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RESEARCH ARTICLES

High dietary salt decreases antioxidant defenses in the liver of fructose-fed insulin-resistant rats

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Abstract

In this study we investigated the hypothesis that a high-salt diet to hyperinsulinemic rats might impair antioxidant defense owing to its involvement in the activation of sodium reabsorption to lead to higher oxidative stress. Rats were fed a standard (CON), a high-salt (HS), or a high-fructose (HF) diet for 10 weeks after which, 50% of the animals belonging to the HF group were switched to a regimen of high-fructose and high-salt diet (HFS) for 10 more weeks, while the other groups were fed with their respective diets. Animals were then euthanized and their blood and liver were examined. Fasting plasma glucose was found to be significantly higher (approximately 50%) in fructose-fed rats than in the control and HS rats, whereas fat liver also differed in these animals, producing steatosis. Feeding fructose-fed rats with the high-salt diet triggered hyperinsulinemia and lowered insulin sensitivity, which led to increased levels of serum sodium compared to the HS group. This resulted in membrane perturbation, which in the presence of steatosis potentially enhanced hepatic lipid peroxidation, thereby decreasing the level of antioxidant defenses, as shown by GSH/GSSG ratio (HFS rats, 7.098±2.1 versus CON rats, 13.2±6.1) and superoxide dismutase (HFS rats, 2.1±0.05 versus CON rats, 2.3±0.1%), and catalase (HFS rats, 526.6±88.6 versus CON rats, 745.8±228.7 U/mg ptn) activities. Our results indicate that consumption of a salt-rich diet by insulin-resistant rats may lead to regulation of sodium reabsorption, worsening hepatic lipid peroxidation associated with impaired antioxidant defenses.
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Keywords: high-salt diet; fructose-fed rats; oxidative stress; steatosis; antioxidant defenses

1. Introduction

Increasing evidence suggests the involvement of oxidative stress in insulin resistance [1] and has generated high interest in the role of free radicals in the maintenance of adequate levels of antioxidant defenses [2,3]. In type 2 diabetes, a significant inverse correlation exists between hepatic fat load and the antioxidant defense system [4], which may prevent generation of an adequate compensatory response for restoration of cellular redox balance [5]. In particular, changes have been demonstrated in some components of the free radical defense system in different models [6–8].

We observed that the expression of genes encoding the antioxidant enzymes glutathione peroxidase (GPx), gamma-glutamylcysteine synthetase and superoxide dismutase (SOD) decreased in the liver tissue of streptozotocin-induced diabetic rats because of increased oxidative stress [9] associated with overproduction of reactive oxygen species (ROS)[10]. Under normal conditions, almost all of the produced superoxide anions (O_2^-) are converted to hydrogen peroxide (H_2O_2) by the action of SOD, which is further detoxified to water by catalase (CAT) or GPx [11]. However hyperglycemia induce the overproduction of O_2^- [12] and dramatic change in the oxidant/antioxidant balance has been postulated to play a role in the pathogenesis of diabetes.

Increasing the sugar intake has been reported to result in dyslipidemia, as indicated by elevated levels of serum triglycerides, cholesterol, and low-density lipoproteins [13,14].These underlying metabolic disturbances appear to induce insulin resistance commonly observed in high-fructose fed human and animal models [15] when fructose consumption causes progressive liver disease stimulated

Abbreviations: AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; CAT, Catalase; GFR, Glomerular filtration rate; GSH, Reduced glutathione; GSSG, Oxidized glutathione; GPx, Glutathione peroxidase (GPx); HOMA, Homeostasis model assessment; ROS, Reactive oxygen species; O_2^-, Superoxide anions; SOD, Superoxide dismutase; TBARS, Thiobarbituric acid reactive substances.

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lipogenesis [16]. Furthermore, despite recent advances in elucidating the pathogenesis of related conditions, studies have shown that presence of insulin resistance and compensatory hyperinsulinemia would lead to sodium retention [17].

