

EFFECT OF DIETARY FRUCTANS AND CHROMIUM(III) SUPPLEMENTATION ON APPARENT FAT DIGESTIBILITY AND BLOOD LIPID INDICES IN RAT*

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Background. Fructans and chromium(III) are believed to influence lipid and carbohydrate metabolism in animal and human organisms, that can be applied in regulation or improvement of lipid indices, however the importance of their effect is still unclear. This study aimed at evaluation of the effect of inulin-type fructans and chromium(III) supplementation on fat digestibility and blood lipid indices in rats.

Material and methods. In a three-factorial design 56 male Wistar rats were fed *ad libitum* semipurified diets (AIN-93) supplemented with two types and levels of fructans (inulin or oligofructose, 5% or 10% of diet) and two levels of chromium(III) (0.5 or 5 mg/kg of diet; as Cr(III) propionate complex) for 10 weeks. Apparent fat digestibility and blood lipid profile were determined.

Results. It was found that experimental factors did not influence apparent fat digestibility, serum total cholesterol, HDL and HDL cholesterol concentrations, however serum TAG level was significantly lower in animals fed oligofructose vs. inulin diets, high-fructan vs. low-fructan diet.

Conclusions. Inulin type fructans and supplementary Cr(III) do not affect apparent fat digestibility, fructans however influence lipid metabolism, lowering serum TAG levels in rat.

Key words: inulin, oligofructose, chromium(III), lipids, rats

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INTRODUCTION

Coronary heart disease, metabolic syndrome, diabetes mellitus and obesity are the major causes of morbidity and disability in many populations worldwide. Their prevalence is strongly related to lifestyle factors, including improper dietary habits. Diets rich in saturated fats, cholesterol, simple sugars, and deficient in certain regulatory nutrients (vitamins and minerals) are considered as risk factors in development of metabolic diseases. Therefore improvement of dietary habits as well as implementation of certain bioactive dietary components is believed to play a crucial role in prevention of civilization diseases.

Lipid and carbohydrate metabolism is regulated by various endogenous factors as well as bioactive substances and micronutrients provided with food.

Dietary fructans are nutritionally interesting oligosaccharides that strictly conform to the definition of prebiotics and exhibit serum and hepatic lipid lowering properties [Jackson et al. 1999, Delzenne and Kok 2001, Delzenne and Williams 2002, Russo et al. 2008]. It was shown that dietary fructans can reduce serum total cholesterol and triacylglycerol (TAG) levels in animals and humans [Davidson and Maki 1999, Anderson and Hanna 1999, Williams 1999, Williams and Jackson 2002].

Chromium(III) is considered as an essential nutrient required for glucose and lipid metabolism [Chen et al. 2009]. Laboratory and clinical studies indicate that Cr supplementation may improve glucose, lipid, and cholesterol metabolism and insulin sensitivity by enhancing intracellular signalling in healthy and diabetic animals and humans [Horvath et al. 2008, Sahin et al. 2007, Lai et al. 2006]. Abraham et al. [1992] reported that Cr deficiency led to significant elevation of HDL cholesterol by 30% in subjects with cardiovascular disease. However individuals who are not Cr deficient do not benefit from Cr supplementation [Vincent 2000, Campbell et al. 1999]. Sun et al. [2002] showed that the trinuclear chromium(III) complex with propionic acid, lowered plasma TAG, total cholesterol, and LDL cholesterol levels after intravenous administration (20 µg Cr per kg body mass daily) in healthy and diabetic type II rats. Taking into account the lipid regulatory potential of dietary fructans and chromium(III), in this work, we studied the synergistic effect of inulin-type fructans and chromium(III) supplementation on fat digestibility and serum lipid indices in healthy rats.

MATERIAL AND METHODS

Animals and diets. Fifty-six male Wistar rats (8 weeks old) were purchased from Laboratory Animals Breeding in Brwinow, Warsaw, Poland. After arrival animals were allocated into 8 groups (7 rats each), with similar average body weight (154 g per animal), and fed *ad libitum* 8 types of semi-purified AIN-93M diets [Reeves et al. 1993] modified according to the three-factorial experimental design. The factors were as follows: factor A – type of fructan: inulin (IN), DP > 10 or oligofructose (OF), DP = 2-8, factor B – dietary fructan level: 5% or 10% of diet (introduced at the expense of wheat starch), and factor C – chromium(III) level: 0.5 mg or 5 mg/kg diet, in the form of trinuclear Cr(III) complex with propionic acid $[\text{Cr}_3\text{O}(\text{O}_2\text{CCH}_2\text{CH}_3)_6(\text{H}_2\text{O})_3]^+ \text{NO}_3^-$. The rats were housed in single cages, and kept in the Animal House with controlled temperature, lighting and humidity (19-22°C, 12 h light/dark cycle, 55-60% of ambient air humidity).

