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Aerobic exercise improves microvascular dysfunction in fructose fed hamsters

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ABSTRACT

Fructose is a major diet component directly related to severe damages to the microcirculation and to diseases such as obesity, diabetes and hypertension to which physical activity is pointed out as an important non-pharmacological treatment since its positive effects precede anthropometric improvements. In this study we have investigated the effects of a light/moderate aerobic exercise training (AET) on microcirculatory dysfunction elicited by carbohydrate overload during a period of 5 months. Male hamsters (*Mesocricetus auratus*) whose drinking water was substituted (F) or not (C) by 10% fructose solution, during 20 weeks, associated or not to AET in the last 4 weeks (EC and EF subgroups) had their microcirculatory function evaluated on the cheek pouch preparation, glucose and insulin tolerance (GTT and ITT) tested. Arterial blood was collected for pO_2, pCO_2, HCO_3^-, pH, total CO_2, saturated O_2 and lactate determinations. Liver fragments were observed using an electron microscope. Microcirculatory responses to acetylcholine [Ach, an endothelium-dependent vasodilator; 10^{-8} M — *123.3 ± 7.5% (C), 119.5 ± 1.3% (EC), *98.1 ± 3.2% (F) and 133.6 ± 17.2% (EF); 10^{-6} M — *133.0 ± 4.1% (C), 135.6 ± 4.3% (EC), *103.4 ± 4.3% (F) and 134.1 ± 5.9% (EF); 10^{-4} M — *167.2 ± 5.0% (C), 162.8 ± 5.4% (EC), *123.8 ± 6.3% (F) and 140.8 ± 5.0% (EF)] and to sodium nitroprusside [SNP, an endothelium-independent vasodilator; 10^{-8} M — 118.8 ± 6.8% (C), 114.0 ± 5.0% (EC), 100.2 ± 2.9% (F), 104.9 ± 4.4% (EF); 10^{-6} M — 140.6 ± 11.7% (C), 141.7 ± 5.5% (EC), 125.0 ± 4.7% (F), 138.3 ± 2.8% (EF); 10^{-4} M — 150.4 ± 10.9% (C), 147.9 ± 6.5% (EC), 139.2 ± 7.3% (F), 155.9 ± 4.7% (EF)] and macromolecular permeability increase induced by 30 min ischemia/reperfusion (I/R) procedure [14.4 ± 3.5 (C), 30.0 ± 1.9 (EC), *112.0 ± 8.8 (F) and *22.4 ± 0.9 leaks/cm^2 (EF)] have shown that endothelium-dependent vasodilatation was significantly reduced and I/R induced macromolecular permeability augmented in sedentary fructose (F) subgroup and both improved after AET. Electron microscopy analysis of the liver showed significant differences between exercised and sedentary subgroups with greater amount of glycogen in F subgroups compared to other ones. No significant changes on mean arterial pressure, heart rate or blood gase between subgroups could be detected. Our results point out that AET could normalize microcirculatory dysfunction elicited by long term substitution of drinking water by 10% fructose solution.

Introduction

Humans tend to choose a more palatable diet and sugars such as fructose and glucose operate as ordinary sweeteners. Given the substantial participation of fructose in Western diet, it appears important to elucidate its metabolic effects and potential cardiovascular risk. For this purpose, the microcirculation could be a primary evaluation spot in individual's health, even in the absence of ill symptoms. The endothelium is essential to autoregulatory mechanisms and nitric oxide (NO) production plays an important role on vascular tone and health (Moncada and Higgs, 1993). Adequate microvascular flow should match organ function and its impairment is associated to organ failure in critically ill patients (Sakr et al., 2004). Endothelial dysfunction (ED) is characterized by reduction in the bioavailability of vasodilators, mainly NO, and activation of endothelial cells elicited by a predominant pro-inflammatory, proliferative and pro-coagulant milieu state (Anderson, 1999). Therewith, altered blood flow and inflammation incite changes on vascular hemodynamic, which in terms of macromolecular permeability is reflected on altered solute diffusion and raise in exchange membrane area. Vascular and microvascular permeability make it possible to correlate extravasation spots to microvascular morphology in several preparations, like for instance the hamster cheek pouch.

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The cheek pouch, an invagination of the oral mucosa that extends under the subcutaneous tissue down to the shoulder region, is an appropriate preparation to study microcirculatory function/dysfunction. Its blood supply comes mainly from the carotid arteries and it remains stable for 5 to 6 h (Duling, 1973). There are several advantages to the use of this preparation (1) ease access, (2) highly vascularized with all classes of microcirculatory vessels, (3) clarity and (4) the possibility to observe either skeletal muscle or subcutaneous microcirculatory beds.

Studies concerning fructose intake have been reported to induce insulin resistance, hyperglycemia, hypertriglyceridemia in rats (Tobey et al., 1982; Zavaroni et al., 1980), to reduce glucose uptake by adipocytes in vitro and endothelium-dependent vasodilation elicited by acetylcholine in aortic strips (Kotchen et al., 1997), to reduce tyrosine phosphorilation of IRS-1 in the soleus muscle (Hyakukoku et al., 2003), to significantly increase fasting plasma insulin without hyperglycemia, to decrease muscarinic receptors and to increase the dependence on nitric oxide and to impair α_2-adrenergic-mediated relaxation (Takagawa et al., 2001). However few novel data have shown its effects on microvascular permeability. Taking these data into account, strategies that set microcirculation as therapeutic target could be of great importance.

