

## Original Article

# Physical training improves body weight and energy balance but does not protect against hepatic steatosis in obese mice

Fabiana S Evangelista<sup>1</sup>, Cynthia R Muller<sup>2</sup>, Jose T Stefano<sup>3</sup>, Mariana M Torres<sup>3</sup>, Bruna R Muntanelli<sup>3</sup>, Daniel Simon<sup>4</sup>, Mario R Alvares-da-Silva<sup>5</sup>, Isabel V Pereira<sup>3</sup>, Bruno Cogliati<sup>3</sup>, Flair J Carrilho<sup>3</sup>, Claudia P Oliveira<sup>3</sup>

<sup>1</sup>School of Arts, Science and Humanities, University of Sao Paulo, Brazil; <sup>2</sup>Experimental Pathophysiology Dept, Faculty of Medicine, University of Sao Paulo, Brazil; <sup>3</sup>Gastroenterology Dept (LIM 07), Faculty of Medicine, University of Sao Paulo, Brazil; <sup>4</sup>Molecular and Cellular Biology Applied to Health Dept, Luterana University of Brazil (ULBRA), Canoas, RS, Brazil; <sup>5</sup>Division of Gastroenterology, Clinics Hospital of Porto Alegre, Federal University of Rio Grande do Sul, Brazil

Received May 16, 2015; Accepted July 6, 2015; Epub July 15, 2015; Published July 30, 2015

**Abstract:** This study sought to determine the role of physical training (PT) on body weight (BW), energy balance, histological markers of nonalcoholic fatty liver disease (NAFLD) and metabolic gene expression in the liver of ob/ob mice. Adult male ob/ob mice were assigned into groups sedentary (S; n = 8) and trained (T; n = 9). PT consisted in running sessions of 60 min at 60% of maximal speed conducted five days per week for eight weeks. BW of S group was higher from the 4<sup>th</sup> to 8<sup>th</sup> week of PT compared to their own BW at the beginning of the experiment. PT decreased daily food intake and increased resting oxygen consumption and energy expenditure in T group. No difference was observed in respiratory exchange ratio, but the rates of carbohydrate and lipids oxidation, and maximal running capacity were greater in T than S group. Both groups showed liver steatosis but not inflammation. PT increased CPT1a and SREBP1c mRNA expression in T group, but did not change MTP, PPAR- $\alpha$ , PPAR- $\gamma$ , and NFKB mRNA expression. In conclusion, PT prevented body weight gain in ob/ob mice by inducing negative energy balance and increased physical exercise tolerance. However, PT did not change inflammatory gene expression and failed to prevent liver steatosis possible due to an upregulation in the expression of SREBP1c transcription factor. These findings reveal that PT has positive effect on body weight control but not in the liver steatosis in a leptin deficiency condition.

**Keywords:** Obesity, energy balance, liver steatosis, gene expression, leptin-deficient mice

## Introduction

The prevalence of obesity, dyslipidemia and diabetes, all of which augment the risk of developing cardiovascular and metabolic disorders, is increasing worldwide and have been caused by the combination of physical inactivity and raised consumption of high-fat diet [1-3]. Impairments in the regulation of lipid metabolism are observed in metabolic disorders which can be associated with tissue lipotoxicity and inflammation [4]. In the liver, the imbalance between lipid storage and oxidation is a key point for the development of nonalcoholic fatty liver disease (NAFLD), which has been closely associated with obesity and diabetes [5, 6].

NAFLD is characterized by accumulation of triglycerides within hepatocytes with reversible feature, but can progress with subsequent inflammation-non-alcoholic steatohepatitis (NASH), which can lead to fibrosis, cirrhosis and liver failure [7, 8]. Evidence in the literature reported the accumulation of lipid in the liver as a consequence of increased supply of lipids from the diet, increased expression of lipogenic proteins such as sterol regulatory element-binding protein 1c (SREBP1c) and fatty acid synthesis (FAS), decreased lipid oxidation and reducing the production of the very-low density lipoproteins [6, 7, 9].

Physical training (PT) has become an important strategy for the prevention and treatment of

## Energy metabolism and leptin-deficient ob/ob mice

obesity, dyslipidemia and diabetes because it promotes a reduction in body weight [10] and adipose mass [11, 12], improves mitochondrial function and increases fatty acid oxidation in the skeletal muscle [13], and induces adipokines secretion responsible to glycemic control and inflammatory response [14, 15]. In the liver, PT is able to increase fatty acid oxidation, to decrease inflammatory state and to reduce NAFLD [6, 16-18].

Experimental models are useful to study cellular and molecular aspects involved in the development and progression of several metabolic dysfunctions. It was recently shown the protective effect of PT in an experimental model of NASH analyzing liver mitochondrial activity during induction of steatosis [16]. In that work, PT was performed after the induction of NASH by fat diet and the result revealed that PT prevents or reverses mitochondrial dysfunction assessed by mitochondrial markers. However, in ob/ob mice, a good model to reproduce the etiology and outcomes of human diabetes and severe obesity [19], the effect of PT on metabolism is contradictory. While acute exercise reduced blood glucose levels associated with improved insulin action [20], PT in treadmill during 4 weeks did not modify hyperglycemia, hypercholesterolemia, hyperinsulinemia, fat mass and body weight [21]. Furthermore, voluntary running improved body weight, but exacerbated plasma glucose and triglyceride levels [22].

