissue inflammation, numerous recent studies have proposed that aerobic training can be used as a non-pharmacological therapy to reduce

chronic low-grade inflammation (Pedersen 2009; Pedersen and Saltin 2015; Goh et al. 2016). In this regard, regular exercise plays an anti-inflammatory role that may mediate a reduction in visceral fat mass via the release of myokines (Pedersen 2009). Most of these studies have focused on mediators produced by skeletal muscle and less attention has been given to adipose tissue-derived factors (Gollisch et al. 2009; Vieira et al. 2009; Kawanishi et al. 2013; Görgens et al. 2015). So far, few studies have shown that aerobic training inhibited macrophage infiltration, decreased tumor necrosis factor alpha (TNF- α) expression in the adipose tissue and attenuated hepatic steatosis in obese mice (Vieira et al. 2009; Kawanishi et al. 2010, 2012, 2013, 2015).

As a novelty, we have visualized the microvasculature of adipose tissue in mice fed the HC diet and subjected to aerobic training using intravital microscopy; this technique allows the quantification of adherent and rolling leukocytes *in vivo* and in real time. Another novel point is that obesity was induced by HC diet in the present study, whereas most of the published studies investigating the effects of aerobic training on low-grade inflammation induced by obesity fed rodents with high-fat diets (Gollisch et al. 2009; Vieira et al. 2009; Kawanishi et al. 2013; Görgens et al. 2015). Carbohydrates and fats are two important caloric sources, and the intake of diets enriched with these macronutrients is becoming more common in recent years, mainly in the Western world. Although the HC and high fat diets increase fat pad mass, the metabolic pathways and transcription factors that drive the adipose tissue expansion are different between these two diets (Oliveira et al. 2013, Ferreira et al. 2014). In this context, little is known about the effects of aerobic training in the adipose tissue of mice fed with the HC diet. Thus, the present study aimed to investigate the effects of aerobic training on adipose tissue inflammatory responses in mice fed a HC diet. Our hypothesis was that aerobic training would decrease the

recruitment of leukocytes in adipose tissue, contributing to lower the levels of proinflammatory cytokines and to improve the metabolic profile in obese mice.

2 Materials and methods

2.1 Ethical approval

Forty male Balb/c mice aged 6 weeks were obtained from the Animal Care Center at the Universidade Federal de Minas Gerais. All the protocols were approved by the Ethics Commission on Animal Use at the same university (protocol number: 194/2013). All the animals were cared for in accordance with the Guide to the Care and Use of Experimental Animals.

2.2 Animals

Mice were kept in a room with a controlled ambient temperature ($25.4 \pm 3.4^{\circ}\text{C}$), with a 12:12 h light/dark cycle, and had free access to tap water and food. They were randomly divided into four groups: (1) control diet/non-trained (C-NT); (2) control diet/trained (C-T); (3) high-refined carbohydrate diet/non-trained (HC-NT) and (4) high-refined carbohydrate diet/trained (HC-T).

2.3 Diet

Mice were individually housed and kept on either the control or the HC diet for 8 weeks to induce obesity according to previous studies (Oliveira et al. 2013; Menezes-Garcia et al. 2014). The HC diet was composed of 45% condensed milk, 10% refined sugar and 45% chow diet. The macronutrient composition of the HC diet was 74.2% carbohydrate, 5.8% fat and 20.0% protein (with a caloric content of 4.4kcal/kg). The macronutrient composition of the

control diet was 65.8% carbohydrate, 3.1% fat and 31.1% protein (with a caloric content of 4.0kcal/kg). The analysis of the HC diet revealed that it contained at least 30% refined sugars (primarily sucrose), showing that this diet contains a higher concentration of refined carbohydrate when compared to the control diet (Oliveira et al. 2013; Menezes-Garcia et al. 2014). Food intake was measured 3 times a week and the average daily intake was calculated.

2.4 Familiarization with running on a treadmill

Following the induction of obesity, the mice were gradually introduced to exercise on the treadmill over 5 consecutive days (Soares et al. 2007; Lacerda et al. 2015). The familiarization protocol was performed to habituate the mice to the treadmill environment. Each daily familiarization session consisted of resting on the treadmill turned off for 5 min. Immediately after, mice ran at speeds of 3-6 m/min for 5 min (Soares et al. 2007; Lacerda et al. 2015).

