MUSCULAR REDOX STATE AND INSULIN SENSITIVITY IN RATS TRAINED FED A DIET HIGH IN FRUCTOSE

ESTADO REDOX MUSCULAR E SENSIBILIDADE À INSULINA EM RATOS TREINADOS ALIMENTADOS COM DIETA RICA EM FRUTOSE

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RESUMO: Nas últimas décadas, o consumo de frutose aumentou cerca de dez vezes. Este nutriente pode causar alterações metabólicas prejudiciais, podendo levar à síndrome metabólica (SM). Assim, o objetivo deste estudo foi analisar os efeitos de uma dieta com alta concentração de frutose sobre a sensibilidade à insulina, biomarcadores de estresse oxidativo e concentrações de triglicerídeos (TG) no músculo gastrocnêmio de ratos sedentários e treinados. **Métodos:** Quarenta e oito ratos Wistar machos (90 dias de idade) foram divididos em quatro grupos: controle sedentário (SC), controle treinado (TC), frutose sedentário (FS) e frutose treinado (FT). Os animais dos grupos FS e FT foram alimentados com dieta rica em frutose (60%) e os animais dos grupos CS e CT com dieta balanceada (AIN-93). Os ratos foram randomizados para os grupos CT e FT e submetidos ao treinamento aos 150 dias de idade; natação 1h dia^{-1}, 5 dias semana^{-1}, por 4 semanas a 80% do limiar anaeróbico. **Resultados:** O grupo FS apresentou menor taxa de remoção de glicose no teste de tolerância à insulina em comparação aos outros grupos. As concentrações de TG no músculo e os biomarcadores de estresse oxidativo: TBARS e CAT não foram alterados pela administração de altas concentrações de frutose na dieta nem pelo treinamento físico, a SOD mostrou sensibilidade ao treinamento apenas no grupo CT. **Conclusão:** O presente estudo mostrou que uma dieta rica em frutose reduz a sensibilidade à insulina. No entanto, o exercício reverteu os efeitos da frutose na sensibilidade à insulina e aumentou a atividade da SOD em ratos treinados.

Palavras-chave: Estado Redox; Limiar Anaeróbico; Frutose.

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ABSTRACT: In recent decades, fructose consumption has increased about tenfold. This nutrient can cause harmful metabolic changes may lead to metabolic syndrome (MS). Thus, the aim of this study was to analyze the effects of a diet with a high concentration of fructose on insulin sensitivity, oxidative stress biomarkers and concentrations of triglycerides (TG) in the gastrocnemius muscle of sedentary and trained rats. **Methods:** Forty-eight male Wistar rats (90 days old) were divided into four groups: sedentary control (SC), trained control (TC), sedentary fructose (FS) and trained fructose (FT). The animals in groups FS and FT were fed a diet high in fructose (60%) and the animals of the groups CS and CT with a balanced diet (AIN-93). The rats were randomized to CT and FT groups and underwent to training at 150 days of age; swimming 1h day^{-1}, 5 days $week^{-1}$, for 4 weeks at 80% of anaerobic threshold. **Results:** The FS group showed lower glucose removal rate in insulin tolerance test compared to the other groups. The TG concentrations in muscle and biomarkers of oxidative stress: TBARS and CAT were not changed by the administration of high concentrations of fructose in the diet nor by exercise training, the SOD showed sensitivity to training only in CT group. **Conclusion:** The present study showed that a diet high in fructose reduces sensitivity to insulin. However, exercise reversed the effects of fructose on insulin sensitivity and increased SOD activity in trained rats.

Keywords: Redox State; Anaerobic Threshold; Fructose.

1 INTRODUCTION

Fructose is a form of sugar found in many foods and, unlike glucose, does not stimulate the secretion of insulin and leptin (Teff *et al*. 2004), which would be highly recommended for type 2 diabetic patients. On the other hand, were found deleterious effects of the fructose, which can harm the body (Alberti *et al*. 2005).

Among the disorders triggered by high fructose intake can highlight changes in glucose metabolism (hyperinsulinemia, insulin resistance and glucose intolerance) and lipid metabolism (increased triglycerides, total cholesterol and Low Density Lipoprotein-LDL and decrease of High Density Lipoprotein-HDL) may lead to a similar framework to the metabolic syndrome (MS) (Alberti & Zimmet, 1998; Balkau & Charles, 1999).