Despite few studies had showed the impact of PT on metabolism, the role of PT on energy balance and hepatic metabolism in ob/ob mice is not completely understood. Thus, the objective of this study was to investigate the effect of PT on body weight, determinants of energy balance, histological markers of NAFLD and metabolic gene expression in the liver in leptin-deficient ob/ob mice.

### Materials and methods

#### *Animals*

Male obese ob/ob mice (8 weeks) (Jackson Laboratories, Bar Harbor, USA), were assigned into groups sedentary (S; n = 8) and trained (T; n = 9) matched for body weight. Mice were housed in a temperature-controlled ( $22 \pm 2^\circ\text{C}$ ) and 12-h light/12-h dark cycle, with free access to tap water and food *ad libitum*. Procedures

were performed in accordance with the guidelines of the Brazilian College for Animal Experimentation and were approved by the Ethics Committee of the School of Medicine of University of Sao Paulo (process number 120/13).

#### *Running test*

Running capacity was assessed before, in the fourth and eighth weeks of PT using a progressive test without inclination on a treadmill as described by Ferreira et al. [23]. The initial speed was 0.4 km/h and the speed was increased by 0.2 km/h every three minutes until exhaustion of the animal, which was characterized by the impossibility of maintaining the running standard. The test variable was quantified as the maximum time to exhaustion (min).

#### *Physical training*

T animals were submitted to PT as described by Ferreira et al. [23]. Animals were trained during the dark cycle (i.e., during their active period) on a motorized treadmill for 1 h/day at 60% of maximal velocity achieved in the running capacity test, five times per week for eight weeks. PT intensity was progressively increased, starting at 0.6 km/h and adjusted after the running capacity test done in the fourth week. S mice were placed on the treadmill for 5 min twice weekly at 0.3 km/h during the experimental protocol to minimize the influence of the treadmill stress.

#### *Body weight and food intake*

Body weight was measured weekly at the same time of day using a digital balance (Gehaka, Model BK4001, Brazil). Body weight gain was calculated as the difference between body weight measured at the beginning and at the end of the PT protocol. The 24-h food intake was determined weekly throughout the study in mice that were housed at four animals per cage.

#### *Indirect calorimetry*

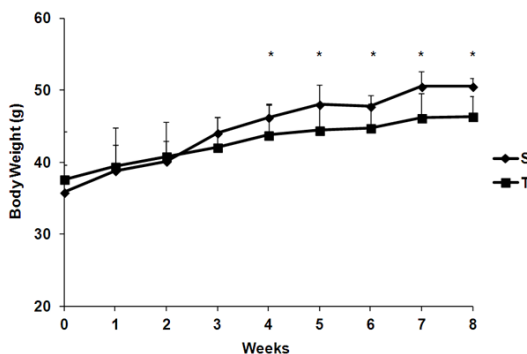
After 24-h of the last session of PT, the animals were submitted to fasting (4-h) and then the volumes of oxygen consumption ( $\text{VO}_2$ ) and carbon dioxide production ( $\text{VCO}_2$ ) during resting were measured using Oxylet System (Panlab,

## Energy metabolism and leptin-deficient ob/ob mice

**Table 1.** Primers used for genes amplification evaluated in the liver from sedentary and trained mice

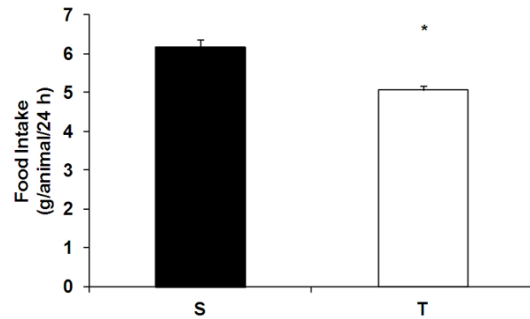
Genes	Sequence
SREBP1c	F: GCG CTA CCG GTC TTC TAT CA R: GGA TGT AGT CGA TGG CCT TG
MTP	F: CCT CTT GGC AGT GCT TTT TC R: ATT TTG TAG CCC ACG CTG TC
PPAR- $\gamma$	F: CAT AAA GTC CTT CCC GCT GA R: GAA ACT GGC ACC CTT GAA AA
PPAR- $\alpha$	F: ATG CCA GTA CTG CCG TTT TC R: TTG CCC AGA GAT TTG AGG TC
CPT1a	F: TGC CTC TAT GTG GTG TCC AA R: TCA AAC AGT TCC ACC TGC TG
NFKB	F: TGT CTG CCT CTC TCG TCT T R: GAG TTT GCG GAA GGA TGT CT
$\beta$ -actin	F: TGT TAC CAA CTG GGA CGA CA R: GGG GTG TTG AAG GTC TCA AA
GAPDH	F: AAT GGT GAA GGT CGG TGT G R: GTG GAG TCA TAC TGG AAC ATG TAG

F = forward; R = reverse.