2.5 Incremental speed exercise

Twenty-four hours after the last day of the familiarization protocol, the first incremental test was performed to evaluate the intrinsic aerobic capacity of the mice and to determine the intensity of training sessions. At the end of the study, this incremental test was performed again to assess the effects of aerobic training on aerobic capacity, as evaluated by the workload and maximal speed attained by the mice.

Briefly, this test consisted of running on a treadmill (incline set at 5%), starting at a speed of 3 m/min with increases of 3 m/min every 3 min, until volitional fatigue. Fatigue was determined as the point at which mice were unable to keep running even when being stimulated by light electrical shocks on the grid at the end of the treadmill for 10 s (Ferreira et al. 2007; Lacerda et al. 2015).

2.6 Physical training protocol

Forty-eight hours after the first incremental exercise, the mice were submitted to the moderate-intensity aerobic training. Each session was performed on a treadmill with a 5% incline at a speed of 60-65% of the maximal speed obtained in the first (or second, as explained below) incremental exercise, 5 days per week for 8 weeks (Ferreira et al. 2007; Lacerda et al. 2015). In the first week, the exercise sessions lasted 30 min. From the second to the eighth week, the mice exercised 60 min per day. An additional (i.e., second) incremental exercise was performed after 4 weeks to adjust the training intensity (Ferreira et al. 2007; Lacerda et al. 2015). Seventy-two hours after the last (i.e., third) incremental exercise, the animals were anesthetized with ketamine (130 mg/kg) and xylazine (0.3 mg/kg). Samples of blood, liver and epididymal white adipose tissue were collected. Mice were euthanized after 4 h of fasting. The experimental design is illustrated in Fig. 1.

2.7 Adipose tissue and histomorphometry

The epididymal adipose tissue (EAT), the retroperitoneal adipose tissue (RAT) and mesenteric adipose tissue (MAT) were collected and weighted separately to calculate adiposity index ($EAT + RAT + MAT \times 100 \div \text{body weight}$), Oliveira et al. 2013; Menezes-Garcia et al. 2014; Lacerda et al. 2015. Samples of epididymal adipose tissue were fixed in a PBS-buffered formaldehyde solution for at least 24 h for histological analysis. These samples were incubated in 70% ethanol prior to processing via routine paraffin embedding and were stained with hematoxylin and eosin. Images of the adipose tissue sections were captured using a digital camera coupled to a microscope. For histomorphometry, the area of fifty cells was measured

under 10x magnification using the Image J software (National Institutes of Health, Bethesda, MD, USA), as described in Oliveira et al. (2013) and Menezes-Garcia et al. (2014).

2.8 Resting oxygen consumption and energy expenditure

Oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were measured 48 h after the last training session in mice previously fasted for 3 h using an open-flow indirect calorimeter (E405 Gas Analyzer, Panlab Harvard Apparatus, Barcelona, Spain). The system was calibrated before use with a certified mixture of gases (20.5% O_2 and 0.5% CO_2). Resting oxygen consumption ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) was continuously recorded on-line using a computerized system (Metabolism, Harvard Apparatus, City, Spain). All measurements were performed during the light phase (10:00–19:00 h). In brief, the mice were placed individually into the chamber. The initial 30 min values were eliminated in order to avoid altered physiological responses because of animal stress due to handling and exposure to the treadmill environment (Freire et al. 2016; Kunstetter et al. 2018). VO_2 and VCO_2 were recorded each min for 3 h and the average of the 10 lowest consecutive measurements were used to determine resting oxygen consumption, as described by Johnson et al. (2007). VO_2 and the respiratory exchange ratio ($\text{RER} = \text{VCO}_2 / \text{VO}_2$) values were used to calculate the resting energy expenditure through the following equation: (1) $\text{EE} = [3.815 + (1.232 \times \text{RER})] \times \text{VO}_2 \times 1.44$ (Freire et al. 2016).

