AN EXPERIMENTAL MODEL TO INDUCE METABOLIC SYNDROME IN RATS. THE FRUCTOSE - ENRICHED DIET

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Abstract
The objective of this study was to induce metabolic syndrome (MS) in rats by means of a fructose-enriched diet (F, 60%). Twenty male Sprague-Dawley rats were randomized into 2 groups and fed for 3 months with standard chow (Control group) or with standard chow supplemented with fructose (F, 60%). After their sacrifice, blood was drawn and the following biochemical parameters were determined from the centrifuged plasma: triglycerides and malondialdehyde (MDA) – a marker of lipid peroxidation. The fructose diet induced a significant increase in glycaemia (5.60 ± 0.07 vs 7.22 ± 0.13 mM, p < 0.001), in systolic blood pressure (SBP 113.66 ± 1.14 vs 130.61 ± 0.80 mmHg, p < 0.001) and in the triglyceride values (61.86 ± 4.86 vs 331.20 ± 23.15 mg/dL, p < 0.001), without significant modification of rat body weight. Moreover, the plasma MDA level was 1.38 times higher in fructose-fed rats. Our results suggest that this experimental model could be used to induce metabolic alterations, such as hypertension, hyperglycaemia and dyslipidemia, alterations associated to lipid peroxidation.

Keywords: metabolic syndrome, rat fructose-enriched diet, research animal model

Rezumat
Obiectivul acestui studiu a fost inducerea sindromului metabolic la șobolani, utilizând o dietă îmbogățită în fructoză (F, 60%). 20 de șobolani masculi Sprague-Dawley au fost randomizați în 2 grupe și au fost hrăniți timp de 3 luni cu dietă standard (C, grupul control), sau cu dietă îmbogățită în fructoză (F, de 60%, grupul F). După sacrificare s-a prelevat sânge, iar din plasma obținută în urma centrifugării s-au determinat următorii parametri biochimici: trigliceridele și malondialdehida (MDA) - un marker al peroxidării lipidade. Dieta îmbogățită în fructoză a induzat o creștere semnificativă a glicemiei (5,60 ± 0,07 vs 7,22 ± 0,13 mM, p <0,001), a tensiunii arteriale sistolice (113,66 ± 1,14 vs 130,61 ± 0,80 mmHg, p <0,001) și a trigliceridelor (61,86 ± 4,86 vs 331,20 ± 23,15 mg/dL, p <0,001), fără a influența greutatea corporală a șobolanilor. De asemenea, nivelul plasmatic al MDA a fost de 1,38 ori mai mare la șobolanii hrăniți cu fructoză. Rezultatele noastre sugerează că acest model experimental ar putea fi folosit pentru a induce modificări metabolice, precum hipertensiune arterială, hiperglycemicie, dislipidemie, modificări asociate cu degradări oxidativă ale lipidelor: peroxidarea lipidică.

Keywords: metabolic syndrome, rat fructose-enriched diet, research animal model
Introduction

There are many definitions of the metabolic syndrome (MS), but, most of the times, it is defined as a cluster of complex symptoms including: obesity, insulin resistance (IR) with impaired glucose tolerance, dyslipidemia (combination of a low level of high-density lipoprotein cholesterol and a high level of triglycerides) or hypertension. The MS is assumed to be present when at least three of the mentioned symptoms are identifiable [1, 2, 6, 7]. In order to better understand MS with all its implications, many studies were performed over time, both experimental, on research animals, as well as human studies.

Despite the significant differences between animal and human metabolism, the research animal model holds the advantage of a more homogeneous study, that can better highlight and quantify the repercussions of the variation of the parameters; respectively diet, obesity, blood pressure etc. Rabbits, rats and mice cannot spontaneously develop atherosclerosis and the typical cardiovascular diseases observed in humans because of their different lipoprotein metabolism (lack of the cholesterol ester transfer proteins - CETP; lipoproteins metabolism primarily based on HDL rather then on LDL; difference in the apolipoprotein B synthesis pathway). Despite the fact that these metabolic differences must be observed in order to reproduce the symptoms as similar to humans as possible, rats, mice and rabbits are currently the animals most widely used in the study of MS [9, 13]. It is common practice that laboratory animals are applied the same eating habits as those of the people from developed countries: diet supplemented with carbohydrates (Western diet) or high fat diet (DIO - diet-induced obesity). Moreover, as many specialized papers have underlined the susceptibility of genetic structures to the action of environmental factors, the interaction between nutrition and the human genome should not be overlooked [8].

