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Increases in peptide Y-Y levels following oat **β**-glucan ingestion are dose-dependent in overweight adults

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Increases in peptide Y-Y levels following oat **β**-glucan ingestion are dose-dependent in overweight adults

Abstract

Peptide Y-Y (PYY) is an anorexigenic hormone implicated in appetite control, and β-glucan is a fiber known to affect appetite. We hypothesized that plasma PYY levels would increase in overweight human adults consuming increasing doses of β-glucan. The objective was to test whether the effect could be seen with β-glucan delivered through extruded cereals containing a high β-glucan oat bran with demonstrated high molecular weight and solubility. Fourteen subjects consumed a control meal and 3 cereals of varying β-glucan concentration (between 2.2 and 5.5 g), and blood samples were collected over 4 hours. Analysis of raw PYY data showed a trend toward significant increases over 4 hours. An increasing dose of β-glucan resulted in higher levels of plasma PYY, with significant differences between groups from 2 to 4 hours post test-meal. Data for the area under the curve analysis also approached significance, with post hoc analysis showing a difference (P = .039) between the control and the highest dose of β-glucan (5.5 g). The PYY levels at 4 hours were significantly different between the control and high-dose meal test (P = .036). There was a significant dose response, with a positive correlation between the grams of β-glucan and PYY area under the curve ($r_2 = 0.994$, P = .003). The optimal dose of β-glucan appears to lie between 4 and 6 g, with the effects on PYY mediated by viscosity and concentration. Meal-test studies examining a range of hormones should measure hormones over a minimum of 4 hours and record meal intake for even longer time frames.

Keywords

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Title: Increases in PYY levels following oat β-glucan ingestion is dose dependant in overweight adults

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Abbreviations:

CCK; cholecystokinin

GLP-1; glucagon-like peptide

MW; molecular weight

netAUC; Area under the curve

NPY; neuropeptide Y

PYY; peptide Y-Y

PYY_{3-36}; peptide $Y-Y_{3-36}$

RMANOVA; repeated measures analyses of variance

Abstract

PYY is an anorexigenic hormone implicated in appetite control and β-glucan is a fibre known to affect appetite. We hypothesized that plasma PYY levels would increase in overweight human adults consuming increasing doses of β-glucan. The objective was to test whether the effect could be seen with β-glucan delivered through extruded cereals containing a high β-glucan oat bran with demonstrated high molecular weight and solubility. Fourteen subjects consumed a control meal and three cereals of varying β-glucan concentration (between 2.2 and 5.5g) and blood samples were collected over 4 hours. Analysis of raw PYY data showed a trend towards significant increases over 4 hours. An increasing dose of β-glucan resulted in higher levels of plasma PYY, with significant differences between groups from 2 to 4 hours post test-meal. Data for the area under the curve analysis also approached significance, with post-hoc analysis showing a difference (P=0.039) between the control and the highest dose of β-glucan (5.5g). PYY levels at 4 hours were significantly different between the control and high dose meal test (P=0.036). There was a significant dose response with a positive correlation between the grams of β-glucan and PYY area under the curve (r^2 =0.994, P=0.003). The optimal dose of β-glucan appears to lie between 4-6 g, with the effects on PYY mediated by viscosity and concentration. Meal test studies examining a range of hormones should measure hormones over a minimum of 4 hours and record meal intake for even longer time frames.

Keywords

1. Introduction

Increasing rates of obesity in the majority of the western world are a source of concern due to related health consequences and the financial burden of a population at risk for diseases including type-2 diabetes, cardiovascular disease and cancer. Strategies which can help reduce energy intake through control of appetite, including the key parameters of satiety and satiation may help individuals control food intake and manage overweight and obesity.