Physical activity has gained visibility as non-pharmacological treatment to obesity and its co-morbidities. The skeletal muscle constitutes approximately 40% of total body weight and is considered the most important determinant of peripheral vascular sensibility to insulin (Smith and Muscat, 2005) and the place for capitation, storage and liberation of glucose (Nuutila et al., 1992). Regular exercise practice is associated to reduction in primary (Myers et al., 2002) and secondary (Piepoli et al., 2010) vascular events, reduction of adiposity, and improvement of several metabolic risk factors including triglycerides (TG), high density lipoprotein-C (HDL-C), insulin and HOMA-IR when both regimens result in similar energy expenditure (Cho et al., 2011). Physical exercise benefits are not necessarily related to adiposity loss but also to improvement on vascular hemodynamic, and as a consequence, an improvement of type 2 diabetes and obesity related cardiovascular risks even without weight loss.

Therefore, our objectives in the present investigation were to evaluate microcirculatory effects, using the hamster cheek pouch preparation, of the substitution of the drinking water by 10% fructose solution during 20 weeks and the possibility of reversing these effects with a light/moderate aerobic exercise training program (AET) applied during the last 4 weeks of carbohydrate overload. Our hypothesis consisted on determining the capacity of reversion of the microvascular damage elicited by carbohydrate overload using a non-pharmacological way of treatment which, in this case, was the AET.

Experimental methods

Experiments were performed on male Syrian golden hamsters (*Mesocricetus auratus*), acclimatized at 20 ± 1 °C, with 12 h cycles day/night, with light from 06:00 to 18:00. On the 21st day after birth, they were randomly divided into two groups, one had the drinking water substituted by 10% fructose solution (Fructose, n = 54) and the other one was kept drinking filtered water (Control, n = 54) during 20 weeks thereafter. In the 16th week, each group was further subdivided into 2 subgroups: sedentary [no aerobic exercise training (AET)] or exercised (subjected to AET). Animals had unrestricted access to food and water or 10% fructose solution and the protocol was approved by the Ethical Committee of the State University of Rio de Janeiro (CEUA/061/2010). The investigation has been conducted according to the Guide for Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised in 1996).

Exercise training

After 16 weeks drinking filtered water or 10% fructose solution, hamsters were randomly assigned to an exercise practice (n = 44). One week before the beginning of the exercise protocol, they were placed in the treadmill machine at lower speed for a short time for adaptation. The exercise training consisted of 4 weeks of running (animals at this time were 6 to 7 months old) on a motor treadmill (INSIGHT – EP 131 – Ribeirão Preto, SP, Brazil), 5 times/week, raising time and speed of the treadmill every week with the limit of 60 min at 18 m/min (Fig. 1) (Ghanbari-Niaki et al., 2007). In the first 3 weeks, the exercise was conducted at 50% of VO_{2max} and in the last week it went to 70% of VO_{2max}, featuring the overall AET protocol as of light/moderate intensity. Measures of Rq (Respiratory quotient) were set at 1 for respiratory exhaustion and conducted in 0.89, indicating the overall exercise as aerobic (see Table 1). Animals did not go through exercise training 24–36 h before the actual experiment was conducted.

Glucose and insulin tolerance test (GTT and ITT)

GTT and ITT were performed from the 15th to the 20th week in four animals of each group and these animals fasted during 6 h before the test. Glucose levels were measured in the blood drawn through saphenous vein puncture (Beeton et al., 2007) at baseline and after intraperitoneal injection of either glucose (2 g/kg BW) or insulin (0.75 U/kg BW) at 30, 60, 90, and 120 min. There was an interval of 5 days between GTT and ITT. Results are presented as total blood glucose after intraperitoneal injection of either glucose or insulin.

Microcirculatory evaluation

On the day of the experiment, anesthesia was induced by an intraperitoneal injection of 0.1–0.2 ml of sodium pentobarbital (pentobarbital sodique, 60 mg/ml, Sanofi Santé Animale, Paris, France) and maintained with α-chloralose [100 mg/kg body weight (Sigma Chemicals, St. Louis MO, USA)] given intravenously. For the cheek pouch preparation, hamsters were placed on a heating pad, controlled by a rectal thermistor and their body temperature was maintained at 37.5 °C (LTB 750 Thermostat System, Uppsala, Sweden). The right femoral vein and the left femoral artery were cannulated (0.28 mm internal/0.61 mm outer diameters) for drug injection, monitoring of mean arterial pressure (MAP), heart rate (HR) and gasometrical measurements (MP 100 Data Acquisition System, BIOPAC Systems, Santa Barbara, CA, USA, Spectramed pressure transducer). A tracheal tube was inserted to facilitate spontaneous breathing (room air).

The cheek pouch was gently everted and mounted on an experimental chamber as previously described (Bouskela and Grampp, 1992). All preparations were superfused at a rate of 4.0 ml/min by a HEPES-supported HCO_3^--saline solution [composition in mM: NaCl 110.0, KCl 4.7, $CaCl_2$ 2.0, $MgSO_4$ 1.2, $NaHCO_3$ 18.0, N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES) 15.39 and HEPES Na^+-salt 14.61] bubbled with 5% CO_2–95% N_2. The pH was kept at 7.4 and the temperature of the animal and the superfusion solution maintained at 37.5 °C throughout the experiment. Preparations were placed under an intravital microscope (Leica DMLFS, Wetzlar, Germany, optical magnification ×600, NA 0.65) coupled to a closed-circuit TV system and allowed to rest for 30 min before measurements were taken. If after this time there was (1) an indication of good vascular tone; (2) brisk blood flow in all parts of the vascular bed including the larger veins (where individual erythrocytes should not be discernible in the image of the blood stream) and (3) no tendency for leukocytes to adhere to the vessel wall (Bouskela and Grampp, 1992) images were recorded in sVHS and analyzed after the experiment.

