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Novel cereal fibre drink as a tool for civilisation disease prevention

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RESEARCH ARTICLE

Abstract

To prevent from civilisation diseases such as obesity, type two diabetes, etc., it is recommended to combine a healthy diet and regular physical activity. An example of such an approach is a development of a cereal fibre drink Actiglucane, high in fibre and β-glucans and low in fat and energy, which physiological effect was tested together with a dance programme. The chemical, physicochemical and nutritional attributes of the Actiglucane were determined and the postprandial as well as long-term effects of its consumption were analysed. The glucose uptake and the consequent release resulting from the postprandial test were significantly slower compared to a standard (P<0.05). The subjective satiety of the Actiglucane drink measured by a questionnaire method was significantly higher than the standard glucose solution (P<0.05) which is a promising characteristic for use in obesity prevention programs. From the long-term point of view, the Actiglucane (daily dose representing 3 g of β-glucans/day) was implemented into a nutritionally balanced diet of 30 healthy women with a higher body fat percentage (more than 29%) for eight weeks. Subjects were divided into two groups: without (D) or with (DE) dance exercise. After the two month long intervention, a significant reduction in the dietary fat (D: 53%; DE: 37%) and the 24 h energy intake (D: 17%; DE: 12%) was observed. These changes in the dietary intake were well manifested in the monitored anthropometric and biochemical parameters. The significant drops of the total body weight, fat mass and visceral fat area as well as an improvement in lipid profile were achieved. A statistically significant synergic effect of the regular dance program Bellylatinofit® (moderate intensity exercise, 75 min, three times per week) added to the above discussed intervention was seen in a body weight reduction.

Keywords: Actiglucane drink, functional food, postprandial test, intervention study

1. Introduction

Intake of cereal grains, mainly in the whole grain form containing dietary fibre, vitamins, minerals and phytochemicals, is associated with lower risk of obesity and other civilisation diseases (Mikušová *et al.*, 2011). One of the dietary fibre components believed to have significant physiological effects is a group of cereal β-glucans. A regular consumption of high β-glucan cereals is related with beneficial effects on blood lipid variables, glucose metabolism, decreased feelings of hunger and prolonged satiation. Generally, it is the ability of viscous fibre to elevate cholesterol catabolism by decreasing bile acid absorption resulting in steroid excretion and increased bile acid secretion (Kaczmarczyk *et al.*, 2012). Furthermore, viscous fibres may interfere also with the absorption of dietary fat (Queenan *et al.*, 2007), what might cause additional effect in obesity prevention programmes. Another

possible mechanism of action is an elevation of short chain fatty acids (SCFA) level when β-glucans are fermented in the large intestine. SCFA, mainly propionate, have demonstrated hypo-cholesterolaemic effect (Hughes *et al.*, 2008) and may be related to the regulation of adipose tissue deposition (Roberfroid *et al.*, 2010). Similar mechanisms of action influence also glucose and insulin metabolism. The physiological effects of β-glucans are mainly attributed to its physicochemical and structural characteristics such as molecular weight or viscosity. Interacting with the gastrointestinal tract (GIT), viscous solutions at low concentrations are formed in the upper part of the GIT which subsequently undergo fermentation in the colon (El Khoury *et al.*, 2012). Increased viscosity in the intestine delays the absorption of glucose, reduces the absorption of cholesterol and consequently re-absorption of bile acids. Although the role of β-glucans as a component raising viscosity in the lumen is important, no standard method for determining the viscosity is available (Anttila *et al.*, 2004).

Since cereal β-glucan, as a dietary fibre component, is being discussed as a potential obesity prevention strategy, the aim of the current contribution was to test the designed novel cereal fibre drink Actiglucane in regard to its physicochemical, chemical and nutritional properties, to evaluate its glycaemic characteristic and satiating effect as well as to determine whether or not Actiglucane added to a nutritionally balanced diet with or without dance intervention (Bellylatinofit®) reduces anthropometric variables and improves lipid profile and glucose level in an eight week long controlled study.

2. Materials and methods

The Actiglucane fibre drink was prepared in two flavours by Essentia Ltd. (Veľký Grob, Slovak Republic) according to their own recipe. The drink was tested with regard to its polysaccharides quality and quantity, physicochemical and nutritional properties as well as a glycaemic characteristic, a satiating ability and a long term effect of its eight week consumption – changes in antropometric and biochemical parameters.