Soluble fibers, such as $(1\rightarrow3)(1\rightarrow4)$-$\beta$-glucan ($\beta$-glucan) from oats, which have a range of positive health benefits including effects on lipidemic [1] and glycemic control [2], can influence appetite by increasing gastrointestinal viscosity. This effect requires solubilization of the fiber in the gastrointestinal tract [3]. There is decreased contact of the food bolus with digestive enzymes disrupting micelle formation and contact with the gastrointestinal wall [4]. Nutrients reach further into the bowel [5] inhibiting the gastric hunger hormone ghrelin, and stimulating the duodenal satiety hormone, cholecystokinin (CCK) along with glucagon-like-peptide-1 (GLP-1) and peptide–YY_{3-36} (PYY_{3-36}), all of which decrease appetite.

Peptide-YY (PYY) belongs to the pancreatic polypeptide family which includes pancreatic polypeptide and neuropeptide-Y (NPY). PYY is primarily secreted by endocrine cells in the distal small bowel and colon [6]. Dipeptidyl peptidase-IV hydrolyzes PYY and converts the precursor PYY_{1-36} to PYY_{3-36}. PYY_{3-36} acts on NPY cells via the NPY Y2 receptor in the medial part of the arcuate hypothalamic nucleus of the brain [7]. In humans, infusions of PYY_{3-36} comparable to those after a meal result in decreased energy intake at subsequent meals compared to a control group [8].

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A landmark study in obesity research [9] showed that obese individuals were not resistant to the anorectic effects of PYY_{3-36}. Obese individuals tend to have lower endogenous PYY_{3-36}. Correction of this anomaly or its effects through pharmacological means became a legitimate pursuit for obesity researchers. In particular, the blunted PYY response to meals in obese subjects demonstrates defects in PYY release in this population [9]. Variation in levels of endogenous PYY and its subtypes between obese and non-obese populations has not always been demonstrated [6]. However, the effects of PYY and PYY_{3-36} in particular, remain of interest due to its anorectic nature and variation in responses to food, with weight loss in obese subjects. Yet to be published animal studies in our laboratory have demonstrated weight loss, increased satiety, increased PYY_{3-36} and down-regulation of mRNA expression for NPY and Y2 receptors with increasing β-glucan dose. Few studies have measured appetite hormones in humans in relation to different fibers, including β-glucan, yet research has indicated different subjective and meal intake responses to different fibers [10]. Hence we sought to identify appetite hormones in relation to β-glucan.

A meal test study in our laboratories recorded acute hormonal and subjective measures of satiety, followed by energy intake from a subsequent meal, after varying doses of β-glucan in extruded breakfast cereals [11]. Subjects consumed different doses of β-glucan and dietary intake was measured after four hours. β-Glucan was found to decrease insulin secretion over 2 hours (RMANOVA, P=0.011) in a dose responsive manner from 2.16 to 5.45 g β-glucan/serving (P=0.007). CCK levels increased linearly over the same range of β-glucan concentrations (P=0.002) in women. Subjective satiety was increased at a β-glucan dose of 2.2g (P=0.039). Subsequent meal intake tended to decrease by greater than 400kJ with higher β-glucan dose (>5g). We further hypothesized that PYY levels would also respond in a dose dependent manner under these conditions. The objective of the study

reported was to test the effects of increasing doses of β-glucan in extruded cereals on PYY levels in this group of overweight adults.

2. Methods and Materials

2.1 Subjects

This analysis was conducted as an extension of the previously described meal-test study [11] with ethical clearance given by the University of Wollongong, Human Ethics Committee (HE06/123). Subjects were recruited via advertisement in local media and institutional email with inclusion of subjects with BMI >25 kgm^{-2}, non-smokers, of general good health and no known diabetes. A total of 41 subjects were screened, with 17 recruited and 3 withdrawals due to time constraints. Participants were male (7) and female (7) subjects aged 29-45 years (mean 38.7 years) with a mean BMI of 29.6 kg/m^2 (25.2-36.6 kg/m^2). All women were tested within the follicular phase of their menstrual cycle. Written informed consent was obtained from all subjects.