**Figure 1.** Body weight evolution during the experimental protocol in sedentary (S, n = 8) and trained (T, n = 9) ob/ob mice. Data are reported as mean  $\pm$  SEM. \* $P \leq 0.05$  S vs. T in the week 0.

Barcelona, Spain). After acclimatization in the system, resting measurements were done during 45 min. The non-protein respiratory exchange ratio (RER), a measurement of metabolic substrate preference, was calculated as the molar ratio of  $VCO_2$  to  $VO_2$ . Energy expenditure (EE) was calculated using the formula:  $EE = [3.815 + (1.232 \times RER)] \times VO_2 \times 1.44$ . Data are expressed as Kcal/day/Kg. The rate of oxidation of carbohydrate (CHO) and lipids (LIP) were calculated as described by Frayn [24]:  $CHO = (4.585 \times VCO_2) - (3.226 \times VO_2)$  and  $LIP = (1.695 \times VO_2) - (1.701 \times VCO_2)$ . Data are expressed as  $mg \cdot min^{-1} \cdot kg^{-1}$ .



**Figure 2.** Food intake in sedentary (S, n = 8) and trained (T, n = 9) ob/ob mice. Data are reported as mean  $\pm$  SEM. \* $P \leq 0.05$  vs. S.

### Euthanasia

Forty-eight hours after the end of the last training session, the animals were anesthetized with an overdose of intraperitoneal ketamine hydrochloride (0.5 mL/kg) and exsanguination was done while the animals were unconscious. The liver were harvested, weighed and processed according to the following experiments.

### Histology

Fragments of liver tissues were fixed in formaldehyde saline (4%) and processed for hematoxylin-eosin (HE) stain. Histological markers of NAFLD activity steatosis (0-3), ballooning (0-2) and lobular inflammation (0-3) were assessed.

### RNA Isolation

After liver tissue pulverization (50 mg) with a dismembrator (B. Braun Biotech International, Melsungen, Germany) at liquid nitrogen temperature, total RNA was prepared using TRIzol® reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) according to the manufacturer's recommendations.

Total RNA was dissolved in RNase-free water and RNA concentration was determined spectrophotometrically. RNA integrity was judged appropriate at a 260/280 nm ratio  $>1.8$  and without signs of degradation on agarose gel. Samples were kept at  $-80^\circ\text{C}$  until processing by reverse transcription quantitative polymerase chain reaction (RT-qPCR) analysis.

### Gene expression by RT-qPCR

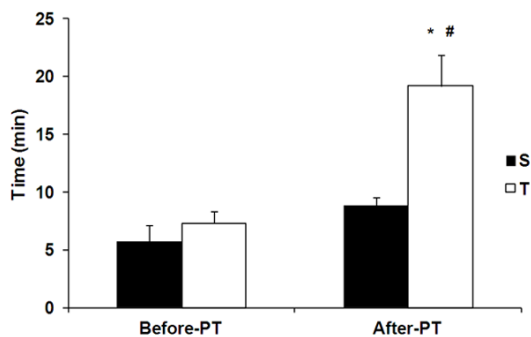
Transcript levels of genes related to lipids metabolism (SREBP1c, MTP, PPAR- $\gamma$ ), mitochon-

## Energy metabolism and leptin-deficient ob/ob mice

**Table 2.** Metabolic parameters measured in the indirect calorimetry

	S (n = 4)	T (n = 6)
VO <sub>2</sub> (mL/min/kg)	19.6 ± 0.3	38.4 ± 1.2*
VCO <sub>2</sub> (mL/min/kg)	18.5 ± 0.7	35.7 ± 1.0*
RER	0.94 ± 0.02	0.93 ± 0.01
EE (Kcal/day/Kg)	141.0 ± 4.0	274.5 ± 8.1*
CHO oxidation (mg/min/Kg)	2.1 ± 0.2	4.1 ± 0.2*
LIP oxidation (mg/min/Kg)	0.2 ± 0.1	0.4 ± 0.04*

Data are reported as mean ± SE. VO<sub>2</sub> = oxygen consumption; VCO<sub>2</sub> = carbon dioxide production; RER = respiratory exchange ratio; EE = Energy expenditure; CHO = carbohydrate, LIP = lipids. \*P≤0.05 vs. S.



