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Effects of treatment with sucrose in drinking water on liver histology, lipogenesis and lipogenic gene expression in rats fed high-fiber diet

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ABSTRACT

We studied the influence of sucrose in drinking water on liver histology, fatty acid profile and lipogenic genes expression in rats maintained on high-fiber. The experimental groups were: control group (water) and sucrose group (sucrose solution in drinking water, 30% w/v). Liver histology of sucrose treated rats revealed steatosis and increased number of αSMA immunoreactive cells without the signs of fibrosis. Sucrose treatment increased *de novo* lipogenesis, lipid peroxidation and MUFA content and decreased PUFA content, C18:2n6 and C20:4n6 content in total phospholipids and phosphatidylethanolamine and C18:2n6 content in cardiolipin. RT-qPCR revealed increase in Δ-9-desaturase and SREBP1c gene expression and decrease in the Δ-5-desaturase and elongase 5 expression. Treatment with sucrose extensively changes fatty acid composition of hepatic lipid and phospholipid classes including cardiolipin, increases oxidative stress and causes pathological changes in liver in rats maintained on high-fiber diet.

1. Introduction

There are three different sources of whole body fatty acids: food, *de novo* lipogenesis and bioconversion. Fatty acids generated *de novo,* as well as fatty acids derived from the food, are bioconverted by series of desaturation, elongation and β-oxidation steps into different SFA, MUFA and PUFA. The regulation of desaturases (Δ9D, Δ6D, Δ5D) and elongases (Elovl2, Elovl5 and Elovl6) is complex and it involves induced expression by different metabolites (glucose), hormones (insulin) and transcriptional factors (peroxisome proliferator-activated receptors α, PPARα; sterol response element-binding protein-1c, SREBP-1c; liver X receptor, LXR; carbohydrate-regulatory element binding protein, ChREBP; MAX-like factor X, MLX) [1,2]. Nutrition (substrate availability) and competition for rate-limiting enzymes as well as lipid oxidation and hormonal status, could substantially contribute or even override other regulatory mechanisms [3].

Metabolic diseases, such as diabetes, obesity or metabolic syndrome, are characterized with changes in lipogenesis and fatty acid concentrations in different tissues. In insulin dependent diabetes mellitus (IDDM), changes includes the decrease in mRNA expression of desaturases [4] and elongases [5]. Moreover, expression of different transcriptional factors, which are part of fatty acid synthesis regulation system, is also changed [1,6]. In experimental insulin resistance and non-insulin dependent diabetes mellitus (NIDDM) fatty acid metabolism is more complex [7] and it depends on experimental model used: spontaneously diabetic rats [8], high fructose [9], high glucose [10], high sucrose [11] or high fat diet and obese animal models [1].

High intake of simple sugars via the beverages is nowadays not always coupled with high fat intake, which is predominant model for metabolic syndrome investigations, but also with dietary restriction and high-fiber diets. Therefore, present work was undertaken to study the influence of long-term treatment with sucrose in drinking water in rats by measuring the fatty acid profile of liver and adipose tissue and the expression of lipogenic genes in rats fed high-fiber diet.

2. Materials and methods

2.1. Animals and diet

The research protocol was approved by the National Ethics Committee (EP 13/2015) and Veterinary Directorate, Ministry of Agriculture, Republic of Croatia. Male Wistar rats with an average initial body weight of 220 ± 10 g were used over the period of 20 weeks. The rats were placed in polycarbonate cages in a controlled environment at a temperature of 22 ± 1 °C and a 12 h cycle of light/dark. The experimental groups were formed according to the following treat-

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Table 1
Nutrient composition of the experimental diet.

Nutrients (% unless otherwise stated)	Experimental diet
Crude protein	14.3
Arginine	0.97
Lysine	0.87
Methionine	0.36
Crude fat	5.15
Crude fiber	19.8
Ash	11.8
ME (MJ)	12.1
Fatty acid composition (% of total fatty acids)	
C14:0	0.29
C16:0	13.24
C16:1	0.29
C18:0	3.59
C18:1n9	24.66
C18:1n7	1.12
C18:2n6	45.37
C18:3n3	10.47
C20:0	0.49
C20:1n9	0.50
Saturated fatty acids	17.60
Polyunsaturated fatty acids	26.56
Monounsaturated fatty acids	55.84
n6/n3 ratio	4.33

ments: control group (water) and sucrose group (sucrose solution in drinking water, 30% w/v). The drinking solutions were prepared daily. Rats were provided with solid high-fiber diet *ad libitum* (Table 1). During the trial, rats were weighed daily at 9.00 h using electronic balance. At the same time, non-fasting, blood glucose levels were determined with a Accu-Chek Go [12]. After 20 weeks, the animals were euthanized, their livers and perirenal adipose tissue were removed and their weight determined. All tissues were frozen in liquid nitrogen and kept at −80 °C until further analyzed.