2.9 Intravital microscopy

In order to check for the recruitment of leukocytes in the adipose tissue, we performed *in vivo* intravital microscopy (Oliveira et al. 2013; Nunes-Silva et al. 2014). Briefly, mice were anesthetized by injecting 130 mg/kg ketamine and 0.3 mg/kg xylazine intraperitoneally. The cell marker, Rhodamine 6G (1.5 mg/kg body weight Sigma, St. Louis, MO), was injected

intravenously to visualize leukocytes in adipose tissue vessels. Adipose tissue was exposed and observed on a fluorescence microscope with a video camera (Nikon H550L, 20x magnification objective lens, Tokyo, Japan). Rolling cells were defined as leukocytes passing through a vessel, 15-30 μm in width and 100 μm in length, over a period of 60 s. Leukocytes were considered adherent if they remained stationary in a vessel for a period of at least 30 s (Oliveira et al. 2013; Nunes-Silva et al. 2014).

2.10 Cytokine analysis

Epididymal adipose tissue was homogenized in cytokine extraction solution (BSA 0.05%, aprotinin 0.02 mg/mL, benzethonium chloride 0.05 mg/mL, NaCl 0.023 mg/mL, EDTA 0.37 mg/mL, PMSF 0.02 mg/mL, 0.5 mL Tween20/PBS) and centrifuged (10,000 rpm, 10 min at 4°C). The supernatant was used for ELISA analysis of interleukin-6 (IL-6), TNF- α , chemokine (C-X-C motif) ligand 1 (CXCL1), interferon- γ (IFN- γ) and interleukin-beta (IL1- β) (DuoSet ELISA development kits, R&D System, Inc., Minneapolis, MN, USA). The ELISA analysis was performed in an Absorbance Microplate Reader (SpectraMax 340PC384, Molecular Devices LLC, San Jose, USA).

2.11 Serum variables

Serum glucose, cholesterol and triglyceride levels were assayed using enzymatic kits (KATAL Belo Horizonte, MG, Brazil). The levels of adiponectin, resistin and insulin were determined by ELISA (DuoSet ELISA development kits, R&D System, Inc., Minneapolis, MN, USA).

2.12 Total lipids in liver

Total lipids were extracted from liver tissue using the method of Folch et al. (1957). In brief, liver was homogenized in a chloroform:methanol solution (2:1) to extract total fat from liver samples. Thereafter, the solution was filtered and mixed with 0.9% saline. The upper phase was aspirated and an aliquot of lower phase was added to a pre-weighed container. Upon evaporation, the extract previously obtained was mixed with 500 μ L of isopropanol. Total lipid content was evaluated using an enzymatic kit (KATAL, Belo Horizonte, MG, Brazil), as described previously by Oliveira et al. (2015).

2.13 Liver histology

Liver fragments were weighed and fixed in a PBS-buffered formaldehyde solution for at least 24 h for histological analysis. These samples were incubated in 70% ethanol prior to processing via routine paraffin embedding and were stained with hematoxylin and eosin. Images of the liver sections were captured using a digital camera coupled to a microscope under 10x magnification (National Institutes of Health, Bethesda, MD, USA). Liver histology was evaluated through a histological score using the following parameters: 0 is none, 1 is mild, 2 is moderate and 3 is severe (adapted from Brunt et al. 1999).

2.14 Statistics

All variables were tested using the Kolmogorov-Smirnov test and were normally distributed. Data are expressed as means $\pm$ SEM and were compared between diets (C vs. HC) and aerobic training status (NT vs. T) utilizing two-way ANOVAs. When these analyses revealed significant effects, the *post-hoc* Fisher's test was conducted to identify differences between pair of means (C-NT, C-T, HC-NT and HC-T). The Fisher test was selected due to the

coefficient of variation of data evaluated ($\geq 15\%$, as previously used by Lacerda et al. 2015). The level of statistical significance was set at $p \leq 0.05$.

