Swim training and the genetic expression of adipokines in monosodium glutamate-treated obese rats

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ABSTRACT

Objective: The aim of this study was to evaluate the genetic expression of adipokines in the adipocytes of monosodium glutamate (MSG)-treated obese rats submitted to physical activity. **Materials and methods:** Obesity was induced by neonatal MSG administration. Exercised rats (MSG and control) were subjected to swim training for 30 min for 10 weeks, whereas their respective controls remained sedentary. Total RNA was obtained from sections of the mesenteric adipose tissue of the rats. mRNA levels of adiponectin (*Adipoq*), tumor necrosis factor alpha (*Tnf*), peroxisome proliferator-activated receptor alpha (*Ppara*), and peroxisome proliferator-activated receptor gamma (*Pparg*) adipokines were quantified by quantitative Real-Time Polymerase Chain Reaction (qRT-PCR). **Results:** In the exercise-trained control group, the expression of *Adipoq* increased compared to the sedentary control, which was not observed in the MSG-obese rats. Increased levels of *Tnf* in MSG-obese rats were not reversed by the swim training. The expression of *Ppara* was higher in sedentary MSG-obese rats compared to the sedentary control. Swimming increased this adipokine expression in the exercise-trained control rats compared to the sedentary ones. mRNA levels of *Pparg* were higher in the sedentary MSG-rats compared to the sedentary control; however, the exercise did not influenced its expression in the groups analyzed. **Conclusions:** In conclusion, regular physical activity was not capable to correct the expression of proinflammatory adipokines in MSG-obese rat adipocytes. Arch Endocrinol Metab. 2015;59(3):210-4

Keywords
Adipoq; *Ppara*; *Pparg*; quantitative RT-PCR; *Tnf*

INTRODUCTION

Neonatal treatment with monosodium glutamate (MSG) causes lesions in the arcuate nucleus, deregulating an important area involved in the body weight regulation and glycemic control (1,2). Rats treated with MSG in the first days after birth develop obesity in adult life and present insulin resistance, glucose intolerance, dyslipidemia and cardiovascular dysfunction, which are typical symptoms of metabolic syndrome carriers (3). Similar to obese subjects, MSG-obese rats present low-grade chronic inflammation, in particular on adipocyte tissue. Studies demonstrated that adipocyte-derived adipokine profile of MSG-obese rats is altered, thereby contributing to insulin resistance in this obesity model (4).

Regular physical activity is an important tool in the obesity control and associated comorbidities, improving inflammatory condition typical of an obese individual. There are no studies with this animal model (MSG-obese rats) reporting the adipokines expression in mesenteric fat and physical activity. Thus, the aim of this study was to analyze the genetic expression profile of some adipokines of the mesenteric adipose tissue of MSG-treated obese rats subjected to swimming.

MATERIALS AND METHODS

Experiments were approved by the Ethical Committee for Animal Experiments of Universidade Estadual de Ponta Grossa (protocol number 02860). The obese group received subcutaneous injections of MSG (4 mg/g of body weight) during the first 5 days of life, whereas the control group received equimolar saline, a protocol adapted by (5-7). At the age of 21 days, ani-

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mals were weaned and divided in four groups (N = 8 rats per group): sedentary controls (CON-SED), exercise-trained controls (CON-EXE), sedentary MSG-treated animals (MSG-SED), and exercise-trained MSG-treated animals (MSG-EXE). All of the exercise-trained animals were subjected to regular swimming 3 times per week and 30 min per day for 10 weeks, according to (5). Each rat had a load weight equivalent to 5% of body mass attached to the base of tail to ensure that animals were in constant swimming activity. Araujo and cols. (8) adapted the lactate minimum test to swimming in rats. Blood lactate concentrations of 5.5 mM are achieved at loads equivalent to 5% body weight, so this swimming program can be considered moderate exercise (8). The CON-SED and MSG-SED groups remained sedentary.

