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EFFECT OF FRUCTANS ON GLUCOSE LEVEL IN BLOOD SERUM OF RATS – A SHORT REPORT

Aneta Kopeć, Ewa Cieślik

Department of Human Nutrition, Faculty of Food Technology, Agricultural University, Cracow

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The study aimed to determine the effect of an experimental dietary supplement of fructo-oligosaccharides (FOS), inulin and flour of Jerusalem artichoke tubers on glucose level in experimental rats. The nutritional experiment was conducted with albino Wistar rats. On the last day of the experiment the animals were anaesthetized and blood was sampled. In the biological material obtained glucose content was determined with the enzymatic method. A statistically significant decrease in glucose level was found in blood serum of rats fed diets containing inulin and Jerusalem artichoke flour. The results obtained demonstrate the purposefulness of further feeding experiments with animals and in the future also of nutritional experiments with people.

INTRODUCTION

Increasing nutritional awareness of consumers is accompanied by their growing interest in food products, which beside satisfying hunger would fulfil functions important for the human health. Such products may influence an improvement in the health state or their components may prevent various diseases, particularly those connected with civilization development (neoplastic diseases, arteriosclerosis, hypertension, diabetes or dental caries). Thus still new types of food are sought which would not only provide nutrients but also positively affect human health. Such functions are, among others ascribed to food containing fructans.

Fructans (inulin and fructooligosaccharides) are not digested in human organism due to a lack of an enzyme hydrolysing these compounds in the digestive system. They are fermented by intestinal bifidobacteria especially in the proximal colon. During colonic fermentation, short-chain fatty acids are produced (acetic, propionic and butyric acids). These acids are likely to produce a positive effect on carbohydrate (glucose) metabolism in animal and human body.

Fructans have sweet taste and are not digested hence they have been widely used in manufacturing of food for diabetics [Barta, 1993; Fontana *et al.*, 1993]. Inulin has been used in diabetic nutrition since the twenties, particularly as a supplement to confectionery products (cakes and desserts), whereas recently it has also been used for low-fat ice-cream and low-energetic beverages production [Polak, 2001], because 1 g of these carbohydrates provides only 1.5 kcal [Roberfroid, 1999]. Cieślik and Kopeć [2003] showed that growing rats fed diets with different levels fructans originating from different sources demonstrated lower body gain compared to rats fed control diets. The same results were reported by Daubioul *et al.* [2002] in an experiment with obese zucker rats.

Together with food also carbohydrates find their way to human organism and are digested in the alimentary tract (starch, glycogen, saccharose) to monosaccharides (fructose, glucose) and fermented by bifidobacteria in the colon (pectins, β-glucans, oligosaccharides, hemicelluloses, gums and plant mucus). Elevated blood serum level of glucose is a stimulus for the pancreas to produce insulin, the hormone regulating glucose level in the organism [Brand-Miller *et al.*, 2002; Leeds, 2002; Ludwig & Eckel, 2002]. Frequent consumption of products of high glycemic index (light bread and pasta or potatoes) causes that the organism gets used to high blood serum glucose level [Brand-Miller *et al.*, 2002; Domensil *et al.*, 2001; Pi-Sunyer, 2002]. Excessive consumption of carbohydrates, particularly monosaccharides leads to overweight and then to obesity, and increases the risk of diabetes [Branca *et al.*, 2001; Brand-Miller *et al.*, 2002; Pi-Sunyer, 2002]. As a result of glucose metabolism to triglycerides the possibility of atherosclerotic changes increases.

Fructans are water-soluble short- and long-chain polysaccharides where fructose is a basic structural unit [Incoll & Bonnet, 1993]. Short-chain oligofructans – fructooligosaccharides – are composed of 3–5 fructopyranose residues bound with β-2-1 glycoside bond where a glucose particle is situated at the end of each chain [Coussement, 1999b; Jackson *et al.*, 1999]. They occur *e.g.* in Jerusalem artichoke (2–3%), leeks (2–5%), onion (2–6%) bananas (0.3–0.7%), and also in garlic and corns [Alles *et al.*, 1999; Niness, 1999].

