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The inflammatory profile and liver damage of a sucrose-rich diet in mice

Liliane Soares C. Oliveira, Daiane A. Santos, Sandra Barbosa-da-Silva, Carlos A. Mandarim-de-Lacerda, Marcia B. Aguila*

Laboratory of Morphometry, Metabolism and Cardiovascular Disease, Biomedical Center, Institute of Biology, State University of Rio de Janeiro, Rio de Janeiro, Brazil

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Abstract

It is still unclear if an isoenergetic, sucrose-rich diet leads to health consequences.
Aims: To investigate the effects of excessive sucrose within an isoenergetic diet on metabolic parameters in male C57BL/6 mice.
Methods: Animals were fed a control diet (10% fat, 8% sucrose — SC group), a high-sucrose diet (10% fat, 32% sucrose — HSu group), a high-fat diet (42% fat, 8% sucrose — HF group) or a high-fat/high-sucrose diet (42% fat, 32% sucrose — HF/HSu group) for 8 weeks.
Results: Mice fed HF and HF/HSu diets gained more body mass (BM) and more body adiposity than SC- or Hsu-fed mice. Despite the unchanged BM and adiposity indices, HSu mice presented adipocyte hypertrophy, which was also observed in the HF and HF/HSu groups ($P<.0001$). The HF, HSu and HF/HSu mice were glucose intolerant and had elevated serum insulin levels ($P<.05$). The levels of leptin, resistin and monocyte chemotactic protein-1 increased, while the serum adiponectin decreased in the HF, HSu and HF/HSu groups ($P<.05$). In the adipose tissue, the HF, HSu and HF/HSu groups showed higher levels of leptin expression and lower levels of adiponectin expression in comparison with the SC group ($P<.05$). Liver steatosis was higher in the HF, HSu and HF/HSu groups than in the SC group ($P<.0001$). Hepatic cholesterol was higher in the HF and HF/HSu groups, while hepatic TG was higher in the HSu and HF/HSu groups ($P<.05$). In hepatic tissue, the sterol receptor element-binding protein-1c expression was increased in the HF, HSu and HF/HSu groups, unlike the peroxisome proliferator-activated receptor-alpha expression that decreased in the HF, HSu and HF/HSu groups in comparison with the SC group ($P<.05$).
Conclusion: A sucrose-rich diet does not lead to a state of obesity but has the potential to cause changes in the adipocytes (hypertrophy) as well as glucose intolerance, hyperinsulinemia, hyperlipidemia, hepatic steatosis and increases in the number of inflammatory cytokines. The deleterious effects of a sucrose-rich diet in an animal model, even when the sucrose replaces starch isocalorically in the feed, can have far-reaching consequences for health.

Keywords: High-sucrose diet; High-fat diet; Adipose tissue; Liver; Adipokines

1. Introduction

Aside from the total energy intake, dietary variety causes different metabolic patterns depending on the sources of energy. The high-fat (HF) diet has been well studied, and periods of weight cycling have been associated with increases in body mass (BM) in each cycle of HF feeding, which have been correlated with disturbances in the oral glucose tolerance test (OGTT), in blood lipids and in the serum and adipose tissue expression of adipokines. These disturbances are not fully recuperated during the lean period of weight cycling. Moreover, the longer the HF feeding is, the more severe the adiposity becomes [1,2].

An increasing recent interest in the role of dietary carbohydrate types (and, by implication, of sugar intake) in preventing or promoting lipid and carbohydrate metabolism disorders has stimulated more research on the association between the metabolic syndrome and the prevalence of type 2 diabetes and cardiovascular disease [3–5]. When sugars, such as abundant as those in many soft drinks (e.g., sucrose and fructose), are consumed in high quantities, the consumption can be associated with an increased prevalence of obesity, diabetes [6] and cardiovascular risk [7].

Sucrose is a disaccharide that consists of a glucose monomer and a fructose monomer connected by glycosidic linkages. Several studies have suggested that diets high in simple sugars exert a high glycemic load and represent a major cause of obesity and metabolic syndrome in humans [8,9]. A considerable number of recent studies have been concerned with fructose consumption, and these studies may have implications for sucrose, which is the major source of dietary fructose worldwide. The conclusions of these studies have been controversial. High fructose consumption has been considered to augment visceral adiposity, lipid dysregulation, and insulin resistance [5], having similar effects to those of sucrose consumption [10]. Usual fructose consumption alone apparently does not lead to adverse effects [11], but when fructose is consumed in caloric excess, adverse effects can

* Corresponding author. Universidade do Estado do Rio de Janeiro, Centro Biomedico, Instituto de Biologia, Laboratorio de Morfometria, Metabolismo e Doença Cardiovascular. Av. 28 de Setembro 87 fds, Rio de Janeiro, 20551-030 RJ, Brasil. Tel.: +55 21 2868 8316; fax: +55 2868 8033.
E-mail address: marciaguila@gmail.com (M.B. Aguila).
URL: http://www.lmmc.uerj.br (M.B. Aguila).