Analyses of mono- and polysaccharides

Isolation of the polysaccharides from the Actiglucane non-flavoured concentrate (Actiglucane drink not diluted with water) was as following: 14.5 ml water and 100 ml of 95% ethanol was added to the Actiglucane concentrate weighing 63.18 g (95% of dry mass). The sample was stirred on a magnetic stirrer 5 min and then centrifuged during 10 min at 1,792×*g*. The precipitate was re-suspended in 50% ethanol (25 ml), centrifuged, and subjected to dialysis in order to remove the low molecular weight substances. Sample free of low molecular weight substances (L-A) was lyophilised with a yield of 1.8993 g. L-A (0.51 g) was further treated with acetate buffer (pH 5.4) at 70 °C for 30 min under stirring and then cooled to 37 °C. The enzymatic digestion of starch was performed with the α-amylase (EC 3.2.1.1; A) from *Aspergillus oryzae* (60.5 U/mg; EC 3.2.1.3) and amyloglycosidase (50 U/mg; AG) from Merck (Darmstadt, Germany) at 37 °C for 48 h under stirring. After inactivation of the enzymes (100 °C for 10 min), the mixture was centrifuged at 2,800×*g* for 15 min. The supernatant was extensively dialysed with deionised water (molecular weight cut-off 3.5 kg/mol; Serva Electrophoresis GmbH, Heidelberg, Germany) and lyophilised (L-A/A+AG; 0.455 g). The sample was divided into water-soluble (ws) (7.8%) and water-insoluble (wis) (92.2%) parts. Hydrolysis of the polysaccharides was performed with 2 M trifluoroacetic acid (TFA) for 2 h under reflux or by the two-step acid hydrolysis with 72% H_2SO_4 (1 h and after dilution to 1.8% H_2SO_4 for 4 h under reflux). The neutral sugar composition of the hydrolysates was determined by gas chromatography on a Hewlett-Packard model 5890 series II chromatograph equipped with a PAS-1701 column (0.32 mm × 25 m) in form of the alditol trifluoroacetates (Hewlett-Packard, Wilmington, DE, USA). The sugars were qualitatively analysed by paper chromatography (Whatman no. 1; W.R. Balston Ltd., Maidstone, UK) using system A: ethyl acetate/pyridine/water (ϕ_r=8:2:1) (neutral sugars) and B: ethyl acetate/acetic acid/formic acid/water (ϕ_r=18:3:1:4) (acidic sugars).

Fourier Transform-Infrared Spectrometry (FT-IR) spectra of polysaccharides were recorded with a Nicolet Magna 750 spectrometer with DTGS detector (Thermo Scientific, Madison, WI, USA) and OMNIC 7.0 software (Thermo Electron Corporation, Madison, WI, USA) at a resolution of 4 cm^{-1}. The sample was pressed into KBr pellet.

Physicochemical properties and nutrition profile of Actiglucane

The molecular weight of the Actiglucane non-flavoured concentrate was determined by a standard method of size-exclusion chromatography (Wood *et al.*, 1991). After dissolution of the soluble part (ws) in 0.3 M NaOH, subsequent filtration and NaOH removal, the suspension was ten times concentrated and a Hema Bio 1000 column with 0.1M $NaNO_3$ with sodium azide as a mobile phase (Tessek, Prague, Czech Republic) was used for analysis. Calibration curve with oat β-glucan standards kit (Putus Macromolecular Sci. & Tech. Ltd., Wuhan, China P.R.) with molecular weight range from 80 to 1,040 kDa was used to calculate the molecular weight of the drink concentrate.

Nutritional composition of the Actiglucane concentrate such as fat, dietary fibre including a soluble and an insoluble form, etc., were determined according to the Slovak STN norms in concordance with international ISO norms. The amount of total fat was analysed by the Soxhlet method

(STN 560116; Slovenské normy, 1974) and the dietary fibre content by the enzymatic-gravimetric method (STN 560031; Slovenské normy, 1999). The content of the total β-glucan in the Actiglucane concentrate was declared by the producer. The energy content of the Actiglucane concentrate was calculated according to the standard formula. The available carbohydrates were calculated by the difference of all other basic nutritional components (amount of dry mass minus the sum of protein, fat, ash and the total dietary fibre content).

Glycaemic characteristic and satiety of Actiglucane

Ten non-smoking healthy women in the age 22-35 years were recruited to participate in the postprandial study aimed to describe glycaemic characteristic of the Actiglucane fibre drink and the satiating ability of this drink compared to the glucose standard. Volunteers were not suffering from any chronic or acute disease and were not taking any medication that could influence the outcomes of the study (Table 1 and 2). Subjects were asked to restrain from food and use of an intense physical activity for 12 h prior to the examination. Upon the arrival to the Institute of Experimental Endocrinology in the morning, blood samples were drawn from antecubital vein to determine lipid profile and fasting glucose of the subjects according to standard laboratory methods described below.

Table 1. General and anthropometric characteristic of participants.

Parameter	Mean ± standard error of the mean
Body mass index (kg/m^2)	24.6±1.3
Body fat percentage (%)[1]	31.4±2.3
Waist circumference (cm)	81.8±3.9
Blood pressure systolic (torr)	115.6±4.4
Blood pressure diastolic (torr)	67.3±2.0
Pulse rate (min^{-1})	65.3±2.3

[1] Measured with OMRON BF 300 (OMRON, Kyoto, Japan).

Table 2. Biochemical characteristic of participants.

