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Aerobic exercise training prevents kidney lipid deposition in mice fed a cafeteria diet

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ABSTRACT

Aim: The objective of this study was to investigate the potential of aerobic exercise training (AET) to prevent kidney lipid accumulation and the contribution of renal metabolism to mediate this response.
Main methods: Male C57BL/6J mice were assigned into groups CHOW-SED (chow diet, sedentary; n = 13), CHOW-TR (chow diet, trained; n = 13), CAF-SED (cafeteria diet, sedentary; n = 13) and CAF-TR (cafeteria diet, trained; n = 13). AET consisted in running sessions of 60 min at 60% of maximal speed conducted five days per week for eight weeks.
Key findings: AET prevented weight gain in both trained groups. Food intake was not different among groups, however water intake, urine output, urine potassium and osmolarity were reduced in CAF-SED and CAF-TR groups. Kidney lipid deposition increased in CAF-SED (4.12 ± 0.5%/area) compared with CHOW-SED (1.7 ± 0.54%/area), and the AET prevented this increase in the CAF-TR group (2.1 ± 0.5%/area). The Bowman's capsule area decreased in CAF-SED and CAF-TR groups while the Bowman' space reduced in CAF-SED compared to CHOW-SED group, which was prevented by AET in the CAF-TF group. We observed a 27% increase in the p-AMPK expression in CAF-TR compared to CHOW-SED group without differences in the SIRT-1, PGC1-α, ACC and p-ACC. β-HAD activity increased in CAF-SED (43.9 ± 4.57 $nmol{\cdot}min^{-1}{\cdot}ug^{-1}$) and CAF-TR (44.7 ± 2.6 $nmol{\cdot}min^{-1}{\cdot}ug^{-1}$) groups compared to CHOW-SED (35.1 ± 2.9 $nmol{\cdot}min^{-1}{\cdot}ug^{-1}$) e CHOW-TR (36.6 ± 2.7 $nmol{\cdot}min^{-1}{\cdot}ug^{-1}$).
Significance: AET prevented kidney lipid accumulation induced by cafeteria diet and this response was not associated with changes in the renal metabolic activity that favors lipid oxidation.

1. Introduction

Changes in lifestyle and dietary habits have led to several health problems, such as obesity, dyslipidemia, Diabetes Mellitus type 2 (DM2) and hypertension. The excess of lipids has been indicated as the main factor triggering pathologies associated with this lifestyle, because they accumulate in non-adipose tissue leading to lipotoxicity. In the kidney, lipid accumulation can cause proteinuria, urinary podocyte loss, insulin resistance, oxidative stress, fibrosis, apoptosis and hypertrophy culminating in chronic kidney disease (CKD) [15]. Thus, excessive lipid deposition is a common mechanism involved in the CKD development in most of pathologies such as obesity, DM2 and metabolic syndrome [3].

Studies have been conducted to better understand the effects of lifestyle change and the ectopically accumulated lipids. In a previous report, it was demonstrated that a high-fat diet induced weigh gain, increased adiposity and renal lipid accumulation, which were associated with inflammation, glomerulosclerosis, mesangial matrix expansion and mitochondrial damage [34]. Our group previously demonstrated that a cafeteria diet increased body weight gain, adiposity, insulin resistance and total cholesterol in mice. Also, we observed that a cafeteria diet increased white adipose lipolysis and circulating free fatty acid (FFA), which may lead to lipotoxicity in peripheral tissues [12]. Thus, mice fed a cafeteria diet can be a good model to study ectopically accumulated lipids in the kidney.

Impairments in the regulation of lipid metabolic activity such as lipogenesis (triacylglycerol biosynthesis and accumulation in the intracellular lipid droplet), lipolysis (triacylglycerol hydrolysis) and fatty acid oxidation may lead to an increase in the renal lipid accumulation. Kume et al. [18] showed that the renal lipid accumulation in a high-fat diet model occurs due to an altered balance between lipolysis and

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lipogenesis, and this modification in renal lipid metabolism can be associated, at least in part, with decrease in AMP-activated protein kinase (AMPK) and increase in acetyl-CoA carboxylase (ACC) activity. Both proteins are crucial for lipid metabolism because while AMPK protein induces pathways that increase ATP (adenosine triphosphate) synthesis by providing more glucose and fatty acid for oxidation [19], the ACC modulates oxidative metabolism by inhibiting the activity of carnitine palmitoyl transferase 1 (CPT-1), an important fatty acid carrier protein in the mitochondria [14].

The aerobic exercise training (AET) has been used to treat and prevent metabolic damage associated with obesity and DM2 [12,21] because it promotes reduction in body weight and white adipose mass [12,23,25], and increases free fatty acid (FFA) oxidation in the skeletal muscle associated with better mitochondrial function [16]. Our previous study demonstrated that AET was able to prevent obesity, glucose intolerance and insulin resistance in mice fed a cafeteria diet by improving white adipose metabolism that favors fat oxidation (higher AMPK and lower ACC expression protein) instead of fat storage [12]. These data show that AET is a good non-pharmacological tool to increase lipid oxidation, however, this effect still needs to be investigated in the kidney.