**Figure 3.** Maximum time reached in the running capacity test. PT = Physical Training. Data are reported as mean ± SEM. S (n = 8); T (n = 9). \*P≤0.05 vs. S after-PT; #P≤0.05 vs. T before-PT.

drial oxidation (CPT1a, PPAR-α) and inflammatory response (NFKB) were determined by quantitative RT-qPCR and the results were normalized according to corresponding values of housekeeping b-actin or GAPDH mRNA. The primers for SREBP1c, MTP, PPAR-γ, CPT1a and PPAR-α were designed to have similar GC contents and annealing temperatures using the Primer 3 Program ([http://frodo.wi.mit.edu/cgi-bin/primer3/primer3\\_www.cgi](http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi)) [25]. The primer sequences for each gene were described in **Table 1**.

mRNA expressions were carried out in a Rotor-Gene RG-3000 (Corbett Research, Sydney, Australia) using SuperScript™ III Platinum® One-Step Quantitative RT-PCR System (Invitrogen Life Technologies, Carlsbad, EUA), according to the instructions provided by the manufacturer. Reactions lacking reverse transcriptase were also run to generate controls for assessment of genomic DNA contamination. Fluorescence changes were monitored after each cycle, and melting curve analysis was per-

**Table 3.** Semi-quantitative analysis of histological parameters in sedentary (S) and trained (T) ob/ob mice

	S (n = 4)	T (n = 6)
Steatosis	3	3
Balloning	2	2
Inflammation	0	0

**Table 4.** mRNA expression in liver in sedentary (S) and trained (T) ob/ob mice

	S (n = 4-5)	T (n = 6)	P value
CPT1a	0.10 ± 0.04	1.10 ± 0.01*	0.024
SREBP1c	0.10 ± 0.01	1.06 ± 0.01*	0.001
MTP	1.89 ± 0.23	1.49 ± 0.12	0.130
PPARγ	0.89 ± 0.07	0.94 ± 0.04	0.585
PPARα	0.67 ± 0.07	0.84 ± 0.03	0.285
NFKB	0.40 ± 0.40	0.55 ± 0.19	0.720

Data are reported as mean ± SEM. \*P≤0.05 vs. S.

formed at the end of cycles to verify PCR product identity (72°C, ramping to 99°C at 0.2°C/sec, with continuous fluorescence readings). Specificity of amplicons was also ensured by agarose gel electrophoresis to visualize a unique product fragment of the appropriate size.

To evaluate the amplification efficiency of each target and housekeeping gene, standard curves were constructed from a control liver RNA sample using duplicate serial dilutions with five different RNA concentrations (500, 100, 20, 4, and 0.8 ng/μL). Relative quantification was calculated using the mathematical model described by Pfaffl [26]. Amplifications of all genes were done in duplicate from each sample.

### Statistical analysis

Data are reported as means ± SEM. The results were compared between the two groups using the Student t-test, except for body weight evolution, which was analyzed using one-way ANOVA for repeated measures. The Bonferroni *post hoc* test was used to determine differences between means when a significant change was observed with ANOVA. A P value of less than 0.05 was considered to be statistically significant.

### Results

No differences in body weight were observed between the S and T groups during PT (**Figure**

1). However the body weight of S group was higher from the 4<sup>th</sup> to 8<sup>th</sup> week of PT compared to their own body weight at the beginning of the experiment ( $P \leq 0.05$ ) (**Figure 1**). In fact, S animals had 41% of weight gain during the protocol while T animals increased 23%, confirming that PT stabilizes body weight gains in ob/ob mice.

As showed in **Figure 2**, food intake in grams assessed over a 24-h period was significantly higher in the S group during the PT period compared with T group ( $6.2 \pm 0.2$  vs.  $5.1 \pm 0.1$  g/animal/24 h).

Resting oxygen consumption and carbon dioxide production measured after the period of PT were significantly higher in the T group compared with S group (**Table 2**). However, no difference was observed in RER between groups. Considering that energy expenditure is calculated based on oxygen consumption, the T group also showed improved energy expenditure during rest. Furthermore, the rates of carbohydrate and lipids oxidation were greater in T group than S group (**Table 2**).

Before PT, no difference in running capacity was present between groups (S =  $5.7 \pm 1.4$  min; T =  $7.3 \pm 1.0$  min; **Figure 3**). However, after PT, the running capacity was increased in the T group ( $19.2 \pm 2.7$  min) compared with S group ( $8.8 \pm 0.7$  min) and compared with itself before PT ( $P \leq 0.05$ ) (**Figure 3**). Data obtained in the 4<sup>th</sup> week were used only to adjust the intensity of PT (not shown).

No differences in liver histology were observed between the S and T groups after 2 months of PT. Semi-quantitative histological analysis including steatosis (0-3), ballooning (0-2) and lobular inflammation (0-3) were not different between the groups. Hepatic steatosis and hepatocellular ballooning were observed in higher grade in both groups. No important inflammation was seen in both groups in this model (**Table 3**).

Our study showed a statistically significant increase in CPT1a and SREBP1c mRNA expression in T group compared with S group (**Table 3**). However, there were no relevant modifications in MTP, PPAR- $\alpha$ , PPAR- $\gamma$ , and NF $\kappa$ B mRNA expression (**Table 4**).

### Discussion

The present study was conducted to determine the effect of PT on energy balance and hepatic metabolism in leptin-deficient ob/ob mice. Our findings provide evidence that PT prevents body weight gains, decreases food intake and increases resting energy expenditure and maximal physical exercise tolerance in ob/ob mice. However, PT does not protect against liver steatosis and does not change inflammatory gene expression, and these responses are consequent of genes expression associated with liver lipid metabolism.