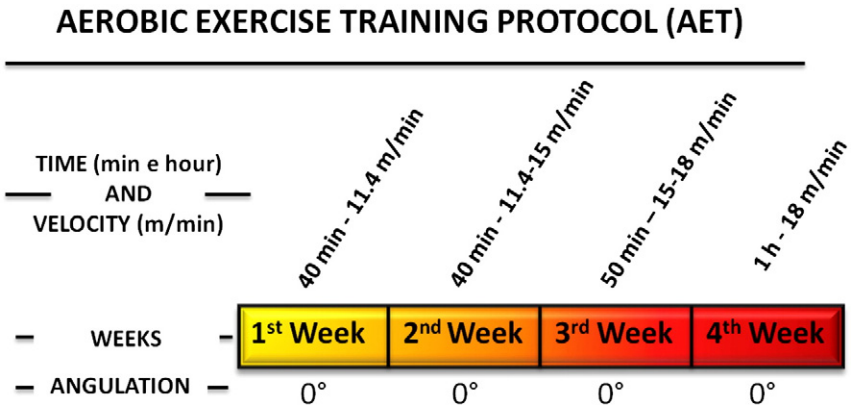

Fig. 1. One week before the aerobic exercise training protocol (AET), animals were placed on the machinery at slower velocity and time for adaptation. The protocol started in the last four of the 20 weeks of carbohydrate overload. Scheme of AET performed by hamsters.

Intravital microscopy

In each preparation, three 2nd or 3rd order arterioles were selected taking into account the possibility to return exactly to the same site (presence of fat cells, bifurcations, etc.) for consecutive measurements. Such choice was important for the response to vasoactive agents. Experiments were performed by taking 3 min videotape recordings of selected microvessels in initial control conditions (before the addition of any drug) and subsequently 10–15 min after each experimental intervention.

Microvascular reactivity

Three arterioles were studied in each cheek pouch preparation, summing up seventy-two 2nd and 3rd order arterioles. Mean internal microvessel diameter was determined using an Image Shearing device (Vista Eletronics, Model 908, San Diego, CA, USA), at baseline and after topical application, 10 min each, with a syringe infusion pump 22 (model 55-2222, Harvard Apparatus, Massachusetts, USA), of three concentrations of freshly prepared acetylcholine (Ach) [Ach — endothelial dependent vasodilator at 10^{-8}, 10^{-6} and 10^{-4} M (Sigma Chemicals, St. Louis, MA, USA)] or sodium nitroprusside [SNP — endothelial-independent vasodilator at 10^{-8}, 10^{-6} and 10^{-4} M (Sigma Chemicals, St. Louis, MA, USA)] in a cumulative dose–response curve.

Macromolecular permeability

Fluorescein isothyocyanate (FITC)-dextran, molecular weight = 150,000 (Bioflor HB, Uppsala, Sweden, 25 mg/100 g body weight, 5% solution) was injected intravenously 20 min after the end of the microvascular reactivity part of the study. Permeability for large molecules was quantified by counting the number of leaky sites (=leaks) in the prepared area using an UV-light microscope (40× magnification). Leaks are defined as visible extravascular spots (diameter ≥ 100 μm) of FITC-dextran in postcapillary venules seen under fluorescent light. The number of leaks was counted at baseline and during reperfusion, after 30 min ischemia, made by an air inflatable tourniquet placed around the neck of the pouch vascular pedicle. Preparations presenting more than 10 spontaneous leaks or petechias at baseline were excluded due to previous damage on the cheek pouch tissue.

Table 1
Data concerning the VO_{2max} test.

Max VO_2 ($ml/kg/min^{-1}$)	Max vel. (m/min)	Weight (g)	% Max vel.	% VO_{2max}	Rq
88.22 ± 4.73	44.8 ± 3.91	150 ± 11	42 ± 0.02%	68 ± 0.05%	0.89 ± 0.1

Data presented as mean ± SD. Measurements were performed on 16 hamsters using the protocol of maximum velocity and intensity utilized. % vel. max and %VO_2 = intensity which data indicated to correspond to 18 m/min. Rq = respiratory quotient.

Blood sample analysis

Blood samples were collected before the experimental protocol from the saphenous vein, using a capillary tube containing heparin (Heparina Perfecta, 75 mm length; São Paulo, SP, Brazil). Collected blood was used for glucose (One touch ultra — Johnson & Johnson, Medical Brazil), triglycerides and cholesterol (Accutrent — Roche Diagnostics, GmbH, D-68298 Mannheim, Germany) determinations.

Before the experimental protocol, few animals [(Control, n = 10); (Fructose, n = 10)] had a catheter introduced in the carotid artery in order to collect a 0.1 ml aliquot of arterial blood for gasometrical measures [pO_2, pCO_2, HCO_3^-, pH, total CO_2 (tCO_2), saturated O_2 ($SatO_2$) and lactate]. On regular times, the arterial line was washed by heparin solution (40 UI ml^{-1}), and the total volume withdrawn did not exceed 0.5 ml. Blood samples were analyzed in a point-of-care blood gas analyzer (CG4 + catridge; i-STAT System Laboratories, Abbott Park, IL, USA) for pH, partial pressure of oxygen (PO_2), partial pressure of carbon dioxide (PCO_2), bicarbonate level (HCO_3^-), base excess (BE), total carbon dioxide content (TCO_2) and arterial oxyhemoglobin saturation ($SatO_2$).