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occur [12]. However, whether isoenergetic, sucrose-rich diets lead to health consequences remains unclear.

Thus, the present study was designed to investigate the effects of excessive sucrose within an isoenergetic diet on metabolic parameters in an animal model compared to an HF diet. Furthermore, we analyzed lipid and carbohydrate metabolism, adiposity and adipokines for their inflammatory responses and liver structure.

2. Materials and methods

2.1. Animals and diet

The animal protocol was approved by the Animal Ethics Committee of the State University of Rio de Janeiro (protocol number CEUA/037/2011), and the procedures were performed in accordance with the guidelines for experimentation with animals [National Institutes of Health (NIH) Publications No. 85-23, revised 1996].

Male C57BL/6 mice (3 months old) were housed under a controlled temperature (21±1°C), humidity (60%±10%) and 12-h light/dark cycle (1:00 AM to 1:00 PM light) with free access to food and water.

The mice were randomly separated into four groups (n=10 per group, total 40) and fed with different diets during an 8-week period, as follows:

a) Standard chow (SC group; 10% energy from fat, 8% energy from sucrose, 3.8 kcal/g diet)
b) HF diet (HF group; 42% energy from fat, 8% energy from sucrose, 4.7 kcal/g diet)
c) High-sucrose diet (HSu group; 10% energy from fat, 32% energy from sucrose, 3.8 kcal/g diet)
d) HF–high-sucrose diet (HF/HSu group; 42% energy from fat, 32% energy from sucrose, 4.7 kcal/g diet)

The diets were manufactured in accordance with the American Institute of Nutrition's recommendations (AIN-93 M) [13] and were produced by PragSolucoes (Jau, SP, Brazil). The composition of the diets is detailed in Table 1.

Table 1
Composition and energy content of the experimental diets

	Groups			
	SC	HF	HSu	HF/HSu
Ingredients (g)				
Casein	140.0	160.0	140.0	160.0
Corn starch	620.692	420.692	320.692	120.692
Sucrose	100.0	100.0	400.0	400.0
Soybean oil	40.0	40.0	40.0	40.0
Lard	–	180.0	–	180.0
Fiber	50.0	50.0	50.0	50.0
Vitamin mix	10.0	10.0	10.0	10.0
Mineral mix	35.0	35.0	35.0	35.0
Cystine	1.8	1.8	1.8	1.8
Choline	2.5	2.5	2.5	2.5
Antioxidant	0.008	0.008	0.008	0.008
Total (g)	1.000.0	1.000.0	1.000.0	1.000.0
Energy (kcal)	3804	4704	3804	4704
Cholesterol (mg)	0.0	438	0.0	438
Sucrose (%)	8	8	32	32
Carbohydrate (%)	76	44	76	44
Protein (%)	14	14	14	14
Lipid (%)	10	42	10	42

The experimental diets were formulated to meet the American Institute of Nutrition AIN 93 M recommendations for rodents.

2.2. BM, food intake and feed efficiency

The mice had free access to food and water during the experimental period, and their intake was monitored daily. In addition, their BM was measured each week. Food consumption of mice was measured by Compulse v 2.7.13 (Harvard/Panlab, Barcelona, Spain) for 72 h, and moreover, the amount of food supplied and the amount of food left in the grid during all the experiments were measured daily. Fresh chow was provided daily, and any remaining chow from the previous day was discarded. Energy intake was the product of food consumption by the energy content of the diet. The energy intake per gram of BM gained, termed the feed efficiency (FE), was calculated as a digestive and metabolic indicator of the ease that the energy consumed was added as BM. We calculated the FE as the ratio between the BM gain in grams and the food consumed in kcal per animal, presented as a percentage.