The feeding with high-fructose, can also promote increased production of reactive oxygen species (ROS) that comes from high lipid oxidation. As consequence of increased ROS, occurs increased inflammatory process, resulting in reduced insulin sensitivity, due to fact that inflammatory substances can inhibit the action of insulin as well as the production and action of adiponectin (Thorburn *et al*. 1989; Faure *et al*. 1997; Basciano *et al*. 2005).

Besides promoting the increase in ROS, a diet high in fructose may develop a deficiency of antioxidant defense systems, specifically in the enzyme superoxide dismutase (SOD), due to the reduction of copper (Busserolles *et* al. 2002; Rajasekar *et al*.

2007). Such changes, generated by fructose-based diet, cause the increased risk of developing cardiovascular disease and type 2 diabetes mellitus (Cornier *et al*. 2008).

On the other hand, physical training becomes an important strategy for reducing the risk of development of MS, there is a direct relationship between physical activity and insulin sensitivity (Schneider *et al*. 1990; Lakka *et al*. 2003), mainly by increasing the concentration of muscle GLUT4 with concomitant decrease in fat tissue (Maarbjerg *et al*. 2011). Furthermore, the aerobic exercise training is able to generate adaptations in the antioxidant system, reducing the deleterious effects of oxidative stress (Radák *et al*. 2000; Powers *et al*. 2010).

Due to the effects caused by fructose in high concentrations in the feed, there is a need to investigate the causes of insulin resistance and the effect of aerobic training in this food condition. Thus, the present study aimed to examine the effects of aerobic training on lipid metabolism, insulin sensitivity, and biomarkers of oxidative stress in rats fed a diet high fructose.

2 METHODS

2.1 ANIMALS

The study was conducted with 40 adult Wistar rats, obtained from the central animal facility of the State University of *São Paulo* (UNESP) - *Botucatu Campus*. The animals were kept in cages polyethylene (4 $cages^{-1}$) room temperature of 25 ± 1 °C, light/dark cycle 12/12 hours with free access to food and water.

The experiment was conducted in accordance with Brazilian law for scientific use of animals (law 11.794, of October 8, 2008). The protocol was approved by the ethics committee on the use of animal (CEUA), the Institute of Biosciences of UNESP - *Rio Claro* (Protocol: 068/2008).

2.2 DIET

According to the randomization the animals received balanced semipurified control diet (C) or high in fructose (F), 60%. The detailed composition of the mixture of minerals and vitamins employed in the preparation of both diets (Table 1) are described in Reeves *et al*. (1993).

Table 1. Content of diets.

Components (g.kg^{-1})	Standard[1]	High in fructose (60%)
Casein[2]	202	202
Starch	397	-
Dextrin	130.5	-
Saccharose	100	27.5
Fructose	-	600
L-cystine	3	3
Soybean oil	70	70
Minerals mix (AIN-93GMX)[1]	35	35
Vitamin mix (AIN-93GVX) [1]	10	10
Dietary fiber	50	50
Choline hydrochloride	2.5	2.5

[1]According America Institute of Nutrition (REEVES et al., 1993).
[2]Values corrected for the protein content of casein.

2.3 EXPERIMENTAL DESIGN

At 90 days of age, animals were randomized into two groups and subjected to dietary treatment control (C) or fructose (F) for 10 weeks. In the sixth week of treatment, the groups were again subdivided to form groups of trained animals (swimming): trained control (TC) and trained fructose (FT), with no change in diet.

2.4 ADAPTATION TO THE WATER

The animals submitted to swimming, firstly, were adapted to the water environment (31±1°C) to minimize stress suffered.

On the first day, the rats were subjected to 15 minutes of swimming in shallow water. On the second day, there was a rise in the water level, as well as the time of duration of the effort for 20 minutes. On the third day, the rats swam in deep water for 20 minutes. On the fourth day a small empty bag, made to house the overload of lead, was tied to the thorax of the animals that swam for 30 minutes. On the fifth day of adaptation the animals swam supporting an equivalent weight to 3% of body weight for 30 minutes. After a rest period of 48 hours the test for maximum lactate steady state (MLSS) was performed.

2.5 AEROBIC-ANAEROBIC METABOLIC TRANSITION AND EVALUATION OF AEROBIC FITNESS

The maximal lactate steady state was determined by the protocol adapted to rats (Gobatto *et al*. 2001). The criterion for stabilization of blood lactate was a difference less or equal to 1.0 mM between 10 and 30 minutes of exercise (Heck *et al*. 1985). The concentration of blood lactate was determined by enzymatic method (Engel & Jones, 1978).