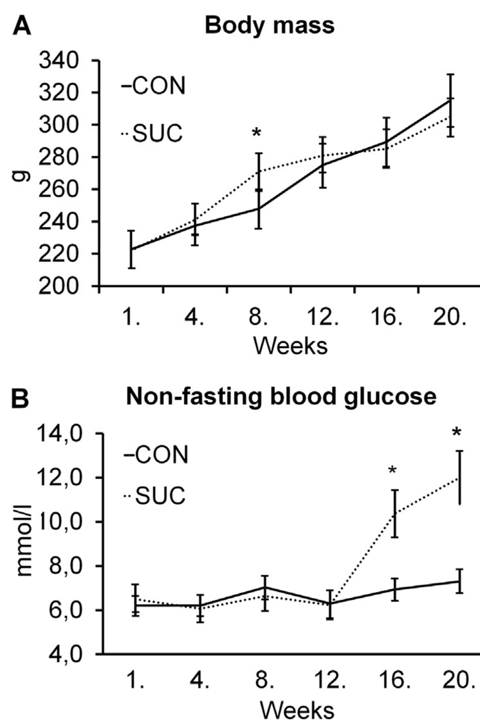

Fig. 1. Effects of sucrose treatment on the body mass of experimental rats (weekly average) (A) and on the non-fasting blood glucose values (weekly average) (B). The data are reported as the mean ± SD. CON, Control; SUC, Sucrose treated group. *P < 0.05.

2.2. Liver histology and alpha smooth actin expression

Liver tissue was harvested and immersed in Zamboni's fixative (4% paraformaldehyde and 0.20% picric acid in 0.1 M PBS at pH 7.4) and post fixation the tissue samples were embedded in paraffin wax. Paraffin blocks sections (5 µm) were deparaffinised and rehydrated in a series of ethanol solutions with decreasing concentrations. Antigen retrieval was done by heating in sodium citrate buffer (pH 6.0) for 12 min at 95 °C in a microwave oven. Incubation overnight with monoclonal mouse anti Alpha Smooth Actin antibody (1:300, M0851; DakoCytomation, Glostrup, Denmark) was performed in a humidified chamber. After washing in PBS, sections were incubated for 1 h with Anti- Mouse IgG-Rhodamine (715-295-151), diluted at 1:300 (Jackson Immuno Research Laboratories, Inc., Baltimore, PA, USA). The sections were then washed in PBS and nuclei were counterstained with 4′, 6-diamidino-2-phenylindole (DAPI). After final rinsing in PBS, all sections were air-dried, mounted (Immuno-Mount, Shandon, Pittsburgh, PA, USA), and coverslipped. Staining controls included omission of primary antibodies from the staining procedure, which resulted in no staining in tissue. Alternatively, after deparaffinization, Mallory trichrome staining was applied, as described previously [13].

Olympus BX51 (Tokyo, Japan) microscope equipped with Olympus DP71 camera was used for examination and photography of sections. Photographs were processed with Cell A Imaging Software for Life Sciences Microscopy (Olympus, Japan).

Sections were examined under a microscope (BX61, Olympus, Tokyo, Japan) and photographed using a cooled digital camera (DP71, Olympus, Tokyo, Japan) under the same magnification (40×), exposition, binning and gain for each image. Images were analyzed using Image J software (National Institutes of Health, Bethesda, MD,

Table 2
List of specific primers used in this study.

Geni	Forward (5′–3′)	Reverse (5′–3′)
Δ5D	TGGAGAGCAACTGGTTTGTG	GTTGAAGGCTGACTGGTGAA
Δ6D	TGTCCACAAGTTTGTCATTGG	ACACGTGCAGGCTCTTTATG
Δ9D	ACATTCAATCTCGGGAGAACA	CCATGCAGTCGATGAAGAAC
Elovl5	TACCACCATGCCACTATGCT	GACGTGGATGAAGCTGTTGA
PPARα	GACAAGGCCTCAGGATACCA	GTCTTCTCAGCCATGCACAA
SREBP-1c	GATTGCACATTTGAAGACATGCTT	GTCCCAGGAAGGCTTCCAGAGA
FASN	AAGCCCTTGGGAGTCAAAGT	TAGACGTCAGCAGGTCGATG
CPT1-liver	TGCCTCTATGTGGTGTCCAA	GGCTTGTCTCAAGTGCTTCC
ACOX1	TCGTTCAGAATCAAGTTCTCAATTTC	GTTGATCACGCACATCTTGGA
Cyclophilin	CTTCTTGCTGGTCTTGCCATTCCT	GGATGGCAAGCATGTGGTCTTTG
ß-Actin	ACTATTGGCAACGAGCGGTT	TGTCAGCAATGCCTGGGTAC

Delta-5-desaturase, Δ5D; Delta-6-desaturase, Δ6D; Delta-9-desaturase, Δ9D; Elongase 5, Elovl5; Peroxisome proliferator-activated receptor alpha, PPARα; Peroxisome proliferator-activated receptor gamma, PPARγ; Sterol regulatory element-binding protein-1c, SREBP-1c; Fatty acid synthase, FASN; Carnitine palmitoyltransferase I liver, CPT1 liver; Peroxisomal acyl-coenzyme A oxidase 1, ACOX1; Beta-actin, ß-Actin.

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