Research Article

Oat β-glucan increases postprandial cholecystokinin levels, decreases insulin response and extends subjective satiety in overweight subjects

Eleanor J. Beck[1], Susan M. Tosh[2], Marijka J. Batterham[1], Linda C. Tapsell[1] and Xu-Feng Huang[1]

[1] School of Health Sciences, University of Wollongong, Wollongong, Australia
[2] Guelph Food Research Centre, Agriculture and Agri-Food Canada, Guelph, ON, Canada

This study recorded acute biochemical and subjective measures of satiety, followed by energy intake from a subsequent meal, after varying doses of β-glucan in extruded breakfast cereals. Molecular weight, solubility and viscosity of β-glucan products were determined. Seven male and seven female subjects (BMI 25 – 36 kg/m) consumed five breakfasts (different doses of β-glucan sourced from two different technological processes) and dietary intake was measured after four hours. Blood was collected to measure glucose, insulin, ghrelin and cholecystokinin, and visual analogue scales measured subjective satiety. Molecular weight, solubility and viscosity indicated products were likely to increase luminal viscosity. β-Glucan was found to decrease insulin secretion over 2 h (RMANOVA, $p = 0.011$) in a dose responsive manner from 2.16 to 5.68 g *per* serving ($p = 0.007$). Cholecystokinin 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 2.2 g ($p = 0.039$). Subsequent meal intake decreased by greater than 400 kJ with higher β-glucan dose (>5 g). β-Glucan improves satiety and release of cholecystokinin is likely to be part of the mechanism. Products with different sources of β-glucan provide similar benefits but each product requires individual testing.

Keywords: Beta-D-glucan / Cholecystokinin / Satiety / Visual analogue scales

Received: July 30, 2008; revised: February 12, 2009; accepted: February 23, 2009

1 Introduction

Numerous studies identify the potential weight control benefits of diets high in fibre. Epidemiological evidence links fibre intakes inversely to population levels of overweight and obesity [1], leanness in individuals with higher fibre intake [2] and obese individuals with lower fibre intakes [3, 4]. Prospective studies also show that consumption of whole grains is inversely related to weight gain over time [5, 6]. Dietary studies usually identify benefits associated with fibre and/or whole grains and postulate mechanisms related to ingestion, digestion and hormones associated with feelings of satiety. Identification of the mechanism by which fibre intake acts acutely, may help establish theoretical positions for undertaking longer-term weight reduction intervention trials.

Oats contain the soluble fibre (1 → 3)(1 → 4)-β-D-glucan (β-glucan). Soluble fibres, such as β-glucan, influence appetite by chemical and physical properties (particularly their bulking action), and increase viscosity in the gastrointestinal tract [7]. β-Glucan improves glucose and insulin control [8], yet a higher insulinaemic response may increase feelings of fullness when subjects are given a set amount of carbohydrate [9]. Any reduction in appetite or intake after ingestion of β-glucan must occur in spite of a lowered insulin response.

Few studies have measured appetite hormones in relation to β-glucan consumption. However, fibre appears to prolong cholecystokinin (CCK) elevation after a meal, which should result in prolonged satiety [10]. Other hormones such as ghrelin, stimulate food intake [11]. There is a premeal rise in ghrelin [12], which then decreases on cessation

Correspondence: Eleanor J. Beck, School of Health Sciences, University of Wollongong, Northfields Ave, Wollongong NSW 2522, Australia
E-mail: eleanor@uow.edu.au
Fax: +61-2-4221-4844

Abbreviations: AUC, area under the curve; **CCK,** cholecystokinin; **HBGO,** high β-glucan; **HBGX,** high β-glucan dose containing extracted β-glucan; **LBG,** low β-glucan; **MBG,** mid-β-glucan; **MW,** molecular weight; **RMANOVA,** repeated measures analysis of variance; **VAS,** visual analogue scales

Table 1. Proximate analysis of test meals

Composition variable	Control[a)]	Oatwell LBG[b)]	Oatwell MBG[b)]	Oatwell HBGO[b)]	HBGX[b)]
Cereal (g)	39	45	45	45	45
Carbohydrate polymer (g)	–	–	4	8	3
Protein powder (g)	3	2	1	–	3
β-Glucan (g/serving)	–	2.16	3.82	5.45	5.65
2% Fat milk (mL)	200	200	200	200	200
total protein (g)	13.3	13.4	13.4	13.5	13.0
Total fat (g)	3.2	3.6	3.8	4.0	3.6
Available carbohydrate (g)	43.6	43.2	42.9	42.6	43.3
Total fibre (g)	1.2	3.7	6.7	9.7	7.8
Energy (kJ)	1080	1098	1106	1115	1157

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. LBG, MBG, HBGO (all containing OatWell), HBGX.

of fasting. A food which could limit elevation of ghrelin either absolutely or via delay of elevation, would be of interest to appetite and obesity investigators.

It is recognized that variability in processing of oats, oat fibre concentrates, and products delivering the β-glucan dose alters key parameters, especially solubility and viscosity [13] which contribute to β-glucan functionality. These parameters have a key role to invoke the mechanism of cholesterol lowering by β-glucan, and therefore most likely the mechanisms of satiety. Any investigation of satiety effects of β-glucan must measure concentration, solubility, viscosity and molecular weight (MW), to ensure the integrity of the β-glucan products used.