2.6 EXERCISE

The animals of the training groups (TC and FT) were underwent to swimming exercise in individual tanks containing water at 31±1°C, 1 h day^{-1}, 5 days $week^{-1}$, with overload of 80% of the MLSS.

2.7 ORAL GLUCOSE TOLERANCE TESTE (GTTO)

After a fasting period of 12 hours, the animals were submitted to oral glucose tolerance test (GTTo). Blood samples (25µl) were taken at 0, 30, 60 and 120 minutes after administration of glucose ([80%]/20% of body weight). The samples were deproteinized at 200µl trichloroacetic acid (TCA) 4%, the glucose determination was made by the method of glucose oxidase using commercial kit (Laborlab®). The glycemic response during GTTo was assessed by the area under curve of the glucose, calculated by trapezoidal method (Mathews *et al*. 1990).

2.7 INSULIN TOLERANCE TEST (ITT)

An insulin solution (150 mU/100g of weight) was administered intraperitoneally. Blood was collected at 0, 4, 8, 12 and 16 minutes after insulin administration at heparinized and calibrated to 25 µl capillaries, to determine the concentrations of glucose, using commercial kits (Laborlab®). The results were analyzed by calculating the rate of removal of serum glucose (Kitt = $(0.0693/t_{1/2}) \times 100$). The removal of blood glucose ($t_{1/2}$) was calculated by curve least squares analysis of the levels of blood glucose in the decay time after insulin administration (Ohkawa *et al*. 1979) using software Origin 6.0.

2.8 LIPID PEROXIDATION

Concentration of products which react to thiobarbituric acid (TBARS) was performed by evaluating the end-products of lipid peroxidation (low molecular weight aldehydes, malondialdehyde and lipid peroxides), which react with the 2-thiobarbituric acid (TBA), forming Schiff bases. The concentrations of the final products were determined by fluorescence at 555nm emission and 515nm excitation or by spectrophotometry at 5nm (Ohkawa *et al*. 1979).

2.9 ANTIOXIDANT DEFENSE SYSTEM

The catalase enzyme activity (CAT) was performed adding phosphate buffer (50mM) and hydrogen peroxide (H_2O_2) (10mM) in the tissue samples, using equation:

(2.3/Δt).(a/b).(logA1/A2) (Aebi, 1984). The decrease in absorbance of H_2O_2 was checked spectrophotometrically at 240nm.

To determine the enzymatic activity of superoxide dismutase (SOD), the tissue samples were immediately washed in phosphate-buffered saline (PBS) at pH 7.4 containing 0.16 mg/mL heparin for removing blood cells. The tissue was homogenized (on ice) in 1mL of HEPES 20mM buffer, pH 7.2 containing: 1mM EGTA, mannitol 210mM and sucrose 70mM. Then, centrifuged at 10.000 xg for 15 minutes at 4°C and the supernatant was stored at -20°C for determination of SOD total (mitochondrial and cytoplasmic), was using commercial kit (Cayman) for analysis of three types of SOD (Cu/Zn^-, Mn^-, and Fe^-SOD).

