FRUCTOSE AFFECTS FATTY ACIDS PROFILE IN LIVER CELLS *IN VITRO* AND *IN VIVO* MODELS IN RATS

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Abstract

It was reported that dietary fructose imposes a number of effects on lipid metabolism including hypertriglyceridemia. The daily intake of fructose in humans is mainly due to sucrose. It was reported that the consumption is still increasing, making a background for health implications. The mechanism of metabolic disorders is poorly understood, but a lot of studies indicate that the liver lipid homeostasis deregulation is essential for a fructose effect on metabolism. The aim of the study is to estimate if fructose affects the profile of fatty acids in in vitro and in vivo models. In this study in vitro and in vivo experiments were conducted to assess the effect of dietary fructose on the fatty acid profile in the cell culture or in the liver of rats. The results showed that in the fructose experimental groups, both in the cell and liver homogenates, the content of the saturated fatty acids were significantly higher than in control groups. According to the obtained data fructose in the medium and in the diet affects saturation of fatty acids in the cell cultures and in the livers of rats. The findings obtained in the experiments support the thesis that fructose influences the homeostasis of lipid metabolism in the liver and may give an opportunity to discuss the limitation of the content of this kind of sugar in food.

Keywords: Fructose, Fatty acids profile, Hep G2 cell culture, Lipid metabolism, Diet.

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Introduction

Dietary fructose may impose a number of disadvantageous effects on lipid metabolism in animals as well as in humans [1, 2, 3]. It was reported that high fructose intake is correlated with hypertriglyceridemia, hyperinsulinaemia and even hypertension [4, 5, 6]. High doses of this sugar lead especially to a significant increase in triglyceride levels, which is mainly linked to hepatic overproduction of very low density protein triacylglycerol (VLDL-TG) [7]. Fructose causes increased hepatic triglyceride synthesis in the liver derived from lipogenesis de novo as well as from amplified estrification of circulating nonessential fatty acids (NEFA). In previous papers the effect of hypertriglyceridemia after addition of 31% of fructose were observed and described in detail (increase in triglycerides level: 86 $p<0.01$) [8, 9].

Busserolles et al. [10] found that a fructose-rich diet induced hypertriglyceridemia in rats after 2 weeks. There was a two-fold increase in plasma triglyceride concentration compared with the control group. The fructose content in the diet applied in the above study was similar (34%) to the model, which was used in this study [7]. Research on rats fed with a high fructose level diet (60%) also reported an increase in triglyceride concentration in comparison to the control group. As mentioned the changes could be caused by overproduction of triglycerides in hepatocytes as a consequence of the fructose metabolism, and therefore increased lipogenesis and synthesis of VLDL. Fructose, unlike glucose, is

metabolized in the liver while the latter is mainly used by extrahepatic tissues. Fructose is phosphorylated by fructokinase and enters the glycolysis pathway due to a specific aldolase action. Fructokinase is the enzyme specific only to the liver. Additionally the activity of fructokinase is very high when compared to liver glucokinase specific for glucose [4]. The synthesis of fatty acids from fructose in liver includes the conversion of pyruvate synthesized in glycolysis to acetyl CoA through Pyruvate Dehydrogenase Complex (PDH). The regulation of PDH is very complex and involves the so called Randle cycle. The main mechanism of this regulation is phosphorylation and dephosphorylation by the action of PDH kinase (PDK) and PDH phosphatase (PDP). PDK is the subject of regulation by dietary components, which was previously reported [11, 12, 13]. Dietary fructose increases the carbon flux through the PDH complex giving substrates to lipid synthesis de novo, which may cause overweight and underlying metabolic disorders.

Because of the high level of triglyceride in plasma after fructose addition to the rats' fodder [7, 8, 9] the present study was designed to assess the impact of fructose addition on the profile of fatty acids in liver and cultured hepatocytes.

Materials and Methods

Chemicals

Dimethyl sulphoxide (DMSO), Trypan Blue reagent for cell culture, fructose were purchased from Sigma. Sterile and non-toxic cell plates, pipets, tips were purchased from Sarstedt and Becton Dickinson. Media and sera were from Gibco. Deionized water was obtained from Milli Ro & Q water purification system (Millipore). Methanol, chlorophorm, n-hexane, heptane, boron trifluoride – methanol complex 20%, acetone were from Merck.

Hep G2 Cell line

Hepatocellular carcinoma cells (ATCC designation: Hep G2, HB-8065) were from American Type Cell Culture collection (ATCC collection). Cells were cultured in Eagle's Minimum Essential Medium (EMEM) with 10% Fetal Bovine Serum and with 1% antibiotic solution (100 IU/ml penicillin, 0.1 mg/ml streptomycin) in incubator and were subcultivated two times a week with Trypsin – 0.05% EDTA solution. Cell morphology was observed by inverted light microscope (Olympus) and viability was assessed with Trypan Blue Exclusion Test. Cells were seeded into plates at a density of 2×10^5 cells/ml of medium. Fructose solution was added to cultures at 5mM while buffered PBS was added to the control cultures.

