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N-acetylcysteine in high-sucrose diet-induced obesity: Energy expenditure and metabolic shifting for cardiac health

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ABSTRACT

To study the effects of N-acetylcysteine (NAC, C_5H_9–NO_3S) on high-sucrose diet-induced obesity and its effects on energy metabolism and cardiac oxidative stress, male *Wistar* 24 rats were divided into four groups ($n = 6$): (C) given standard chow and water; (N) receiving standard chow and 2 g/l N-acetylcysteine in its drinking water; (HS) given standard chow and 30% sucrose in its drinking water, and (HS-N) receiving standard chow, 30% sucrose and N-acetylcysteine in its drinking water.

After 30 days of the treatment, obesity was evidenced in HS rats from enhanced body weight, respiratory quotient, hypertriglyceridemia. As well depressed resting metabolic rate, and oxygen consumption per surface area. HS rats had triacylglycerol accumulation, oxidative stress and metabolic shifting in cardiac tissue. NAC enhanced fat oxidation and energy expenditure, normalizing these adverse effects, comparing HS-N and HS rats. The beta-hydroxyacyl coenzymne-A dehydrogenase activity was higher in HS-N animals, indicating higher heart fatty acid degradation than in HS. NAC normalized myocardial glycogen and lactate dehydrogenase activity, comparing HS-N and HS rats, but had no effects on calorimetric and biochemical parameters in standard-fed rats, comparing N and C groups.

In conclusion, N-acetylcysteine offers promising therapeutic value in prevention of high-sucrose induced-obesity and its effect on cardiac tissue. N-acetylcysteine reduced the oxidative stress and prevented the metabolic shifting in cardiac tissue, enhancing fatty acid oxidation and reducing anaerobic metabolism in high-sucrose-fed conditions. The application of this agent in food system via exogenous addition may be feasible and beneficial for antioxidant protection and energy metabolism in cardiac tissue.

1. Introduction

Cardiac diseases represent one of the main health problems associated with premature mortality. Although the mechanisms that are triggered are not yet well established, several alterations in energy metabolism of the heart have been associated with cardiovascular pathologies [1].

Cardiac muscle utilizes a variety of substrates to produce energy, and the heart can shift from one substrate to another depending on food intake and pathophysiological state [2]. The pathways involved in the energy metabolism are especially susceptible to changes in the diet compound [3], and an increasingly preference for high-sucrose diet has been associated with enhanced incidence of obesity [4], and cardiovascular disease [5]. However, the mechanisms for these actions are yet poorly understood.

There is a growing awareness that during energy metabolism, the mitochondrial respiratory chain represents a major intracellular source of reactive oxygen species (ROS). Alterations in food constituents [3,6], or substrate for energy generation may result in higher ROS, thus inducing oxidative stress, an imbalance between oxidants and antioxidants systems in favor of the former. The oxidative stress depresses myocardial energy [7,8], and promotes an inhibitory effect on myocardial function [9]. Therefore, any strategy to reduce oxidative damage would have significant health protective effects.

N-acetylcysteine (NAC, $C_5H_9NO_3S$), as a precursor of reduced glutathione (GSH), an important compound of the antioxidant system has been successfully used in various pathological conditions [10–14], including inhibition of sucrose-induced insulin resistance and serum oxidative stress [15,16]. Researches in our laboratory

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showed that low concentration of NAC prevented dyslipidemic profile and hyperglycemia, reducing the hepatic oxidative stress in high-sucrose fed rats [17]. However, it was not studied whether NAC intake can inhibit the myocardial oxidative stress and can improve the energy metabolism in cardiac tissue of high-sucrose-fed rats, which certainly would bring new insights on high-sucrose related cardiovascular disease control.

Studies on NAC actions have been reported in a scattered fashion, but its effects on cardiac energy metabolism and oxidative stress had drawn little attention until recently. To the best of our knowledge this is the first study that evaluated the NAC effects on oxidative stress and the association with some markers of metabolic pathways in cardiac tissue of rats previously submitted to experimental obesity due high-sucrose intake.

Thus, the major purpose of the present study was to investigate the effects of N-acetylcysteine supplementation on high-sucrose diet-induced obesity and its action on energy expenditure, as well on myocardial oxidative stress and energy metabolism in cardiac tissue of high-sucrose fed rats.

2. Methods

2.1. Animals and experimental procedure

All experiments and procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals of U.S. National Institute of Health and were approved by the Ethical Committee, Institute of Biological Sciences, São Paulo State University, UNESP, SP, Brazil. Twenty-four male *Wistar* rats, 75 days old were individually housed in polypropylene cages in an environmentally controlled clean-air room, $22 \pm 3\,^{\circ}C$, 12 h light and dark cycle, $60 \pm 5\%$ relative humidity. The rats received water and a rodent chow (Purina, Campinas, SP, Brazil) containing 20.0% protein, 46.5% carbohydrate, 3.8% fat, 4.5% fibre (by weight), and 12.55 kJ/g total metabolizable energy. The animals were randomly assigned to one of four groups ($n = 6$/group). Rats in the control group (C) were given free access to a standard chow and water. The (N) group received standard chow and 2 g/l N-acetylcysteine (NAC, $C_5H_9NO_3S$) in its drinking water. The (HS) group received the standard chow and 30% sucrose in its drinking water. The (HS-N) group was fed standard chow and received both 30% sucrose and N-acetylcysteine (2 g/l) in its drinking water. Food and drinking solutions consumption were measured daily at the same time (9:00–10:00 h). The body weights were determined once a week. The body weight was used to determine the surface area = body weight$^{0.7}$ [18]. Based on food intake, sucrose consumption, caloric value of rodent chow (12.55 kJ/g) and sucrose (16.74 kJ/g), the total energy intake (EI) was calculated; EI = [mean food consumption/day (g) × 12.55] + [Amount of sucrose intake/day (g) × 16.74] = kJ/day.