This study used an acute meal test to identify (i) acute satiety effects of β-glucan in extruded breakfast cereal foods; (ii) the dose responsiveness of these effects and (iii) any differences which may exist in the acute effects of β-glucan concentrated by different processes. To ensure the integrity and clearly characterize the β-glucan used in the study, concentration, solubility, viscosity and MW within the test food was also measured.

2 Materials and methods

2.1 Subjects

Overweight subjects aged 19 – 45 years (BMI range from 25 – 36kg/m^2) were sought by paid advertisement. Smokers and those with known food allergies were excluded. Female subjects were tested within the follicular phase of their menstrual cycle. Height, weight, waist circumference and percentage body fat (Tanita Scales Model no. UM-019) were recorded. A diet history and three-day weighed food records were collected to design familiar meals within the subject's taste preferences. Twenty-four hour dietary recalls were collected for the day prior to each study visit. Subjects were instructed to fast for a minimum of 10 h prior to presentation for study appointments. The study was approved by the University of Wollongong Human Research Ethics Committee, application no. HE06/123.

2.2 Test foods

Subjects consumed five different breakfast meals (Table 1) on five occasions after overnight fast with a minimum of three days between visits. Meals consisted of a bowl of cereal served with 200 mL reduced-fat milk and a glass of water. The test cereals were a corn-based control cereal, three cereals with varying levels of β-glucan (low-β-glucan dose (LBG), mid-β-glucan dose -(MBG) and high β-glucan dose (HBGO)) sourced from OatWell™ (CreaNutrition, Switzerland), an oat bran with high β-glucan content and one cereal with an oat β-glucan concentrate (52.02% β-glucan) produced by an ethanolic extraction process (high β-glucan dose containing extracted β-glucan (HBGX)). HBGO and HBGX were designed to have similar β-glucan contents. Extruded test cereals were formulated from oat flour, maize flour, sugar, maltodextrin, sodium bicarbonate, salt, water and the β-glucan ingredient. An APV-MPF50 twin-screw extruder (Baker Perkins Inc, MI) was used. To complete the test breakfast, available carbohydrates and proteins were matched by dissolving glucose polymer (Poly-Joule®, Nutricia, Australasia) and protein powder (Beneprotein®, Novartis, United States) in the milk.

Total β-glucan was measured by the method of McCleary and Glennie-Holmes [14] using a kit from Megazyme (Megazyme International, Ireland). The β-glucan was extracted at 37°C following an *in vitro* digestion protocol [15]. Viscosity of the extract was determined using a controlled strain rheometer (TA Instruments, NJ) and apparent viscosity at 30 s was reported. For the HBGX ingredient, the β-glucan content was too high to allow 5 g to be hydrated in 100 mL of buffer, so a 2 g sample was used. The concentration of β-glucan was determined by flow injection analysis following the method of Jørgensen [16]. MW of β-glucan was determined by size exclusion HPLC

[17] except that the columns were Shodex OHpak KB806M and Waters Ultrahydrogel (Waters, Milford, MA). Moisture was determined by drying a weighed sample in a vacuum oven at 80°C for 5 h and measuring weight loss.

2.2.1 Appetite markers and measures

On arrival at the clinical laboratory, subjects had a cannula inserted into the forearm and initial fasting blood samples were collected. The subject then ate one of the five test breakfast meals within a 10-min period. Fifteen minutes after completion of this meal, further samples were taken. Blood samples were then collected at 30, 60, 120, 180 and 240 min from completion of the meal. Glucose (Glucose hexokinase method, Roche Diagnostics, Australia) and insulin (Electrochemluminescence method, Roche Diagnostics, Australia) analyses were performed at an accredited pathology laboratory. Collection, preparation and analysis for ghrelin and CCK were according to standard protocols for the respective assays. Ghrelin analysis used Linco Research™ ELISA (EZGAC-86K) for measurement of the active octanoyl-modified ghrelin. Phoenix Peptides™ RIA (RK-069-04) for CCK octapeptide (CCK 26-33) was used on extracted peptides.

At each of the described time-points for blood collection, subjects completed a four question visual analogue scale (VAS) related to appetite adapted from Flint *et al.* [18]. The current study used the questions – How hungry do you feel? How satisfied do you feel? How full do you feel? How much do you think you can eat? Subjects recorded their feelings on individual forms and results along each line were measured in millimetres on a 100 mm scale. Participants were also asked to complete the 'Three-Factor Eating Questionnaire' [19] to determine any presence of restrictive eating in normal eating behaviours. Four hours after completion of breakfast, a buffet lunch was served, consisting of sandwiches (cut into bite-size pieces), dried fruit, nuts, yoghurt and juice (totalling approximately 7500 kJ – 50% carbohydrate, 20% protein and 30% fat). Foods were weighed or measured before the meal and at completion.

2.2.2 Statistical analysis

Previous studies identified that to detect biochemical differences, as few as seven subjects of each sex may be sufficient [10], while 18 subjects should identify statistically significant changes using a paired design and a study power of 0.8 if VAS ratings vary by at least 5 mm [18]. Using a repeated measures design would decrease this to as few as 8 subjects [20]. A target of 15 – 18 subjects was set for recruitment based on these studies. Results for blood analysis, VAS values and second-meal dietary intake were entered into SPSS for windows, Version 15.0 and trapezoidal area under the curve (AUC) where values were corrected for baseline but areas below the baseline were also subtracted (netAUC as described by Wolever) [21]. Data was analysed for individual and combined sexes as past studies have identified some differences between sexes [10]. AUC differences in glucose, insulin, CCK, ghrelin and VAS results between the breakfasts were identified using repeated measures analysis of variance (RMANOVA) with post-hoc Bonferroni adjustments. For meal intake values, RMANOVA of kilojoules consumed was performed. Student's *t*-test was used to make comparisons between test meals. Regression analysis analysed relationships between dose, biochemical, subjective and meal intake data and 24 h dietary recalls. Biochemical results corrected for baseline and peak values were reviewed. VAS data from individual questions as well as the sum of four questions (converted from a measure of fullness to hunger where necessary) were analysed using RMANOVA of AUC.