PT can be used as a kind of non-pharmacological intervention for the prevention and/or treatment of various diseases such as obesity, diabetes, hypertension, cancer, and other metabolic disorders. It is known that PT modulates the dynamics of intra and extracellular fatty acid and can be used 30-80% for the production of energy according to nutritional status and physical fitness of the practitioner, and intensity and duration of exercise. The amount of fatty acid is used during mild or moderate exercise is three times higher than in resting conditions [17]. Thus, PT is an important tool to control and to reduce body weight and fat mass [27]. In our study, PT stabilized body weight gain in ob/ob mice starting at 4 weeks, which seems to have an important metabolic impact. Also, in previous studies by our group using another model, we showed that PT controls body weight gain in wild-type mice [28] and in animals fed cafeteria and high-fat diets [29, 30].

Although reductions in body weight gain can improve life quality and prevent the development of obesity-related complications such as insulin resistance, type 2 diabetes and hypertension [31], these associations are contradictory in ob/ob mice. While the reduction in body weight gains after a period of dietary methionine restriction was associated with improvement in HOMA index, a good marker of insulin resistance [32], Broderick et al. [22] showed that PT decreased body weight but also worsened the metabolic state of ob/ob mice with hyperinsulinemia, hyperglycemia and elevated lipids compared with sedentary counterparts. We do not know if our animals had damage in metabolic blood markers because the focus of



## Energy metabolism and leptin-deficient ob/ob mice

the present study was to understand the body weight response, but it is important to consider the differences between our PT protocol (involuntary running) and the voluntary wheel running protocol applied in the Broderick et al. [22] study.

Considering that body weight is closely related to the energy balance [33], we studied some determinants of the latter. Food intake assessed over a 24-h period was significantly smaller in the T group during the PT period. Considering that leptin deficiency could display higher food intake [34] which is suppressed after treatment with leptin [34, 35], and that PT increases [28, 36] or does not change food intake [37], our result was quite interesting because PT down-regulated food intake in ob/ob mice. Thus, it is possible that reduction in food intake may be mediated by signals and neural pathways independent of leptin. Additional studies are needed to better understand PT-associated food intake in ob/ob mice.

The resting oxygen uptake assessed by indirect calorimetry was increased in T group. Considering that energy expenditure is calculated based on oxygen uptake, we also observed higher energy expenditure in T group compared with S group. This result is interesting because ob/ob mice have profoundly decreased energy expenditure due to the lack of leptin action on energy metabolism [38], which is associated with increases in body weight and fat mass. It is important to notice that despite some determinants of daily energy expenditure were not evaluated in this study, such as energy expenditure during PT and the thermogenic effect of food, and that the assessment of resting energy expenditure refers only to a period of 45 min, our results indicate that body weight control in trained mice is due to an increase in resting metabolic rate associated with lesser caloric intake.

The respiratory exchange ratio was not different between groups, but the rates of carbohydrates and lipids oxidation were greater in T group than S group. These results revealed that PT does not promote substrate switch for oxidation, but improves peripheral metabolic substrate utilization in T group. If increases in substrate oxidation instead of storage are responsible to reduce fat mass and body weight [29, 39], our results provide evidence that PT pre-

vents body weight gains in ob/ob mice via improvement in substrate oxidation.

Although we did not measure markers of skeletal muscle metabolism, the better performance in the maximal running capacity test showed by T group suggest an improvement in oxidative capacity of skeletal muscle. In fact, skeletal muscle oxidative capacity is a key determinant of exercise tolerance [40]. Possible mechanisms induced by PT that result in better oxidative metabolism are increases in the rate of glucose transport into skeletal muscle [41], in fatty acid transport into the mitochondria [42], in the expression of metabolic and mitochondrial genes [43], in enzymes responsible to substrate oxidation [44], and type 1 fiber content [45].

It has been demonstrated that ob/ob mice develop hepatic steatosis associated with obesity, insulin resistance and dyslipidemia [19]. Furthermore, physical inactivity increases the risk for a positive energy balance and inactivity/low fitness/overnutrition contribute to develop peripheral insulin resistance and ectopic lipid storage in the liver due to increased fatty acid uptake, activated *de novo* lipogenesis, and suppressed triglyceride export. Thus, we hypothesized that in ob/ob mice, PT could improve hepatic mitochondrial content and/or function resulting in better oxidative capacity and lesser lipid accumulation. However, in the present study, histological analysis of hepatic tissue confirmed that hepatic steatosis and hepatocellular ballooning were observed in higher grade in both groups. Thus, although PT improves metabolic parameters, genes related to fatty acid oxidation and lipogenesis [29, 46], PT failed to prevent liver steatosis.