2.3. Blood glucose test analysis

A blood glucose test, the OGTT, was conducted after 7 weeks of diet feeding. For blood glucose levels, the blood was obtained by milking the tail after a small incision had been made (glucometer Accu-Chek; Roche Diagnostic, Manheim, Germany). The OGTT was performed using a 25% solution of glucose in sterile saline (0.9% NaCl) at a dose of 1.0 g/kg. The glucose was administered by orogastric gavage after a 6-h fasting period. The blood glucose concentration was measured before glucose administration (0 min) and 15, 30, 60 and 120 min after administration. Changes in blood glucose levels during the OGTT were analyzed by calculation of the total "area under the curve" (AUC) of glucose using the trapezoidal method [14].

2.4. Euthanasia and biochemistry

On the day before euthanasia, after 8 weeks on a diet, the animals were deprived of food for 6 h prior to being anesthetized (intraperitoneal sodium pentobarbital, 150 mg/kg). Then, blood samples were obtained by cardiac puncture through the right atrium. Plasma was obtained by centrifugation at room temperature (120g for 15 min). Total cholesterol (TC) and triglycerides (TGs) were assayed using an enzymatic colorimetric method according to the manufacturer's instructions. Biochemical analyses were performed in a semiautomatic spectrophotometer (Bioclin, Belo Horizonte, MG, Brazil).

The inguinal fat pad located between the lower part of the rib cage and the midthigh was considered subcutaneous fat. The retroperitoneal fat (connected to the posterior abdominal wall near the kidneys and the abdominal portion of the ureters) and the epididymal fat (located in the lower part of the abdomen and connected to the epididymis) were considered intra-abdominal fat pads. Therefore, after the animals were euthanized, the subcutaneous and intra-abdominal fat pads were carefully dissected and weighed. The adiposity index was determined as the ratio between the sum of the masses of the intra-abdominal and subcutaneous fat pads divided by the total BM, presented as a percentage. In addition, at the time of euthanasia, the liver was rapidly removed and weighed.

2.5. Milliplex Map immunoassay

The serum concentrations of insulin, leptin, resistin and monocyte chemotactic protein-1 (MCP-1) were measured using Multiplex Biomarker Immunoassays for Luminex xMAP technology (Millipore, Billerica, MA, USA; cat. no. MMHMAG-44 K-08), and the concentrations of serum adiponectin were evaluated with MADPK-71 K-01. The reading was performed using Luminex 200 Equipment — with Xponent/Analist software version 4.2. The ratio of insulin and fasting plasma glucose (I/G) was then calculated to evaluate insulin resistance.

2.6. Adipocyte quantification

Epididymal adipose tissue samples were fixed in freshly prepared formaldehyde in 4% wt/vol 0.1 M phosphate buffer (pH 7.2) for 48 h and then embedded in Paraplast plus (Sigma-Aldrich, St. Louis, MO, USA), sectioned at 5 μm and stained with hematoxylin and eosin. Digital images (TIFF format) were acquired with a light microscope (Leica DMRBE microscope; Leica Microsystems GmbH, Wetzlar, Germany) and an Infinity 1-5c camera (Lumenera Co., Ottawa, ON, Canada). Ten nonconsecutive microscopic fields were analyzed per animal, and the diameters of at least 50 adipocytes per animal were measured, blindly and at random (Image Pro plus software v7.01 for Windows; Media Cybernetics, Silver Spring, MD, USA).

2.7. Liver structure and biochemistry

The liver was carefully removed, weighed and sliced into several minor fragments, and the fragments were maintained in fixative [freshly prepared formaldehyde (4% wt/vol) in 0.1 M phosphate buffer, pH 7.2] for 48 h at room temperature. Then, the liver fragments were embedded in Paraplast plus (Sigma-Aldrich), sectioned at 5 μm and stained with hematoxylin and eosin. The digital images were acquired blindly and at random using the same system described previously. The volume density (Vv) of liver steatosis was assessed by point counting using a test system made up of 36 test points on at least 10 fields per animal, as described elsewhere [15,16]. The test system was produced using the STEPanizer Web-based system (www.stepanizer.com) [17].

The hepatic cholesterol and TG levels were measured in accordance with the protocols used in our laboratory [18]. Briefly, 50 mg of frozen liver tissue was placed in an ultrasonic processor with 1 ml of isopropanol. The homogenate was centrifuged at 2000×g, and 5 μl of the supernatant was analyzed in an automatic analyzer (K55; Bioclin System II, Quibasa, Belo Horizonte, MG, Brazil) using a kit for measuring TGs or cholesterol.

2.8. Western blots

The total protein content from the epididymal adipose tissue and liver was extracted in homogenizing buffer containing protease inhibitors. The homogenates