Feed intake was measured daily, while body weight gain was monitored weekly. During the experimental period the 10-day balance study was performed to collect faeces. At the end of the study, after 12 h starvation, rats were sacrificed (under anaesthesia with intra peritoneal thiopental injection), than blood was drawn from heart into non-heparinized tubes. Blood serum was obtained after 10 min centrifugation at 4000 rpm. The experimental protocol was approved by the Animal Bioethics Committee of Poznan, Poland (No 93/2001).

Parameters and analytical methods. The total dietary and faecal fat content was determined by the Soxhlet method. Apparent digestibility of fat (ADF) was calculated on the basis of dietary fat intake (a) and fat faecal output (b), according to the equation: $ADF (\%) = [(a - b)/a] \times 100$. The serum total cholesterol (TC), high-density lipoproteins cholesterol (HDL) and triacylglycerol (TAG) concentrations were determined by the enzymatic method on auto-analyzer (KONE-PRO Selective Chemistry Analyser, Finland). The concentration of cholesterol in the low-density lipoproteins (LDL) was calculated using the Friedwald's equation (1972)

Statistical analyses. The results are presented as arithmetic means and standard deviations. For statistical evaluation of results multifactorial ANOVA and Tukey's test were applied at significance level $p < 0.05$, using computer program Statistica ver. 7.0.

RESULTS AND DISCUSSION

The results are presented in Tables 1 and 2, as main effects (for each factor: A, B, and C, independently on other factors, so each data represent average values calculated for 28 animals) and interaction effects (presented as Fisher's coefficients – F values, according to multifactorial ANOVA methodology). It was found that non of experimental factors (independently and in interaction) influenced diet intake, faecal fat excretion and apparent fat digestibility. Also experimental factors did not affect serum total cholesterol, HDL cholesterol and LDL cholesterol concentrations in rats. However, the serum TAG concentration was influenced by the type of dietary fructan and its amount in the diet. The oligofructose-diet significantly ($p < 0.001$) reduced serum TAG level by 25% in comparison with the inulin-diet. Also the high-fructan diet (10%) markedly decreased serum TAG by 17.3% compared to the low-fructan diet (5%). Moreover, rats fed the high-fructan (10%) and low-chromium diet (0.5 mg/kg) had significantly lower ($p < 0.05$) serum TAG concentration compared to the group fed low-fructan diet (interaction BC). In literature information about the influence of inulin, oligofructose and chromium on macronutrient digestibility is scanty. Hesta et al. [2001] showed reduced protein digestibility in cats fed diet supplemented with 3, 6 and 9% oligofructose as a result of higher bacterial nitrogen content of the faeces. Ellegard et al. [1997] investigated the influence of inulin (7 g/day) and oligofructose (17 g/day) on nutrient utilization, digestibility and energy excretion with faeces in patients with ileostomy. Dry mass excretion increased by 14.4 g on inulin and by 14.7 g on oligofructose and energy excretion increased by 245 kJ on inulin and 230 kJ on oligofructose diet compared to control diet ($p < 0.05$). However, cholesterol, bile acids, nitrogen, fat, and minerals excretion were not affected by these fructans. Kornegay et al. [1997] showed that chromium picolinate (CrPic) (200 µg/kg diet) increased dry mass digestibility and the rate of nitrogen excretion from the small intestine in pigs. In our study experimental factors

Table 1. Effect of experimental factors on diet intake, fat intake and excretion and apparent fat digestibility in rats (ANOVA, values are means for 28 rats)

Parameter in serum	Experimental factors			Interactions (F value)			
	A type of fructans IN vs. OF	B amount of fructans 5% vs. 10%	C Cr level 0.5 vs. 5.0 mg/kg	AB	AC	BC	ABC
Feed intake ^a , g/day	21.6	21.3	21.2	NS	NS	NS	NS
	21.1	21.5	21.5				
Stool output ^b , g/d.m./day	0.98	0.89	0.96	NS	NS	NS	NS
	0.95	1.03	0.96				
Faecal fat excretion ^b , g/day	0.09	0.08	0.08	NS	NS	NS	NS
	0.08	0.09	0.09				
Apparent fat digestibility ^b , %	94.6	95.3	95.3	NS	NS	NS	NS
	95.1	94.3	94.4				

NS – non significant.