Liver electronic microscopy evaluation

Liver fragments, fixed with glutaraldehyde (2% solution), were obtained from 4 different animals from each experimental subgroup in order to evaluate possible changes elicited by the substitution of the drinking water by 10% fructose solution, after 5 h fasting and observed using an electron microscope (transmission electron microscope, TEM — Zeiss EM 900, magnification 21.560×).

Data analysis

Kolmogorov–Smirnov normality test was performed, data with normal distribution passed through one way ANOVA analysis, and data without normal distribution were analyzed using Kruskal–Wallis test. Besides microcirculatory assessments, all data were analyzed using the one way ANOVA with the Tukey's post-hoc test, Kruskal–Wallis test, with the Dunn post-hoc test. Data are expressed as mean ± standard error of the mean (S.E.M.), unless otherwise noticed. No differences between groups concerning basal internal arterial diameters were observed. Microvessel diameters are presented as relative changes compared to baseline, considered as 100%. Group differences in arteriolar diameter responses to Ach were determined by Friedman's test. Statistical

analysis was performed using Prism 5.01 (GraphPad Inc., San Diego, CA, USA). Results were considered statistically significant when $p < 0.05$.

Results

One hundred and eight animals entered into this study and were randomly assigned to two experimental groups: Control [C (n = 54)] and Fructose [F (n = 54)], and at a later time, subdivided into two different protocols, sedentary or exercised: Sedentary control [C (n = 27)]; Exercised Control [EC (n = 27)], Sedentary Fructose [F (n = 27)] and Exercised Fructose [EF (n = 27)].

Weight evaluation

Data from weight evaluation did not show any difference between subgroups on the day of the experiment (Table 2).

Blood sample analysis

On the day of the experiment, blood sample analysis has shown that glycaemia and triglycerides were significantly different between C and F and EF subgroups as seen in Table 2.

GTT and ITT

After 6 h fasting, GTT and ITT were not significantly different between F and EF subgroups although we could observe a tendency towards reduction in the EF subgroup. In some weeks, exercise improved plasma glucose in EC and EF subgroups in comparison to C and F sedentary subgroups as shown in Fig. 2.

MAP, HR and blood gasses

On the day of the experiment, animals had a catheter inserted in their carotid artery in order to evaluate MAP, HR and blood gasses (see Tables 2 and 3). Although it was possible to observe discrete differences between groups, none of them were significant.

Microcirculatory evaluation

Microvascular reactivity

The vasomotor activity was studied in more detail on seventy-two 2nd and 3rd order arterioles with internal diameter ranging from 39.8 to 90.5 μm. All of them presented robust flow and single flowing red blood cells were not discernible at any time. No differences could be detected between groups concerning basal internal arteriolar diameter (Supplementary table). Topical application of acetylcholine showed a significant reduction in vasodilatation in F compared to C [(10^{-8} M − *123.3 ± 7.5% (C), 119.5 ± 1.3% (EC), *98.1 ± 3.2% (F) and 133.6 ± 17.2% (EF); 10^{-6} M − *133.0 ± 4.1% (C), 135.6 ± 4.3% (EC), *103.4 ± 4.3% (F) and 134.1 ± 5.9% (EF); 10^{-4} M − *167.2 ±5.0% (C), 162.8 ± 5.4% (EC), *123.8 ± 6.3% (F) and 140.8 ± 5.0% (EF) − (Fig. 3)]. Significant differences could also be detected between F and EF groups with the application of 10^{-6} M of Ach (103.4 ± 4.3% and 134.1 ± 5.9%). Topical application of sodium nitroprusside (SNP) did not elicit significant differences between subgroups as seen in Fig. 3 [10^{-8} M − 118.8 ± 6.8% (C), 114.0 ± 5.0% (EC), 100.2 ± 2.9% (F), 104.9 ± 4.4% (EF); 10^{-6} M − 140.6 ± 11.7% (C), 141.7 ± 5.5% (EC), 125.0 ± 4.7% (F), 138.3 ± 2.8% (EF); 10^{-4} M − 150.4 ± 10.9% (C), 147.9 ± 6.5% (EC), 139.2 ± 7.3% (F), 155.9 ± 4.7% (EF)].

Macromolecular permeability increase induced by ischemia/reperfusion

Ten minutes after the onset of reperfusion, macromolecular permeability was significantly higher in F compared to C and EC subgroups as demonstrated in Fig. 4 (T_0, immediately after the onset of reperfusion; T_5, 5 min after and T_{10}, 10 min after tourniquet release). The EF subgroup had the number of leaks totally restored to values similar to C and EC subgroups [14.4 ± 3.5 leaks/cm^2 (C), 30.0 ± 1.9 leaks/cm^2 (EC), *112.0 ± 8.8 leaks/cm^2 (F) and *22.4 ± 0.9 leaks/cm^2 (EF)].

Liver electron microscopy evaluation

Liver analysis has shown significant differences between exercised and sedentary subgroups as well as between control and fructose ones. The sedentary fructose subgroup showed greater amount of glycogen in comparison to control and exercised ones and both exercised subgroups have shown an increased area of the rough endoplasmic reticulum as shown in Fig. 5.

Discussion

Our initial hypothesis was confirmed since increased ischemia/reperfusion induced microvascular permeability and endothelial dysfunction elicited by the substitution of drinking water by 10% fructose solution was significantly attenuated by chronic light/moderate aerobic exercise training program (AET).

In order to evaluate AET's effects, the chosen microcirculatory assessment technique was the cheek pouch preparation. This preparation represents a trustworthy technique since it displays robust, stable blood flow and responsiveness to topic vasoactive drugs. Its transparence based on translucent conjunctive tissue makes it very practical as it remains stable generally for 5–6 h, as shown by Duling (1973) for arteriolar reactivity to acetylcholine (ACh); Svensjo (1990) for macromolecular permeability increase induced by bradykinin and Bouskela

Table 2
Characteristics of investigated animals.

