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Long-term fructose-enriched diet introduced immediately after weaning does not induce oxidative stress in the rat liver

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ABSTRACT

Increased fructose consumption is correlated with the rising prevalence of obesity, metabolic syndrome, and type 2 diabetes. It is believed that reactive oxygen species contribute to the development and progression of metabolic disturbances, especially those associated with insulin resistance. Dietary fructose produces both pro-oxidative and antioxidative effects, depending upon the experimental conditions, dosage, duration of treatment, and pathophysiological milieu. The effects of fructose overconsumption on young populations, which have an increased risk of developing metabolic disorders in adulthood, have not been fully elucidated. We have previously shown that rats subjected to a long-term fructose-enriched diet immediately after weaning display impaired hepatic insulin sensitivity. In this study, we tested the hypothesis that long-term fructose consumption induces alterations in the redox setting of the liver. Starting from the 21st day after birth, male Wistar rats were maintained for 9 weeks on a standard diet (control) or a fructose-enriched diet that consisted of standard food and 10% fructose solution instead of drinking water. The expression and activity of antioxidant enzymes as well as lipid peroxidation and protein damage markers were measured. The results showed that a fructose-enriched diet led to an increased expression of mitochondrial manganese superoxide dismutase but did not affect antioxidant enzymes activity, lipid peroxidation, thiol content, and the level of protein oxidation. Therefore, our results suggest that the decrease in hepatic insulin sensitivity that was previously observed in rats that were kept on the same diet regime might be attributed to molecular mechanisms other than redox disbalance. A possible fructose-related micronutrient deficiency should be examined.

1. Introduction

High-calorie diets and sedentary lifestyles have been correlated with the rising prevalence of obesity, metabolic syndrome, type 2 diabetes, and coronary diseases [1,2]. Widespread overconsumption of solid fats and added sugars, especially fructose-sweetened beverages, is the hallmark of modern lifestyles and particularly prominent in young populations [3]. Although young organisms and adults differ largely by their metabolic and physiological profiles, most of the previous studies

Abbreviations: CAT, catalase; iNOS, inducible nitric oxide synthase; GPx, glutathione peroxidase; GR, glutathione reductase; Hsp, heat shock protein; SOD1, copper-zinc superoxide dismutase 1; SOD2, mitochondrial manganese superoxide dismutase 2; TBARS, thiobarbituric acid reactive substances.

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investigated fructose-induced metabolic disturbances in adults. Therefore, the link between increased fructose consumption in childhood and the development of metabolic disorders in adulthood is still not clear.

The fructose-fed rat represents a commonly used animal model for studying diet-induced metabolic disturbances [4]. Previous animal studies have shown that fructose-rich diets can induce most of the features of metabolic syndrome, including hypertension, insulin resistance, abdominal obesity, hepatic steatosis, endothelial dysfunction, and inflammation [5,6]. It has been suggested that oxidative stress participates in the development and progression of these metabolic disturbances [7,8].

Oxidative stress is a condition that occurs when the prooxidants (radicals) overwhelms the antioxidant defense. The antioxidant defense system includes antioxidant enzymes, such as superoxide dismutase (SOD); catalase (CAT); glutathione peroxidase (GPx); glutathione reductase (GR); and nonenzymatic low-molecular weight compounds, including reduced glutathione; vitamins A, E, and C; beta-carotene; and bilirubin. In addition, numerous proteins, including heat shock proteins (Hsp): Hsp70 and Hsp90, assist in the repair of oxidatively damaged cellular biomolecules.

The induction of some metabolic syndrome features by chronic fructose feeding or by a single dose of fructose [9] was associated with oxidative stress and disruption of antioxidant mechanisms [10]. However, a large discrepancy in the course and the intensity of fructose-induced alterations in the functioning of antioxidant enzymes has been observed. Protective effects of fructose and its phosphorylated forms after short-term application were also demonstrated in oxidative stress–related conditions, and several studies have reported their antioxidative and cytoprotective effects [11-14]. In general, it appears that negative effects of fructose mostly emerge after long-term exposure, whereas its acute application seems to protect the cells and can be beneficial under some pathophysiological conditions [11].