Biochemical parameter	Reference range	Mean ± standard error of the mean
Fasting blood glucose (mmol/l)	3.6-6.1	4.5±0.1
Cholesterol (mmol/l)	3.9-5.2	3.9±0.2
Triglycerides (mmol/l)	0.45-1.92	1.07±0.28
High density lipoproteins-cholesterol (mmol/l)	1.0-1.7	1.3±0.1
Low density lipoproteins-cholesterol (mmol/l)	1.3-4.0	2.1±0.1
Very low density lipoproteins (mmol/l)	0.3-0.9	0.5±0.1

A catheter (Meditrade, Ltd., Bratislava, Slovakia) was placed in the antecubital vein for blood sampling early in the morning. 25 g of available carbohydrate portions of the Actiglucane concentrate or glucose respectively, were served on two separate sessions, mixed with 250 ml of water. Blood was taken before the consumption of the tested drink (0 min) and 15, 30, 45, 60, 90, 120 min after ingestion (ISO 26642:2010(E); ISO, 2010). Blood samples were placed into ice, centrifuged and stored at minus 20 °C until analysed. At the same time points, subjective satiety feeling was evaluated on a scale from -3 (extremely hungry) to +3 (totally full) (adapted from Quilez *et al.*, 2007). Pure water consumption was allowed during the whole meal test.

Study of the long term consumption/intervention study of Actiglucane

Thirty women with higher body fat percentage (≥29%) were recruited for the study and assigned into two treatment groups: D (Actiglucane drink; n=15) and DE (drink plus dancing exercise; n=15). Required criteria for inclusion into the study were: generally healthy, non-smokers, age 19-34 years, body fat percentage ≥29% and no intake of medication that could negatively affect variables of this study. Ten women in the same age group, but with normal body fat percentage (<29%) with adherence to a healthy lifestyle and regular exercise (150 min moderate activity weekly as recommended by WHO, 2011) for more than one year, were recruited as a control group into this study (C). No statistically significant changes in anthropometric or biochemical parameters were observed between all participants at the beginning of the study (Table 3).

For all anthropometric measurements a direct segmental multi-frequency bioelectrical impedance method was used (body composition analyser InBody 230; Biospace Ltd., Centennial, CO, USA). Biochemical analyses such as glucose, triglycerides (TAG), total cholesterol (TC) and high density lipoprotein (HDL) cholesterol content in the blood were performed using standard laboratory methods on an automatic analyser Vitros 250 (Johnson & Johnson, New Brunswick, NJ, USA). Low density lipoprotein (LDL) cholesterol was calculated according to the Friedewald formula. Atherogenic index (AI) was calculated as a ratio of TC and HDL cholesterol (Krajcovicova-Kudlackova *et al.*, 2004). Very low density lipoprotein (VLDL) cholesterol content was calculated as an amount of TAG in mmol/l divided by 2.2. Fasting insulin levels were measured using ELISA immunoassay kit (Alpco Diagnostics, Salem, NH, USA). Homeostasis model assessment-insulin resistance (HOMA-IR) was calculated as fasting glucose multiplied by fasting insulin levels and divided by factor 22.8.

Table 3. Baseline general, anthropometric and biochemical characteristics of the study population participating in the long term intervention study (means ± standard error of the mean).[1]

Baseline parameter	D group	DE group	C group
Body mass index (kg/m^2)	27.4±1.1	26.6±0.9	19.7±0.9
Body fat percentage (%)[2]	37.8±1.5	37.6±1.4	24.6±1.4
Waist circumference (cm)	88.6±1.8	87.3±2.6	69.5±2.0
Fasting glucose (mmol/l)	5.00±0.13	5.09±0.16	4.67±0.09
Cholesterol (mmol/l)	5.25±0.21	5.61±0.40	4.27±0.17
High density lipoproteins-cholesterol (mmol/l)	1.49±0.08	1.49±0.15	1.76±0.11
Low density lipoproteins-cholesterol (mmol/l)	3.21±0.18	3.40±0.41	2.06±0.20
Triglycerides (mmol/l)	1.22±0.13	1.57±0.33	1.00±0.05
Very low density lipoproteins (mmol/l)	0.56±0.06	0.71±0.15	0.45±0.02

[1] D group = β-glucan fibre drink; DE group = β-glucan fibre drink with exercise (dance programme Bellylatinofit®); C group = controls, without any lifestyle change.
[2] Measured with InBody 230 (Biospace Ltd., Centennial, CO, USA).

The cereal Actiglucane fibre drink was tested as a part of a dietary programme based on balanced nutrition combined with new type of dance exercise, Bellylatinofit®. After completion of the 2-month long initial phase (diet and exercise regime adaptation), β-glucan fibre drink was included into the diet for 2 months. Participants in D and DE group were instructed and encouraged to lower consumption of high energy and high saturated fat food, to enhance consumption of fruit and vegetables, as well as to control their daily portions (size and frequency), to achieve nutritionally balanced diet. Subjects were asked to attend group meeting sessions every two weeks, and also one individual dietary counselling pro month was available for every participant. Food diaries were collected electronically each week and diet analyses were completed using nutrition software Alimenta (Food Research Institute, Bratislava, Slovakia). Participants of DE group were advised not to change their physical activities regime (if any) except to the exercise protocol of the study. D group participants were asked not to change their physical activity regime (if any) at all. Participants of the control group were advised not to change their lifestyle at all.