	C (n = 22)	EC (n = 22)	F (n = 22)	EF (n = 22)
	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
Body weight (g)	131.9 ± 4.2	137.5 ± 2.2	135.2 ± 2.1	137.2 ± 2.4
Weekly ingestion (g)	109.9 ± 12.7	185.9 ± 23.4	81.1 ± 7.2	125.6 ± 14.9
Weekly ingestion of water and fructose solution (ml)	235 ± 45.9	237.5 ± 12.9	280 ± 29.1	305.6 ± 25.8
Blood cholesterol (mg/dl)	170 ± 2.8	165.8 ± 1.6	161.7 ± 3.8	163.4 ± 1.6
Heart rate	304.7 ± 4.2	299.7 ± 16.9	343.7 ± 71.7	364.6 ± 30
Mean arterial pressure	118.9 ± 4.8	115.5 ± 5.9	125.3 ± 5.5	133.0 ± 7.9
Blood triglycerides (mg/dl)	174 ± 9.0	165.5 ± 22.7	170.5 ± 10.1	$^{+}$132.7 ± 7.3
Fasting blood glucose (mg/dl)	93.1 ± 5.7	96.6 ± 7.2	*124.9 ± 8.2	*123.9 ± 7.8

Measurements performed on the day of the experiment: weight, glycaemia, triglycerides and cholesterol. All values are presented as mean ± standard error of the mean. $^{+}$Significantly different from sedentary control and fructose groups ($p < 0.05$); *Significantly different from the control and exercised control groups ($p < 0.01$). C = animals that had drunk filtered water for 20 weeks (control group); EC = animals that had drunk filtered water for 20 weeks, exposed during the last four weeks to exercise (exercised control); F = animals that had drunk 10% fructose solution for 20 weeks (fructose group); EF = animals that had drunk 10% fructose solution for 20 weeks, exposed during the last four weeks to exercise (exercised fructose).

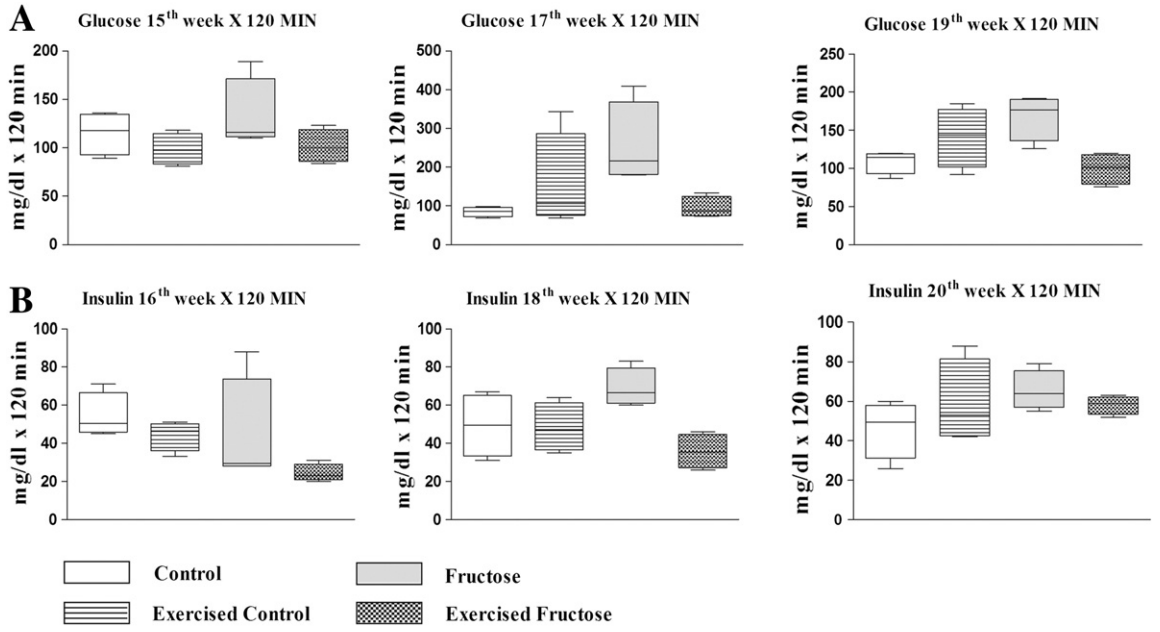

Fig. 2. Glucose and insulin tolerance tests. (A) Blood glucose measurements were performed at baseline and followed at 30, 60, 90 and 120 min after intraperitoneal injection of glucose (2 g/kg body weight). (B) Blood glucose measurement performed at baseline and followed at 30, 60, 90 and 120 min after intraperitoneal injection of insulin (0.75 U/kg body weight) in hamsters whose drinking water has been substituted or not by 10% fructose solution (Fructose and Control, Exercised Fructose and Control; n = 4 in each one). Measurements were made from the 15th to the 20th week of carbohydrate overload, interspersing glucose and insulin tolerance tests. Results are presented as total blood glucose after injection of either glucose or insulin. Data are expressed as mean ± S.E.M. and represented by vertical bars.

and Grampp (1992) for spontaneous arteriolar vasomotion. Although the microcirculatory bed observed in the cheek pouch in this investigation was the cutaneous part, it is conceivable to consider that it represents systemic alterations. Experiments in humans using laser Doppler and intravital videomicroscopy to examine the skin of the dorsum of the middle finger have shown a good correlation between the cutaneous microcirculation and systemic alterations (Antonios et al., 2001; Ijzerman et al., 2003). Microvascular alterations observed on cutaneous tissue attributed to microvascular dysfunction are not only a local, but a systemic process, and studies concerning the cutaneous vascular bed can reproduce alterations on noble tissues as the muscular one (Sax et al., 1987).