Our previous studies show that a 9-week fructose-enriched diet, applied immediately after weaning, led to a decrease in insulin sensitivity, alterations of lipid metabolism, and low-grade inflammation in rat livers [15,16]. The question now though is whether the same diet regime promotes oxidative stress, which may be responsible for previously observed metabolic disturbances, such as decreased hepatic insulin sensitivity. In this study, we tested the hypothesis that long-term fructose consumption in the period from weaning to adulthood induces alterations in the redox setting of the liver, as the main fructose-metabolizing tissue. To test this hypothesis, we measured the expression and the activity of antioxidant enzymes as well as the level of markers of lipid and protein damage in the liver of rats subjected to a fructose-rich diet immediately after weaning.

2. Methods and materials

2.1. *Animals and diets*

Male Wistar rats (21 days old) were randomly divided into 2 experimental groups with nine rats each. The control group (C) was provided with standard chow and drinking water, and the fructose group (F) was provided with the same chow but 10% (wt/vol) fructose solution instead of the drinking water. Both experimental groups had access to food and drinking fluid for 9 weeks ad libitum. The detailed composition of the diets is presented in Table 1. The choice of fructose concentration was based on data stating that 10% fructose solution closely resembles the intake of sweet beverages that are characteristic of the Western diet [17]. Rats were kept under standard conditions, at 22°C with a 12-hour light/dark cycle. During the 9 weeks of treatment, food and liquid intake was measured daily. Caloric intake was calculated as the sum of calories consumed as both food and liquid. Body mass was measured weekly, whereas the livers were weighed immediately after sacrifice. The procedures complied with the EEC Directive (86/609/EEC) on the protection of animals used for experimental and other scientific purposes, and they were approved by the Ethical Committee for the Use of Laboratory Animals of the Institute for Biological Research "Siniša Stanković," University of Belgrade.

2.2. *Tissue collection and determination of glucose concentration in the blood*

After overnight fasting, rats were euthanized by rapid decapitation. Trunk blood was quickly collected into EDTA-containing tubes and agitated slowly. Glucose concentration in the blood was immediately measured with MultiCare strips (Biochemical Systems International, Arezzo, Italy). After blood collection, the livers were perfused with cold 0.9% NaCl, excised, and stored in liquid nitrogen.

2.3. *Tissue preparation and determination of antioxidant enzymes activity*

For preparation of whole cell extracts, the livers were homogenized in 10 vol (wt/vol) of Tris buffer (50 mM Tris, 0.25 M sucrose, 1 mM EDTA, pH 7.4) and sonicated (3 × 10 s at 10 MHz on ice),

Table 1 – Diet composition

Ingredient	Standard diet	Fructose-enriched diet
Fructose solution (g/L)	–	100
Protein	20%	20%
Lysine	0.90%	0.90%
Methionine + cystine	0.75%	0.75%
Cellulose	8%	8%
Phosphorus	0.50%	0.50%
Sodium	0.15%-0.25%	0.15%-0.25%
Calcium	1%	1%
Manganese (mg/kg)	30	30
Copper (mg/kg)	20	20
Zinc (mg/kg)	100	100
Iron (mg/kg)	100	100
Iodine (mg/kg)	0.5	0.5
Selenium (mg/kg)	0.1	0.1
Vitamin A (IU/kg)	10000	10000
Vitamin D_3 (IU/kg)	1600	1600
Vitamin E (mg/kg)	25	25
Vitamin B_{12} (mg/kg)	0.02	0.02

Control animals were fed with commercial standard chow and drinking water, and rats on fructose-enriched diet were fed with the same chow and 10% (wt/vol) fructose solution instead of drinking water. Animals were exposed to these diets during 9 weeks.