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3 Results

3.1 Physical training reduces adiposity and increases resting energy expenditure in obese mice

At the beginning of the aerobic training or control procedure, the mice of all groups had similar body mass (C-NT: 28.4 ± 1.0 g; C-T: 29.8 ± 1.2 g; HC-NT: 29.8 ± 2.0 g; HC-T: 29.6 ± 1.8 g). Mice fed the HC diet showed a higher adiposity index, increased weight of epididymal adipose tissue and enhanced adipocyte area when compared with mice fed the control diet (Fig. 2A-D, $p < 0.01$). Physical training reduced both the adiposity and adipocyte size in obese mice, but not in mice fed the control diet (Fig. 2A-D $p < 0.01$). Analysis of the adipocyte size distribution demonstrated that mice fed the HC diet had a greater number of enlarged adipocytes, when compared with mice fed the control diet (Fig. 2E, $p < 0.05$). In contrast, HC-T mice had smaller adipocytes when compared with HC-NT animals (Fig. 2E, $p < 0.05$). Likewise, C-T mice showed smaller adipocytes relative to C-NT mice (Fig. 2E, $p < 0.05$), indicating that aerobic training reduced adipocyte size irrespective of diet. Subcutaneous adipose tissue weight was also higher in obese mice. Aerobic training reduced the subcutaneous adipose tissue in obese mice and tended to reduce in mice fed the control diet (Fig. 2F, $p < 0.05$). There were no alterations in body mass (Fig. 2G) or food intake (Fig. 2H) caused by either the HC diet or aerobic training.

Obese mice displayed lower resting oxygen consumption and energy expenditure than sedentary mice fed the control diet (Fig. 2H-I, $p < 0.05$). Physical training increased resting oxygen consumption and resting energy expenditure in obese mice, although no changes were observed in these variables when comparing C-T and C-NT mice (Fig. 2I-J). As expected, the

aerobic training protocol efficiently increased the work and maximal running speed in trained groups regardless of the diet composition (Supplementary Figure S1).

3.2 Physical training reduces the recruitment of leukocytes in the adipose tissue of obese mice

The number of immune cells was increased in the blood of obese mice, an effect that was prevented after 8 weeks of aerobic training (Fig. 3A, $p < 0.05$). In order to visualize leukocyte-endothelial cell interactions in the microcirculation of adipose tissue, we performed *in vivo* intravital microscopy. Similar to the number of immune cells in the blood, aerobic training reduced the number of rolling (Fig. 3B, $p < 0.01$) and adherent cells (Fig. 3C, $p = 0.01$) in the adipose tissue vessels of obese mice. CXCL-1 levels were also reduced in obese mice subjected to aerobic training (Fig. 3D, $p = 0.01$). Representative intravital microscopy images of the microvasculature of adipose tissue are shown in Fig. 3D and Supplementary Video (S1-S4). No changes were observed in C-T mice relative to C-NT mice in any of the above-mentioned variables (Fig. 3A-E).

3.3 Obese trained mice show lower cytokine concentrations in adipose tissue

To evaluate whether physical training influences the production of adipose tissue cytokines, the levels of IL-6, TNF- α , IFN- γ and IL-1 β were measured by ELISA (Fig. 4). The concentrations of IL-6 (Fig. 4A, $p < 0.05$), TNF- α (Fig. 4B, $p < 0.05$) and IFN- γ (Fig. 4C, $p < 0.05$) were higher in obese mice than in mice fed the control diet. Aerobic training reduced these cytokine concentrations in obese mice, without affecting concentrations in mice fed the control

diet (Fig. 4A-C, $p < 0.05$). No changes were found in concentrations of IL-1 β caused either by the HC diet or aerobic training (Fig. 4D).

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3.4 Aerobic training attenuates increased glycemia, but not increased total cholesterol levels, in obese mice

Glucose levels were higher in obese mice (Fig. 5A, $p < 0.01$) and this increased glycemia was attenuated by aerobic training (Fig. 5A, $p < 0.05$). No changes were observed in C-T mice relative to C-NT mice (Fig. 5A), indicating that training had no effects on glucose levels in mice fed the control diet. There were no significant changes in the levels of insulin (Fig. 5B), adiponectin (Fig. 5C), resistin (Fig. 5D), triglycerides (Fig. 5E) and free fatty acids (Fig. 5F) caused by either the HC diet or aerobic training. Serum total cholesterol levels were higher in HC mice than in mice fed the control diet. In contrast, no alterations in total cholesterol levels were found in trained animals, irrespective of their diet; this result means that training has failed to reverse the higher cholesterol levels induced by obesity (Fig. 5G, $p < 0.05$).