The experimental protocol for both studies was approved by a local ethic committee. All volunteers were fully informed of the nature and purpose of the studies and a written informed consent was obtained before participation.

3. Results and discussion

The novel cereal fibre drink Actiglucane was characterised in terms of its chemical and nutritional composition and effect of its short-term and long-term consumption was evaluated.

Analysis of mono-and polysaccharides

Purified fractions of the non-flavoured Actiglucane were subjected to chemical and spectral analyses to estimate their polysaccharide composition. The monosaccharide composition of the water soluble fraction L-A/A+AG ws showed that apart from xylose (Xyl; 62%) and arabinose (Ara; 25%), only traces of glucose (Glc; 6%), galactose (Gal; 6%) and mannose (Man; 1%) were present. The presence of 4-*O*-methyl-β-D-glucuronic acid (MeGlcA) was confirmed by paper chromatography (system B). The results suggested that the water soluble fraction (7.8% of Actiglucane) comprised predominantly of arabinoglucuronoxylan (AGX).

Two methods were used to hydrolyse the water insoluble fraction L-A/A+AG wis. The hydrolysates contained mainly Glc (TFA method: 18%; H_2SO_4: 47%) and Xyl (TFA method: 69%; H_2SO_4: 51%). Lower content of Ara was detected using both hydrolysis methods which could have partially originated from the presence of less branched AGX in this fraction. The high content of Glc predominantly originates from the (1,3/1,4)-β-D-glucans abundantly present in cereals (hence starch was removed), and/or cellulose. Presence of (1,3/1,4)-β-D-glucans was confirmed by NMR analysis (data not shown) and their content in the Actiglucane concentrate was 3 g/100 g of fresh weight.

Infrared spectroscopy is a quite extensively applied method in plant cell wall analysis. In the spectrum of L-A/A+AG ws (Figure 1), the bands at 1,650 and 1,550 cm^{-1} were due to the stretching of NH groups of amide I and amide II indicating the presence of proteins. Small bands at 2,852 cm^{-1} show the presence of lipids (Alonso-Simon *et al.*, 2011; Kačuráková *et al.*, 1999), present only in the water soluble fraction. Low content of phenolics in all samples was