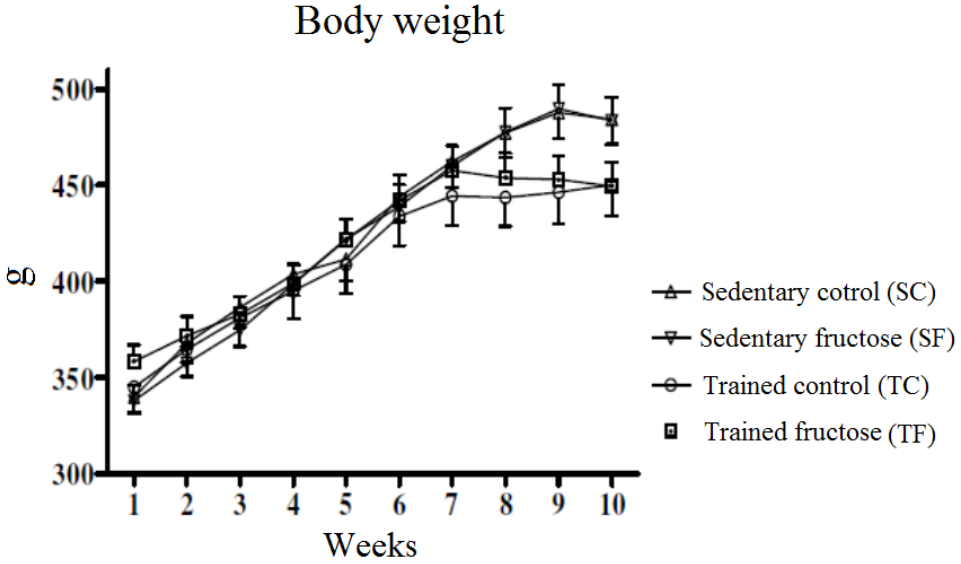
2.10 STATISTICS

Data normality was verified using the Shapiro-Wilk test, the homogeneity of variance was analyzed by Bartlett's test. The results were analyzed using the Student's t test or by analysis of variance two-way (ANOVA) where appropriate. When necessary, we used post hoc Bonferroni test. In all analyzes, the level of significance was pre-set at $P < 0.05$.

3 RESULTS

No significant difference was observed, between all groups, in body mass of animals within ten weeks (Figure 1). Body weight and food intake were recorded once a week throughout the experiment.

Figure 1. Evolulção body weight in grams during the experimente.



Dietary intake during the 10 weeks was 10% lower for the groups fed a diet high in fructose and there no interaction with exercise (Table 2).

Tabel 2. Area under the curve of food intake.

	Sedentary		**Trained**	
	Control	Fructose	Control	Fructose
ASC – food intake	251.3 ± 5.5	219.7 ± 5.3*	227.0 ± 8.3	209.7 ± 8.9*

ASC: Area under the curve; *Difference to the control groups; $p < 0,05$.

Triglyceride concentrations in gastrocnemius showed no difference between groups. The total weight of the epididymal fat was 50% lower in trained groups and no interaction with diet. The Animals underwent to four weeks training showed difference in FFA concentrations compared with their respective sedentary. We also observed an interaction between diet and training, and the sedentary fructose group showed higher values of FFA when compared to other groups. However, training reversed this situation, because the fructose trained group showed no difference compared to controls. Regarding the triglycerides, diet induced an increase in this parameter and the physical training was not able to reverse this situation (Table 3).

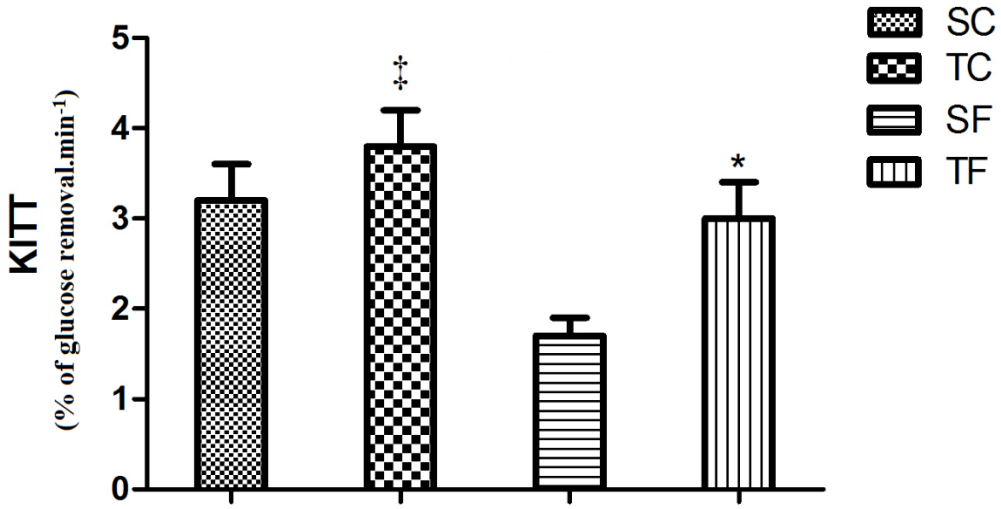
Tabel 3. Values of the concentrations of triglyceride in muscle and serum and concentrations of FFA and weight of epididymal fat at end of the experiment.