3 Results

3.1 Subjects

A total of 41 subjects were screened, with 17 recruited and 3 withdrawals due to time constraints. Seven male and seven female subjects were aged 29 – 45 years (mean 38.7 years) with an average BMI of 29.6 kg/m^2 (25.2 – 36.6 kg/m^2). Average waist circumference was 76.0 cm ± 11.2 with body fat 34.7 ± 6.0%. Average fasting glucose was 4.2 ± 0.9 mmol/L with fasting insulin 9.5 ± 5.4 mU/L (0.4 – 24.4 mU/L). Two individuals demonstrated elevated fasting insulin and overall hyperinsulinaemia. Data related to glucose metabolism was reviewed including and excluding these subjects. Results from the 'Three Factor Eating Questionnaire' indicated the absence of restrained eaters amongst study subjects.

3.2 Test food analysis

Results for analyses of test foods for total β-glucan content, solubility, viscosity and MW are detailed in Table 2. There was a slight decrease in peak MW with increase in concentration of β-glucan. Size exclusion chromatography shows the shift in the distribution of MW as the β-glucan concentration was increased (Fig. 1). As the dose was increased, the peak became narrower, resulting in a gradual shift of the peak. However, the MW of the β-glucan would still be considered high enough to ensure functionality (>10 00 000 g/mol) [22]. To estimate the effect of β-glucan on luminal viscosity, an *in vitro* digestion protocol was used. The cereal was treated sequentially with amylase (pH 6.9), pepsin (at pH 2) and pancreatin (at pH 6.9) at 37°C. In the HBGX cereal, solubility of β-glucan was much higher than in the ingredient (72 *vs.* 39%) and comparable to the solubility of β-glucan in all cereals made with Oatwell (CreaNutrition, 68 – 78%). As expected, soluble β-glucan concentration correlated ($R^2 = 0.95$) with viscosity of the extract. Concentration strongly influenced viscosity, with doubling of concentration resulting in a 15-fold increase in viscosity.

Table 2. Physico-chemical characteristics of β-glucan in test meals

Name of test food	Moisture (%)	Total β-glucan (% dwb)	Soluble β-glucan[a)] (% dwb)	Viscosity of extract (mPa.s)	MW[b)] (g/mol)
Oatwell oat bran[c)]	4.09	21.03	13.09	766	1 689 000
Extracted β-glucan[c)]	6.55	52.02	20.36	532[d)]	1 513 000
LBG	4.65	5.04	3.42	5.8	1 681 000
MBG	4.91	8.92	6.96	32.0	1 378 000
HBGO	4.09	12.62	8.89	76.6	1 213 000
HBGX	3.83	13.05	9.37	84.8	1 222 000

a) Grams of extractable β-glucan *per* 100 g of ingredient or cereal.
b) Peak MW.
c) Raw ingredients
d) Viscosity of extract from 2 g; other samples were 5 g.

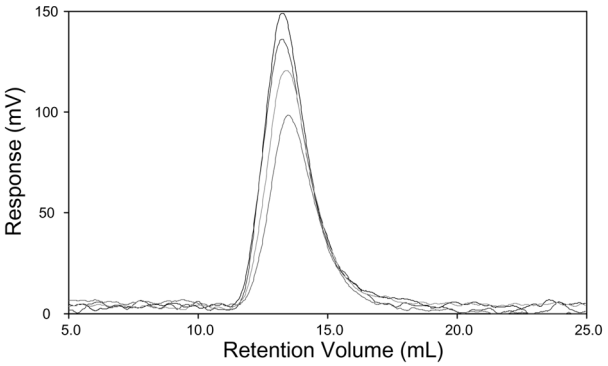

Figure 1. Size exclusion chromatograms of β-glucan MW in the *in vitro* extracts. Peaks represent the distribution of β-glucan MW in, from top to bottom, OatWell (CreaNutrition) oat bran (raw ingredient), LBG, MBG and HBGO. (Peak heights were adjusted to allow better comparison and do not reflect concentration in the original extract.)

3.3 Baseline measurements

Dietary recalls for the 24 h prior to each visit, averaged 9428 kJ (3623 – 1 6845 kJ). Female average intake was 7428 kJ and for males 11 426 kJ. As expected, regression analysis indicated some contribution of total energy intake consumed in the previous 24 h to the prediction of total lunchtime intake ($R^2 = 0.132$, $p = 0.002$). No relationship was identified between the 24 h recalls and baseline measurements of individual VAS questions or the combined questions, fasting insulin, glucose or ghrelin levels. A relationship ($p = 0.018$) was identified between 24 h energy intake data and fasting CCK but this prediction was extremely weak ($R^2 = 0.08$).

3.4 Clinical indices

Insulin AUC data corrected for baseline indicated a significant difference between responses over the first 2 h ($p = 0.011$) using RMANOVA (Table 3). Regression analysis comparing soluble and total β-glucan to 2 h AUC insulin, showed significant inverse relationships with $R^2 = 0.95$ ($p = 0.005$) and $R^2 = 0.97$ ($p = 0.007$) respectively. When the control meal was compared to each individual dose using a *t*-test, significant results were noted for the MBG ($p = 0.029$), HBGO ($p = 0.021$) and HBGX doses ($p = 0.014$). Removing results for subjects with hyperinsulinaemia showed improvement in insulin response for the LBG dose (Table 3).