At the age of 90 days, the naso-anal length (NAL) and body weight were measured to assess the obesity degree in the animals using the Lee index [$\sqrt[3]{}$(body weight (g)/naso-anal length (cm)], which is an indicator of obesity used for rodents (9). Additionally, visceral fat (mesenteric) deposits were removed, washed and weighed. Data are reported considering body weight (g/100 g of body weight). All values are reported as means ± standard error (SEM). The Student T-test was used with significance of $P < 0.05$.

For the analysis of genetic expression by qRT-PCR, sections of mesenteric fat were used for total RNA extraction and, subsequently cDNA synthesis. The amplified adipokines were: *Adipoq*, *Tnf*, *Ppara* and *Pparg*. The adipokines primer sequences were synthetized according to (4). The 18S rDNA was used as an internal control. Relative change in genetic expression was presented as $2^{-\Delta\Delta Ct}$ (10).

RESULTS

According to table 1, body weight and NAL were 27.7 and 13.4% lower in the MSG-SED group, respectively, compared to the CON-SED group ($P < 0.05$). As for the Lee index, the value was 4.7% higher in the MSG-SED animals when compared to the CON-SED ones ($P < 0.05$). MSG-SED rats presented a significantly increase of 109.9% of mesenteric fat in comparison with CON-SED rats. Swimming had no effect in rat NAL. However, there was a reduction of approximately 8.7 and 6.7% of body weight and 18.5 and 20.6% of mesenteric fat accumulation in CON-EXE and MSG-EXE, respectively, when compared to their respective sedentary groups ($P < 0.05$). Physical activity significantly reduced the Lee index in 4.2% only in animals treated with MSG compared to the MSG-SED.

There was no statistical difference in the expression of *Adipoq* between MSG-SED and CON-SED groups. The expression level of adiponectin was approximately 6-fold higher in CON-EXE rats compared to the CON-SED rats ($P < 0.05$) (Figure 1A). *Tnf* expression significantly increased approximately 4 times in the MSG-SED group in comparison with the CON-SED group. Nevertheless, in both groups, exercise did not alter the expression of *Tnf* in the mesenteric adipose tissue (Figure 1B). The expression levels of *Ppara* were on average 3.7 times higher in the MSG-SED group in comparison with the CON-SED group ($P < 0.05$). The practice of physical activity resulted in a 4.1-fold increase in the expression of *Ppara* in CON-EXE rats when compared to the CON-SED rats ($P < 0.05$) (Figure 1C). The levels of *Pparg* mRNA were around 4.5 times higher in the MSG-SED group, compared to the CON-SED ($P < 0.05$). However, exercise did not influenced the expression of *Pparg* in the groups assessed (Figure 1D).

DISCUSSION

Rodents treated with MSG develop several obesity characteristics (1-3), probably caused by the reduction in the energy metabolism (11) and a dysfunction in the autonomic nervous system, which is characterized by a high parasympathetic and a low sympathetic activities, as consequences of hypothalamic lesion caused by MSG (12).

Table 1. Biometric parameters in MSG-obese and control rats submitted at swimming training

Parameters	CON-SED	CON-EXE	MSG-SED	MSG-EXE
Body weight (g)	$359.5 \pm 3.79^{c,d}$	328.4 ± 7.7^{d}	260.0 ± 9.8^{a}	$242.6 \pm 11.3^{a,b}$
NAL (cm)	$23.1 \pm 0.1^{c,d}$	$22.9 \pm 0.3^{c,d}$	$20.0 \pm 0.5^{a,b}$	$20.4 \pm 0.5^{a,b}$
Lee index	0.298 ± 0.002^{c}	$0.283 \pm 0.013^{c,d}$	$0.312 \pm 0.003^{a,b,d}$	$0.299 \pm 0.005^{b,c}$
Mesenteric fat (g/100 g)	$0.81 \pm 0.05^{c,d}$	$0.66 \pm 0.03^{c,d}$	$1.70 \pm 0.12^{a,b,d}$	$1.35 \pm 0.10^{a,b,c}$

Data are presented as mean ± SEM obtained from 8 rats by group. Letters above of numbers represent statistical difference ($P < 0.05$) in Student T-test. [a] CON-SED; [b] CON-EXE; [c] MSG-SED; [d] MSG-EXE.

