DOI 10.1002/mnfr.201100095 *Mol. Nutr. Food Res.* 2011, *55*, 1118–1121

FOOD & FUNCTION

Diet high in oat β-glucan activates the gut-hypothalamic ($PYY_{3–36}$-NPY) axis and increases satiety in diet-induced obesity in mice

Xu-Feng Huang[1], Yinghua Yu[1], Eleanor J. Beck[1], Tim South[1], Yulin Li[1], Marijka J. Batterham[2], Linda C. Tapsell[1] and Jiezhong Chen[1]

[1] School of Health Sciences, University of Wollongong, NSW, Australia
[2] School of Mathematics and Applied Statistics, University of Wollongong, NSW, Australia

This study tested the effects of (1→3),(1→4) β-D-glucan from oats, on activation of the gut-hypothalamic ($PYY_{3–36}$-NPY) axis, satiety, and weight loss in diet-induced obesity (DIO) mice. DIO mice were fed standard lab chow diets or varied doses of β-glucan for 6 weeks. Energy intake, satiety, body weight changes and peptide Y-Y_{3-36} (PYY_{3-36}) were measured together with a satiety test and measurement of neuropeptide Y (NPY) mRNA expression in the hypothalamic arcuate nucleus (Arc). The average energy intake (-13%, $p<0.05$) and body weight gain was lower with increasing β-glucan over 6 wk with acute suppression of energy intake over 4 h. The highest β-glucan diet significantly increased plasma PYY_{3-36}, with suppression of Arc NPY mRNA.

Received: February 15, 2011
Revised: April 9, 2011
Accepted: May 2, 2011

Keywords:
β-Glucan / Energy intake / Neuropeptide Y / Obesity / Satiety

Obesity is one of the most common and rapidly growing metabolic disorders in the western world. Although environmental factors are important, physiologically, energy intake is primarily regulated by the central nervous system, which senses satiety or hunger signals from peripheral organs. Hormones associated with satiety and satiation signal the brain and so manipulation of hormones such as peptide Y-Y $(PYY)_{3–36}$ could be used in the treatment of obesity [1].

PYY is a 36-amino acid hormone in the pancreatic polypeptide (PP) family, which includes functionally related neural peptide Y (NPY) and PP. PYY is cleaved by dipeptidyl peptidase IV (DPP-IV) to produce $PYY_{3–36}$ which can pass through the blood–brain barrier to act on the hypothalamic arcuate nucleus (Arc) NPY-Y2 receptors [2, 3] to suppress food intake [4, 5]. $PYY_{3–36}$-NPY axis is an important neuroendocrine pathway regulating food intake and in turn, energy balance [3, 6, 7].

Oats contain significant amounts of (1→3) (1→4)-β-D-glucan (β-glucan), with associated functional properties including lipid lowering, effects on insulin and glycaemic [8] control and enhancement of post-meal satiety through elevation of plasma PYY [9] and other hormones [10]. Studies investigating the action of β-glucan on hormones associated with weight control are consistent with scientific review of fiber intake related to obesity as a risk factor for Metabolic Syndrome, whereby numerous studies identify the positive effects of fiber on hormones related to obesity [11]. However, the studies with β-glucan have been small such as a pilot study using oat and barley sourced β-glucan as a dietary supplement in a weight reducing diet, which showed weight loss, increased fasting PYY, and glucagon-like-peptide-1 (GLP-1) as well as increased satiety in a meal test after 14 wk of supplementation [12]. Our recent work has also shown cholecystokinin (CCK) release increases with β-glucan dose [10] and so it would seem that hormonal changes associated with a decreased appetite are all activated by β-glucan.

Correspondence: Dr. Eleanor J. Beck, School of Health Sciences, University of Wollongong, Northfields Avenue, New South Wales 2522, Australia
E-mail: eleanor@uow.edu.au
Fax: +61-2-4221-4096

Abbreviations: ANOVA, analysis of variance; **Arc,** hypothalamic arcuate nucleus; **DIO,** diet-induced obesity; **HG,** high β-glucan; **LC,** lab chow; **LG,** low β-glucan; **MG,** medium β-glucan; **NPY,** neural peptide Y; **PP,** pancreatic polypeptide; **PYY,** peptide Y-Y

In order to elucidate the mechanisms associated with these hormones, the current study investigated different concentrations of oat β-glucan in the diet on levels of $PYY_{3–36}$ and expression of Arc NPY mRNA in diet-induced obesity (DIO) mice. We aimed to detect the effects of oat β-glucan on the $PYY_{3–36}$-NPY axis as a possible intervention in the treatment of obesity.