Fructose is a carbohydrate with a lower glycemic index than glucose, which does not induce insulin secretion like glucose does, thus representing a good alternative sugar for diabetic patients. Nevertheless, it seems that frequent usage of a fructose as additive in solid and liquid foods (soft drinks) leads to dyslipidemia, obesity, diabetes mellitus type 2 and hypertension in young adults [11, 12, 15].

The aim of our work was to study the influence of high fructose diet on the metabolic parameters and oxidative stress in rats.

Materials and Methods

Animals and experimental protocol

This study was approved by the Ethics Committee of the University of Medicine and Pharmacy “Iuliu Hatieganu”, Cluj-Napoca, Romania. All the
procedures were designed in accordance with international and institutional guidelines for the care and use of laboratory animals.

Twenty male Sprague-Dawley rats (7 weeks old), weighing 200–250 g were purchased from “Cantacuzino” National Institute of Research and Development, Bucharest, Romania. The rats were randomly divided into 2 groups, and had ad libitum access to water and specific chow.

The control group (C, n = 10) received regular chow (“Cantacuzino” National Institute of Research and Development). The standard chow diet was composed mainly of 18% sheer proteins, 1.5% sheer fats and 5% sheer fibers.

The high fructose-fed group (F, n = 10) received chow supplemented with fructose 60% (Ssniff, Germany). The high fructose diet was mainly composed of 17.9% sheer proteins, 6.1% sheer fats, 6% sheer fibers, 5.3% sheer ash, 60.7% sugars, about 4% minerals and vitamins (vitamins A, D3, E, K3, C and cooper).

The rats’ body weight was monitored every 7 days. Moreover, food and water consumption was determined.

The rats’ glycaemia and blood pressure were evaluated at the beginning of the study (W0) and after 12 weeks (W12).

The rats were euthanized 12 weeks (3 months) after the beginning of the study. Before being sacrificed, the rats were anesthetized with Nesdonal® (50 mg/kg body weight, i.p.) and the blood was collected by retro-orbital sinus puncture in heparin coated tubes and in tubes without anticoagulant. Plasma and serum, obtained by low-speed centrifugation of blood, were stored at – 80°C until analysis. The livers were also excised and their weight was determined.

Heart’s functional parameters measured in vivo

Systolic blood pressure (SBP) and heart rate (HR) were evaluated at the beginning of the study (W0) and after 3 months on specific diet by means of a non-invasive tail-cuff technique (Non-invasive Blood Pressure Recorder 58500, Hugo Basil, Italy). The rats were handled repeatedly and allowed to adapt to the restraint chamber for one week before the beginning of the study. The rats were prewarmed at 30°C for minimum 30 minutes before measurements were performed. The average of ten consecutives readings was recorded as the individual SBP and HR.

Biochemical investigations

Glycaemia was evaluated by a glucometer (Accu-Check, Roche) at the beginning of the study and after 3 months on the specific diet. The triglycerides (TG) concentrations were measured on a Konelab 20i Clinical Chemistry Analyzer (Thermo Scientific, Finland).
**Oxidative stress parameters**

**Malondialdehyde (MDA)**

Measurement of plasma lipid peroxidation was done by a colorimetric reaction with thiobarbituric acid (TBA). The total MDA is the sum between the bound and the free forms of MDA. The bound malondialdehyde (MDA) from plasmatic lipoproteins was determined in the protein precipitate obtained after adding the trichloroacetic acid [10] while the free MDA had been determined in the supernatant [3]. The TBA was added in the supernatant / into the protein precipitate (precipitate washed with a solution of H$_2$SO$_4$ 0.05 M). The solutions were incubated for 10 minutes in boiling water bath and the absorbance of the pink solution obtained was recorded at 530 nm after cooling. In the case of bound MDA, the complex formed with TBA was extracted with n-butanol; the two phases were separated and the absorbance was determined. In both cases, MDA concentration (nMol/mL) was calculated based on a calibration curve obtained in the same working conditions with malondialdehyde bis-diethyl-acetate (1,1,3,3-tetraethoxypropan).