Therefore in the present study we examined whether a high-salt diet could impair antioxidant defenses in the liver of fructose-fed rats due activation of enhanced renal sodium reabsorption potentiating oxidative stress.

2. Materials and methods

2.1. Animal and diets

Forty-five male 12-week-old Fischer rats, weighing approximately 300 g, were individually housed in a temperature-and humidity-controlled room under a 12 h light/dark regimen. Initially, the rats were randomly assigned to three experimental groups (n=10–12) as follows: the control group (CON), fed with the AIN93M diet [18] and water; the high-salt group (HS), fed with the AIN93M diet plus 8% w/w NaCl and water; and the high-fructose group (HF), fed with the AIN93M diet and a 20% w/v fructose solution as drinking water. After 10 weeks of treatment, the animals belonging to the HF group were further divided into 2 groups: rats that continued to be fed on the fructose solution (HF) and rats that were switched to a high-fructose + high-salt regimen (HFS) for 10 more weeks. Details of the experimental diets are given in Table 1. Food and water were provided ad libitum and their intake was measured. At the end of the experimental period, the rats were fasted for 12 hours, anesthetized with isoflurane and euthanized by total blood collection from the brachial plexus. The blood was centrifuged at 1500g for 15 min. One liver lobule from each animal was separated for histological analysis and the rest was frozen at −80°C until further analysis. All the procedures were approved by the Ethical Committee for Animal Care and Use of the Federal University of Ouro Preto.

2.2. Biochemical determinations

Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) activities and plasma glucose concentration were determined using commercial kits from Labtest Diagnostica SA (Lagoa Santa, MG, Brazil) # 108, 109 and 84, respectively, by following the manufacturer's instructions. ELISA was utilized to quantify plasma insulin and leptin levels using commercial kits from Ultra Sensitive Rat Insulin ELISA, Crystal Chem Downers Grove, IL, USA, and Rat Leptin ELISA Kit, Linco Research, USA, (Catalog #90060 and #90040, respectively). The homeostasis model assessment (HOMA), described by Matthews et al. [19] as a measure of insulin resistance, was calculated using the formula [insulin (µmol/ml)×glucose (mM/L)/22.5]. Hepatic fat was extracted using a chloroform-methanol mixture (2:1, v/v) according to the method of Folch et al. [20] and the total lipids were quantified gravimetrically by evaporating the solvents in the extract. Sodium concentrations were measured by flame photometry (Olidef model C-71 apparatus; São Paulo, Brazil).

2.3. Antioxidant defenses and oxidative stress

Liver SOD activity was measured by the method of Marklund and Marklund [21]. One unit of SOD activity was defined as the amount of enzyme that inhibited the rate of autoxidation of pyrogallol by 50%, which was determined at 570 nm. Catalase activity was measured according to Aebi [22] and was expressed in units per milligram of protein using the extinction coefficient of 0.0394 L/mmol/L/cm. The rate of H_2O_2 decomposition was followed by monitoring absorption at 240 nm in 50 mM phosphate buffer, pH 7.0, containing 5 mM H_2O_2. Tissue protein content was determined according to the method developed by Lowry et al. [23] using bovine serum albumin as the standard. The total glutathione (GSH + GSSG) was measured after precipitation of proteins with an equal volume of 4% sulfosalicylic acid using the enzymatic method previously described [24]. Oxidized glutathione (GSSG) was determined after derivatization of total GSH with 2-vinylpiridine. Oxidative stress index was calculated from the GSH/GSSG ratio and by lipid peroxidation status through of levels of thiobarbituric acid reactive substances (TBARS) as described by Buege and Aust [25].

Table 1
Diets composition

Ingredient	Composition (g/kg diet)			
	CON	HS	HF[3]	HFS[3]
Starch	622.5	542.5	622.5	542.5
Casein	140.0	140.0	140.0	140.0
Sucrose	100.0	100.0	100.0	100.0
Salt	-	80.0	-	80.0
Cellulose	50.0	50.0	50.0	50.0
Fat	40.0	40.0	40.0	40.0
[1]Minerals	35.0	35.0	35.0	35.0
[2]Vitamins	10.0	10.0	10.0	10.0
Choline	2.5	2.5	2.5	2.5
Energy content (kcal/kg)	3810	3490	3810	3490

[1]Mineral mixture for AIN93M; [2]Vitamin mixture for AIN93M; [3]D-Fructose (SynthLab-synth, São Paulo, Brazil).

