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Exercise training and taurine supplementation reduce oxidative stress and prevent endothelium dysfunction in rats fed a highly palatable diet

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ABSTRACT

Aim: Few studies have analysed, from a nutritional point of view, the influence of exercise in minimizing detrimental diet-related health effects. This study evaluated the effectiveness of exercise and taurine supplementation in preventing vascular and metabolic disorders caused by highly palatable diet intake.
Main methods: Thirty-two male Wistar rats (255–265 g) were divided into 4 groups: Sedentary (SD); Sedentary + 2% taurine (SDTAU), Trained (TR) and Trained + 2% taurine (TRTAU). Exercise (treadmill, 60% maximum speed, 60 min, 5 days/week) started after 4 weeks of highly palatable diet feeding and was carried out for 7 weeks.
Key findings: Exercise effectively reduced insulin (61% and 68%), glucose (30% and 7%) and leptin levels (75% and 67%) in TR and TRTAU groups, respectively. All groups showed a reduction in hepatic triglyceride infiltration (74% for SDTAU, 82% for TR and 85% for TRTAU) but only exercise reduced TBARS (50% for TR and 41% for TRTAU). Impaired relaxation was seen in SD (E_{max} = 67%) and improved with taurine (E_{max} = 86%) and exercise (E_{max} = 90% for TR and TRTAU). Increased expression of EC-SOD (32%) was seen in the aortas from all treated groups. Exercise, in the absence of taurine, increased Cu–Zn SOD (44%) and reduced $gp91^{phox}$ (34%). Superoxide formation in the aorta was reduced in supplemented (75% in SDTAU) and in trained groups (64% and 77% for TR and TRTAU, respectively).
Significance: Exercise and taurine supplementation were effective in preventing endothelial dysfunction induced by highly palatable diet intake, through a decrease in vascular oxidative stress.

1. Introduction

Excessive fat-mass accumulation, a feature of obesity, is closely related to hypertension and insulin resistance [23,33]. Insulin resistance plays a crucial role in the development of obesity co-morbidities such as type II diabetes mellitus, cardiovascular complications and non-alcoholic fat liver disease [13,17]. Moreover, there is a cross-talk between an increase in fat-mass and obesity-related health complications due to inflammation and oxidative stress [31].

Environmental and cultural behaviours, such as reduced levels of daily physical activity and overconsumption of energy-dense foods, can contribute to an increase in body fat mass and metabolic disorders. On the other hand, exercise can prevent weight gain, improve insulin signalling and reduce oxidative stress [8]. Nutritional strategies can also minimize these detrimental effects. Taurine (2-aminoethanesulfonic acid), an amino acid involved in bile production, osmoregulation, immune system modulation and a potential antioxidant, has been considered to be a good asset in nutritional therapies [2,18]. The beneficial effects of exercise on vascular and metabolic disorders have been reported; however, very few studies have analysed the effects of exercise, from a nutritional point of view, in minimizing diet-related detrimental health effects. The aim of this study was to analyse the effectiveness of exercise and taurine supplementation in preventing vascular and metabolic disorders caused by highly palatable diet intake.

2. Material and methods

2.1. Animals and experimental protocol

All procedures were reviewed and approved by the Ethics Committee on Animal Use in Research (CEUA/PUSP-RP protocol number 10.1.1290.53.5) in compliance with the "Principles of laboratory animal care" (NIH publication No. 86-23, revised 1985) and the national law (CONCEA publication No. 11.794, 2008).

Thirty-two male Wistar rats (255–265 g) were divided into 4 groups: sedentary (SD); sedentary supplemented with taurine solution (2%) in drinking water (SDTAU); trained (TR) and trained

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Table 1
Standard AIN-93 and High-palatable diet ingredients.

Ingredient (g/kg)	AIN-93	High-palatable diet
AIN-93	–	330
Whole Condensed Milk (Nestlé™)	–	330
Casein	200	92
Cornstarch	397	–
Dextrinized cornstarch	131	–
Sucrose	100	156
L-Cystine	3	–
Soybean oil	70	72
Mineral mix (AIN-93)[a]	35	23
Vitamin mix (AIN-93)[a]	10	7
Fibre	50	–
Choline bitartrate	2.5	–

[a] [28].

supplemented with taurine solution (2%) in drinking water (TRTAU). Taurine was purchased from Ajinomoto Food Ingredients (Chicago, IL, USA) and the concentration used was determined according to Nandhini and Anuradha [24]. Animals were housed in polypropylene cages (41 × 34 × 30 cm) containing three animals in each and kept on a 12 h light/dark cycle with unlimited access to a highly palatable diet [27,28]. The energy content of the diet derived from 56% carbohydrate, 18% protein and 26% fat. A full list of ingredients can be found in Table 1.

The experiment lasted 11 weeks. Animals were fed for 4 weeks prior to exercise training and/or taurine supplementation. Exercise training consisted of treadmill run at 60% maximum speed (ms) for 60 continuous minutes, 5 days a week, for 7 weeks. Training speed was determined after a maximum incremental exercise test, which began at 11.6 m/min and increased by 1.6 m/min every 2 min until 20 m/min. Subsequently, the speed was increased by 3.2 m/min and rats ran until exhaustion (determined when the animal touched the bottom of the bay five times within 1 min). The speed at which exhaustion occurred was considered as the maximum speed. Training intensity progressively increased from 40%$_{ms}$ on the first week, 50–55% from the 2nd to 4th week and 60%$_{ms}$ from the 5th to 7th week.