Animals and diet formulation.

Twelve Wistar male rats (mean weight 245.4±7.0 g) were kept in cages with 12 hours dark/12 hours light period in the Animal House of Jagiellonian University. The rats had unlimited access to fodder and tap water. The protocols for animal experiments were approved by the Animal Experimentation Committee of Jagiellonian University.

The diet contained 31% of fructose for 5 weeks. At the end of the experiment livers were collected and kept in -80°C. Experimental diets were formulated according to the following scheme: [g/kg fodder]: casein 200, rapeseed oil 50, chalk 28, calcium monophos-

phate 29, lecithin 10, sodium chloride 3, cellulose 50, mixture of vitamins and micro-elements 10 (Premix LPM, BASF, Poland). Two groups were given either standard fodder, which contained 620 g/kg corn starch or a fodder in which 310 g/kg of corn starch was substituted with 310 g/kg of fructose, respectively. The energy density, total amount of proteins, fat, and carbohydrates in the diets were published previously [2].

Determination of fatty acids profile

Fatty acids from cells and livers after homogenization in PBS were extracted with chlorophorm – methanol solution (2:1 v/v). Fatty acid methyl esters (FAME) were synthesized with use 20% BF_3 w in methanol at the 100°C. FAME analyses were done by gas chromatography using Agilent 6890N equipped with capillary column DB- 23 (50%-Cyanopropyl)-methylpolysiloxane, 60m, ID 0,25mm, film 0,25 μm) and FID detector. FID 260°C, injector 250°C, split ratio 50:1. Oven 140°C for 5 min., 140-190°C at 4°C/min., 190°C for 15 min., 190-240°C at 2,75°C/min, 240°C for 4min. Carrier gas - Helium at 43cm/sec, constant pressure mode. For the identification of fatty acids, retention times of standards FAME from Supelco (47801) were used.

For all fatty acids the mean value in percent was calculated. The total sum of saturated (SAT) and unsaturated (UNSAT) fatty acids in each probes was presented on graphs.

Statistical analysis

After logarithmic transformation the data were normally distributed and significant differences in the fatty acids content between groups were assessed by use of a one-way analysis of variance (ANOVA) using Tukey's test at the 95% confidence level ($p<0,05$).

Statistical calculations were carried out using the commercially available packages Statistica 8.0 (StatSoft, Ic., Tulsa, USA).

Results And Discussion

In the control cell 36% of saturated fatty acids and 64% of unsaturated fatty acids were observed. In the cultures treated with fructose, the SAT index was statistically higher (59%) while UNSAT index was lower compare to the control (Fig 1.). Statistically the highest value of palmitic acid was observed (35%). Also other saturated acids, like lauric or stearic were significantly higher in the experimental group than in the control samples (Fig 2.).

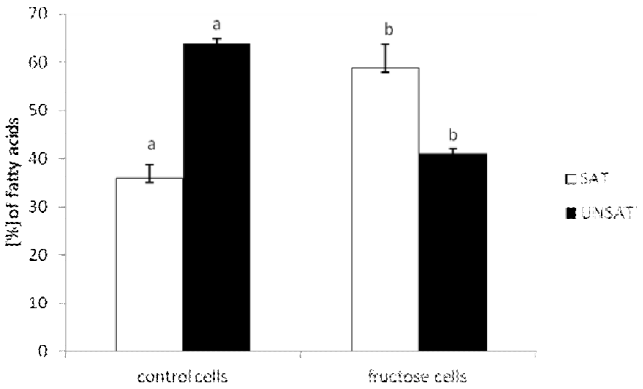

Fig. 1 SAT and UNSAT fatty acids, [%] of the total lipids from HepG2 cells – control and treatment groups. Mean values ±SD, N=5, different letters denote significant statistical differences between groups, $p<0,05$

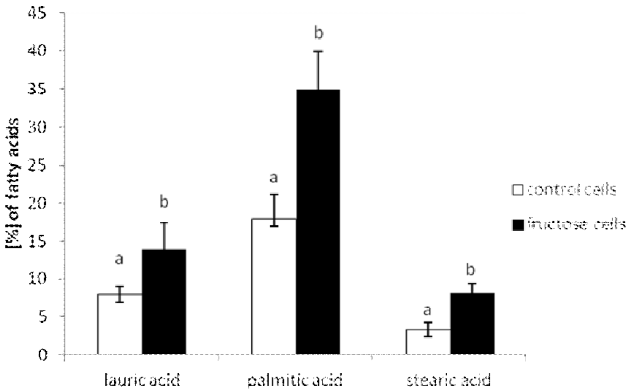

Fig. 2. The main saturated fatty acids [%] in the control and fructose treatment cell cultures. Mean values ±SD, N=5, different letters denote significant statistical differences between groups, $p<0,05$

After 5 weeks of keeping animals on a high fructose diet, the lipid profile changed comparing to the control group. The index of SAT in the liver from treatment animals were higher then in the control samples (Fig 3).