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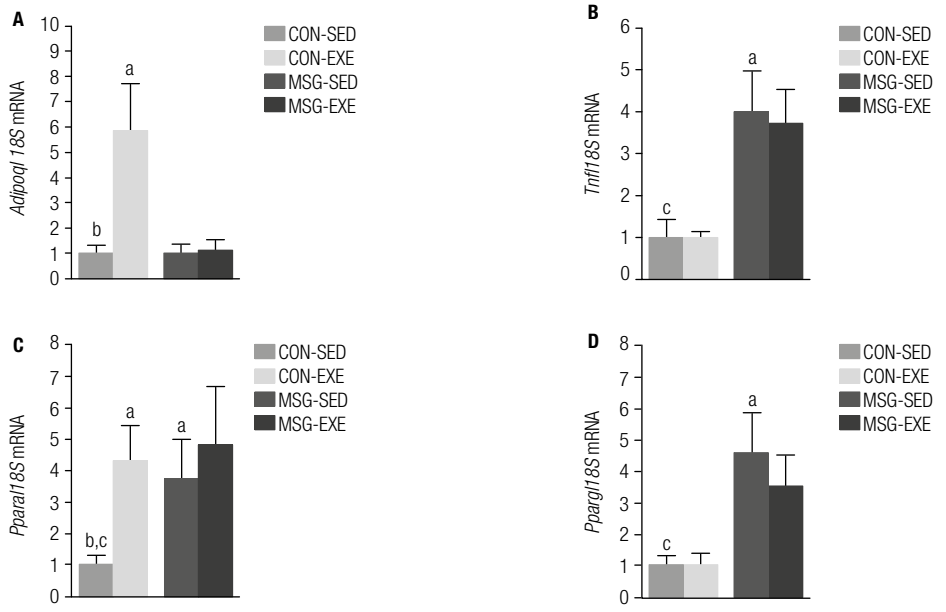

Figure 1. mRNA expression levels of the mesenteric fat tissue samples of MSG-obese and control rats submitted at swimming training (N = 8 rats per group). (**A**) *Adipoq*, (**B**) *Tnf*, (**C**) *Ppara* and, (**D**) *Pparg*. Letters above the bars represent statistical difference in Student T-test ($P < 0.05$). [a] CON-SED; [b] CON-EXE; [c] MSG-SED; [d] MSG-EXE.

The increase in the Lee index associated with a major accumulation of mesenteric fat in MSG-treated obese rats confirmed the efficacy of MSG neonatal administration in obesity induction in the animals of our study. However, MSG-obese rats presented reduction in body weight and in NAL due to the delay in bone and muscle development, which is a consequence of a reduction in the growth hormone (GH) levels resulting from the arcuate nucleus lesion (13).

Corroborating with previous reports (5,6), our study showed that swimming 30 min/3 times per week with a load weight equivalent to 5% of body mass was effective in promoting reduction in mesenteric fat deposits in MSG-treated animals, without causing collateral effects. Regular swimming corrects hyperinsulinemia and resistance to insulin and favors lipolysis triggered by the activation of the sympathetic nervous system (5). Similarly, Shima and cols. (14) using run in rats showed that frequency of 2 to 3 times/week is sufficient to prevent development of type 2 diabetes in OLETF rats (spontaneous model of type 2 diabetes). The training protocol used in this study began immediately after weaning, when rodents may be treated in terms of development like children. Atlantis and cols. (15) revised the beneficial effects of body fat reduction in obesity/overweight children submitted to exercise and showed that good results in training can be obtained with exercises in lower intensity and frequency, compared with those recommended in the literature.