2.2. Concentration–response curves in isolated aorta

The thoracic aorta was carefully removed and placed in a freshly prepared Krebs solution containing (mM): NaCl, 118; $NaHCO_3$, 25; glucose, 5.6; KCl, 4.7; KH_2PO_4, 1.2; $MgSO_4$, 1.17 and $CaCl_2$, 2.5. All adherent tissue was removed and the arteries were cut into 3 mm rings. Each ring was suspended between two wire hooks and mounted in 5 ml organ chambers containing Krebs solution (pH 7.4) at 37 °C and continuously aerated with a mixture of 95% O_2 and 5% CO_2 under a resting tension of 1.5 g. The tissues isometric tension was recorded by a force displacement transducer (UgoBasile, Varese, Italy) connected to a PowerLab 400™ data acquisition system (ADInstruments Pty Ltd., Castle Hill, Australia).

After 1 h of equilibration, intact aorta rings were pre-contracted with phenylephrine (2 μM) and endothelium-dependent relaxation was assessed by cumulative concentration–response curves to acetylcholine (ACh, 10 nM–100 μM). Cumulative concentration–response curve to sodium nitroprusside (SNP, 100 pM–100 nM) was also calculated in pre-contracted rings. The following equation was used to determine whether the concentration–response data fit into a logistic function: $E = E_{max} / ((1 + (10^c / 10^x)^n) + \Phi)$, where E corresponds to the response; E_{max} to the maximum response that the agonist can produce; c to the logarithm of the EC_{50}, the concentration of agonist that produces half-maximum response; x to the logarithm of the concentration of agonist; the exponential term, n, to a curve fitting parameter that defines the slope of the concentration–response line and Φ to the response observed in the absence of added agonist. Nonlinear regression analysis to determine E_{max}, log EC_{50} and n was performed using GraphPad Prism (GraphPad Software, San Diego, CA, USA) with the constraint that Φ = zero.

2.3. Reactive oxygen species (ROS) detection

The oxidative fluorescent dye dihydroethidium was used to evaluate in situ superoxide production [6]. Transverse aorta sections (10 μm) obtained using a cryostat were incubated in phosphate buffer at 37 °C, for 10 min. Subsequently, fresh phosphate buffer containing hydroethidine (2 μM) was applied to each tissue section and incubated in a light-protected humidified chamber at 37 °C, for 30 min. Negative control sections received the same volume of phosphate buffer without hydroethidine. Images were obtained with an optical microscope (Olympus BX60, Olympus, Center Valley, PA, USA) equipped with rhodamine filter and camera (Olympus DP-72) using a 20× objective. The fluorescence was quantified using Image J software (National Institutes of Health, Bethesda, MD, USA).

2.4. Western blot analysis

The aortic tissue was homogenized in RIPA lysing buffer (Upstate, Temecula, CA, USA) and protein concentration determined using the Bradford method [3]. Samples containing 50 μg protein were loaded into gels prior to electrophoresis and proteins subsequently transferred by electroblotting onto polyvinylidene difluoride membranes and incubated in mouse anti Cu/Zn SOD (1:1500, SIGMA, St. Louis, MO, USA) primary antibody. Chemiluminescent signals (ECL plus Amersham, Piscataway, NJ, USA) were captured on X-ray film (Hyperfilm Amersham,

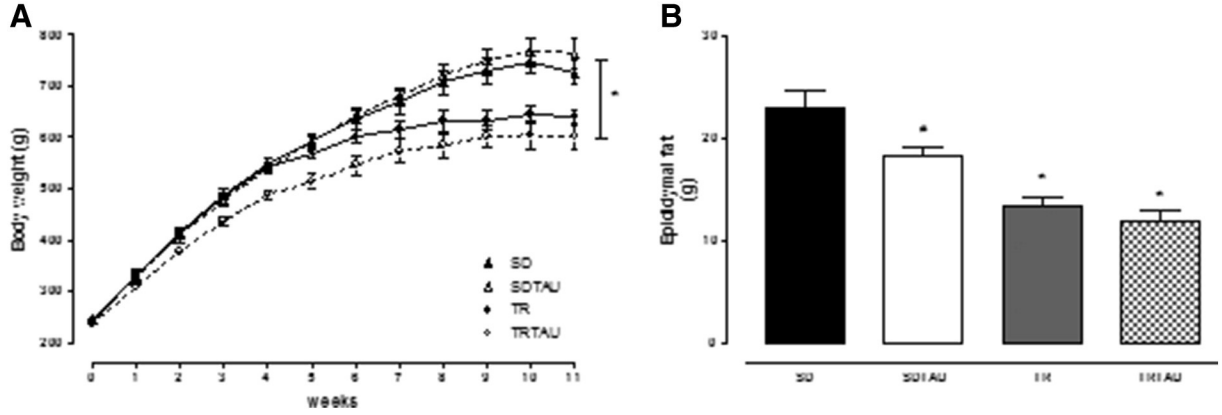

Fig. 1. Body weight gain (A) and epididymal fat mass (B) of sedentary (SD), sedentary supplemented with taurine (SDTAU), trained (TR) and trained supplemented with taurine (TRTAU) Wistar rats. Data are expressed as mean ± SEM of $n = 6–7$ per group. Two-way ANOVA, Tukey post-test ($P < 0.05$). #Taurine effect, *exercise effect.