3.5 Physical training reduces fat deposition, but does not change cytokine concentrations in the liver of obese mice

Total fat in the liver and steatosis score were both higher in obese mice when compared with mice fed the control diet (Fig. 6A-C, $p < 0.05$ and $p < 0.01$, respectively). Physical training reduced fat content and steatosis score in obese, but not in control, mice (Fig. 6A-C, $p < 0.05$). No changes were observed in IL-6 and CXCL-1 concentrations in the liver caused by either the HC diet or aerobic training (data not shown).

4 Discussion

The high consumption of refined carbohydrates enhances the risk of developing a variety of chronic diseases, including obesity, insulin resistance and hepatic steatosis (Aller et al. 2011; Oliveira et al. 2013; Lumeng and Saltiel 2011). Our previous reports have demonstrated that mice fed the HC diet show obesity, inflammation, metabolic dysfunction and liver steatosis (Oliveira et al. 2013; Oliveira et al. 2015). These outcomes occurred regardless of the caloric intake, highlighting that dietary composition may dictate the adipose tissue size and metabolic disarrangements. Herein, we demonstrated that aerobic training reduced adiposity and increased resting energy expenditure in obese mice. Furthermore, we visualized *in vivo* that obese mice subjected to aerobic training showed reduced immune cell infiltration in the adipose tissue and lower cytokine levels in the same tissue. In addition, physical training reduced glycemia and ameliorated the hepatic fat accumulation in obese mice.

A large body of evidence supports the association between regular physical exercise and weight loss (Thompson et al. 2012; Swift et al. 2014; Bae et al. 2017). Exercise is a key component of the energy balance that increases energy expenditure, ultimately leading to reduced fat mass (Thompson et al. 2012). We found that 8 weeks of aerobic training reduced visceral and subcutaneous adiposity in obese mice. In particular, the trained animals fed the HC diet showed a greater number of smaller adipocytes, probably due to the negative balance induced by training sessions and the increased resting energy expenditure. Furthermore, no compensations in food intake were observed, as trained mice ingested a similar amount of food to non-trained mice, irrespective of the diet.

Obesity is characterized by the accumulation of diverse immune cells in the adipose tissue. Notably, macrophage infiltration leads to an increased production of pro-inflammatory cytokines (Lumeng et al. 2007). Regarding the diet used in the present study, an earlier study reported that mice fed the HC diet exhibited higher adiposity that was associated with rapid and sustained adipose tissue inflammation, characterized by higher number of macrophages and neutrophils and cytokine production. Current evidence points towards a reduction in adipose tissue inflammation induced by chronic exercise (Kawanishi et al. 2010; Baynard et al. 2012; Kawanishi et al. 2013; Pedersen and Saltin 2015, Goh et al. 2016). Indeed, Kawanishi et al. (2010) demonstrated that chronic exercise might contribute to inhibit inflammation in adipose tissue via downregulation of toll like receptor (TLR) 4 and a reduction in TNF- α levels. Moreover, Kawanishi et al. (2015) showed that 16 weeks of aerobic training decrease the numbers of macrophages and neutrophils in adipose tissue of mice fed a high-fat diet; these authors have suggested that the ability of exercise to suppress macrophage infiltration might result from the reduced secretion of elastase by neutrophils. Herein, obese mice subjected to physical training exhibited a reduced number of blood leukocytes, rolling cells and adherent cells in adipose tissue. Thus, our study supports the anti-inflammatory effect of aerobic training in adipose tissue in mice fed the HC diet. Herein, we visualized the microvasculature of adipose tissue in obese mice *in vivo* after aerobic physical training; these mice showed reduced leukocyte recruitment and cytokine/chemokine concentrations. However, additional studies are necessary to better understand which leukocytes are less recruited following aerobic training in obesity induced by a HC diet.