Inulin is a long-chain fructan. The carbohydrate is a reserve substance in many plants of *Compositae* and *Liliacae* families, its highest amounts are found in chicory (15–20%) and in Jerusalem artichoke (17–22%) [Coussement, 1999a; Hirayama *et al.*, 1993].

Available literature data suggest that fructans and

Author's address for correspondence: Aneta Kopeć, Department of Human Nutrition, Agricultural University, al. 29 Listopada 46, 31-425 Kraków, Poland; tel. (48 12) 662 50 18; e-mail: rrciesli@cyf-kr.edu.pl

Jerusalem artichoke tuber flour are characterised by hypoglycaemic effect [Kok *et al.*, 1998; Varlamova *et al.*, 1996]. Thus the undertaken studies aimed at determining the effect of a supplement of fructooligosaccharides, inulin and flour of Jerusalem artichoke tubers added to hypercholesterolemic diet (lard) on glucose level in experimental rat blood serum.

MATERIALS AND METHODS

Flour of Jerusalem artichoke tubers (*Helianthus tuberosus* L.), Topstar cv., fructooligosaccharide (FOS) preparations – "Raftilose" and inulin preparation "Raftiline" produced by a Belgian company Orafti were used in the study. Jerusalem artichoke tubers were cultivated at the Experimental Station of Eggenburg Agricultural University, Vienna, Austria. The contents of basic components (protein, saccharose, fiber and ash) were determined in the Jerusalem artichoke flour with standard analytical methods [AOAC, 1997] and total fructans – with the enzymatic method [Boehringer-Mannheim, 1989] (Table 1).

TABLE 1. Levels of selected ingredients in Jerusalem artichoke flour.

Ingredient	g/100 g d.m.
Proteins	7.4
Saccharose	15.0
Dietary fiber	14.5
Ash	7.2
Fructans	44.1

Experimental animals. Nutritional experiments were conducted with albino laboratory Wistar rats, bred by the Department of Animal Nutrition, Institute of Animal Husbandry in Krakow. Growing males aged between 5-6 weeks with mean body weights of 90–120 g were used for the experiment. The studies were carried out in compliance with ethical requirements and were approved by the local Ethical Commission. During the adaptation period (7 days) the animals had standard GLM-1 granulate and drinking water *ad libidum*. After the acclimatisation period the animals in experiments 1, 2, 3 were divided into 4 groups (n=6) and fed experimental diets. Experimental diets were prepared of: corn starch (ICN, OH. USA), casein (95% N x 6.25, Sigma ST. Louis, MO. USA), lard (Baso, Olkusz, PL), cellulose (ICN, OH. USA), choline bitartrate (Sigma ST. Louis, MO. USA), tert-butylhydroquinone (Sigma ST. Louis, MO. USA) and vitamin and mineral mixtures according to Reeves *et al.* [1993]. The composition of basal (control) diet is shown in Table 2. The addition of FOS in Exp. 1 and inulin in Exp 2. was 0%, 4%, 8%, and 12%. In Exp. 3 rats were fed diets with the addition of Jerusalem artichoke (0%, 5%, 10%, and 15%). During the experiment the animals were kept in individual metabolic cages with floors made of rustless steel mesh, in a room with constant temperature and air humidity, and 12-h day-and-night cycle. The rats had free access to water and food. Diet intake was checked daily, whereas the increase in their body weights was monitored at the outset and at the end of the experiment. On the day before the end of the experimental period the animals were fed in the way allowing blood sampling for analyses on empty stomachs, *i.e.* after 14–16 h since the last feeding. The next day, the animals were anaesthetised by intraperitoneally injected 25 mg/100 g of weight body dose of Thiopenthal (Biochemie, Vienna, Austria) and blood was sampled directly from the heart. The biological material obtained was collected to single tubes and centrifuged for 10 min at 4000 g to get blood serum. In the biological material obtained glucose was assayed with the enzymatic method by means of Bio Vendor analytic kit No. 11601.