No significant differences were found with insulin reviewed over 4 h (Table 3), although the same trend existed with decrease in insulin with increase in fibre. Raw data for

Table 3. Post prandial insulin (mU/L X min) responses for 2 and 4 h for all subjects and excluding subjects with obvious hyperinsulinaemia.

Meal/Dose	Insulin AUC (0–2 h) all subjects[a)]		Insulin AUC (0–2 h) excluding subjects with hyperinsulinaemia[a)]		Insulin AUC (0–4 h) all subjects		Insulin AUC (0–4 h) excluding subjects with hyperinsulinaemia	
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD
Control	14	3559 ± 1478	12	3179 ± 1107	14	4511 ± 1914	12	4016 ± 1459
LBG	14	3643 ± 1926	12	3079 ± 1332	14	4801 ± 2794	12	3990 ± 1962
MBG	14	3072[b)] ± 1432	12	2628[b)] ± 903	14	4351 ± 2395	12	3683 ± 1706
HBGO	14	2952[b)] ± 1664	12	2431[b)] ± 1079	14	4085 ± 2348	12	3353 ± 1543
HBGX	14	2959[b)] ± 1533	12	2471[b)] ± 944	14	4247 ± 2484	12	3514 ± 1652

a) RMANOVA $p < 0.05$ for overall analysis.
b) $p < 0.05$ for *t*-test comparison with control dose of fibre.

Table 4. Post prandial glucose (mmol/L X min), ghrelin (pg/mL X min) and CCK (pg/mL X min) responses

Meal/Dose	Glucose AUC (0–2 h)		Ghrelin AUC (0–4 h)		CCK AUC combined sexes (n = 14)		CCK AUC female (n = 7)[a)]	
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD
Control	14	505 ± 142	12	5320 ± 2850	14	3052 ± 1451	7	2213 ± 1227
LBG	14	493 ± 96	12	3860 ± 3217	14	3455 ± 1708	7	2670 ± 796
MBG	14	505 ± 83	12	5300 ± 4428	14	3680 ± 1652	7	3061 ± 960
HBGO	14	488 ± 109	12	5041 ± 3007	14	3831 ± 1872	7	3190 ± 1049
HBGX	14	493 ± 101	12	4408 ± 3724	14	3814 ± 1800	7	3478 ± 1289

a) RMANOVA $p < 0.05$ for overall analysis.

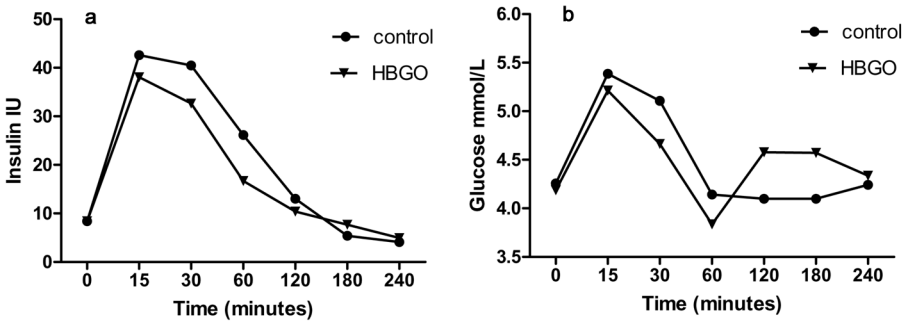

Figure 2. (a) Postprandial insulin responses for control and high β-glucan (>5 g) cereal sourced from OatWell (CreaNutrition) for subjects without obvious hyperinsulinaemia. (b) Postprandial glucose responses for control and high β-glucan (>5 g) cereal sourced from OatWell (CreaNutrition).

post-prandial insulin responses for subjects (without hyperinsulinaemia) consuming the control and HBGO meals is depicted in Fig. 2a. No analyses showed any significant difference between the two HBG meals.

Raw data for post-prandial glucose responses is depicted in Fig. 2b showing minimal differences between even the control and HBGO dose. There were no significant differences in glycaemic responses between test meals. This included analyses using RMANOVA of AUC data (Table 4) and comparison of peak glucose responses. Calculation of incremental AUC (method of Wolever *et al.* [23]) also showed no statistical difference.

RMANOVA of AUC for ghrelin showed no significant results (Table 4). RMANOVA of AUC for CCK trended towards a dose relationship ($p = 0.11$) (Fig. 3). When data for each sex was analysed separately, this relationship was significant for women ($p = 0.048$) but not men ($p = 0.691$) (Fig. 3 and Table 4). No significant differences were noted between HBGO and HBGX.

A dose response for CCK was noted (Table 4). Regression analysis for the dose of soluble or total β-glucan compared to the CCK responses identified significant relationships ($R^2 \geq 0.97$, $p = 0.002$ for both). Student's t-test comparing the control meal to fibre doses identified results approaching significance for the combined sexes for MBG, HBGO and HBGX doses ($p = 0.062$, 0.071 and 0.063 respectively). When only female results are reviewed, the same doses produced significantly different results to the control ($p = 0.036$, 0.032, 0.006 respectively). No differences were identified between HBGO and HBGX for combined sexes or for women alone.