Xu et al. (2003) and Weisberg et al. (2003) were the first to demonstrate that the production of TNF- α in adipose tissue in obese mice was almost entirely derived from cells that

have migrated. Indeed, cytokine production is elevated in obese individuals and is positively and strongly associated with the infiltration of cells (Guzik et al. 2017). Consistent with the reduced leukocyte recruitment to the adipose tissue, we observed a decrease in IL-6, TNF- α , CXCL-1 and INF- γ levels in obese trained mice. The latter finding suggests that the reduced influx of leukocytes into the adipose tissue may underlie the decreased cytokine concentrations in this tissue induced by physical training that, in turn, would reduce the systemic pro-inflammatory milieu. Furthermore, the pro/anti-inflammatory balance is strongly associated with the expansion of the adipocytes (Guzik et al. 2017). In this respect, fat loss induced by chronic exercise may also lead to reductions in inflammatory biomarkers (Thompson et al. 2012). Altogether, these data highlight physical training-induced health improvements that contribute to restore homeostasis in adipose tissue during obesity.

Another mechanism that could explain the benefits of aerobic training in obese animals would be the enhanced secretion of myokines (You et al. 2013; Golbidi and Lahen 2014). Several studies have reported that the anti-inflammatory effects caused by exercise bouts are likely mediated by myokines and independent of fat loss (Balducci et al. 2010, 2012; Arikawa et al. 2011; Balducci et al. 2012; Kawanishi et al. 2015). However, this does not seem to be the case of the present study. We did not observe significant changes in IL-6 concentrations in muscle tissue 72 h after the third incremental exercise in obese mice subjected to aerobic training (*unpublished observations*); this finding reinforces the idea that reduced adiposity and the resulting lower adipose tissue inflammation have played a major role in training-induced health benefits.

Insulin resistance has frequently been associated with increases in inflammation and cytokine production (Lumeng and Saltiel 2011; Bluher 2016). TNF- α , which has been proposed as a link between obesity and type 2 diabetes, is overexpressed in the adipose tissue of obese animals (Lumeng and Saltiel 2011). In fact, exercise has been largely used to revert insulin resistance and is an essential component of lifestyle management in type 2 diabetes patients (Pedersen 2009; Pedersen and Saltin 2015; Way et al. 2016). In this study, we demonstrated that aerobic training decreased the influx of cells, decreased IL-6 and TNF- α concentrations in adipose tissue and improved serum glycemia in obese mice; these outcomes are probably associated with the reduced adipose tissue inflammation. Despite these findings, we did not show alterations in serum concentrations of resistin and adiponectin, indicating that improved serum glucose control occurred regardless of changes in these adipokines. In agreement with previous observations (Oliveira et al. 2013), we also have shown that the HC diet increased serum total cholesterol. Interestingly, aerobic training could not prevent the increased cholesterol concentrations caused by obesity. Most likely, longer or more intense training protocols may be required to revert some metabolic dysfunction in obese mice, including hypercholesterolemia.

Large adipocytes may indicate that adipose tissue is saturated with lipids (Asrih and Jornayvaz 2015), which leads to increased lipid storage in other organs, including the liver. The elevation in hepatic lipid content is strongly associated with insulin resistance (Asrih and Jornayvaz 2015). In fact, Oliveira et al. (2015) showed that the HC diet leads to an acute and sustained fat accumulation in the liver and moderate steatosis. Herein, we showed that aerobic training decreased liver fat deposition, an outcome that has probably contributed to explain the improved insulin resistance in obese mice. Kawanishi et al. (2012) demonstrated that exercise in mice fed a high-fat diet not only suppressed adipose tissue and hepatic inflammation, but also

inhibited the development of nonalcoholic steatohepatitis. Therefore, aerobic training seems to be an effective non-pharmacological intervention to treat nonalcoholic liver disease caused by obesity.

