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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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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: Oat β-glucan; Peptide Y-Y; Satiety; Appetite; Fiber; Human obesity
Abbreviations: CCK, cholecystokinin; MW, molecular weight; netAUC, area under the curve; NPY, neuropeptide Y; PYY, peptide Y-Y; $PYY_{3\text{-}36}$, peptide $Y\text{-}Y_{3\text{-}36}$; RMANOVA, repeated measures analysis of variance.

1. Introduction

Increasing rates of obesity in the majority of the western world are a source of concern because of related health consequences and the financial burden of a population at risk for diseases including type 2 diabetes, cardiovascular disease, and cancer. Strategies that 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→3)(1→4)-β-glucan (β-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

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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 and peptide Y-Y_{3-36} (PYY_{3-36}), all of which decrease appetite.

Peptide Y-Y (PYY) belongs to the pancreatic polypeptide family, which includes pancreatic polypeptide and neuropeptide Y (NPY). Peptide Y-Y (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}. Peptide Y-Y_{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 with a control group [8].

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 pharmacologic 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 nonobese populations have not always been demonstrated [6]. However, the effects of PYY and PYY_{3-36} in particular remain of interest because of 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 4 hours. β-glucan was found to decrease insulin secretion over 2 hours (repeated measures analysis of variance [RMANOVA], $P = .011$) in a dose-responsive manner from 2.16 to 5.45 g β-glucan per serving ($P = .007$). The CCK levels increased linearly over the same range of β-glucan concentrations ($P = .002$) in women. Subjective satiety was increased at a β-glucan dose of 2.2 g ($P = .039$). Subsequent meal intake tended to decrease by greater than 400 kJ with higher β-glucan dose (>5 g). 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 body mass index greater than 25 kg m^{-2}, who are nonsmokers, of general good health, and with 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 to 45 years (mean, 38.7 years), with a mean body mass index of 29.6 kg/m^2 (range, 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 served with 200 mL of reduced-fat milk and a glass of water. Subsequent samples were collected at 15, 30, 60, 120, 180, and 240 minutes postbreakfast. Extruded test cereals were formulated from oat flour, maize flour, sugar, maltodextrin, sodium bicarbonate, salt, water, and the β-glucan ingredient. Available carbohydrate and 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, low dose, medium dose, 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 after an in vitro digestion protocol [13]. Viscosity of the extract was determined using a controlled strain rheometer (TA Instruments, New Jersey), 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]. Molecular weight (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 MW expected to produce desired physiological outcomes [3].

Table 1
Composition and nutrient analysis of test meals

	Control	LBG	MBG	HBGO
Meal composition				
Cereal (g)	39 [a]	45 [b]	45 [b]	45 [b]
Carbohydrate polymer (g)	–	–	4	8
Protein powder (g)	3	2	1	–
2% fat milk (mL)	200	200	200	200
Nutrient profile				
Total protein (g)	13.3	13.4	13.4	13.5
Total fat (g)	3.2	3.6	3.8	4.0
Available carbohydrate (g)	43.6	43.2	42.9	42.6
Total fiber (g)	1.2	3.7	6.7	9.7
β-Glucan (g/serving)	–	2.16	3.82	5.45
Energy (kJ)	1080	1098	1106	1115

LBG indicates low β-glucan dose; MBG, mid β-glucan dose; HBGO, high β-glucan dose.

[a] Ingredients: corn (90%), sugar, barley malt extract, salt, vitamins and minerals.

[b] Ingredients: maize flour, β-glucan source, maltodextrin, sugar, calcium carbonate, bicarbonate soda, salt.

2.3. Peptide Y-Y determination

Blood samples were collected in an S-Monovette tube containing potassium EDTA (to achieve a concentration of 1/2 to 2 mg EDTA/mL of blood after collection) and aprotinin equivalent to 0.6 trypsin inhibitor units per milliliter of blood (Aprotinin Solution, NZ, manufactured by Serologicals, sourced from Chemicon Australia: activity 5-10 trypsin inhibitor units per milliliter). The samples were then centrifuged at 4°C for 15 minutes at 1500 × *g*. Plasma was collected and stored at −80°C for further use. Samples from the control breakfast and from 3 different doses of β-glucan–enriched oat bran cereals were tested for total PYY. Peptide Y-Y 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, Mo).

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, required as few as 8 subjects. The measurements 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 RMANOVA with post hoc Bonferroni adjustments. Results were tested for differences between genders because previous studies have identified differences in hormone responses between the genders [16]. Results for varied periods were also compared. Regression analysis was applied to test for relationships between dose and PYY values. Results for the control compared with each dose were also reviewed at individual time points using Student *t* test.