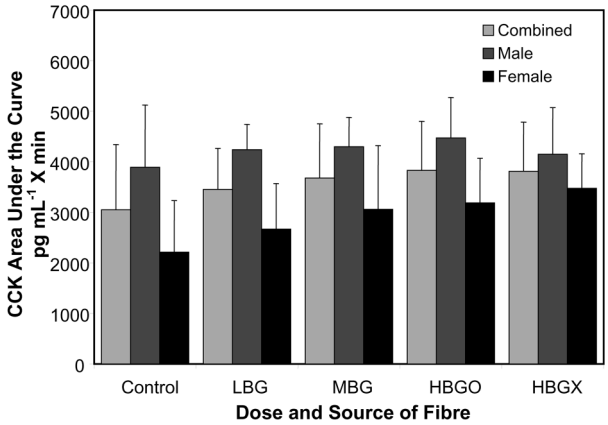

Figure 3. CCK response to various levels of β-glucan (combined sexes n = 14; male, n = 7 and female, n = 7).

3.5 Subjective satiety

Responses to individual VAS questions (Table 5) for varying OatWell (CreaNutrition) doses showed significant variation for Question 3 (How full do you feel?) ($p = 0.017$, RMANOVA for AUC). Other questions approached a significance level of 0.05 ($p = 0.071$, 0.101 and 0.099 for Questions 1, 2 and 4 respectively). Pairwise comparisons using Bonferroni adjustments indicated differences primarily between the control and all doses of fibre. Comparison between HBGO and HBGX responses showed some variations. Question 3 showed a difference ($p = 0.013$), where the HBGO product appeared to make participants feel more full. Data for all other questions showed a similar trend of

Table 5. VASs score for area under the curve over 4 h, AUC (mean ± SD)

	Control	LBG	MBG	HBGO	HBGX
Question 1 How hungry do you feel?	13 600 ± 3900	11 100 ± 4500	11 420 ± 4080	10 660 ± 5180	11 700 ± 3840
Question 2 How satisfied do you feel?	9 350 ± 4630	11 400 ± 5060	10 750 ± 4400	11 530 ± 6080	10 440 ± 5110
Question 3[a)] How full do you feel?	8 600 ± 4690	11 470 ± 5310	10 480 ± 4750	11 460 ± 6540	9 630 ± 5320
Question 4 How much could you eat?	15 000 ± 4100	13 230 ± 4730	12 840 ± 4440	13 350 ± 5090	13 530 ± 4560
Combined[a)] (Q2 & Q3 reversed)	58 640 ± 16 580	49 510 ± 19 150	51 030 ± 16 480	49 020 ± 22 310	53 170 ± 17 920

a) RMANOVA $p < 0.05$.

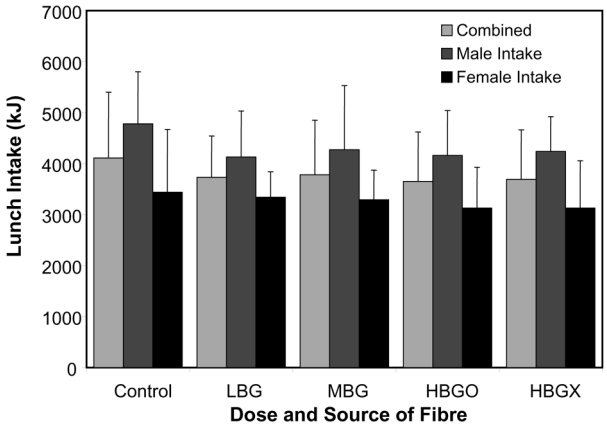

Figure 4. Average ($n = 14$) energy intake at lunch meal for males, females and combined sexes.

increased satiety with HBGO, but no significant results were noted (Question 1, $p = 0.263$; Question 2, $p = 0.101$; Question 4, $p = 0.794$).

When the responses to questions were analysed as a single response (a measure of hunger with Questions 2 and 3 responses reversed), an overall effect was noted using RMANOVA ($p = 0.039$). Pairwise comparisons showed no difference between fibre doses (LBG, MBG and HBGO) but the overall effect resulted from differences between the control and all other fibre doses. No gender effect was noted. RMANOVA of the two HBG doses showed less hunger/greater fullness with the OatWell (CreaNutrition) product but this only tended towards significance ($p = 0.085$). Student's *t*-test analyses of the control meal compared to other doses showed differences between the control and all OatWell (CreaNutrition) doses ($p = 0.013$, 0.026, 0.015, 0.086 for LBG, MBG, HBGO and HBGX respectively).

3.6 Second meal intake data

RMANOVA of lunch energy intake data showed non-significant results ($p = 0.22$) although the trend was as expected (Fig. 4). Overall, the lunch time energy difference between the control and HBGO meal was 460 kJ. Less than 40 kJ difference was noted between the HBGO and the HBGX meals. Student's *t*-test analyses showed significant difference between the control and HBGX groups' meals ($p = 0.033$) and approaching significance for comparison between the control and HBGO groups' meals ($p = 0.097$). No gender/fibre interaction could be identified. However, results for males showed a drop in intake between the control and all doses of β-glucan, while the female response was more attenuated, more evenly decreasing with increase in fibre dose (Fig. 4).

4 Discussion

The current study combined a variety of measures for appetite and satiety within a single experimental design. Content analysis of the organoleptically acceptable products found that β-glucan was stable to the extrusion processes and maintained characteristics for attributable metabolic effects. MW remained high, with good water solubility at body temperature, ensuring maximum viscosity within the aqueous gut environment. Variability in the cholesterol-lowering action of β-glucan has been attributed to low MW or viscosity after certain forms of processing [24, 25].