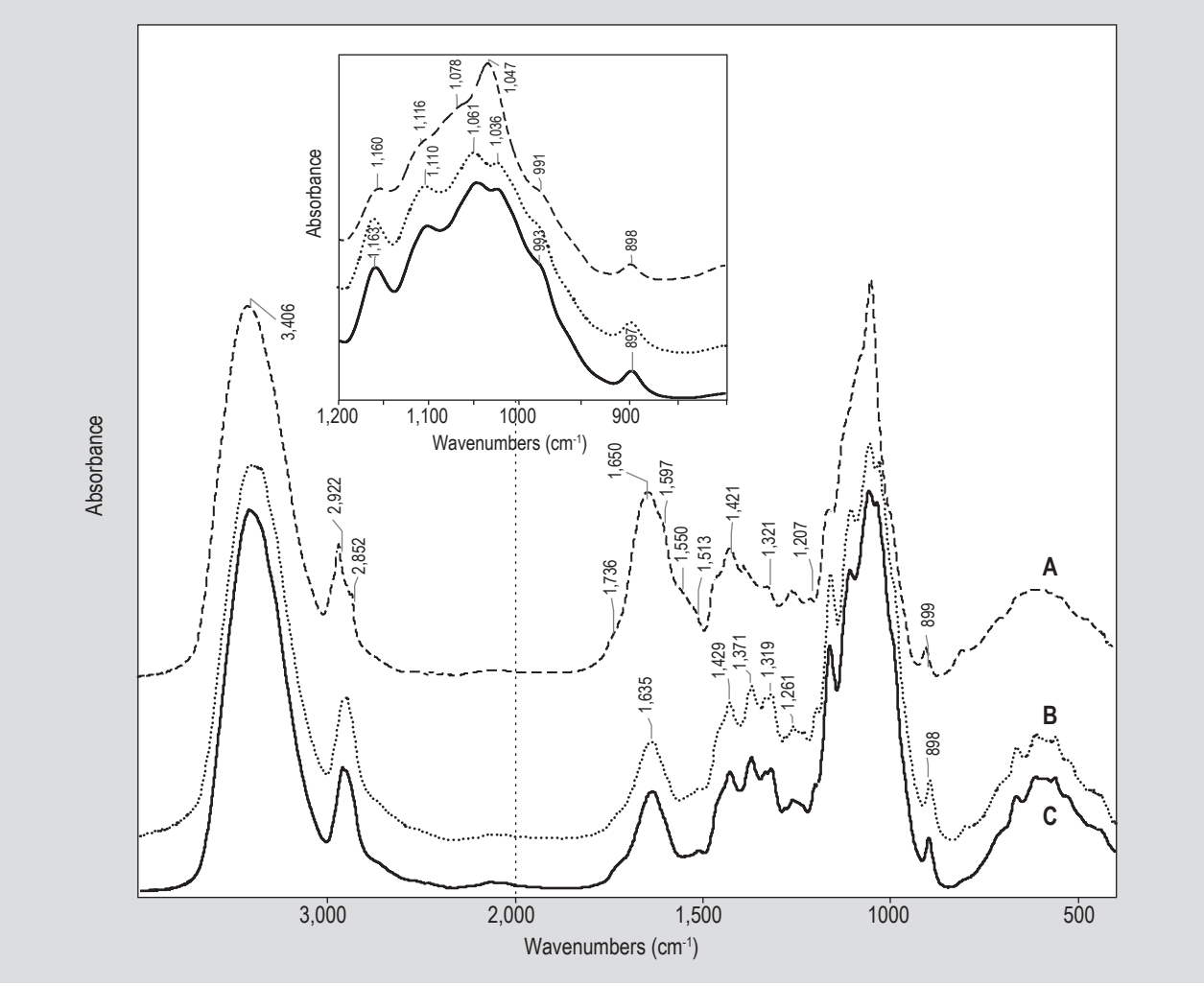

Figure 1. FT-IR spectra of water-soluble (line A) and water-insoluble (line B) fractions of L-A/A+AG (line C) Actiglucane.

indicated by absorption bands at 1,500-1,520 cm^{-1} in the FT-IR spectra. The presence of polysaccharides is manifested by absorption bands in the finger-print region 800-1,200 cm^{-1} (Kačuráková *et al.* 1999). The complete removal of starch from the samples was confirmed by absence of α-glycosidic absorption band at 850 cm^{-1}.

In the FT-IR spectrum of L-A/A+AG ws (Figure 1A), the bands at 1,078, 1,047, and 898 cm^{-1} are typical for xylan-type polysaccharides (Hromádková and Ebringerová, 2003; Robert *et al.*, 2005). The acid or salt form of glucuronic acid side chains were indicated by a band at either at 1,730 or 1,600 cm^{-1}, respectively. The absorption bands at 897 and 1,160 cm^{-1} correspond to β-glycosidic linkages. As seen in Figure 1 (lines B and C), the FT-IR spectra of Actiglucane fractions L-A/A+AG wis and L-A/A+AG show similar patterns with bands at 1,036, 1,061, 1,110, and 1,104 cm^{-1} in accord with the prevailing sugar components xylose and glucose. The all-equatorial (OH) group positions in glucose showed the lowest frequency maximum at 1,035 cm^{-1} in the IR spectrum (Kačuráková *et al.*, 1999). As observed in Figure 1, the spectra of Actiglucane fractions B and C showed this band, similarly as the standard β-glucans (Megazyme, Wicklow, Ireland) (Mikkelsen *et al.*, 2010). The differences between the spectra preliminarily indicated that the Actiglucane contains next to β-glucans also acidic heteroxylans.

Physicochemical properties and nutritional profile of Actiglucane

The results of Wolever *et al.* (2010) showed that the effect of oat β-glucan on LDL levels is significantly related to its viscosity which is in turn determined by molecular weight. The molecular weight of the Actiglucane (1091 kDa) belongs to the group of high molecular β-glucans, which were demonstrated to have a higher physiological effect on regulating glucose metabolism (Regand *et al.*, 2011) and on lipid parameters compared to the medium molecular weight β-glucans (Wolever *et al.*, 2010).

The Actiglucane was analysed in terms of basic nutrients and nutritionally important parameters such as dietary fibre. The daily dose (2×45 g of concentrate diluted in 250 ml of water) consisted of 3 g of β-glucan, which is a minimal effective dose for cardiovascular disease prevention according to the Food and Drug Administration (FDA, 1997) and European Food Safety Authority (EFSA, 2011). Complete nutritional profile is discussed elsewhere in detail (Mikušová *et al.*, 2012). Based on the average Actiglucane composition, we can conclude that the concentrate is low in fat (0.1 g/100 g fresh weight; fw), high in dietary fibre (3.6 g/100 g fw) comprising mainly of insoluble fibre (2.7 g/100 g fw) and low in energy (123.5 kcal/100 g fw), which are important properties for dietary interventions.

Glycaemic characteristic and subjective satiety

According to the nutrition composition table (Mikušová *et al.*, 2012), amount of available saccharides in the Actiglucane concentrate was calculated. The amount of drink concentrate corresponding to the 25 g of available saccharides was used for the study. The glycaemic characteristic of the Actiglucane fibre drink compared to the glucose standard is shown in the Figure 2.

A significantly lower glycaemic response was seen in the 45th min after the Actiglucane consumption compared to the glucose standard (P<0.016). It is known that the intake of dietary fibre could support the energy intake regulation and satiety (Lyly *et al.*, 2009). Therefore, the next aim of this study was to compare the satiety of the Actiglucane fibre drink to the glucose standard. The results of a subjective satiety measured according to Quilez *et al.* (2007) in the same time points after the Actiglucane vs glucose consumption are shown in the Figure 3.