The past two decades were marked by transition on the nutritional state, in which fructose consumption in the diet, under the form of sucrose, increased exponentially on industrialized and developing countries. With that, the evaluation of carbohydrate overloads constitutes an important piece of information for the public health system. Fructose is an energy generating molecule that helps to build and maintain body mass. Although it is documented in the literature the potential effect of fructose intake on weight gain, our results did not show any significant difference between groups suggesting that the damage to the microcirculation precedes body mass alterations. Félétou and coworkers, also using the cheek pouch preparation and substitution of the drinking water by 10% fructose solution for 18 weeks, did not find a significant increase on body weight compared to control hamsters either (Félétou et al., 2003a).

Glucose homeostasis in fasting state results from the balance between glucose production by the liver and glucose utilization by muscle and fat. Although the fine tuning of glucose metabolism may be influenced by many hormones and metabolic intermediates, normal glucose disposal depends primarily on 4 factors: (1) ability of the body to secrete insulin acutely and in sustained fashion, (2) ability of insulin to inhibit hepatic glucose output, (3) ability of glucose to promote its disposal or on insulin sensitivity and (4) ability of glucose to enter cells in absence of insulin (Bergman et al., 1992). The pathogenesis of type 2 diabetes appears to involve at least 2 defects in this regulatory system. In our study, animals subjected to substitution of drinking water by 10% fructose solution showed a significant increase in glycaemia, similar to findings reported by Huang et al. (2004) and Félétou et al. (1996b). In the present investigation, however, the extent of metabolic changes elicited by fructose were far more severe than those reported under similar experimental conditions (Félétou et al., 2003) where only glycaemia was significantly different from controls. In various animal models, including hamsters, several alterations such as hyperinsulinemia, hypertriglyceridemia, hypercholesterolemia, and insulin resistance have been reported with fructose-enriched diet (Kasim-Karakas et al., 1996; Taghibiglou et al., 2000). In contrast, fasting glycemic levels were not affected in those studies. These discrepancies in relation to the present investigation could be explained primarily by the severity of the diet. Most studies have been performed using food containing fructose that was 60% of the total caloric intake (Hwang et al., 1987) leading to daily intake of approximately 16 g of fructose/hamster (Iyer and Katovich, 1996) instead of 1 to 2.4 g ingested in the present investigation. Our data showed increased and sustained triglycerides and fasting glucose levels in F and EF subgroups (Table 3), despite AET. These differences could be probably related to the intensity

Table 3
Characteristics of investigated animals: blood gasses.

	Control groups (C and EC)	Fructose groups (F and EF)
pH	7.391 ± 0.013	7.383 ± 0.014
PO_2 (mm Hg)	78.3 ± 4.1	69.6 ± 4.3
PCO_2 (mm Hg)	49.52 ± 1.63	54.46 ± 3.41
HCO_3^- (mmol l^{-1})	30.10 ± 1.09	32.32 ± 1.07
BE (mmol l^{-1})	5.1 ± 1.2	7.0 ± 1.0
TCO_2 (mmol l^{-1})	31.6 ± 1.1	33.8 ± 1.1
$SatO_2$ (%)	94.4 ± 0.9	92.4 ± 1.6

Blood gasses measured on the day of the experiment. PO_2 = partial pressure of oxygen; PCO_2 = partial pressure of carbon dioxide; HCO_3^- = bicarbonate level. BE = base excess; TCO_2 = total carbon dioxide content; $SatO_2$ = arterial oxyhemoglobin. C = animals that had drunk filtered water for 20 weeks (control group); EC = animals that had drunk filtered water for 20 weeks, exposed during the last four weeks to exercise (exercised control); F = animals that had drunk 10% fructose solution for 20 weeks (fructose group); EF = animals that had drunk 10% fructose solution for 20 weeks, exposed during the last four weeks to exercise (exercised fructose).