The decrease in MW noted with increasing β-glucan is most likely secondary to the heat generated during extrusion. The more viscous the raw ingredients (higher concentrations of β-glucan) the slower the flow rate through the extruder and the greater the likelihood of heat degradation during processing. However, if these results are compared to others utilizing a variety of processing techniques [25] extrusion still compares favourably. As the proportion of β-glucan in the cereal increased, the solubility of the β-glucan also increased, probably as a result of increased pressure in the extruder. Therefore, the increase in solubility, more than made up for the decrease in MW and the overall viscosity in the *in vitro* extract actually increased as the β-glucan dose increased. It will always be critical to measure parameters such as MW, viscosity and solubility in a new product but it would seem extrusion is acceptable downstream processing for β-glucan products.

Previous research using VAS suggests no need to standardize meals prior to meal-tests [18]. This was confirmed

here, where no true correlations were found between 24 h recall data prior to testing and fasting VAS scores, ghrelin or CCK. This does not mean that fasting measures of these scores or hormones are unrelated to longer-term dietary behaviours, particularly as these behaviours impact on weight control.

A range of soluble fibres, including β-glucan have been shown to improve glycaemic responses [8, 26], with each gram of β-glucan found to decrease glycaemic index by four units [8]. The food vehicle, however, may be important. Studies have found that high doses of β-glucan (>5 g) in pasta [27] and in rye bread [28] did not improve glycaemic responses. β-Glucan depolymerization has been shown to increase during processing of pasta and bread [25]. However, β-glucan from barley, served as a hot cereal, produced a significant decrease in glucose with only 2 g of β-glucan in women [29]. All of the test meals here produced blunted glucose responses when compared to a similar study [30]. It was expected that the 43 g of available carbohydrate here would produce a greater elevation in blood glucose from baseline, particularly in the overweight/obese study sample. The small response for the control was unexpected but the overall low energy content of test meals may have made differentiation between meals difficult. It is also recognized that venous sampling of blood glucose results in more attenuated responses compared to capillary samples [31].

Regardless of glucose response, the observation of decreased insulin release was a positive outcome, possibly through delayed rate of glucose delivery or specifically through actions of CCK [32]. The role of hyperinsulinaemia in the development of obesity or indeed hyperinsulinaemia as a consequence of obesity may be multifactorial. Karpe and Tan [33] argue that hyperinsulinaemia may lead to lipolytic inhibition in adipose tissue; so any ingredient which decreases insulin secretion is helpful in minimizing obesity-promoting effects. This is particularly promising, given that other authors have demonstrated increased insulin responses with dairy products (included here with our cereal), yet increasing doses of β-glucan still produced lowered insulin responses [34]. Our results indicated a dose response to β-glucan with a dose greater than 3 g demonstrating a consistent decrease in insulin secretion. This is consistent with other recently published data identifying a dose of 4 g β-glucan was necessary to show insulin decreases [35]. Differences in insulin results for subjects with hyperinsulinaemia indicate that future studies should examine glucose, insulin and appetite responses in subjects with and without insulin resistance. Given the trend of insulin responses over four hours, it is likely that greater subject numbers would have also shown a significant difference over the longer time frame.

A decrease in ghrelin is associated with food intake, but here the β-glucan did not suppress ghrelin secretion in a dose responsive manner. Other studies [36] investigated the effect of insoluble wheat and insoluble oat fibres on secretion of ghrelin. The oat-fibre bread showed no decrease in ghrelin compared to controls. It would seem that both the insoluble fibre [36] and the soluble fibre used in this study do not alter ghrelin secretion at the levels provided.

The dose-response relationship between β-glucan and CCK in our study demonstrates a possible mechanism of satiety associated with increase in fibre intake. The link between fibre and CCK secretion has been previously established [10, 37]. The greater sensitivity in females compared to males is also consistent with the literature [10, 38] and use of fibre in foods targeted specifically at increasing satiety in women may be justified. The effective dose of close to 4 g demonstrated here is consistent with advice from Malkki and Virtanen [39] relating to gastrointestinal effects of oat bran. Given the *t*-test results for males were close to significant, and the overall RMANOVA for combined sexes shows increasing CCK with fibre close to significance ($p = 0.110$), it is likely that greater subject numbers would have allowed elucidation of the precise level of β-glucan required to show CCK differences in men, and a subject group of combined sexes.

Overall the VAS scores to rate hunger/appetite indicate that even relatively low doses of β-glucan (>2 g) will give a decreased feeling of hunger. Of interest is the marginal differences identified between the OatWell (CreaNutrition) and the extracted β-glucan HBG meals. While biochemical and subjective measures, measure different things, the majority of results here support higher β-glucan improving all markers and measures of satiety, and it is only the VAS results for the HBGX fibre here which are inconsistent.

The wide variety of factors affecting meal intake [40] may limit the ability of small studies to identify differences in dietary intake related to a single nutrient. Our study manipulated nutrient composition of meals to focus on the effect of fibre and in particular oat β-glucan as a variable in appetite control, but wide SDs (up to 29% in males) may have diluted results. Based on the differences in energy intake in this study a sample size of 37 would be required for this result to reach statistical significance in a paired design (80% power, alpha 0.05). Although overall the RMANOVA of results was not significant, post-hoc *t*-test analysis indicated differences with the HBGX ($p < 0.05$) and perhaps the HBGO ($p < 0.1$) meals. The fact that female intake produced flat responses demonstrates that large numbers of females would be required to demonstrate differences and, that other factors may attenuate intake. In particular, the widely held social belief that women are more likely to show restraint in a buffet situation (regardless of appetite) may be demonstrated here.