Results were expressed as means ±SEM. Data were analysed by one-way ANOVA and Duncan's multiple range test [Duncan, 1965]. The analysis of linear regression was carried out in order to find the interrelations between the dietary amount of fructans and concentrations of selected biochemical indices in rat blood serum. The differences between means were tested for significance at $p \leq 0.05$.

TABLE 2. Composition of basal (control) diet.

Ingredient (g)/Group	I – control
Corn starch	533.97
Casein	200
Sucrose	100
Fibre	50
Lard	70
Vitamin mix	10
Mineral mix	35
Choline bitartrate	1.017
Tertbutylohydroquinone	0.014

RESULTS

Average diet intake was: in Exp. 1 – 1548 g, in Exp. 2 – 1494 g, and in Exp. 3 – 1512 g.

In experiment 1, where diets were supplemented with FOS, it was found that glucose concentration in rat blood serum in group I was 12.44 mmol/L and in group II it increased to 13.26 mmol/L. On the other hand, in groups III and IV it decreased to 8.90 mmol/L and 8.75 mmol/L, respectively (Table 3). Despite such big differences ANOVA did not reveal any statistically significant changes between control and the other experimental groups.

It was found that glucose level in rat blood serum was decreasing with an increasing dietary inulin content (Exp. 2). The highest level was demonstrated in group I – control (12.44 mmol/L), in group II it decreased to 10.02 mmol/L, whereas in groups III and IV it accounted for 9.16 mmol/L and 8.37 mmol/L, respectively.

TABELE 3. Glucose level in serum of rats (mmol/L).

Group / % of fructans	FOS*	Inulin*	Jerusalem artichoke flour**
I	12.44 ± 1.6^{a}	12.44 ± 1.6^{b}	12.20 ± 0.47^{c}
II	13.26 ± 1.58^{a}	10.02 ± 0.53^{ab}	8.42 ± 1.52^{b}
III	8.90 ± 1.25^{a}	9.16 ± 0.37^{a}	8.26 ± 0.84^{a}
IV	8.75 ± 1.17^{a}	8.37 ± 0.47^{a}	11.84 ± 0.25^{c}
SE***	0.95	0.72	0.87

*FOS and inulin have been added to diets at doses of 4%, 8%, 12%. Jerusalem artichoke meal** has been added to diets at doses of 5%, 10%, 15%. Values in a row with different letters (a, b, c) are significantly different ($p \leq 0.05$), ± SE,*** standard error

Glucose concentration in experiment 3, where the diets were supplemented with 5%, 10% and 15% of Jerusalem artichoke tuber flour, was decreasing with a growing percentage of flour in diets. In the experimental rat blood serum in group I it was 12.2 mmol/L. In groups II and III, where the dietary supplements were respectively 5% and 10%, it diminished significantly and reached 8.42 and 8.26 mmol/L. On the other hand, in group IV glucose concentration in rat blood serum raised again to 11.84 mmol/L and was lower than glucose content in control rat blood serum (Table 3).

DISCUSSION

Glucose level in rodent blood serum decreased in experiment 2 and 3 with an increasing amount of fructans in experimental diets (Table 3). In the experiment with FOS, the lowest level of glucose was reported for group IV (8.75 mmol/L), which was lower in comparison with the control group by 30%. Despite considerable differences between the control and experimental groups, ANOVA analysis did not reveal any statistically significant differences. It might have been influenced by biological variability causing big differences in blood serum glucose concentrations among individual organisms in groups. Kok *et al.* [1998] observed a similar hypoglycaemic effect in an experiment where the experimental diet was supplemented with 10% of FOS. Blood serum glucose concentration decreased by 1.76 mmol/L.