Studies in rodents have shown that PT increases the hepatic fatty acids oxidation reducing hepatic steatosis [6, 16, 18], improves insulin sensitivity and decreases inflammatory response in the adipose tissue [29, 47]. In humans, St George et al. [48] reported that PT has a favorable effect in NAFLD patients, reducing aminotransferase levels and other metabolic parameters, independent of weight loss. The differences observed between our results and others may be explained by the animal model studied here. In this case, we first demonstrated that PT was not able to prevent liver steatosis in leptin deficiency condition.

Considering that two main factors responsible for the accumulation of triglycerides within hepatocytes are increased fatty synthesis and decreased fatty acid oxidation, some genes involved in both process were investigated. PPAR- $\alpha$  and CPT1a genes mediate fatty oxidation, and in the present study, PT increased CPT1a gene expression which suggests an increase in liver oxidative capacity. However, this response was counter-balanced by the increase in SREBP1c transcription factor in the T group, which is responsible to enhance lipogenic activity inducing genes such as fatty acid synthase (FAS) and stearoyl-CoA desaturase (SCD-1) [6]. Thereby, the liver steatosis maintained in T group may be mediated, in part, by increased lipogenic activity associated with leptin deficiency. In fact, Jiang et al. [49] showed that leptin suppressed the expression of lipogenic enzymes ATP-citrate lyase (ACL) and FAS in the liver and white adipose tissue. However, the mechanism involved in this response still needs to be better understood.

PT can reduce inflammatory response associated with obesity, insulin resistance and type 2 diabetes [4, 5]. This response improves hepatic metabolism and decreases disorders associated with NAFLD [6]. However, we did not observe difference in NF $\kappa$ B mRNA expression between groups. This data corroborates the liver histology which was not found differences in lobular inflammation between groups. It is possible that our animals did not develop inflammation or the tool to measure inflammation was not sensitive enough to detect the response. Furthermore, our PT protocol was not able to change gene expression of inflammatory marker.

Altogether, our study provides evidence that PT prevented body weight gain in ob/ob mice by inducing negative energy balance and increased physical exercise tolerance. However, PT did not change inflammatory gene expression and failed to prevent liver steatosis possible due to an upregulation in the expression of SREBP1c transcription factor. These findings reveal that PT has positive effect on body weight control but not in the liver steatosis in a leptin deficiency condition.

### Acknowledgements

This study was supported by grants from the São Paulo Research Foundation (FAPESP) number 2013/06020-3.

### Disclosure of conflict of interest

None.

**Address correspondence to:** Dr. Fabiana S Evangelista, School of Arts, Science and Humanities, University of Sao Paulo, Av. Arlindo Bettio, 1000 Ermelino Mattarazzo-São Paulo-SP, CEP 03828-000-BRAZIL. Tel: (5511) 3091-8855; E-mail: fabiana\_evangelista@yahoo.com.br

### References

- [1] Song M, Um J, Jang H, Lee, B. Beneficial effect of dietary Ephedra sinica on obesity and glucose intolerance in high-fat diet-fed mice. *Exp Ther Med* 2012; 3: 707-712.
- [2] Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. *Lancet* 2005; 365: 1415-1428.
- [3] Misra A, Singhal N, Khurana L. Obesity, the metabolic syndrome, and type 2 diabetes in developing countries: role of dietary fats and oils. *J Am Coll Nutr* 2010; 29: 289S-301S.
- [4] Jocken JW, Blaak EE. Catecholamine-induced lipolysis in adipose tissue and skeletal muscle in obesity. *Physiol Behav* 2008; 94: 219-30.
- [5] Joanna WJ, Krzysztof K, Jacek C, Katarzyna P, Jacek S, Henry S, Jan K. Impact of vertical banded gastroplasty on body weight, insulin resistance, adipocytokine, inflammation and metabolic syndrome markers in morbidly obese patients. *J Endocrinol* 2011; 62: 109-119.
- [6] Schultz A, Mendonca LS, Anguila MB, Mandarim-De-Lacerda CA. Swimming training beneficial effects in a mice model of nonalcoholic fatty liver disease. *Exp Toxicol Pathol* 2012; 64: 273-282.
- [7] Oliva J, French SW, Jun LJ, Bardag-Gorce F. Proteasome inhibitor treatment reduced fatty acid, triacylglycerol and cholesterol synthesis. *Exp Mole Pathol* 2012; 93: 26-34.
- [8] Jun H, Kim J, Hoang M, Lee S. Hepatic lipid accumulation alters global histone H3 lysine 9 and 4 trimethylation in the peroxisome proliferator-activated receptor alpha network. *PLoS One* 2012; 7: e44345.
- [9] Postic C, Girard J. The role of the lipogenic pathway in the development of hepatic steatosis. *Diabetes Metab* 2008; 34: 643-648.
- [10] Malin SK, Kirwan JP. Fasting hyperglycaemia blunts the reversal of impaired glucose tolerance after exercise training in obese older adults. *Diabetes Obes Metab* 2012; 14: 835-841.
- [11] Nassis GP, Papantakou K, Skenderi K, Triandafilopoulou M, Kavouras SA. Aerobic exercise training improves insulin sensitivity