Satiety of the Actiglucane drink was significantly higher in the 30, 45, 60, 90th min compared to the glucose standard.

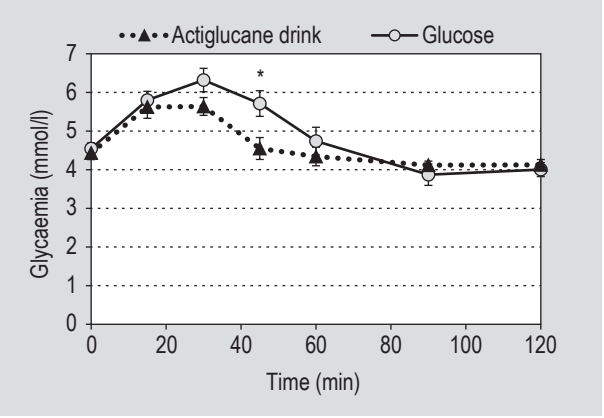

Figure 2. Glycaemic response measured at 0, 15, 30, 45, 60, 90, 120 min after Actiglucane fibre drink (black triangles) or glucose (white circles) consumption (*P<0.05).

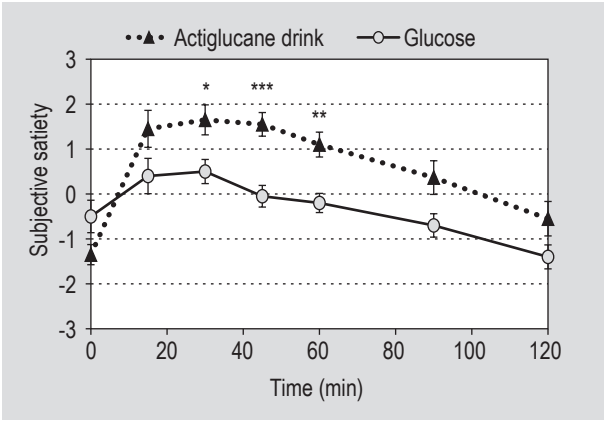

Figure 3. Subjective feeling of satiety after the Actiglucane fibre drink (black triangles) or glucose (white circles) consumption, evaluated on the scale from -3 (extremely hungry) to +3 (not hungry at all, totally full) (significant change: *P<0.05; **P<0.01; ***P<0.001).

The results are consistent with Lyly *et al.* (2009) who compared four types of beverage (control, wheat bran, guar gum, oat fibre addition) varying in the amount of fibre present (from 0 to 10.5 g/100 g). The results support the idea, that a fibre drink can enhance the perceived satiety and lower the desire to eat more than a beverage without fibre. Owing to these beneficial properties, the consumption of the drink may lead to the decrease of the food intake, what may be reflected in anthropometry and biochemical variables in the long term study.

Intervention study of Actiglucane

The health beneficial potential of the Actiglucane fibre drink was tested also from the long term aspect. Moreover, exercise in a form of the dance programme Bellylatinofit® was implemented into the design of the study (DE group). Adaptation to the dietary regime (nutritionally balanced diet itself) or the Bellylatinofit® dance programme alone (eight weeks) did not show significant differences in main anthropometric parameters (data not shown). The β-glucan fibre drink addition into the diet of D and DE group with a midmorning and afternoon snack caused changes in the dietary intake of nutrition components such as proteins, carbohydrates, total fat and also lowered the 24 h energy intake (Table 4).

Total fat and energy intake reduction are significantly different from the baseline for both diet intervention groups (D, DE) consuming β-glucan fibre drink. No changes in the diet composition were observed in the control group during the study (data not shown). Total fat and energy intake reduction obtained by β-glucan fibre drink addition into the food regime may be the cause of achieved anthropometric differences presented in following part of the article. As the dietary breakfast, lunch and dinner regime was not

Table 4. Diet composition changes in both diet intervention groups consuming β-glucan fibre drink with a midmorning and afternoon snack (2×45 g of drink concentrate equals 3 g of β-glucan fibre per day).[1]

Diet composition changes (baseline difference)	D	p_D (pre/post)	DE	p_{DE} (pre/post)	$p_{D/DE}$ (between groups)
Δ Protein (g/day)	-12.6	0.105	-26.6	0.068	0.258
Δ Carbohydrate (g/day)	-34.2	0.295	-45.9	0.163	0.749
Δ Total fat (g/day)	-34.3	0.016	-24.0	0.017	0.361
Δ Dietary fibre (g/day)	+0.4	0.627	+0.84	0.806	0.552
Δ Energy (kcal/day)	-375	0.034	-268	0.049	0.494

[1] D = β-glucan fibre drink; DE = β-glucan fibre drink with exercise (moderate intensity dance Bellylatinofit®); p_D = paired student t-test in D group; p_{DE} = paired student t-test in DE group; $p_{D/DE}$ = one way ANOVA between groups comparison.