2.2. Indirect calorimetry

After 28 days of the treatments, rats were placed into metabolic chambers (airflow = 1.0 l/min) of a computer-controlled indirect calorimeter (CWE, Inc, St. Paul, USA) to determine the calorimetric parameters of fed state. The determinations were made in the morning (8:00–11:00 h). For assessment of fasting calorimetric parameters, rats were fasted overnight (12–14 h) and so placed into metabolic chambers. Respiratory quotient (RQ) and energy expenditure, namely resting metabolic rate (RMR) were measured using a respiratory-based software program (software MMX, CWE, Inc., USA). Average oxygen consumption (VO_2) and average carbon dioxide production (VCO_2) were integrated over periods of 15 min. Carbohydrate and fat oxidation were calculated from the no protein oxygen consumption, their relative oxidative proportions and the amount of oxygen consumed per gram of substrate oxidized [19]: carbohydrate oxidation = $VO_2 \times (RQ - 0.707)/0.293 \times 0.746$; fat oxidation = $VO_2 \times (1 - RQ)/0.293 \times 0.746$. Where VO_2 is measured in liters per minute, 1.00 is the RQ for total carbohydrate oxidation, 0.707 is the RQ for total fat oxidation, 0.293 is the difference between 1.000 and 0.707, 0.746 is the number of liters of oxygen consumed per gram glucose oxidized.

2.3. Biochemical determinations

After 30 days of treatment, rats were fasted overnight (12–14 h), anaesthetized with 0.1 ml ip of 3% sodium pentobarbital and sacrificed by decapitation. Blood was placed into a centrifuge tube and allowed to clot to obtain the serum. The serum was separated by centrifugation at $1400 \times g$ for 10 min. Triacylglycerol (TG) was determined in serum by enzymatic method (test kit CELM, Modern Laboratory Equipment Company, São Paulo, Brazil).

The heart was rapidly removed and the cardiac adipose tissue was discarded. The heart was weighed and the left ventricle was divided into three parts. Cardiac samples of 200 mg of left ventricle were homogenized in 0.6 M perchloric acid and the amount of free glucose was determined using glucose oxidase procedure (test kit CELM, Modern Laboratory Equipment Company, São Paulo, Brazil). Tissue glycogen was then hydrolyzed to glucose directly in homogenates by treatment with amyloglucosidase, and total glucose released was measured. Glycogen concentration was calculated as the difference between total and free glucose [20]. The myocardial triacylglycerol was extracted with chloroform:methanol at a ratio of 2:1, from the second piece of the left ventricle [21].

Cardiac samples of 200 mg were homogenized in 5 mL of a cold 0.1 M phosphate buffer, pH 7.4. Tissue homogenates were prepared in a motor-driven Teflon-glass *potter elvehjem* tissue homogenizer (1 min, $100 \times g$). The homogenate was centrifuged at 10,000 *g*, for 15 min and the supernatant was used for total protein [22], lipid-hydroperoxide, antioxidant capacity, or total antioxidant substances and metabolic enzymes determinations.

The total antioxidant substances (TAS), or total antioxidant capacity was quantified in cardiac tissue by the amount of ABTS [2,2′-azinobis-(3-ethylbenzthiazoline-6-sulfonate)] radical anions that was able to react with a thermolabile azo compound, 2,2′-azobis-(2-amidinopropane) (ABAP), and expressed as the percentage of trolox antioxidant capacity (test-kit Randox Laboratories, Crumlin, Co., Antrim, United Kingdom).

The lipid hydroperoxide (LH) was measured through hydroperoxide-mediated oxidation of Fe^{2+}, with 100 μl of sample and 900 μl of a reaction mixture containing 250 μM $FeSO_4$, 25 mM H_2SO_4, 100 μM xylenol orange and 4 mM butylated hydroxytoluene (BHT) [23].

The cardiac energy metabolism was assessed by lactate dehydrogenase (LDH; E.C.1.1.127.), β-hydroxyacyl coenzyme-A dehydrogenase (OHADH; E.C.1.1.1.35.) and citrate synthase (CS; E.C.4.1.3.7.) determinations [24]. The assay medium for LDH contained 50 mM Tris-HCl buffer pH 7.5, 0.15 mM nicotinamide adenine dinucleotide reduced-form (NADH) and 1 mM pyruvate. OHADH was assayed in a medium containing 50 mM Tris-HCl buffer pH 7.0, 5 mM EDTA, 0.45 mM NADH, and 0.1 mM acetoacetyl-coenzyme-A. For CS activity the assay medium consisted of 50 mM Tris-HCl buffer pH 8.1, 0.3 mM acetyl-CoA, 0.1 mM 5,5′-dithiobis-(2-nitrobenzoic) acid (DTNB) and 0.5 mM oxaloacetate.

Enzyme activities were performed at 25 °C using a micro-plate reader (μQuant-MQX 200 with Kcjunior software to computer system control, Bio-Tec instruments, Winooski, Vermont, USA.). The spectrophotometric determinations were performed in a Phar-