	Sedentary		**Trained**	
	Control	Fructose	Control	Fructose
Triglycerides in gastrocnemius (mg/g)	3.0 ± 0.48	2.3 ± 0.29	3.0 ± 0.62	2.78 ± 0.37
Triglicerídeos no serum (mmol/L)	1.3 ± 0.18	2.4 ± 0.33*	1.5 ± 0.13	2.3 ± 0.32*
FFA (mg/L)	88.9 ± 10.2	230 ± 27.7‡	91.4 ± 7.8	70.7 ± 6.6
Total weight of fat epididymal (mg/100g)	924.7 ± 73	1113 ± 125	600.5 ± 64*	632.7 ± 59*

FFA: Free fatty acids, *Difference to the control groups; ‡Difference between all groups; $p < 0,05$.

Was observed a decrease in insulin tolerance in animals fed a diet high in fructose and an increase in animals subjected to training (Figure 2).

Figure 2. KITT: Decay constant of the glucose in tolerance test insulin.



*Difference to the control groups; ‡Difference between all groups; p < 0,05.

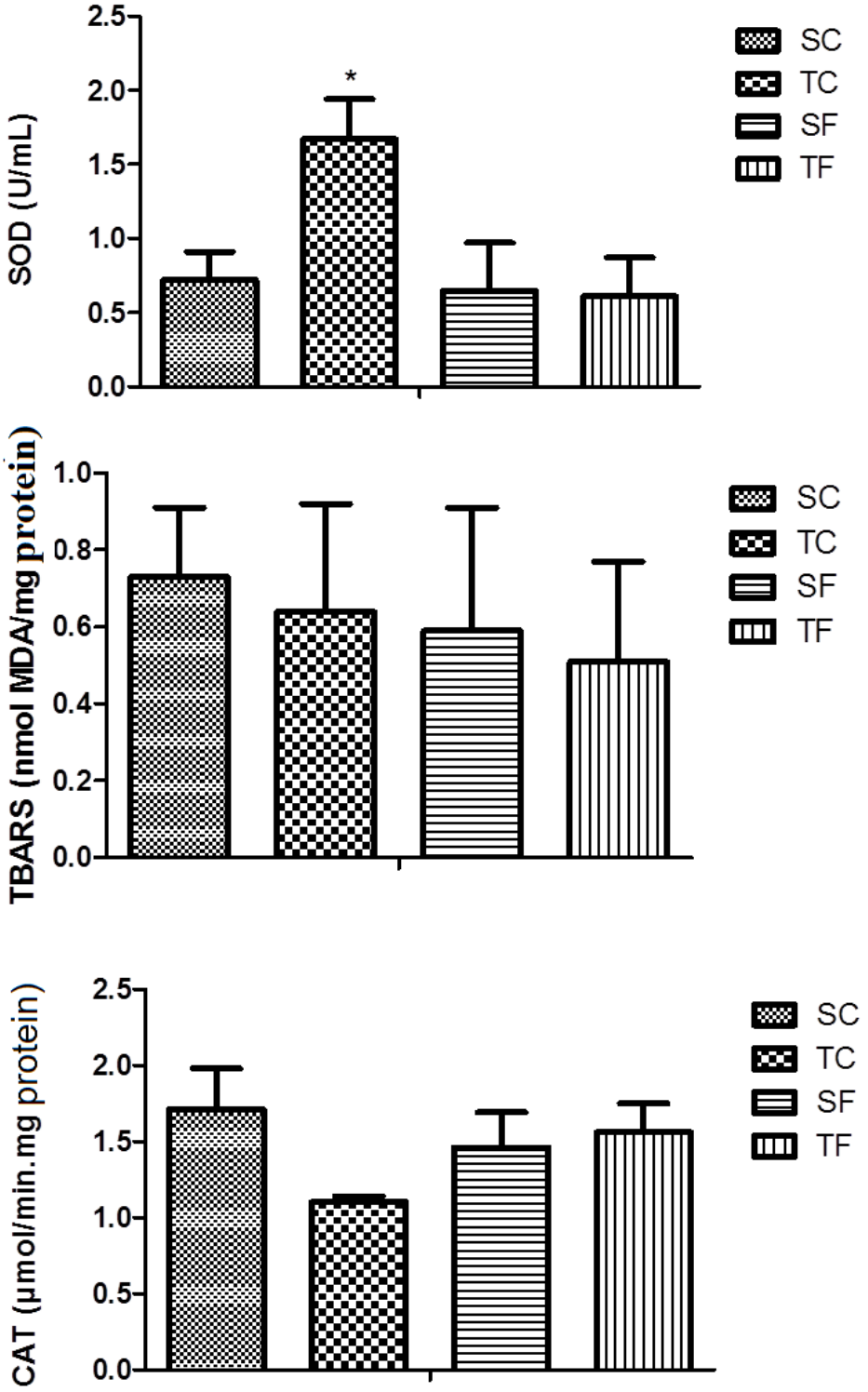
There was a significant increase in SOD enzyme in trained control, when compared to the other groups. No significant difference was found between groups in biomacador of oxidative stress (TBARS). The same was observed for the antioxidant enzyme catalase (Figure 3).

