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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.