2.4. Liver histology

After removal from each animal, the livers were immediately fixed in 10% buffered formaldehyde, embedded in paraffin, cut (4-µm thickness) and mounted on glass slides. The sections were deparaffinized in xylene, stained with hematoxylin and eosin (H&E) using the standard technique and then examined. Histological examination of the slides was performed by using concentrated light microscope equipped with photographic digital camera (DM5000; Leica) with software Qwin Plus. Scoring of the slides was performed using a semi-quantitative method reported by Brunt et al. [26]. Fat degeneration was graded according to the percentage of fat-containing hepatocytes. Grade of vesicular steatosis according to the original system involved 10 grades, whereas in this system, steatosis was graded from 0–4 based on the percentage of hepatocytes involved in the biopsy (0, none; 1, 10%; 2, 10–33%; 3, 33–66% and 4, >66%).

2.5. Statistical analysis

Normality of the sample distribution for each continuous parameter was tested with the Kolmogorov–Smirnov test. The significance of any differences in proportions of medians was tested with Kruskal–Wallis test and in means by one-way analysis of variance (ANOVA), followed by Dunns and Tukey tests, respectively. Correlation analysis was used to measure the degree to which 2 variables were related. Significance for all measures was defined when $P<.05$. GraphPad Prism version 5.00 for Windows (San Diego, CA, USA) was used for statistical analyses.

3. Results

Notably, the mean food intake was significantly different amongst different dietary groups. In relation to the control group, high dietary NaCl resulted in a lower caloric value leading to higher food intake in HS rats ($P<.01$). On the other hand, fructose supplementation led to lower food intake ($P<.001$) due to its energy content (Fig. 1A). Mean values for liquid intake were significantly higher in the high-salt groups ($P<.001$) than in the CON and HF groups (Fig. 1B). Higher fructose intake lowered the energetic demand for solid food ($P<.001$) in HFS rats than in other groups (Fig. 1C). The high-salt diet led to increased liquid intake, thus compelling HS and HFS rats to drink approximately 4.0 and 3.0 times the liquid volumes consumed by the CON animals, respectively, resulting in the corresponding differences in the liquid calorie intake of HFS rats drinking the fructose solution ($P<.001$) in relation to HF rats (Fig. 1D). There were no significant differences in the total energy intake (Fig. 1E). Moreover, plasma leptin concentrations in HF rats was higher ($P<.01$) compared to that in other groups (Fig. 1F).

The average final body weight was lower in HFS rats than in HF and CON rats ($P<.05$). Relative liver weights were higher ($P<.05$) in fructose-fed rats (HF and HFS) and a corresponding increase was observed in the liver lipid content and plasma glucose in fructose-fed rats in relation to CON ($P<.05$) and HS ($P<.001$). The highest insulin concentration was found in HFS rats ($P<.05$) and HOMA analysis revealed that this group had significantly higher values than the controls animals ($P<.01$), thereby indicating that the combination of dietary fructose with NaCl in HFS rats impaired insulin response. For determination of whether the dietary treatment induced liver injury, serum AST and ALT activities were examined, and ALT but not AST in the HF group were found to be significantly higher than that of the control group ($P<.05$). Augmented natriuretic response to a high-salt diet significantly decreased serum sodium in HS rats compared to that in other groups ($P<.05$) and particularly HFS showed a substantial increase in relation to the HS group (Table 2).

Photomicrographs of hepatic specimens stained with H&E are shown in Fig. 2A–D, and the scores of histological variables are presented as medians in Fig. 2E. Mild or no hepatic steatosis occurred in CON rats (Fig. 2A). HS rats did not show predominant occurrence of steatosis, but hyperemic vessels were observed in the parenchyma

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