4 DISCUSSIONS

The literature has shown that a high intake of fructose may trigger changes in glucose and lipid metabolism, leading to establishment of metabolic syndrome and changes in the homeostasis of the organism (Alberti & Zimmet, 1998; Balkau & Charles, 1999; Elliott *et al*. 2002; Alberti *et al*. 2005). The present study shows differences in levels of food intake between control and fructose groups. The FC and FT group had a lower intake indicating greater efficiency of the diet with this nutrient, however, no difference in body mass related to diet. This result does not corroborate the hypothesis that fructose can lead to changes in the central control mechanism of hunger, to generate inhibition of satiety (Teff *et al*. 2004).

In this study, diet high in fructose promoted an increase TG and FFA in sérum and reduced sensitivity to insulin. There are several hypotheses that feeding with high fructose may influence insulin resistance (Shulman, 2000; Ueno *et al*. 2000). Among the existing

Figure 3. TBARS: thiobarbituric acid, SOD: superoxide dismutase, CAT: catalase.



*Difference between all groups; p < 0,05.

hypotheses, we highlight the increased content of TG and FFA at serum. According Basciano et al. (Basciano *et al*. 2005), the increase in FFA and TG are primarily responsible for the disturbance in insulin signaling. In a study by Ueno et al. (Ueno *et al*. 2000), it was found that after 28 days of feeding with high-fructose no changes in concentration of the insulin receptor, but self insulin-stimulated phosphorylation was

reduced about 70% in the liver. The FFAs act on insulin resistance due to reduced substrate phosphorylation of the insulin receptor 1 (IRS-1) in tyrosine and its association with PI3-kinase, causing a reduction in the signaling pathway with less translocation of GLUT-4 to the periphery of the cells, thereby reducing the uptake of glucose into cells (Shulman, 2000; Ueno *et al*. 2000).

The training was unable to promote significant reductions of the TG in neither of the groups. On the other hand, physical training to 80% of MLSS was able to reverse the insulin resistance (Figure 2) promoted by feeding high fructose, such benefit may have occurred through the reduction in the concentrations of FFAs (Table3).

Exercises performed between 25 and 65% of maximal oxygen uptake (mild to moderate) are associated with an increased 5-10 times in the oxidation of lipids, compared to rates of rest, given the increase in muscle energy demand (Wolfe *et al*. 1990) increasing using of FFA and its sérum reduction. Corroborating the findings of this study, Solomon, et al. (Solomon *et al*. 2009), found that 12 weeks of aerobic exercise training (1h day^{-1}, 5 times $week^{-1}$, at an intensity of 65% of maximal oxygen consumption) leads to a reduction in the FFA and a concomitant increase in insulin sensitivity independent of association with dietary restriction and weight reduction. Taking a direct binding of insulin sensitivity by reducing the concentration of FFAs, thus demonstrating the importance of reducing the concentration of FFAs and insulin resistance not only the reduction of body weight.

However, the state of insulin resistance can also be generated by the oxidative stress, activation of stress-sensitive pathways such as NF-κB and p38 MAPK (Evans *et al*. 2003). Furthermore, oxidative stress damages cells, directly, by oxidation of DNA, proteins and lipids (Halliwell & Gutteridge, 1989).

Several studies have shown that a diet high in fructose may promote an increase in reactive oxygen species, generating an imbalance and subsequent state of oxidative stress (Faure *et al*. 1997; Busserolles *et al*. 2002; Rajasekar & Anuradha, 2007; Lin *et al*. 2011).