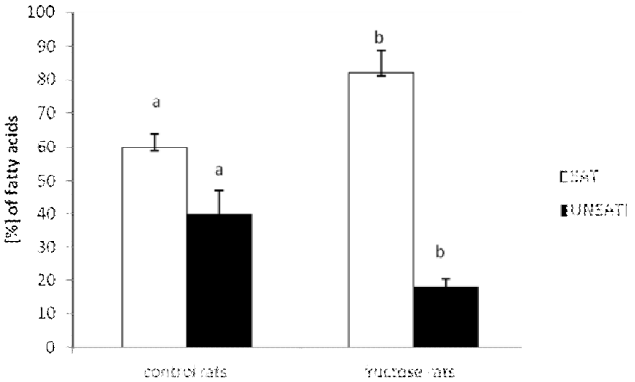

Fig. 3. SAT and UNSAT fatty acids, [%] of the total lipids from rats' livers – control and treatment groups. Mean values ±SD, N=5, different letters denote significant statistical differences between groups, p<0,05

An increase of saturated acids, like palmitic (p<0,05), stearic (p<0,05) or lauric compared to the control group was observed (Fig 4).

Our results proved that fatty acid profile had changed in hepatic cell lines as well as in rat's livers after administration of fructose. The precise evaluation of biological activity of a diet is essential for recommendation as not toxic and safe for human. Apart from that it is important to find out if dietary components affect metabolic homeostasis, especially in liver, which plays a crucial role in the regulation the carbohydrate and lipid pathways. A lot of clinical trials have focused on modified diet and nutritional supplements. The use of fructose as naturally occurring sugar as well as in sucrose is still very popular in human diet. Due to a significant influence of dietary fructose on lipid homeostasis, the limitation in consumption of fructose should be considered.

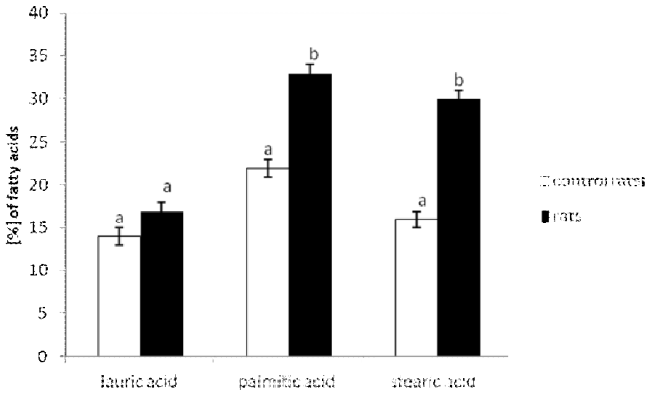

Fig. 4. The main saturated fatty acids [%] in the control and fructose treatment rats' livers. Mean values ±SD, N=5, different letters denote significant statistical differences between groups, p<0,05

Conclusion

Taking into consideration obtained results, fructose seems to be an arguable sugar compound of human diet. The negative effect of the changing lipid profile was observed in vitro, in Hep G2 cells as well as in vivo in the livers of rats. It is was previously suggested that the effect of fructose on lipid homeostasis may be disadvantageous, while the changes in the lipid profile (increase of saturated fatty acids content) seem to be an important observation associated with the influence of fructose on the mammal organism. However, to obtain a more specific understanding of the influence of fructose, advanced experiments will be continued in in vivo as well as in vitro models.

Resumo

Estis konata ke dieta fruktozo havas diversajn efikojn al la lipida metabolismo, kiuj inkluzivas hipertrigliceridemion. La homa ĉiutaga provizado de fruktozo dependas ĉefe de sukrozo. Estis raportita ke la konsumado estas ankoraŭ kreskanta, kiu gravas por pensoj pri sano. La mekanismo de metabolaj malsanoj estas nur malbone komprenita, sed multaj studoj montras ke la malsuprenregulado de la hepata homeostazo de lipidoj gravas por la efiko de fruktozo al metabolismo. La celo de tiu ĉi esploro estas eltrovi ĉu fruktozo influas la profilon de grasaj acidoj en in vitro kaj in vivo modeloj. En tiu ĉi esploro in vitro kaj in vivo eksperimentoj estis faritaj por taksi la efikon de dieta

fruktozo al la profilo de grasaj acidoj en ĉelaj kulturoj aŭ en la hepatoj de ratoj. La resultoj montris ke en la fruktozo eksperimentaj grupoj, kaj en la ĉelaj kaj en la hepataj homogenaĵoj, la enhavo de saturitaj grasaj acidoj estis pli alta ol en la kontrolaj grupoj. Laŭ la trovitaj datenoj fruktozo en la mediumo kaj en la dieto influas la saturadon de grasaj acidoj en ĉelaj kulturoj kaj en la hepatoj de ratoj. La resultoj de la eksperimentoj apogas la tezon ke fruktozo influas la homeostazon de lipida metabolismo en la hepato kaj ofertas okazon pridiskuti la neceson de limigo de la kvanto de tiu sukero en nutraĵoj.

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