## Energy metabolism and leptin-deficient ob/ob mice

- without changes in body weight, body fat, adiponectin, and inflammatory markers in overweight and obese girls. *Metabolism* 2005; 54: 1472-1479.
- [12] Ross R, Bradshaw AJ. The future of obesity reduction: beyond weight loss. *Nature reviews. Nat Rev Endocrinol* 2009; 5: 319-325.
- [13] Kelley GA, Sharpe Kelley K. Aerobic exercise and resting blood pressure in older adults: a meta-analytic review of randomized controlled trials. *J Gerontol* 2001; 56: M298-M303.
- [14] Zeng Q, Isobe K, Fu L, Ohkoshi N, Ohmori H. Effects of exercise on adiponectin and adiponectin receptor levels in rats. *Life Sciences* 2007; 80: 454-459.
- [15] Sahin-Efe A, Katsikeris F, Mantzoros CS. Advances in adipokines. *Metabolism* 2012; 61: 1659-1665.
- [16] Rector RS, Thyfault JP, Laye MJ, Morris RT, Borengasser SJ, Uptergrove GM, Chakravarthy MV, Booth FW, Lbdah JA. Cessation of daily exercise dramatically alters precursors of hepatic steatosis in Otsuka Long-Evans Tokushima Fatty (OLETF) rats. *J Physiol* 2008; 586: 4241-4249.
- [17] Gonçalves IO, Passos E, Rocha-Rodrigues S, Diogo CV, Torrella JR, Rizo D, Viscor G, Alves ES, Aleixo IM, Oliveira PJ, Ancensão A, Magalhães J. Physical training prevents and mitigates non-alcoholic steatohepatitis-induced liver mitochondrial structural and bioenergetics impairments. *Mitochondrion* 2014; 15: 40-51.
- [18] Cintra DE, Ropelle ER, Vitto MF, Luciano TF, Souza DR, Engelmann J, Marques SO, Lira FS, Pinho RA, Pauli JR, Souza CT. Reversion of hepatic steatosis by exercise training in obese mice: The role of sterol regulatory element-binding protein-1c. *Life Sciences* 2012; 10: 1016.
- [19] Larter CZ, Yeh MM. Animal models of NASH: getting both pathology and metabolic context right. *J Gastroenterol Hepatol* 2008; 23: 1635-1648.
- [20] De Souza CT, Frederico MJS, Luz G, Cintra DE, Ropelle ER, Pauli JR, Velloso LA. Acute exercise reduces hepatic glucose production through inhibition of the Foxo1/HNF-4 $\alpha$  pathway in insulin resistant mice. *J Physiol* 2010; 588: 2239-2253.
- [21] Pons S, Martin V, Portal L, Zini R, Berdeaux DMA, Ghaleh B. Regular treadmill exercise restores cardioprotective signaling pathways in obese mice independently from improvement in associated co-morbidities. *J Mole Cell Card* 2013; 54: 82-89.
- [22] Broderick TL, Wang D, Jankowski M, Gutkowska J. Unexpected effects of voluntary exercise training on natriuretic peptide and receptor mRNA expression in the ob/ob mouse heart. *Regul Pept* 2014; 188: 52-59.
- [23] Ferreira JC, Rolim NP, Bartholomeu JB, Gobatto CA, Kokubun E, Brum PC. Maximal lactate steady state in running mice: effect of exercise training. *Clin Exp Pharmacol Physiol* 2007; 34: 760-65.
- [24] Frayn KN. Calculation of substrate oxidation rates in vivo from gaseous exchange. *J Appl Physiol* 1983; 55: 628-634.
- [25] Rozen S, Skaletsky H. Primer 3 on the www for general users and for biologist programmers. *Methods Mole Biol* 2000; 132: 365-386.
- [26] Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* 2001; 29: e45.
- [27] Swift DL, Johannsen NM, Lavie CJ, Earnest CP, Church TS. The role of exercise and physical activity in weight loss and maintenance. *Prog Cardiovasc Dis* 2014; 56: 441-447.
- [28] Higa TS, Bergamo FC, Mazzucatto F, Fonseca-Alaniz MH, Evangelista FS. Physical training prevents body weight gain but does not modify adipose tissue gene expression. *Braz J Med Biol Res* 2012; 445: 988-994.
- [29] Higa TS, Spinola AV, Fonseca-Alaniz MH, Evangelista FS. Remodeling of white adipose tissue metabolism by physical training prevents insulin resistance. *Life Sciences* 2014; 103: 41-48.
- [30] Gollisch KS, Brandauer J, Jessen N, Toyoda T, Nayer A. Effects of exercise training on subcutaneous and visceral adipose tissue in normal- and high-fat diet-fed rats. *Am J Physiol Endocrinol Metab* 2009; 297: E495-504.
- [31] Vieira VJ, Valentine RJ, Wilund KR, Antao N, Baynard T, Woods JA. Effects of exercise and low-fat diet on adipose tissue inflammation and metabolic complications in obese mice. *Am J Physiol Endocrinol Metab* 2009; 296: E1164-E1171.
- [32] Malloy VL, Perrone CE, Mattocks DAL, Ables GP, Caliendo NS, Orentreich DS, Orentreich N. Methionine restriction prevents the progression of hepatic steatosis in leptin-deficient obese mice. *Metab Clin Exp* 2013; 162: 1651-1661.
- [33] Muller MJ, Bosy-Westphal A, Later W, Haas V, Heller M. Functional body composition: insights into the regulation of energy metabolism and some clinical applications. *Eur J Clin Nutr* 2009; 63: 1045-1056.
- [34] Ribeiro AC, Ceccarini G, Dupre C, Friedman JM, Pfaff DW, Mark AL. Contrasting Effects of Leptin on Food Anticipatory and Total Locomotor Activity. *PLoS One* 2011; 6: e23364.
- [35] Baicy K, London E, Monterosso J, Wong M, Delibasi T, Sharma A, Licinio J. Leptin replacement alters brain response to food cues in ge-