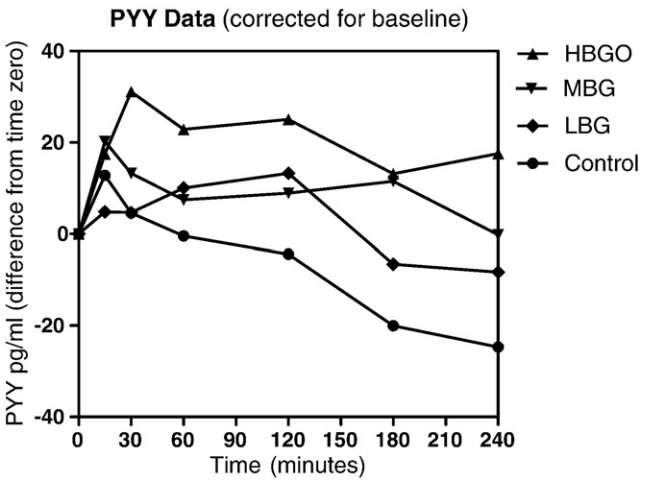

Fig. 1. Peptide Y-Y responses corrected for baseline. Repeated measures analysis of variance 0-120 minutes, $P = .435$; 120-240 minutes, $P = .035$ (Student *t* test, control vs HBG at 240 minutes, $P = .006$).

3. Results

Results from only 13 of 14 subjects were included in this analysis because some values obtained for 1 subject were between 10- and 50-fold greater than other subjects. The data

Table 2
Physicochemical characteristics of β-glucan in test meals

Name of test food	Total β-glucan (% dwb)	Soluble β-glucan[a] (% dwb)	Viscosity (mPa s)	MW (g/mol)[b]	Total β-glucan/ serve (g)
LBG	5.04	3.42	5.8	1 681 000	2.16
MBG	8.92	6.96	32.0	1 378 000	3.82
HBG	12.62	8.89	76.6	1 213 000	5.45

LBG indicates low β-glucan dose; MBG, mid β-glucan dose; HBG, high β-glucan dose; dwb, dry weight basis.

[a] Grams of extractable beta-glucan per 100g of cereal.

[b] Peak molecular weight.

Table 3
Peptide Y-Y responses to test meals (netAUC analysis)

Test meal	AUC ± SD (pg/mL × minute)
Control	−1932 ± 7772
LBG	780 ± 8346
MBG	2152 ± 6094
HBG[a]	4120 ± 7958

LBG indicates low β-glucan dose; MBG, mid β-glucan dose; HBG, high β-glucan dose.

[a] Repeated measures analysis of variance, $P = .102$; post hoc Bonferroni adjustments, control vs HBG, $P = .039$.

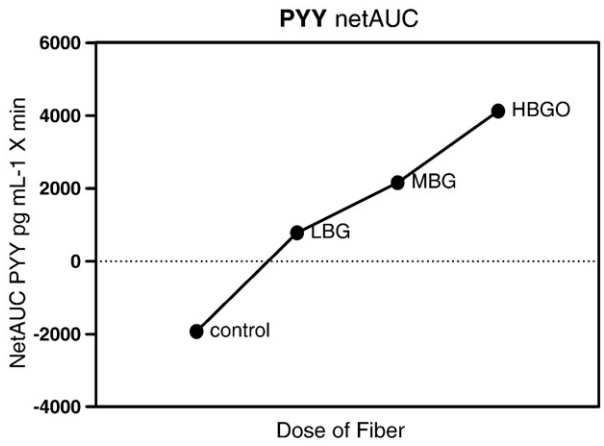

Fig. 2. Peptide Y-Y netAUC for each test meal. $R^2 = 0.994$ for dose vs PYY netAUC ($P = .003$).

for this subject were 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 toward significance ($P = .131$), where an increasing dose of β-glucan resulted in a greater release of PYY (Fig. 1). Post hoc Bonferroni adjustments showed the majority of this trend was caused by differences between the control and highest dose of β-glucan ($P = .072$). NetAUC (area under the curve [18]) results showed a similar overall trend ($P = .102$), whereas the post hoc calculations showed a significant difference between the control and HBG dose ($P = .039$; Table 3 and Fig. 2). Regression analysis showed a significant correlation ($P = .003$) between plasma PYY and total β-glucan content ($R^2 = 0.994$).

The RMANOVA calculations of the difference in PYY values at the time-point immediately before the lunch meal (4 hours) showed a significant dose response ($P = .023$), with post hoc identification of a significant difference between the control and HBG meal tests ($P = .036$). Student t tests between the control and each dose at this time show a trend toward a difference between the control and mid β-glucan dose ($P = .074$) and a statistically significant difference between the control and HBG dose ($P = .006$). If data are analyzed 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 ($P = .435$). 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 ($P = .035$).

4. Discussion

This study found that total levels of plasma PYY increase in a linear fashion, with increasing concentration of β-glucan (up to 5.45 g 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 HBG dose of β-glucan; this is consistent with the literature, which discusses a minimum level of between 4 and 6 g 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 (2 to 4 hours). In particular, the single data point at 4 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 emphasizes 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 vs high-viscosity β-glucan [20] may show lower hormone responses over 2 to 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; therefore, 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, Ref. [11]), where 8 to 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 where significant results were expected. The large standard deviations in responses indicate that interindividual 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 that can only be compared with 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 and 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 4 hours and collect dietary data over even longer time frames.

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