Glucose content diminished with an increasing percentage of dietary inulin (Table 3). It was the highest in group I – control, in group II it decreased by 20% and in groups III and IV – by 26% and 33%, respectively. On the basis of ANOVA, significant differences were demonstrated between groups I and III and I and IV. Whereas Diez *et al.* [1988] in an experiment with dogs showed that dietary inulin did not induce any changes in glucose concentration in plasma of these animals.

Glucose level in blood serum of animals fed flour was the lowest in the group receiving 10% flour supplement and amounted to 8.26 mmol/L. It diminished in comparison with the control group by 32.3%. On the other hand, in the group where 15% flour supplement was used it increased to 11.84 mmol/L but was lower than this index concentration in the control group (Table 3). On the basis of ANOVA, significant differences were demonstrated between groups I and II and I and III. A decrease in blood serum glucose might have been affected by quickened intestinal peristalsis. Faster intestinal passage might have hampered digestion and absorption of carbohydrates and in this way blood serum glucose level might have been decreased. The experiments were conducted with growing rats whose bodies needed energy for anabolic processes. It might have been the reason why glucose level kept diminishing in the animal blood serum. Varlamova *et al.* [1996] obtained a similar hypoglycaemic effect in an experiment with rats.

The hypoglycaemic effect of fructans (FOS, inulin) has not been explained so far. It is likely that short-chain fatty acids (acetic, propionic and butyric acids) positively affect the level of glucose in blood. Another possible hypoglycaemic action of fructans is the secretion of intenstinal hormones (glucose-dependent insulinotropic polypeptide and glucagon-like peptide 1) [Kok *et al.*, 1998].

CONCLUSIONS

A statistically significant decrease in blood serum glucose level was observed in animals fed diets supplemented with inulin and flour of Jerusalem artichoke tubers. In prevention and treatment of diabetes and obesity it is recommended to consume high amounts of products abundant in dietary fiber (especially soluble dietary fiber, *i.e.* fructans) at a decreased consumption of monocarbohydrates (10–15% of total supply of energetic substrates). Thus vegetables and fruit, which are a source of inulin and FOS (chicory, onion, garlic, leeks, banana), should be a common component of everyday diets of all people.

The results obtained demonstrate the purposefulness of further nutritional experiments with people to explore the effect of various amounts and sources of fructans on carbohydrate management.

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WPŁYW FRUKTANÓW NA POZIOM GLUKOZY W ORGANIZMACH SZCZURÓW DOŚWIADCZALNYCH – KRÓTKI KOMUNIKAT

Aneta Kopeć, Ewa Cieślik

Katedra Żywienia Człowieka, Wydział Technologii Żywności, Akademia Rolnicza w Krakowie, Kraków

Doświadczenia żywieniowe przeprowadzono z udziałem 72 rosnących szczurów albinotycznych szczepu Wistar. Po siedmiodniowym okresie aklimatyzacji zwierzęta podzielono losowo na grupy eksperymentalne. Gryzonie żywiono dietami z dodatkiem FOS (fruktooligosacharydów – doświadczenie 1), inuliną (doświadczenie 2), mączką z bulw topinamburu (doświadczenie 3) przez 21 dni. Po zakończeniu doświadczenia szczurom w stanie narkozy pobierano z serca krew. W otrzymanej krwi oznaczano enzymatycznie zawartość glukozy. Zaobserwowano statystycznie istotne obniżenie poziomu glukozy w surowicy krwi zwierząt karmionych dietami z dodatkiem inuliny oraz mączki z bulw topinamburu (tab. 3).

Otrzymane wyniki wskazują na celowość prowadzenia dalszych doświadczeń żywieniowych w organizmie człowieka, dotyczących wpływu różnych ilości i źródeł fruktanów na gospodarkę węglowodanową w organizmach.