Studies have found an increase of free radicals and TBARS in rats fed fructose, possibly by a hyperglycemia mediated-mechanisms such as self-oxidation of glucose, advanced glycation and altered pathway the polyols (Faure *et al*. 1997; Busserolles *et al*. 2002; Rajasekar & Anuradha, 2007; Lin *et al*. 2011). Contrary to the literature, this study showed no significant difference in TBARS values between fructose groups. Our findings do not corroborate those of Rajasekar & Anuradha (2007) and Lin *et al*. (2011), due to a possible difference in the composition of the control diet. Rajasekar & Anuradha (2007),

used in their work a commercial diet (Karnataka State Agro Corporation Ltd., Agro feeds division, Bangalore, India) as a control diet with 60% fructose to verify disturbances in insulin signaling, Lin *et al*. (2011), also followed the same dietary pattern. In our work was used a diet proposed by the American Institute of Nutrition in 1993 (AIN-93). However, these two diets have calorie different (commercial diet: 3028.0 kcal/kg and AIN-93: 3802.7 kcal/kg) than are normally neglected. Busserolles et al. (Busserolles *et al*. 2002) also observed an increase in lipid peroxidation marker, however, the insulin resistance was induced diet composed of 65% fructose, promoting an increase in the amount of fructose consumed daily per rat (as compared to our method 60% fructose in the diet).

As can be seen in these papers there is a big difference in calories and/or composition of the diets, and according Dandona et al. (Dandona *et al*. 2005), a simple increase in macronutrient intake can induce a state of oxidative stress. Thus ingestion of 75g glucose may be able to induce a 140% increase in superoxide generation, compared to baseline levels, capable of increasing subunits of the NADPH oxidase enzyme, responsible for converting it into oxygen molecule radical superoxide (Mohanty *et al*. 2002). Thus, our results contradict the literature possibly by effects caused by increased caloric of chow used as standard diet, finding no evidence in increased oxidative stress promoted by fructose intake.

Regarding the antioxidant enzymes, we found no effect of physical training or diet high in fructose on catalase activity between groups, confirming the findings of Busserolles et al. (Busserolles *et al*. 2002), who also found no effect of fructose intake on CAT.

Miyazaki et al. (Miyazaki *et al*. 2001), performed exercise training in humans for 12 weeks , (running 60 minutes at 80 % of maximal oxygen consumption, five times a week), in order to investigate whether training decreased oxidative stress in red cells and if this decrease was caused by induction of antioxidant enzymes and/or decrease production of antioxidants by neutrophils. The aerobic training was able to significantly increase the activity of SOD and glutathione-peroxidase (GSH-GPx) at rest, but not of CAT. Araújo *et al*. (2009), and Araújo *et al*. (2010), using a treadmill, did not find significant increases in the activity of catalase in the gastrocnemius muscle of the trained groups compared to the sedentary group. Due to several existing results, the issue of interference of training in the activity of enzymes of the antioxidant system has not been fully elucidated. While some authors have demonstrated an increase in antioxidant

enzyme activities (CAT, SOD and GSH-GPx) in skeletal muscle induced by exercise training (Ohkawa *et al*. 1979; Kinnunen *et al*. 2005), others found no significant changes in the activity of these enzymes (Alessio *et al*. 1988; Araújo *et al*. 2009; Araújo *et al*. 2010).

Among the antioxidant enzymes present in skeletal muscle, the activity of SOD have been more sensitive to adaptations of physical training regardless of the form and intensity of training (Powers *et al*. 1994; Powers *et al*. 2010; Powers *et al*. 2011). The aerobic exercise training used in this study was able to increase the concentrations of SOD in the gastrocnemius muscle of control animals trained, but this did not occur in animals subjected to diets high in fructose.

According to some authors (Gore *et al*. 1998; Gomez-Cabrera *et al*. 2008; Radak *et al*. 2008) an adaptation of the antioxidant system by training protocol occurs by an increase in mRNA levels of SOD, activated by stresses proteins such as NFκB sensitive. Ho et al. (Ho *et al*. 2005), reported an increased expression of this protein leading to higher activation of the enzyme SOD. In contrast, physical training did not increase this enzyme in rats with diet high in fructose, which may be associated with reduced copper, promoted by diet with high concentrations of fructose (Busserolles *et al*. 2002). Therefore, future research should evaluate the effect of protocols of long-time physical training on biochemical adaptations of the markers of oxidative stress and antioxidant capacity.

5 CONCLUSIONS

According to the data obtained, we conclude that a diet with high concentrations of fructose led to a state of insulin resistance, being reversed by physical training. However, physical training was not able to increase the activity of SOD in muscle of rats subjected to a diet high in fructose.

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