In summary, we have demonstrated that aerobic training reduces adiposity, the recruitment of immune cells and cytokine levels in adipose tissue of mice fed the HC diet. These findings were accompanied by improved hyperglycemia control and lower fat deposition in the liver. Our study sheds light on the role of physical training-mediated changes in adipose tissue that are relevant for the treatment of obesity and metabolic dysfunction induced by HC diets.

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Conflicts of interest statement

The authors declare that there are no conflicts of interest.

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Figure captions

Fig. 1: Experimental procedures. Legend: C: control, HC: high refined carbohydrate diet, S_{MAX} : maximal speed obtained in the incremental exercise, VO_2 : oxygen consumption.

Fig. 2: Adiposity and variables associated with the energy balance. A. Adiposity index. B. Epididymal adipose tissue (expressed as a percentage of epididymal adipose tissue weight), $p < 0.01$. C. Adipocyte area (μm^2) in the epididymal adipose tissue, $p < 0.01$. D. Histological analysis of the epididymal adipose tissue (haematoxylin and eosin staining; scale bars represent 50 μm). E. Frequency distribution of adipocytes in the cell surface area of the epididymal adipose tissue $p < 0.05$. F. Subcutaneous adipose tissue (expressed as a percentage of epididymal adipose tissue), $p < 0.05$. G. Body mass of the mice collected on the day they were euthanized. H. Food intake ($g \cdot day^{-1}$). Food intake was measured 3 times a week and the average daily intake was calculated. I. Resting VO_2 , $p < 0.05$. J. Resting energy expenditure, $p < 0.05$. Results are expressed as means $\pm$ SEM and $n = 8$ mice per group. #Significantly different, HC-NT versus C-NT and HC-T versus C-T. *Significantly different, HC-NT versus HC-T and C-NT versus C-T.

Fig. 3: Leukocyte rolling and adhesion in the microvasculature of epididymal adipose tissue assayed by *in vivo* intravital microscopy. A. Total blood cells, $p < 0.05$. B. Number of rolling cells, $p < 0.01$. C. Number of adherent cells, $p = 0.01$. D. CXCL-1 production in the epididymal adipose tissue, $p = 0.01$. E. Intravital microscopy images of the microvasculature of adipose tissue. The black arrow indicates the labeled leukocytes after the intravenous administration of Rhodamine 6G-Sigma. Rolling cells were defined as leukocytes passing through a vessel, 15-30 μm in width and 100 μm in length, over a period of 60 s. Leukocytes were considered adherent if

they remained stationary in a vessel for a period of at least 30 s. Results are expressed as means $\pm$ SEM and $n = 8$ mice per group. #Significantly different, HC-NT versus C-NT and HC-T versus C-T. *Significantly different, HC-NT versus HC-T and C-NT versus C-T.

Fig. 4: Concentration of cytokines in the epididymal adipose tissue. A. Interleukin-6 (IL-6), $p < 0.05$. B. Tumor necrosis factor-alpha (TNF- α), $p < 0.05$. C. Interferon- γ (INF- γ), $p < 0.05$. D. Interleukin 1 beta (IL-1 β). Results are expressed as means $\pm$ SEM and $n = 8$ mice per group. #Significantly different, HC-NT versus C-NT and HC-T versus C-T. *Significantly different, HC-NT versus HC-T and C-NT versus C-T.

Fig. 5: Serum concentrations of metabolic/hormonal variables. A. Glucose, $p < 0.05$. B. Insulin. C. Adiponectin. D. Resistin. E. Triglycerides. F. Free fatty acids. G. Total cholesterol, $p < 0.05$. Results are expressed as means $\pm$ SEM and $n = 8$ mice per group. #Significantly different, HC-NT versus C-NT and HC-T versus C-T. *Significantly different, HC-NT versus HC-T and C-NT versus C-T.

Fig. 6: Liver variables. A. Total liver fat determined by the Folch method, $p < 0.05$. B. Hepatic steatosis score, $p < 0.01$. C. Histological images of liver (haematoxylin and eosin staining - 400x). Results are expressed as means $\pm$ SEM and $n = 8$ mice per group. #Significantly different, HC-NT versus C-NT and HC-T versus C-T. *Significantly different, HC-NT versus HC-T and C-NT versus C-T.

Fig.5