The kilojoule difference between control and HBG doses may not show statistical significance, but the absolute difference of greater than 400 kJ at a single meal is of clinical significance if these results could be repeated in a more powerful study. Future studies should record intake over the entire day. If no compensatory intake took place later in the

day, the difference would equate to a 100 g weight loss each week if maintained daily.

Overall, the use of β-glucan in foods with a target market of individuals wishing to maintain or lose weight through appetite control is justified. Appetite suppressants such as CCK are released in response to β-glucan at a minimum dose of approximately 3.8 g. Subjective ratings of hunger are improved at a minimum dose of 2.2 g of β-glucan. Insulin responses relevant to the development of Type 2 diabetes are significantly decreased at a dose of at least 3.8 g of β-glucan. Although minor differences may exist in the clinical effectiveness of β-glucan, either when extracted or from oat bran as in OatWell (CreaNutrition), both show favourable results. However, variation in results between foods tested here and in other studies, necessitates individual testing of all β-glucan products.

The authors wish to acknowledge the funding of this project by Cereal Partners Worldwide. In addition the assistance of Associate Professor Ken Russell from the Statistical Consulting Service at the University of Wollongong is greatly appreciated, as are comments on the manuscript by Ruedi Duss of CreaNutrition.

The authors have declared no conflict of interest.

5 References

[1] Howarth, N. C., Saltzman, E., Roberts, S. B., Dietary fiber and weight regulation, *Nutr. Rev.* 2001, *59*, 129 – 139.

[2] Miller, W. C., Niderpruem, M. G., Wallace, J. P., Lindeman, A. K., Dietary fat, sugar, and fiber predict body fat content, *J. Am. Diet. Assoc.* 1994, *94*, 612 – 615.

[3] Alfieri, M. A. H., Pomerleau, J., Grace, D. M., Anderson, L., Fiber intake of normal weight, moderately obese and severly obese subjects, *Obes. Res.* 1995, *3*, 541 – 547.

[4] Lovejoy, J., DiGirolamo, M., Habitual dietary intake and insulin sensitivity in lean and obese adults, *Am. J. Clin. Nutr.* 1992, *55*, 1174 – 1179.

[5] Koh-Banerjee, P., Franz, M., Sampson, L., Liu, S., *et al.*, Changes in whole-grain, bran, and cereal fiber consumption in relation to 8-y weight gain among men, *Am. J. Clin. Nutr.* 2004, *80*, 1237 – 1245.

[6] Liu, S., Willet, W., Manson, J., Hu, F. *et al.*, Relation between changes in intakes of dietary fiber and grain products and changes in weight and development of obesity among middle-aged women, *Am. J. Clin. Nutr.* 2003, *78*, 920 – 927.

[7] Burton-Freeman, B., Dietary fibre and energy regulation. Symposium: Dietary composition and obesity: Do we need to look beyond dietary fat? *J. Nutr.* 2000, *130*, 272S – 275S.

[8] Jenkins, A. L., Jenkins, D. J., Zdravkovic, U., Wursch, P., Vuksan, V., Depression of the glycemic index by high levels of beta-glucan fiber in two functional foods tested in type 2 diabetes, *Eur. J. Clin. Nutr.* 2002, *56*, 622 – 628.

[9] Flint, A., Moller, B. K., Raben, A., Sloth, B., *et al.*, Glycemic and insulinemic responses as determinants of appetite in humans, *Am. J. Clin. Nutr.* 2006, *84*, 1365 – 1373.

[10] Burton-Freeman, B., Davis, P. A., Schneeman, B., Plasma cholecystokinin is associated with subjective measures of satiety in women, *Am. J. Clin. Nutr.* 2002, *76*, 659 – 667.

[11] Hosoda, H., Kojima, M., Kangawa, K., Ghrelin and the regulation of food intake and energy balance, *Mol. Interv.* 2002, *2*, 494 – 503.

[12] Cummings, D. E., Purnell, J. Q., Frayo, R. S., Schmidova, K. *et al.*, A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans, *Diabetes* 2001, *50*, 1714 – 1719.

[13] Anttila, H., Sontag-Strohm, T., Salovaara, H., Viscosity of beta-glucan in oat products, *J. Agric. Food Sci.* 2004, *13*, 80 – 87.

[14] McCleary, B. V., Glennie-Holmes, M., Enzymatic quantification of (1→3) (1→4) – β-D-glucan in barley and malt, *J. Inst. Brew.* 1985, *91*, 285 – 295.

[15] Beer, M. U., Wood, P. J., Weisz, J., Fillion, N., Effect of cooking and storage on the amount and molecular weight of (1→3) (1→4)-β-D-glucan extracted from oat products by an in vitro digestion system, *Cereal Chem.* 1997, *74*, 705 – 709.

[16] Jørgensen, K. G., Quantification of high molecular weight (1→3) (1→4) – β- D – glucan using calcofluor complex formation and flow injection analysis. Analytical principle and its standardization, *Carlsberg Res. Commun.* 1988, *53*, 287 – 296.

[17] Wood, P. J., Weisz, J., Mahn, W., Molecular characterization of cereal β- glucans. II. Size-exclusion chromatography for comparison of molecular weight, *Cereal Chem.* 1991, *68*, 530 – 536.