2.2 Subject Protocols

Subjects attended our laboratory for an initial appointment to familiarize themselves with the surroundings, as well as for collection of background dietary data. On meal-test days, subjects arrived fasted (minimum of 10 hours) and a cannula was inserted for collection of a fasting blood sample. Subjects consumed breakfast with β-glucan incorporated into extruded cereals and served with 200mL of reduced-fat milk and a glass of water. Subsequent samples were collected at 15, 30, 60, 120, 180 and 240 minutes post-breakfast. Extruded test cereals were formulated from oat flour, maize flour, sugar, maltodextrin, sodium bicarbonate, salt, water and the β-glucan ingredient. Available carbohydrate and

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protein were matched by dissolving glucose polymer (Poly-Joule®, Nutricia Australasia) and protein powder (Beneprotein®, Novartis, United States) in the milk. The nutrient composition of the test meals is described in Table 1. The precise dose of β-glucan (control – C, low dose – LBG, medium dose – MBG and high dose – HBG), was measured by the method of Glennie-Holmes and McCleary [12] using a kit from Megazyme (Megazyme International, Ireland). The β-glucan was extracted at 37°C following an *in vitro* digestion protocol [13]. Viscosity of the extract was determined using a controlled strain rheometer (TA Instruments, NJ) and apparent viscosity at 30 s^{-1} was reported. The concentration of β-glucan was determined by flow injection analysis following the method of Jørgensen [14]. MW of β-glucan was determined by size exclusion high performance liquid chromatography [15] except that the columns were Shodex OHpak KB806M and Waters Ultrahydrogel (Waters, Milford, MA). Measurements are detailed in Table 2, with increasing viscosity with concentration and maintenance of molecular weight expected to produce desired physiological outcomes [3].

2.3 PYY determination

Blood samples were collected in a S-Monovette™ tube containing potassium EDTA (to achieve a concentration of ½-2mg EDTA/ml of blood after collection) and aprotinin equivalent to 0.6 trypsin inhibitor units (TIU) per mL of blood. (Aprotinin Solution, NZ, manufactured by Serologicals, sourced from Chemicon Australia: Activity 5-10 TIU/mL.) The samples were then centrifuged at four degrees Celsius for 15 minutes at 1500xg. Plasma was collected and stored at -80 degrees Celsius for further use. Samples from the control breakfast and from three different doses of β-glucan enriched oat bran cereals were tested for total PYY. PYY in these samples was tested using an enzyme linked immunosorbent assay

(EZHPYYT66K-Millipore Human PYY (Total)) kit according to the standard protocols of the manufacturer (Millipore, St Charles, Missouri, USA).

2.4 Statistical Analyses

Power for this study was calculated based on previous meal test studies indicating differences in biochemical markers of appetite [16] and subjective measures of appetite [17] in a repeated measures design may require as few as 8 subjects. The measurement of PYY described here were subsequent to the initial study design and so additional power calculations could not include interpretation from previous studies involving PYY. Results for PYY values were entered into SPSS for windows, (Version 15.0) for both raw data and trapezoidal area under the curve (netAUC- where values were corrected for baseline but areas below the baseline were also subtracted [18]). Differences in raw data and netAUC results ± SD, between the different doses of β-glucan were identified using repeated measures analysis of variance (RMANOVA) with post-hoc Bonferroni adjustments. Results were tested for differences between sexes as previous studies have identified differences in hormone responses between the sexes [16]. Results for varied time periods were also compared. Regression analysis was applied to test for relationships between dose and PYY values. Results for the control compared to each dose were also reviewed at individual time points using Students' t-test.

3. Results

Results from only 13 of 14 subjects were included in this analysis as some values obtained for one subject were between ten and fifty-fold greater than other subjects. The data for this

subject was excluded from the overall analysis on the basis of its implausibility. No significant differences were identified between the sexes for any data analysis.

Review of raw PYY values corrected for baseline indicated a trend towards significance (P=0.131) where an increasing dose of β-glucan resulted in a greater release of PYY (Figure 1). Post-hoc Bonferroni adjustments showed the majority of this trend was due to differences between the control and highest dose of β-glucan (P=0.072). NetAUC results showed a similar overall trend (P=0.102) while the post-hoc calculations showed a significant difference between the control and the HBG dose (P=0.039) (Table 3 and Figure 2). Regression analysis showed a significant correlation (P=0.003) between plasma PYY and total β-glucan content (R^2=0.994).

