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METABOLIC CHANGES IN EXPERIMENTAL MODEL OF METABOLIC SYNDROM - INDUCED BY HIGH-FRUCTOSE DIET IN RATS

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ABSTRACT

The global epidemic of metabolic syndrome (MS) correlates with changes in the environment, feeding, behavior and lifestyle, leading to obesity, glucose intolerans, dyslipidemia and elevated cardiovascular risk. AIM: The aim of our study was to develop an experimental model of the MS in rat that imitate the investigated metabolic disorders using high-fructose diet. METHODS: We used two groups: control group (C)- rats, maintained on plain water (n=6); fructose group (FRU)- rats received 12.5% high-fructose corn syrup in drinking water for 12 weeks (n=6). The main markers of metabolic abnormalities (glucose, total cholesterol, triglycerides, uric acid, body and organs weight), the markers of oxidative stress (malondialdehyde (MDA), total thiols) and C-reactive protein (CRP) - inflammatory marker were measured. RESULTS: Our data showed hypercholesterolemia, hyperglycemia, hyperuricemia and significant elevated levels of CRP, MDA, body and organs weight, and inhibited antioxidant defense in fructose- drinking rats. CONCLUSION: The experimental model will support our studies associated with pathophysiology and pharmacology of MS.

Key words: experimental model of metabolic syndrome, fructose, liver, oxidative stress, rat

INTRODUCTION

The metabolic syndrome is defined as the presense of three or more of the listed criteria - visceral adiposity, dyslipidemia (combination of low levels of high-density lipoprotein cholesterol and high levels of triglyceride), high fasting glucose and hypertension (1). Development of these metabolic abnormalities predispose to high risk of oxidative stress, type-2-diabetes, cardiometabolic risk and nonalcoholic fatty liver disease (NAFLD) (4-7). The global epidemic of MS is related with changes in the environment, feeding, behavior and lifestyle. An important explanation for about is elevation the consumption of high- fructose corn syrup (HFCS), widely used in food industry as a sweetener in soft drinks and many other foods (2,3).

Fructose-fed rats also develop features of MS. Different animal models of MS, were created characterizing a particular aspects of MS. Major methodological differences in terms of:

1. Species of animals used: normal Wistar rats; normotensive and nonglucose tolerant Sprague Dawley rats (SDRs); spontaneously hypertensive rats (SHRs) and other.
2. Type of carbohydrate intake: fructose or sucrose.

Mor Oron-Herman et al.(2008) compare two animal models of MS: the high-sucrose diet given to spontaneously hypertensive rats (SHRs) and high- fructose diet given to Sprague Dawley rats (SDRs) and thus demonstrate specific genetic factors. They found that feeding fructose affects more SDRs in terms of hyperinsulinemia, hypertriglyceridemia, and hypercholesterolemia, while SHRs develop mainly hypertension (8). Other authors reported the central role of nutritional factors in the development of MS. They applied in their research a high carbohydrate diet of normal rats.The results of Busserolles et al. (2002) indicate that consumption of a high-sucrose diet causes mainly oxidative stress (11), whereas Thirunavukkarasu et al. (2004) reported that high-fructose feeding to normal rats causes equally oxidative tissue damage and hyperglycemia, hyperinsulinemia, dyslipidemia, obesity, deposition of lipids in liver, kidney and skeletal muscle (9,10). We chose this model as appropriate for our future research.

The aim of our study was to develop an experimental chronic model of MS indused by high- fructose diet on male Wistar rats. The model of MS plays an important role in our investigation on relationships between liver metabolic disorders, oxidative stress and inflammation.We are data in the literature that liver is the magor target in MS (12).

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MATERIAL AND METHODS

Animal models

Male albino Wistar rats were housed in a 20+2°C room temperature and with a standard 12-h light/ dark cycle. They all received a standard diet and water ad libitum. The standard diet was composed of starch - 50%, protein - 20%, fat - 4,5%, 5% cellulose, standard vitamins and mineral mix. At the beginning of experiment the body weight of rat was 140-180 g. After acclimatization (two weeks) the animals were divided randomly into two groups: control group (C)- rats, maintained on plain water (n= 6); fructose group (FRU)- rats received 12.5% high-fructose corn syrup in drinking water for 12 weeks (n= 6). Food intake was recorded daily and their weight was monitored weekly. At the end of the experiments, the rats were killing with lethal dose of thiopental. All manipulations were performed at 4-8°C. Analysis was performed immediately after thawing of the samples. The experimental procedures were approved by the Home Office for Care and Use of Laboratory Animals and performed with a strong consideration for ethics of animal experimentation according to the International Guiding Principles for Animal Research approved in Bulgaria.

Blood and tissue collection

At the termination of the experiment blood was collected from the tail vein of rats after pre-narcosis with Thiopental at a dose of 15 mg / kg i.p. Serum obtained (subject to rules of good practice Lab) used to determine some biochemical indicators on the same day, and froze the rest. Under general anesthesia with Thiopental 30mg/kg (rapid intravenous) conducted a laparotomy. We took different organs for research, preparation of homogenate, and then was quickly dissected and frozen.The weight of heart, liver, kidney, testis and retroperitoneal fat were measured. Tissue extracts were prepared by homogenisation with buffer PBS- 50 mM, pH = 7.4 (1 g tissue, 9 ml of buffer) in ice using a teflon glass homogenizer (2000 rpm / 3 min). Homogenates centrifuged in refrigerated centrifuge (4000 rpm / 10 min). Supernatant was used for analysis after having identified the concentration of protein.