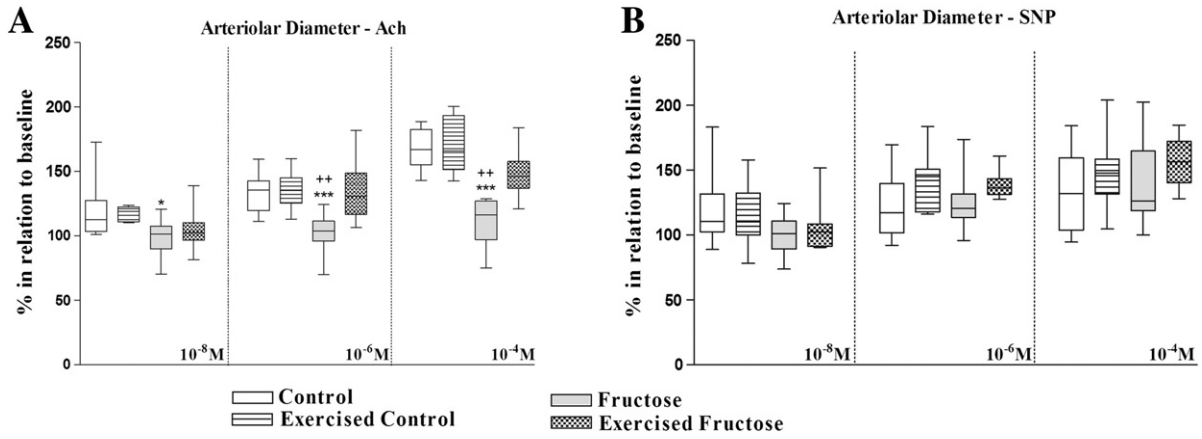

Fig. 3. Mean arteriolar diameters after topical application of three concentrations of (A) acetylcholine (endothelial-dependent vasodilator — 10^{-8}, 10^{-6} and 10^{-4} M) and (B) sodium nitroprusside (endothelial-independent vasodilator — 10^{-8}, 10^{-6} and 10^{-4} M) on the cheek pouch of hamsters whose drinking water has been substituted by 10% fructose solution (F and EF, n = 18 each) or not (C and EC, n = 18 each) during 20 weeks. Data are shown as changes relative to baseline considered as 100%, expressed as mean ± S.E.M. and represented by vertical bars. (A) *Significantly different from sedentary control group ($p < 0.01$); #Significantly different from sedentary fructose ($p < 0.01$); + Significantly different from exercised control ($p < 0.05$).

of the program, chronically applied to animals in the present investigation (Fig. 1).

No significant differences were found between groups concerning GTT and ITT tests, possibly because there was a small number of animals per group (n = 4), due to the need to reduce EC and EF groups, subjected to several measurements. One possible mechanism by which fructose causes alterations on glycaemia was confirmed by the liver analysis, which has shown the beginning of hepatic insulin resistance, leading to future systemic alterations since F groups indicated altered hepatic glucose metabolism.

Blood gasses, although not different between groups, showed a tendency to decrease on fructose drinking ones. It is possible that the light hyperglycemia [124.9 ± 8.2 mg/dl (F group) and 123.9 ± 7.8 mg/dl (EF group)] observed in the fructose groups was not high enough to elicit significant changes in these parameters. On the other hand, intravital microscopy observation of the spinotrapezius muscle of Goto–Kakizaki type II diabetic rats (blood glucose 263 ± 34 mg/dl compared to 105 ± 5 mg/dl in healthy male Wistar rats) has shown that the percentage of red blood cell perfused capillaries, capillary tube hematocrit, red blood cell velocity, red blood cell flux and O_2 delivery per unit of muscle were all decreased (Padilla et al., 2006). The difference in glycemic levels between the Goto–Kakisaki type II diabetic rats and the fructose drinking hamsters could account, at least in part, for the observed discrepancy. It is however important to consider that other systemic changes besides metabolic ones could lead to impairment in capillary hemodynamics and consequently in a decrement of O_2 delivery to tissues, like for instance chronic heart failure (Kindig et al., 1999; Richardson et al., 2003).

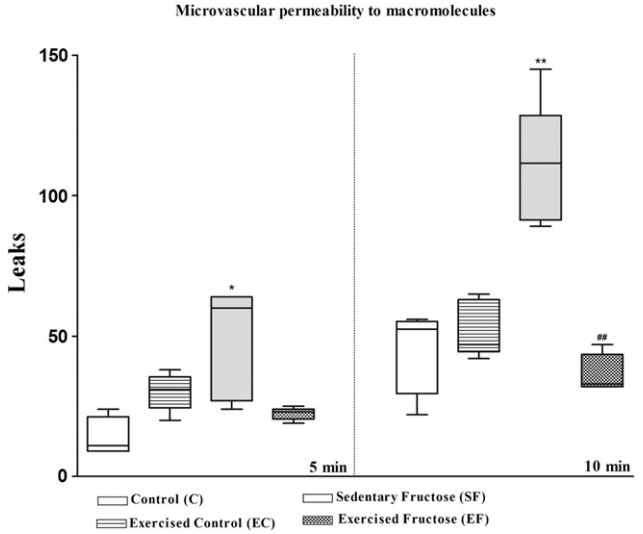

Fig. 4. Macromolecular permeability at postcapillary venules after 30 min ischemia of the cheek pouch of hamsters whose drinking water has been substituted by 10% fructose solution (F and EF each, n = 18) or filtered water (C and EC, n = 18) during 20 weeks. T_5, 5 min after and T_{10}, 10 min after tourniquet release. Data are expressed as mean ± S.E.M. represented by vertical bars. *Significantly different from the C group ($p < 0.01$), #Significantly different from the F subgroup ($p < 0.01$).

Fructose behaves differently from glucose since its phosphorylation in the liver occurs in higher rate than glucose, with fewer control checkpoints as allosteric modulations, altering glycolysis and gluconeogenesis metabolism. The hepatic metabolism of fructose is characterized by its rapid conversion to triose-P, independently of insulin and of the negative feedback by ATP and citrate. With that, hepatocytes transform fructose into many products, being glycogen one of them. The association of high levels of fructose and glucose propitiates the accumulation of glycogen in the liver, as seen on histological observations performed by our group, in which accumulation of glycogen could be detected and directly related to higher levels of blood glucose. After AET, glycogen accumulation spots were significantly reduced (Fig. 5), suggesting an improvement on glucose metabolism. Thus, disturbances on glucose and lipid metabolism could be important factors to impair NO bioavailability and its vasodilator effects (Galili et al., 2007). Several mechanisms might be involved on microvascular impairment in type 2 diabetes, like for instance, chronic reduction of NO bioavailability secondary to increased oxidative stress (Hansel et al., 2004) and/or reduction in both convective O_2 delivery and diffusive O_2 transport (Padilla et al., 2006), increased activity and expression of protein kinase C (Bohlen, 2004) and/or reduction in tetrahydrobiopterin (BH_4) (Meininger et al., 2004). Analysis of microcirculatory reactivity showed that 10% fructose solution causes alterations on endothelial responses in all concentrations of acetylcholine tested (10^{-8} M, 10^{-6} M and 10^{-4} M) in the F group, indicating that endothelial dysfunction could occur as consequence of fructose consumption, even in the absence of co-morbidities such as type 2 diabetes. After AET, the EF group presented higher responses (increased vasodilatation) to acetylcholine, equalizing itself to EC and C subgroups (Fig. 3). There was no difference between groups regarding SNP (endothelium-independent vasodilator).