^aValues are mean for 7-weeks experiment.^bValues are mean for 10-d balance study.

Table 2. Effect of experimental factors on serum lipid indices in rats (ANOVA, values are means for 28 rats)

Parameter in serum	Experimental factors			Interactions (F value)			
	A type of fructans IN vs. OF	B amount of fructans 5% vs. 10%	C Cr level 0.5 vs. 5.0 mg/kg	AB	AC	BC	ABC
Total cholesterol level, mg/dl	72.5	73.3	72.9	NS	NS	NS	NS
	70.6	69.8	70.2				
HDL cholesterol level, mg/dl	19.3	19.7	19.1	NS	NS	NS	NS
	19.3	19.0	19.5				
LDL cholesterol level, mg/dl	38.9	40.0	40.9	NS	NS	NS	NS
	40.9	39.9	39.0				
TAG level, mg/dl	71.2**	68.2*	64.4	NS	NS	*	NS
	53.4	56.4	60.2				

NS – non significant.

*, **Statistically significant differences at $p < 0.05$, $p < 0.001$, respectively.

did not affect diet intake, stool output and apparent fat digestibility. This is probably due to the fact that inulin and oligofructose are almost completely degraded by the colonic bacteria to soluble short-chain fatty acids, thus does not affect stool dry mass and does not interfere with absorption of fat from the gastrointestinal tract. Effect of various

fructans on lipid metabolism was studied in human subjects and animal models. In healthy human subjects, short-term studies have shown that the addition of inulin-type fructans to diet may have a plasma lipid-lowering effect, while in long-term studies no such beneficial effect was observed [Forcheron and Beylot 2007]. Many authors observed lipo-suppressive effect of fructans due to the fatty acids synthesis reduction in the liver. Delzenne et al. [2002] obtained 25% reduction of triacylglycerol and lipoprotein synthesis in rat's liver after feeding diets enriched (10%) with inulin or oligofructose. Delzenne and Kok [2001] reported similar reduction of serum triacylglycerol and lipids levels in rats after feeding diets with 10% of oligofructose or inulin. Yamashita et al. [1984] and Hidaka et al. [1991] observed serum triacylglycerol and LDL-cholesterol level reductions in subjects eating standard diets enriched with inulin and oligofructose.

In the present study the only blood lipid parameter affected by experimental factors (type of fructan and its amount) was serum TAG concentration. Oligofructose had stronger reducing effect on serum TAG compared with inulin. Also high-fructan diet was more efficient in reducing TAG concentration vs. the low-fructan diet. In our previous study [Krejpcio et al. 2007] the high-fructan (10% inulin) diet was more efficient in improving blood biochemistry indices (decreased glucose and insulin resistance, increased beta-oxidation of fatty acids in white blood cells) of STZ-induced diabetic Wistar rats vs. the low-fructan diet.

In our earlier paper [Józefiak et al. 2005], we reported that Wistar rats fed high-inulin diet (10%) had markedly higher concentration of volatile fatty acids, especially butyrate in caecum. The short chains fatty acids that are produced in colon during fructan fermentation, especially propionic acid, could slow down the gene expression that is responsible for lipogenic enzymes synthesis.