Biochemical assays

The blood concentrations of glucose, total cholesterol and triglycerides (TG) were determined by commercial kits (F.Hoffmann-La Roche Inc.), CRP by ELISA kit (Helica biosystems, INC.). Uric acid (Folin reagent), MDA(method of Porter (1976) and total thiols groups (method of Ellman) in serum and tissue by spectrophotometric assays was measured. Liver fat was extracted by method of Folch et al. and hepatic triglycerides were measured as above.

Statistical analyses

All results were expressed as means ± SEM as indicated in the figures and table. Statistical significance of the studied parameters analyzed by Student's t test. P values of less than 0.05 were regarded as significant. The statistical procedure was performed with GraphPad InStat software.

RESULTS AND DISCUSSION

The results indicate metabolic disorders of experimental chronic model of MS indused by high- fructose diet and present changes in experimental group compared to control group.

Table.1. Markers of metabolic abnormalities measured in the control and experimental groups.

Groups	Control (C)	Fructose (FRU)
Body weight (g) *initial* *final*	 152 ±7.2 226 ±4.9 a	 138 ±3.9 239 ±7.8 [a b]
Glucose / serum (mmol/l)	6,08 ±0.88	8.14 ±0.5 [b]
Total cholesterol /serum (mmol/l)	0,81 ±0,24	1,29 ±0,24
Triglyceride / serum (mmol/l)	0,89 ±0.1	0,86 ±0.09
Triglyceride / liver (mg/g)	11.8 ±1.2	15.7 ±0.98 [b]
CRP /serum (ng/ml)	24.91 ±1.16	27.75 ±0.42 [b]
Uric acid / serum (μmol/l)	111.48 ±6.02	145.16 ±14.49 [b]
Uric acid / liver (μmol/l)	28.32 ±1.8	33.46 ±5.64

Mean levels ±SEM; n= 6; C- control group rats; FRU-fructose-drinking rats; $P^a < 0.0001$ - statistical significance between initial and final body weight; $P^b < 0.05$ - statistical significance between treated and control groups.

Of the metabolic parameters statistically significant differences were found for glucose, CRP, uric acid in serum , and not significant increase of total cholesterol in fructose

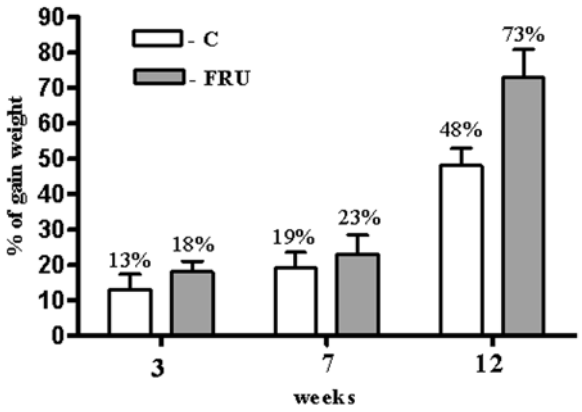

Fig. 1. Percentage of weight gain during the development of experimental model of MS

drinking rats compared with the control group (Table 1). FRU rats developed signs of MS, including significant elevated hepatic fat deposition (Table 1). At 12 weeks fructose drinking rats increased body weight, statistically significant

both between the FRU and control groups and between initial and final weight in each group (Table 1). Figure 1 presents the steady weight gain in the experimental rats during the development of nutritional model of MS.
Figure 2 shows the increase in weight of various organs, particularly statistically significant for retroperitoneal fat.

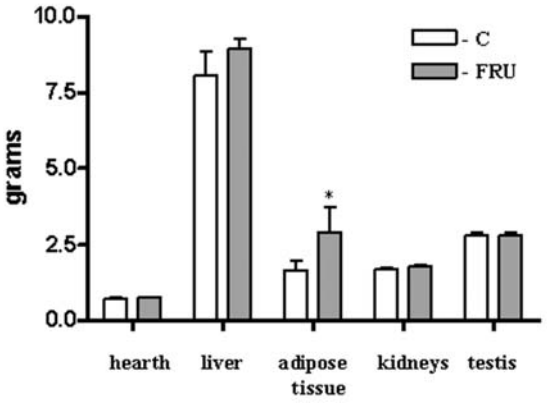

Fig. 2. Change of organ weights in experimental group versus the control group

Unexpected levels of uric acid in serum and liver was increased, confirming the data of other authors that the uric acid has a causal role in fructose-induced MS (Table 1) (13).
We found marked oxidative stress in the group of FRU, considering the increased lipid peroxidation (MDA) and altered activities of antioxidant enzymes (Total thiols groups) (Table 2).

Table.2. Markers of oxidative stress and antioxidative capacity measured in the control and experimental groups.

Groups	Control (C)	Fructose (FRU)
MDA / serum (mmol/l)	2,18 ± 0,2	2,38 ± 0.5
MDA / liver (μmol /g pr.)	0,11 ± 0,02	0,15 ± 0,02
Total thiol groups / serum (mmol/l)	0,89 ± 0.1	0,86 ± 0.09
Total thiol groups / liver (μmol/l)	612,5 ± 28,1	566,6 ± 32,3

Mean levels ± SEM; n= 6; C- control group rats; FRU-fructose-drinking rats

Likely overproduction of free radicals and decreased antioxidant activity as a result of hyperglycaemia in FRU rats may suppress the activation of insulin receptor (10).
In conclusion, the results of our studies show that high-fructose diet causes metabolic disorders in rats associated with MS. Experimental models will support our future research on the pathophysiology and pharmacology of MS.

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