[18] Flint, A., Raben, A., Blundell, J. E., Astrup, A., Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies, *Int. J. Obes.* 2000, *24*, 38 – 48.

[19] Stunkard, A. J., Messick, S., The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger, *J. Psychosom. Res.* 1985, *29*, 71 – 83.

[20] Raben, A., Agerholm-Larsen, L., Flint, A., Holst, J., Astrup, A., Meals with similar energy densities but rich in protein, fat, carbohydrate, or alcohol have different effects on energy expenditure and substrate metabolism but not on appetite and energy intake, *Am. J. Clin. Nutr.* 2003, *77*, 91 – 100.

[21] Wolever, T. M., Effect of blood sampling schedule and method of calculating the area under the curve on validity and precision of glycaemic index values, *Brit. J. Nutr.* 2004, *91*, 295 – 300.

[22] Tosh, S. M., Brummer, Y., Wolever, T. M. S., Wood, P. J., Glycemic response to oat bran muffins treated to vary molecular weight of beta-glucan, *Cereal Chem.* 2008, *85*, 211 – 217.

[23] Wolever, T. M., Brand Miller, J., Abernethy, J., Astrup, A., *et al.*, Measuring the glycaemic index of foods – interlaboratory study, *Am. J. Clin. Nutr.* 2008, *87*, 247S – 257S.

[24] Kerckhoffs, D., Hornstra, G., Mensink, R., Cholesterol-lowering effect of beta-glucan from oat bran in mildly hypercholesterolemic subjects may decrease when beta-glucan is incorporated into bread and cookies, *Am. J. Clin. Nutr.* 2003, *78*, 221 – 227.

[25] Aman, P., Rimsten, L., Andersson, R., Molecular Weight Distribution of beta-glucan in oat-based foods, *Cereal Chem.* 2004, *81*, 356 – 360.

[26] Makelainen, H., Anttila, H., Sihvonen, J., Hietanen, R.-M., *et al.*, The effect of beta-glucan on the glycemic and insulin index, *Eur. J. Clin. Nutr.* 2007, *61*, 779 – 785.

[27] Holm, J., Koellreutter, B., Wursch, P., Influence of sterilization drying and oat bran enrichment of pasta on glucose and insulin responses in healthy subjects and on the rate and extent of *in vitro* starch digestion, *Eur. J. Clin. Nutr.* 1992, *46*, 629 – 640.

[28] Juntunen, K., Niskanen, L., Liukkonen, K., Poutanen, K., *et al.*, Postprandial glucose, insulin, and incretin responses to grain products in healthy subjects, *Am. J. Clin. Nutr.* 2002, *75*, 254 – 262.

[29] Kim, H., Behall, K. M., Vinyard, B., Conway, J., Short-term satiety and glycemic response after consumption of whole grains with various amounts of beta-glucan, *Cereal Foods World* 2006, *51*, 29 – 33.

[30] Lan-Pidhainy, X., Brummer, Y., Tosh, S. M., Wolever, T. M., Wood, P. J., Reducing beta-glucan solubility in oat bran muffins by freeze-thaw treatment attenuates its hypoglycemic effect, *Cereal Chem.* 2007, *84*, 512 – 517.

[31] Wolever, T. M., Bolognesi, C., Source and amount of carbohydrate affect postprandial glucose and insulin in normal subjects, *J. Nutr.* 1996, *126*, 2798 – 2806.

[32] Chandra, R., Liddle, S. A., Cholecystokinin, *Curr. Opin. Endocrinol. Diabetes* 2007, *14*, 63 – 67.

[33] Karpe, F., Tan, G. D., Adipose tissue function in the insulin-resistance syndrome, *Biochem. Soc. Trans.* 2005, *33*, 1045 – 1048.

[34] Bjorck, I., Liljeberg, H., Ostman, E. M., Low glycemic-index foods, *Brit. J. Nutr.* 2000, *83*, S149 – S155.

[35] Granfeldt, Y., Nyberg, L., Bjorck, I., Muesli with 4 g oat [beta]-glucans lowers glucose and insulin responses after a bread meal in healthy subjects, *Eur. J. Clin. Nutr.* 2008, *62*, 600 – 607.

[36] Weickert, M. O., Spranger, J., Holst, J. J., Otto, B., *et al.*, Wheat-fibre-induced changes of postprandial peptide YY and ghrelin responses are not associated with acute alterations of satiety, *Brit. J. Nutr.* 2006, *96*, 795 – 798.

[37] Bourdon, I., Yokoyama, W., Davis, P., Hudson, C., *et al.*, Postprandial lipid, glucose, insulin, and cholecystokinin responses in men fed barley pasta enriched with beta-glucan, *Am. J. Clin. Nutr.* 1999, *69*, 55 – 63.

[38] Burton-Freeman, B., Davis, P. A., Schneeman, B., Interaction of fat availability and sex on postprandial satiety and cholecystokinin after mixed-food meals, *Am. J. Clin. Nutr.* 2004, *80*, 1207 – 1214.

[39] Malkki, Y., Virtanen, E., Gastrointestinal effects of oat bran and oat gum – A review, *Lebensm. – Wiss. u. – Technol.* 2001, *34*, 337 – 347.

[40] Mattes, R. D., Hollis, J., Hayes, D., Stunkard, A. J., Appetite: Measurement and manipulation misgivings, *J. Am. Diet. Assoc.* 2005, *105*, S87 – S97.