changed through the eight week long study, drink addition twice a day resulted in a fat and total energy intake drop. Decrease in energy intake may be explained by a low energy content of the Actiglucane drink (average daily energy intake was 111 kcal, consumed in two portions) and by a demonstrated high satiating effect that caused no other traditional snack food consumption in a midmorning or afternoon time. The fall in fat intake might be partly caused by the sweet taste of the β-glucan fibre drink, what resulted in decreased consumption of other sweets such as cookies and chocolate, which are besides sugar also rich sources of dietary fat. Additionally, consumption of fatty snack food was eliminated by the satiation ability of the drink. Diet components intake was significantly improved in the eight week long study compared to the recommended daily intake in both, D and DE, group. To sum it up, a significant drop of 53% in D group (P<0.016) and 37% in DE group (P<0.017) in fat consumption was observed during β-glucan fibre drink consumption. We achieved a 17% energy decline in D group (P<0.034) and 12% energy decline in DE group (P<0.049), where additional energy deficit was caused by regular dance exercise Bellylatinofit®.

After implementation of the Actiglucane drink into the diet, changes in most of monitored variables (weight, body-mass index (BMI), fat percentage, waist-to-hip ratio (WHR index) and visceral fat) in both diet intervention groups (D, DE) were seen. The effect of β-glucan drink combined with diet regime, with or without exercise (DE, D) on selected anthropometric variables compared to controls is shown in Figure 4 and 5. No significant changes in anthropometric values before and after treatment were seen in the control group. However clear decreases were observed in all anthropometric parameters in the intervention groups (weight and fat mass what was well reflected in WHR and BMI indices), statistical significance at the 95% confidence level between groups DE, D and control was seen only in the total body weight (DE group: P<0.016), fat mass (DE

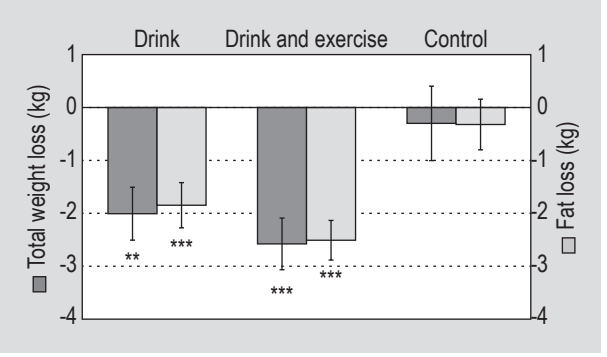

Figure 4. Reduction of total body weight (dark bars) and body fat (light bars) in both treatment groups after 2 months of the study. Groups D and DE consumed Actiglucane drink comprising of 3 g β-glucan fibre/day. Moreover, DE attended 3 times a week Bellylatinofit® dance classes. Significant changes before and after study intervention: **P<0.01, ***P<0.001.

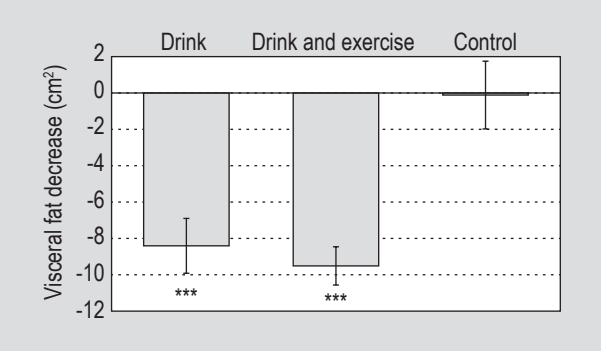

Figure 5. Reduction of visceral fat in both treatment groups after 2 months of the study. Groups D and DE consumed Actiglucane drink comprising of 3 g β-glucan fibre/day. Moreover, DE attended 3 times a week Bellylatinofit® dance classes. Significant changes before and after study intervention: ***P<0.001.

group: $P<0.003$ and D group: $P<0.017$) and visceral fat loss (DE and D group: $P<0.016$).

It is noteworthy to mention, that there were no statistically significant changes between D vs. controls, whereby DE group vs. control lost weigh significantly compared to the control group ($P<0.016$ for total weight; $P<0.003$ for fat mass). This may indicate that moderate intensity dance exercise combined with dietary regime and β-glucan fibre drink has a synergic effect, hence D group alone did not differ in weight compared to controls at the 95% confidence level. Still, no difference was seen between DE an D itself, what may indicate that all beneficial effect of exercise on monitored parameters cannot be clearly detected in such a short time. We can sum up that the current study demonstrated that a midmorning and afternoon consumption of cereal β-glucan fibre drink Actiglucane lowered significantly the fat intake and consequently the total energy intake resulting in a decrease of the most evaluated anthropometric parameters.