RMANOVA calculations of the difference in PYY values at the time-point immediately before the lunch (4 hours) showed a significant dose response (P=0.023) with post-hoc identification of a significant difference between the control and HBG meal tests (P=0.036). Students' t-tests between the control and each dose at this time, show a trend to difference between the control and MBG dose (P=0.074) and a statistically significant difference between the control and HBG dose (P=0.006). If data is analysed for the first 2 hours, although the HBG dose elicits the greatest peak change in PYY (31 pg/mL at 30 minutes), no significant difference between results is shown (P=0.435). However, if the results for the second 2 hour period (2 to 4 hours post-meal) are reviewed, a significant difference is noted for the RMANOVA analysis (P=0.035).

4. Discussion

This study found that total levels of plasma PYY increase in a linear fashion with increasing concentration of β-glucan (up to 5.45g of β-glucan) in the first 4 hours after a meal. The strong correlation between meal-test PYY response and concentration of β-glucan indicate that it is affected acutely by the amount of soluble fiber, or at least the concentration of the tested oat bran. Post-hoc analysis indicated a difference between the control and the HBG dose of β-glucan and this is consistent with the literature which discusses a minimum level of between 4 and 6g as necessary for the gastrointestinal effects of β-glucan [19].

Examining the data at different time-points shows a significant difference in PYY secretion over the longer time frames (two to four hours). In particular the single data point at four hours shows the greatest PYY level for the HBG dose. This is consistent with the secretion of PYY in the distal gut and colon and emphasises the longer lasting effects of anorexigenic hormones such as PYY. Studies over shorter time frames may be suitable for measuring glycemic and insulinemic advantages of β-glucan ingestion, however longer time frames may be required to show the full satiety effect of highly viscous fibers such as β-glucan.

The time frame for PYY increases also explains how studies of low viscosity versus high viscosity β-glucan [20] may show lower hormone responses over 2-3 hours, where the faster transit of the low-viscosity fiber causes higher levels of hormones such as PYY to be released initially. However, if results are reviewed for the entire day, a lower kilojoule intake is shown with high viscosity fiber ingestion [20]. This is also consistent with the trends shown in our original work for dietary intake [11] where the greater viscosity seen with the higher concentrations of β-glucan gave the lowest second-meal energy intake. It is likely that the undigested nutrients in the large bowel caused by soluble fibers creating a viscous bolus, result in long lasting satiety through the action of hormones such as PYY.

They do not necessarily show increased satiety initially, so future studies should include dietary intake measured over an entire day. In addition, the fact that insulin secretion is decreased by β-glucan ingestion over 2 hours [2, 11] may result in a transient decrease in satiety [21] surpassed by the increase over longer time frames. This means that the benefits of β-glucan are wide ranging, where satiety hormone responses compensate for glycemic mechanisms of control over intake.

The primary limitation of this study is the power, however it was originally calculated to detect hormone changes such as CCK, as well as subjective satiety (reported elsewhere, [11]), where 8-15 subjects should have identified differences between doses [17]. It was however expected that changes in PYY may be similar in magnitude to the other hormonal changes significant results were postulated. The large standard deviations in responses indicate that inter-individual variation is large and greater numbers are required to identify statistically significant results at all levels of analysis. All meal-test studies are limited by the creation of an artificial environment which can only be compared to the free living population. However, the repeated-measures design of this study means comparisons between doses and knowledge of the physical properties of the β-glucan provides useful data for identifying mechanisms of satiety.

Previous unpublished animal data from our laboratory showed increased circulating PYY_{3-36} with increasing β-glucan dose ingested chronically. It would seem that acute exposure to β-glucan changes the levels of PYY released in humans consistent with the animal studies. Combining this new knowledge with previous studies we conclude that the optimal dose of β-glucan affecting satiety and other markers of appetite regulation would be between 4-6 g. The effects on satiety related hormones appear to be mediated through both viscosity and

concentration. Acute studies relating to appetite should determine hormone levels for a minimum of four hours and collect dietary data over even longer time frames.

5. Acknowledgment

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