Regular physical activities, even if there is no loss of weight, are related to a substantially reduction of total and visceral fat, besides leading to a significantly improvement in obesity (16,17). MSG-SED rats have hypertrophic adipocytes (5), which presented an altered inflammatory profile directly related to insulin resistance. Carvalho Leite and cols. (5) demonstrated that swim training program was effective in attenuating morphological alterations in the adipose tissue and pancreatic islets in MSG-treated obese rats. Histological analyzes in adipose tissue deposits, demonstrated that physical training reduced the adipocyte diameter and increased the number of adipocytes in MSG-EXE and CON-EXE groups, compared with their sedentary counterparts (5).

It is known that the expression of adiponectin mRNA is inversely related to obesity, type 2 *diabetes mellitus* (T2DM) and cardiovascular diseases (18). Our study showed that there was no difference in adiponectin expression between MSG-obese rats and control animals. Other studies have already shown that MSG obesity model did not reflect hyperadiponectinemia or adiponectin resistance, a phenomenon observed in other obesity models (4,19). Our research showed that swimming stimulated the genetic expression of adiponectin in adipocytes of CON-EXE rats. High plasma

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levels of adiponectin were also found in humans subjected to aerobic exercises (20). On the other hand, exercised MSG-obese rats did not present alteration in adiponectin expression, which may reinforce that adiponectin levels in the MSG model is normal.

MSG rats presented a higher expression of *Tnf*, when compared to control animals, corroborating with Roman-Ramos and cols. (4). Indeed, expression levels of *Tnf* increased in obesity, T2DM and cardiovascular disease conditions (18). TNF promotes reduction in adiponectin expression and secretion (18), contributing to metabolic syndrome development. Together, these data indicate that high levels of *Tnf* expression found in adipocytes of MSG-obese rats are probably due to chronic inflammation of the adipose tissue with macrophage infiltration (21). Probably this adipokine contributes to insulin resistance, which is typical for this model. According to our data, exercise does not correct the inflammatory profile in this obesity model.

Corroborating Roman-Ramos and cols. (4), *Ppara* and *Pparg* had their mRNA levels increased in the MSG-treated animals, which may indicate an inflammatory condition due to obesity. *Pparg* is mainly expressed in the adipose tissue and its activation in MSG-treated rat adipose tissue may lead to the increase of adipocyte size, probably causing hypertrophy (5). Roman-Ramos and cols. (4) suggested that the activation of PPARs is responsible for the deregulated inflammatory profile of MSG animals; however, some authors reported that *Ppara* and *Pparg* activation may improve insulin resistance induced by obesity (22). Our results indicate that *Pparg* may participate directly in the increase of the visceral fat content and adipocyte size that occurs in MSG-obese animals (5). On the other hand, exercise was not able to correct its levels in obese animals. Probably, *Pparg* function is not only metabolic, but it may also be involved in the inflammatory control, participating in the proinflammatory cytokine pathway and in the modulation of acute phase of the inflammatory response by independent mechanisms of lipoprotein changes (23).

Genes regulated by *Ppara* participate in the key-protein regulation involved in lipid metabolism, fatty acid oxidation, hemostasis and inflammation (24). High levels of *Ppara* mRNA may be related to the increase of body weight in MSG-treated animals. Physical activity did not have influence over this gene expression in these animals. The higher expression of *Ppara* mRNA in CON-EXE animals can be explained by a high mitochondrial activity and peroxisomal beta-oxidation. PPARA is capable of upregulating the expression of genes to enzymes such as carnitine-palmitoyltransferase-I (CPT-I), which transports fatty acids into the mitochondria for beta-oxidation. Expression of PPARA also increases expression of acetyil-CoA synthase, a mitochondrial enzyme necessary for beta-oxidation (25). However, more studies are needed to determine which exactly the role of *Ppara* is and its relation to obesity.

Regular physical activity was not capable to correct proinflammatory adipokine expression in adipocytes of MSG-treated obese rats. Other factors or adipokines cannot be discarded. More studies are necessary to determine which adipokines have altered expression by physical activity and the resulting effect in energy homeostasis. Furthermore, studies to analyze the resulting proteins of the analyzed genes are necessary, although cytokines may have autocrine and paracrine actions, which may affect the metabolism of adipose tissue without necessarily affecting plasma levels.

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