Maki *et al.* (2010) conducted a similar study, where an effect of whole grain ready-to-eat oat cereal containing 3 g of β-glucan (the same daily dosage as in the current study) compared to a low-fibre breakfast snack food with similar energy and macronutrient content, as a part of a 12 weeks dietary weight loss programme, was investigated. Although the weight loss -2.2±0.3 kg vs. -1.7±0.3 kg did not show significant changes between groups, the waist circumference decreased more with whole grain oat cereal (Maki *et al.*, 2010). Similar weight differences (though significant) were found in the current study, where the DE group total weight decrease was 2.6±0.5 kg and D group lost 2.0±0.5 kg after the eight week long study. Since the diet regime itself did not demonstrate significant changes (preparation part of the study, data not shown), the main influence on weight and fat mass loss can be attributed to the Actiglucane. On the contrary, oat β-glucan supplementation in two different daily dosages (5-6 g/8-9 g) was examined in a 3 month long study with overweight women following energy restricted diet with no additional effect. The authors concluded that in general, oat β-glucan did not enhance the effect of energy restricted diet on weight loss, although some inter-individual changes can play a role (Beck *et al.*, 2010).

It is difficult to discuss the decrease of fat mass after β-glucan supplementation, because no specific measures of body fat percentage have been evaluated before (to authors' best knowledge), except one study summarizing, that no previous data exist that evaluate the effect of β-glucan on visceral fat (Shimizu *et al.*, 2008). In the above mentioned trial, significant changes for visceral fat, BMI and waist circumference after diet supplementation with high β-glucan (7 g/day) pearl barley have been found.

Glucose and insulin metabolism is very closely related to the feelings of hunger, what directly improves strategies for obesity prevention. Delayed gastric emptying resulting in slower absorption of nutrients may lead to the greater insulin sensitivity, what is an important factor in the prevention of complications closely related to obesity such as diabetes mellitus type 2 or metabolic syndrome. The effect of β-glucan fibre drink on the postprandial level is discussed at the beginning of the article. The influence of the Actiglucane consumption on the level of fasting glucose in the long-term perspective compared to the control group was also determined. A significant decrease in fasting serum glucose after vs. before intervention in all groups was found. However, also in a control group, hence no statistically significant differences between groups were seen. Insulin fasting levels did not differ significantly after completion of the study within any group, as well as between groups. HOMA-IR did not differ significantly after completion of the study, although a decreasing trend was seen in all intervention groups compared to the increasing trend seen in the control group (data not shown).

There are many studies considering if β-glucan is helpful in controlling blood sugar or insulin levels in people with diabetes and in the general population (Kim *et al.*, 2006). Many of them are dealing with postprandial effects of β-glucan consumption, reporting mostly positive results (Beck *et al.*, 2009; Behall *et al.*, 2006; Brennan and Cleary, 2005; Casiraghi *et al.*, 2006). One of the factors responsible for a low glycaemic response to oat and barley food is the β-glucan viscosity, which directly depends on solution concentration and β-glucan molecular weight (Wood, 2007). On the other hand, many studies did not show any changes of blood sugar levels at all (Li *et al.*, 2003; Lovegrove *et al.*, 2000). Supporting the results of the current study with no obviously significant effect on glucose or insulin, Queenan *et al.* (2007) also did not find any effect on fasting glucose in a six week long study; although other clinical changes such as LDL decrease were observed. To summarise it up, in people with normal glucose tolerance, oat and barley β-glucan appear to have no significant effect on blood sugar level (Kim *et al.*, 2006).

A regular consumption of oat, barley and cereals rich β-glucans is positively correlated with lowering cholesterol effect and hence decreased cardiovascular disease risk. The potential cardiovascular risk of the subjects is often expressed as an AI, known also as a TC/HDL ratio. Actiglucane cholesterol lowering effect (without dancing intervention) is discussed in detail elsewhere (Mikušová *et al.*, 2012). Briefly, a significant LDL decrease ($P<0.02$) and AI drop ($P<0.004$) were seen in the D group. This parameter was significantly improved also in the combined DE group ($P<0.046$), indicating a beneficial potential in the cardiovascular prevention programmes. Although there is

no significant drop in TC of D and DE groups compared to the control, both groups consuming β-glucan fibre drink reduced these values under the critical reference limit (5.2 mmol/l) in the eight week long study. Still, there is no statistically significant difference between DE and D group itself at the 95% confidence level. A decreasing trend was seen in other parameters such as TAG and VLDL. However, these parameters were not significantly different after vs. before treatment neither in DE nor in D group.

4. Conclusions

We can conclude that acute Actiglucane consumption lead to a consequent slower glucose uptake and a higher subjective satiety compared to a standard glucose solution. Eight weeks of Actiglucane consumption by 30 healthy women with higher body fat percentage lead to a significant reduction in the dietary fat and total energy consumption. This reduction was well reflected in positive changes in the total and visceral body fat percentage as well as in an improvement of lipid profile. Synergic effect of the dance program Bellylatinofit® added to the dietary intervention was observed. The combined programme has more pronounced health beneficial effects and therefore represents a promising interventional tool in the prevention and treatment of obesity, its related health complications and other civilisation diseases.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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