Basal macromolecular permeability was not different between groups, but after 30 min ischemia it increased significantly in the F

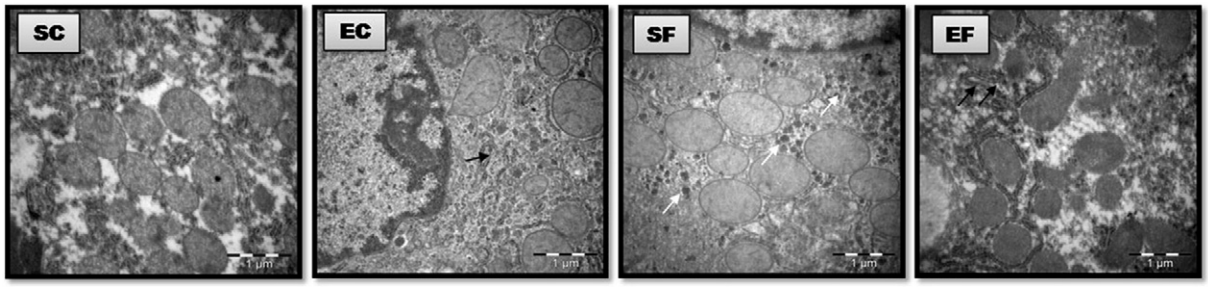

Fig. 5. Liver electron microscopy evaluation. White arrows show increased amount of glycogen in the liver of SF subgroup. Black arrows point to development of the rough endoplasmic reticulum on exercised subgroups. Microscope magnification was 21.560×.

group, 5 and 10 min after the onset of reperfusion, characterizing a state of microvascular endothelial dysfunction (Fig. 4). After AET, most of the damage elicited by the carbohydrate overload was reversed, providing new data concerning fructose studies.

Exercise adaptive responses are associated with the endothelium, a dynamic interface between the intima of the vasculature and the luminal flow of blood. Endothelial cells are aligned in the direction of laminar blood flow which enables them to respond to physical forces induced by it, such as shear stress. Endothelial properties are affected by mechanical stress and macromolecular permeability increase induced by ischemia/reperfusion demonstrated opposite effects when acute and chronic shear stress occurred. Acute stimulus is considered as promoter of increased macromolecular permeability count in endothelial cell monolayer (Warboys et al., 2010). The cross-talk between exercise and endothelial cells is important to explain how shear stress acts on the cell surface. This mechanical agonistic stimulus possesses an important effect wherein augment target endothelial genes and enhanced blood flow are observed. The subsequent stages are related to upregulation of pivotal genes responsible for beneficial effects of physical activity, such as eNOS and Ca^{2+}-dependent K^{+} channel 4 (KCNN4)(Zhang and Friedman, 2012). According to our results on microvascular reactivity, it is possible to infer higher gene expression and protein activity of key genes, such as eNOS and GMPc whereas vasodilator effects provoked by acetylcholine were significantly different between sedentary and exercised fructose groups. There are also a number of studies that indicate analog or synergic effects of exercise other than shear stress. Exercise may also influence sarcolemmal K^{+} and L-type Ca^{2+} channels (Bowles and Woodman, 2000a; Bowles, 2000; Laughlin et al., 1996a). Large-conductance Ca(2+)-activated K(+) (BKCa) channels provide a negative feedback that regulate vascular tone. Experiments conducted on arterial smooth muscle cells demonstrated that aerobic exercise may be related to enhanced BKCa channel activity in cerebral arterial myocytes (Li et al., 2013), with upregulation of β-1 receptors of BKCa channels on Wistar rats' mesenteric arteries (Shi et al., 2013), most likely by changing its biophysical properties. Further mechanisms that could explain the improvement on endothelial function due to physical activity are reduction of free radical species since exercise influences anti-oxidant defenses (Marcelino et al., 2013; Radak et al., 2013) resulting in increased expression of endothelial NO synthase (Hodges et al., 2010). Furthermore mechanical alterations of the endothelium result in decreased β-adrenergic vascular responsiveness, and consequently in attenuated sympathetic outflow (Laughlin et al., 2008).

Limitations of this study versify animal choice. The hamster enables a trustworthy microcirculatory analysis; however it was not possible to collect any drop of blood during the exercise protocol for the lactate curve since this animal has no tail. With that, the conclusion that this was indeed an aerobic protocol was based on the Rq value. Attempts to introduce catheters on either carotid or femoral arteries failed since on the first access animals presented cerebral damage and were not able to perform the exercise protocol and the second access hindered the possibility of execution of the AET. Besides those limitations, the catheter itself is useful only in the first two to three days after its insertion on animal's vessel, making it impossible to perform a before–after AET gasometrical analysis. Another limitation concerns lack of serum analysis, because methods such as ELISA are limited on the *Mesocricetus auratus* species. Concluding, GTT and ITT did not show significant differences between groups most likely due to the small number of animals per group ($n = 4$).

In summary, we have presented evidence suggesting that AET could be a recommended non-pharmacological way of treatment for microvascular/endothelial dysfunction since its practice during 4 weeks reversed most observed damages and hepatic alterations elicited by the substitution of drinking water by 10% fructose solution.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.mvr.2014.02.012.

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