Chromium(III) supplementation is believed to improve serum total cholesterol, LDL cholesterol concentrations and TAG levels in experimental animals [Sahin et al. 2007, Yang et al. 2006, Króliczewska et al. 2004]. However, the results are highly variable, contradictory and depend on the Cr compound and its dose, Cr bioavailability, duration of experiment, animal model (healthy, diabetic), and species researchers used. For instance Lai et al. [2006] reported that supplemental Cr yeast (600 mcg Cr/kg body mass/day) given to diabetic type 1 male Wistar rats had decreased the plasma LDL-cholesterol level as compared to controls. Sahin et al. [2007] observed that high-fat diet supplemented with CrPic (80 mcg/kg body weight/day) lowered serum glucose (by 63%), total cholesterol (by 9.7%) and TAG (by 6.6%) in male Sprague-Dawley rats intraperitoneally injected with STZ. Kim et al. [2002] obtained a significant decrease of serum TAG level, although no differences in cholesterol concentration, in dexamethasone-treated rats supplemented for 14 days with 30 mcg Cr/kg/day as CrPic, in comparison to the reference group of rats. Cefalu et al. [2002] observed lower plasma total cholesterol and higher HDL cholesterol levels in obese rats supplemented for three months with 80 mcg Cr/day as CrPic, in comparison to obese rats with placebo. Sun et al. [2002] demonstrated that the Cr propionate complex (injected intravenously at the dose of 10-20 mcg/kg body weight) significantly lowered plasma TAG, total cholesterol, LDL cholesterol, and insulin levels after 24 weeks of supplementation in healthy Sprague-Dawley and Zucker obese rats. However, a significant decrease of plasma TAG levels in healthy rats appeared after 12 weeks the earliest, and only in the group receiving the highest dose of the compound (20 µg/kg body weight/day). Our earlier study [Kuryl et al. 2006] on healthy male rats fed the same diets (supplemented with

fructans and Cr) showed that supplementary Cr(III) (5 mg/kg diet) significantly decreased serum insulin (by 15%) level, while elevated beta-oxidation of fatty acids in white blood cells (by 77%).

In the light of meta-analysis [Balk et al. 2007], Cr(III) supplementation significantly improves glycemia among patients with diabetes, however no such effect is found on lipid and glucose metabolism in healthy subjects.

In this study we did not observe significant effects of Cr (III) supplementation (5 mg/kg diet) as Cr propionate complex on serum lipid indices in rats, probably due to the fact that animals were healthy and chromium sufficient, while the positive pharmacologic effects of Cr is usually observed in metabolic disturbances, like hyperglycemia hyperlipidemia and hypercholesterolemia, as well as in Cr-deficiency state in animals or humans.

CONCLUSIONS

1. Inulin-type fructans and supplementary Cr(III) (as propionate complex), in the applied doses, do not affect digestibility of fat in rat.
2. Dietary supplementation with inulin-type fructans influence lipid metabolism, lowering triacylglycerols formation, while supplementary chromium(III) does not affect blood lipid indices in healthy rats.

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WPLYW SUPLEMENTACJI DIETY FRUKTANAMI I CHROMEM(III) NA STRAWNOŚĆ POZORNĄ TŁUSZCZU ORAZ WSKAŹNIKI LIPIDOWE KRWI U SZCZURA

Wstęp. Fruktany i chrom(III) uważa się za czynniki wpływające na metabolizm lipidów i węglowodanów w organizmie zwierząt i ludzi, z tego względu mogą być wykorzystane do regulacji lub poprawy profilu lipidowego, jednakże w pełni nie jest wyjaśnione ich znaczenie profilaktyczne. Celem tej pracy było określenie wpływu suplementacji diety szczura fruktanami typu inuliny oraz chromem(III) na strawność tłuszczu oraz profil lipidowy krwi.

Materiał i metody. W trzyczynnikowym doświadczeniu, 56 samców szczurów Wistar żywno przez 10 tygodni *ad libitum* dietami AIN-93 wzbogaconymi fruktanami typu inuliny na dwóch poziomach (inulina lub oligofruktoza, 5% lub 10% diety) oraz Cr(III) (0.5 lub 5 mg/kg diety w postaci kompleksu z kwasem propionowym). Określono strawność pozorną tłuszczu oraz profil lipidowy krwi.

Wyniki. Stwierdzono, że czynniki doświadczalne nie wpływały istotnie ani na strawność pozorną tłuszczu, ani profil lipidowy krwi, z wyjątkiem poziomu triacylogliceroli, który był istotnie niższy w grupach żywionych dietami z dodatkiem oligofruktozy vs. inuliny oraz wysokofruktanowych vs. niskofruktanowych.

Wnioski. Fruktany typu inuliny oraz suplementacja Cr(III) nie wpływają na strawność pozorną tłuszczu, natomiast fruktany oddziałują na metabolizm lipidów, obniżając poziom TAG we krwi szczurów.

Słowa kluczowe: inulina, oligofruktoza, chrom(III), lipidy, szczury

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