Evidence in the literature has confirmed that AET provides improvement in renal function and morphology. In a previous study, Silva et al. [31] showed that AET decreased proteinuria in animals with DM induced by streptozotocin. Agarwal et al. [1] have shown that AET has beneficial morphofunctional effects on the kidney. Considering the role of lipid accumulation in the development and progression of kidney damage, and the effect of AET to improve lipid metabolism by increasing fatty acid oxidation, the objective of this study was to investigate the potential of AET to prevent kidney lipid accumulation and the contribution of renal metabolism to mediate this response. Our hypothesis is that the AET prevents kidney lipid accumulation induced by a cafeteria diet and this response is associated with the improvement of renal metabolic activity that favors lipid oxidation.

2. Materials and methods

2.1. Animals

Eight-week-old male C57BL/6J mice were assigned in groups CHOW-SED (chow diet, sedentary; n = 13), CHOW-TR (chow diet, trained; n = 13), CAF-SED (cafeteria diet, sedentary; n = 13) and CAF-TR (cafeteria diet, trained; n = 13). Animals were maintained under the same housing conditions (12-h light/12-h dark cycle, temperature 22 ± 2 °C) with free access to tap water and food ad libitum. All procedures were performed in accordance with the guidelines of the Brazilian College for Animal Experimentation and were approved by the Ethics Committee of the Faculty of Medicine of University of Sao Paulo (#18/2014).

2.2. Diets and aerobic exercise training

The standard chow diet contained 4% of kilocalories from fat, 55% from carbohydrate and 22% from proteins (Nuvilab®, Paraná, Brazil). The cafeteria diet contained 18.8% of kilocalories from fat, 55% from carbohydrate and 14.8% from proteins [11]. Diet and AET were started simultaneously. CHOW-TR and CAF-TR animals were submitted to AET as described by Ferreira et al. [7]. Animals were trained during the dark cycle (i.e., during their active period) on a motorized treadmill for 1 h/day at 60% of maximal velocity achieved in the running capacity test, five times per week for eight weeks. AET intensity was progressively increased and adjusted after the running capacity test done in the fourth week. To minimize the influence of the treadmill stress, sedentary mice were placed on the treadmill for 5 min twice weekly at 0.3 km/h during the experimental protocol.

2.3. Running capacity test

Running capacity was assessed before, in the fourth and eighth weeks of AET using a progressive test with a 0% incline on a treadmill as described by Ferreira et al. [7]. The initial speed was 0.4 km/h and the speed was increased by 0.2 km/h every 3 min until exhaustion of the animal, which was characterized by the impossibility of maintaining the standard rate. The test variable was quantified as maximum time to exhaustion (min).

2.4. Body weight control

Body weight was measured at the beginning and at the end of the AET protocol using a digital balance (Gehaka, Model BK4001, Brazil). Body weight gain was calculated as the difference between beginning body weight and final body weight.

2.5. Metabolic cages

In the 6th week of the protocol, the animals were housed individually in metabolic cages (Tecniplast, Buguggiate, VA, Italy) for 48 h. The first 24-h were used to adapt the mice to the environment and the following 24-h were used to collect urine. Food consumption and water intake were also monitored. The water balance was calculated through the water to urine ratio. Urine samples collected during 24-h period were used to determine urine output, creatinine, sodium, potassium chloride, protein excretion and osmolarity. Creatinine and total protein were quantified in spectrophotometer using colorimetric method (LABTEST Biochemical Kit, Brazil). In addition, electrolytes ($Na+$, $K+$ and $Cl-$) and osmolarity were measured in a gas analyzer (ABL800 FLEX, Radiometer Copenhagen) and osmometer (Vapor Pressure Osmometer 5520 USA), respectively. The creatinine clearance for assessing the Glomerular Filtration Rate (GFR) was calculated using the formula [(Urine (Creatinine) × Urine Vol) / Serum (Creatinine)]. The calculation of the water balance was performed using the formula Water intake / Urine output (mL/24-h).

2.6. Tissue and blood collection

Forty-eight hours after the end of the last training session, the animals were killed with an intraperitoneal injection of sodium pentobarbital (4 mg/100 g body weight). Kidneys were harvested, weighed and stored at −80 °C (right kidney) or fixed in 10% neutral-buffered formalin (left kidney) for subsequent histological analyses. The vena cava blood was collected and centrifuged at 4 °C (10.000*g* for 10 min) and serum was sent for creatinine analysis as described above.

2.7. Histological analysis

Glomerular injury was measured in paraffin sections of kidney (5 μm) stained with Picrossirius Red (Sigma). Digital images from thirty glomeruli per animal were obtained using a light microscope (Leica) at 200× magnification. After digitalization, Bowman's capsule area, glomerular tuft area, Bowman's space area and glomerular diameter were traced and calculated using a computerized morphometric analysis system (Image Pro-Plus 4.1; Media Cybernetics, Silver Spring, MD, USA).

Lipid content was measured using quantitative histochemistry of Oil Red O (Sigma-Aldrich) staining of kidney. Tissue sections (thickness 10 μm) obtained in a cryostat were examined by light microscopy at 200× magnification and analyzed by a computerized morphometric analysis system (Image Pro-Plus 4.1; Media Cybernetics, Silver Spring, MD, USA). The slides were counterstained with hematoxylin to visualize the nuclei. Lipid accumulation was determined in 20 images per animal based on the percentage of area occupied by lipid droplets. Histological analyses were conducted by CR Muller, blinded to mice