## Energy metabolism and leptin-deficient ob/ob mice

- netically leptin-deficient adults. *Proc Natl Acad Sci* 2007; 104: 18276-18279.
- [36] Mazzucatto F, Higa TS, Fonseca-Alaniz MH, Evangelista FS. Reversal of metabolic adaptations induced by physical training after two weeks of physical detraining. *Int J Clin Exp Med* 2014; 15: 2000-2008.
- [37] Eguchi R, Cheik NC, Oyama LM, do Nascimento CMO, MelloMT, Tufik S, Dâmaso A. Effects of the chronic exercise on the circulating concentration of leptin and ghrelin in rats with diet-induced obesity. *Braz J Sports Med* 2007; 14: 182-187.
- [38] Morton GJ, Kaiyala KJ, Fisher JD, Ogimoto K, Schwartz MW, Wisse BE. Identification of a physiological role for leptin in the regulation of ambulatory activity and wheel running in mice. *Am J Physiol Endocrinol Metab* 2011; 300: E392-E401.
- [39] Gaidhu MP, Anthony NM, Patel P, Hawke TJ, Ceddia RB. Dysregulation of lipolysis and lipid metabolism in visceral and subcutaneous adipocytes by high-fat diet: role of ATGL, HSL, and AMPK. *Am J Physiol Cell Physiol* 2010; 298: C961-971.
- [40] McGee SL, Hargreaves M. AMPK-mediated regulation of transcription in skeletal muscle. *Clin Sci* 2010; 118: 507-518.
- [41] Kim HJ, Lim JS, Kim CK. Effect of exercise training on muscle glucose transporter 4 protein and intramuscular lipid content in elderly men with impaired glucose tolerance. *Eur J Appl Physiol* 2004; 93: 353-35.
- [42] Fu L, Liu X, Niu Y, Yuan H, Zhang N, Lavi E. Effects of high-fat and regular aerobic exercise on global gene expression in skeletal muscle of C57BL/6 mice. *Metabolism* 2011; 61: 146-152.
- [43] Ljubicic V, Joseph AM, Saleem A, Ugucioni G, Collu-Marchese M, Lai RY, Nguyen LM, Hoo DA. Transcriptional and post-transcriptional regulation of mitochondrial biogenes in skeletal muscle: Effects of exercise and aging. *Biochim Biophys Acta* 2010; 1800: 223-234.
- [44] Leick L, Hellsten Y, Fentz J, Lyngby SS, Wojtaszewski JF, Hidalgo J, Pilegaard HA. PGC-1alpha mediates exercise-induced skeletal muscle VEGF expression in mice. *J Physiol Endocrinol Metab* 2009; 297: 92-103.
- [45] Dastmalchi M, Alexanderson H, Loell I, Stahlberg M, Borg K, Lundberg IE, Esbjörnsson M. Effect of physical training on the proportion of slow-twitch type I muscle fibers, a novel non-immune-mediated mechanism for muscle impairment in polymyositis or dermatomyositis. *Arthritis & Rheumatology* 2007; 57: 1303-1310.
- [46] Sakurai T, Endo S, Hatano D, Ogasawara J, Kizaki T, Oh-ishi S, Izawa T, Ishida H, Ohno H. Effects of exercise training on adipogenesis of stromal-vascular fraction cells in rat epididymal white adipose tissue. *Acta Physiologica* 2010; 200: 325-338.
- [47] Bradley RL, Jeon JY, Liu FF, Maratos-Flier E. Voluntary exercise improves insulin sensitivity and adipose tissue inflammation in diet-induced obese mice. *Am J Physiol* 2008; 295: E586-E594.
- [48] St. George SA, Bauman A, Johnston A, Farrell G, Chey T, George J. Independent Effects of Physical Activity in Patients with Nonalcoholic Fatty liver Disease. *Hepatology* 2009; 50: 68-76.
- [49] Jiang L, Wang Q, Yu Y, Zhao F, Huang P, Zeng R, Qi RZ, Li W, Liu Y. Leptin contributes to the adaptive responses of mice to high-fat diet intake through suppressing the lipogenic pathway. *PLoS One* 2009; 4: e6884.