**Statistics**

All data are expressed as average ± S.E.M. To compare the groups, statistical analyses were performed with the one-factor analysis of variance (ANOVA) test; ANOVA was followed, where necessary, by a Newman Keuls test. Significance was established at a value of $p < 0.05$.

**Results and Discussion**

Both rat groups gained weight during the 3 months of the study. In our experiment, the fructose intake did not influence the rats’ body weight (Figure 1). Also, the body weight and the food consumption have followed approximately the same pattern.

![Figure 1](image)

Evolution of the body weight of the rats fed with standard chow (C, n = 10) and the rats fed with a fructose-enriched diet (F, n = 10), during the 3 months of the study.
At the beginning of the study the average of systolic blood pressure (SBP) was 107 mmHg and the glycaemia 5.62 mM, in both groups of rats. After 12 weeks of fructose-enriched diet, both physiological parameters were significantly (p < 0.001) higher than in control group. The SBP had increased by 14.92% and the glycaemia by 28.93% (Figure 2). No significant difference was noticed in heart rate (HR) between the two groups of rats. Also, at the end of the study, the plasmatic triglycerides level was 5.35 times higher in the F group as compared to the control group. The plasmatic lipid profile alteration was associated with a significant (p < 0.01) increase in liver weight (Figure 3).

**Figure 2**
A: The Systolic blood pressure (SBP) and B: The glycaemia (mM) of the rats fed with standard chow (C, n = 10) and the rats fed with a fructose-enriched diet (F, n = 10), after 12 weeks (3 months) of diet. Results are expressed as average ± S.E.M. *** p < 0.001.

**Figure 3**
A: Plasma triglycerides (mg/dL) and B: Liver weight (g) of the rats fed with standard chow (C, n = 10) and the rats fed with a fructose-enriched diet (F, n = 10), after 3 months of diet. Results are expressed as average ± S.E.M. ** p < 0.01; *** p < 0.001.
After 12 weeks of diet, the lipid peroxidation, expressed as nmol/mL MDA, was 1.38 times higher in the rats fed with fructose as compare to the lipid peroxidation in the control group (Figure 4).

![Figure 4](image)

Plasma MDA (nmol/mL) of the rats fed with standard chow (C, n = 10) and the rats fed with a fructose-enriched diet (F, n = 10), after 3 months of diet. Results are expressed as average ± S.E.M. * p < 0.05.

Our study showed that the high fructose diet given to rats was associated with the development of metabolic disorders such as hypertension, hyperglycemia and dyslipidemia (hypertriglyceridemia) and that the metabolic perturbations are accompanied by significant plasma lipid peroxidation, expressed as MDA. The free radicals - formed on account of increased blood pressure and/or glycaemia - have predominantly oxidized the lipid structures, leading to the formation of hydroperoxides, which then decompose to MDA [4].

Our results are in accordance with a recent study [5] that indicated that fructose-fed Sprague Dawley rats manifest major characteristics of human metabolic syndrome. However, this experimental model is not recommended when aiming for a significant increase in body weight. It was found that, in order to achieve a significant weight increase, the type of feeding protein must be taken into account. Thus, it seems that, compared to a diet rich in casein, a diet rich in soy protein rather induces weight loss [5, 14].

Conclusions

The fructose-enriched diet (60%) used in our experimental model induced the development of pathophysiological characteristics associated with the metabolic syndrome, involving hypertension, hyperglycemia and dyslipidemia. We have also found that chronic fructose (60%) feeding is
associated with an increase in lipid peroxidation, expressed as plasma MDA. This model could be considered a good experimental model in the study of hypertension, hyperglycemia and dyslipidemia, but not in the study of obesity.

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References


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