Forty-eight, 12-wk-old, C57BL/6 male mice were obtained from the Animal Resource Centre, Perth, Australia. Mice experienced DIO using a previously validated method (Supporting Information), followed by a 2-wk lab chow (LC) diet to washout the possible effect of high fat diet. As is suitable in rodent studies [13] a 6-wk dietary intervention ensued with the standard LC diet or low (0.7%, LG), medium (3.5%, MG), and high (7.0%, HG) oat β-glucan derived from OatWell^{TM} oatbran (CreaNutrition, Switzerland) (Supporting Information Table 1). All diets were controlled (approximately) for total dietary fiber using wheat fiber (VITACEL-WF600, Rosenberg, Germany). The physicochemical characteristics of the oat-bran ingredient used in this study including molecular weight and solubility have been characterized previously [10], showing a molecular weight of approximately 1.7 million g/mol and solubility of around 13% dwb.

Twenty-four-hour energy intake and body weight were measured weekly during the dietary intervention. A satiety test was carried out at the end of the dietary intervention. Mice were deprived of food for 5 h followed by ad libitum access to their respective test foods for 4 h. Food eaten was weighed at 0.5, 1, 2, and 4 h.

Tissue and blood preparation is described in the Supporting Information. After dissection, an adiposity index was calculated by visceral fat (epididymal, perirenal, and omental) divided by final body weight. Plasma $PYY_{3–36}$ was determined using a $PYY_{3–36}$ Radioimmunoassay (RK-059-04, Phoenix Pharmaceuticals, Belmont, CA, USA). The specific antisense hybridization oligoprobes for NPY were the same as that used previously (Supporting Information).

Data were analyzed using the SPSS 13.0 statistical package (SPSS, Chicago, IL, USA). Two-way repeated analysis of variance (ANOVA) (dietary intervention × repeated measure of weeks) assessed energy intake and body weight change during intervention. The refeeding energy intake after 5-h food deprivation, average energy intake during intervention, body weight prior and after the intervention, body weight gain, adiposity index, fat mass, plasma $PYY_{3–36}$, Arc NPY mRNA expression were assessed by one-way ANOVA followed by Post-Hoc Tukey–Kramer–HSD tests. Pearson's correlations were used to determine the relationship between β-glucan concentration and metabolic parameters and Arc NPY mRNA expression. Values are expressed as means ± SEM and *p*-values of less than 0.05 were regarded as statistically significant.

At the week 6, the satiety test showed after 5 h fasting, the energy intake for 1 h was significantly lower in the HG group than the LG and LC group (LG: −34.3%, $p = 0.012$; LC: −33.1%, $p = 0.018$; Supporting Information Fig. 1A) and similarly after 2 h (LC: −26.6%, $p = 0.006$; LG: −34.3%, $p < 0.001$). After 4 h, both HG and MG groups had a lower energy intake than the LG group (−33%, $p < 0.001$ versus HG; −28%, $p < 0.001$ versus MG). A decrease in energy intake was shown between HG group and the LC group (−22%, $p = 0.006$). The energy intake from 0.5 to 4 h in β-glucan diet-fed mice was negatively correlated with β-glucan concentration (Table 1).

The average 24-h energy intake over the 6-wk intervention was significantly affected by diet type ($F_{3,46} = 20.336$, $p < 0.001$), dietary intervention duration ($F_{5,230} = 59.680$, $p < 0.001$), and interaction between the two factors ($F_{15,230} = 4.611$, $p < 0.001$). The average energy intake was significantly different ($F_{3,49} = 25.524$, $p < 0.001$) among the four groups (Table 2). The average 24-h energy intake of HG-fed mice was significantly lower than the LG (−18.5%, $p < 0.001$), MG (−13.6%, $p < 0.014$), and LC (−12.8%, $p < 0.020$) groups (Supporting Information Fig. 1B). There was a strong negative correlation between β-glucan concentration and average energy intake of β-glucan diet-fed mice (Table 1).

A two-way repeated ANOVA revealed that body weight change during the intervention was significantly affected by diet type ($F_{3,46} = 20.336$, $p < 0.001$) and weeks ($F_{5,230} = 59.680$, $p < 0.001$) and there was a significant interaction between the two factors ($F_{15,230} = 4.611$, $p < 0.001$). After the dietary intervention, body weight gain was significantly different among the four groups ($F_{3,49} = 25.524$, $p < 0.001$; Supporting Information Fig. 2). The body weight gain of the HG group was significantly lower than that of the LG groups ($p = 0.032$) and there was a trend for lower body weight gain compared with the LC group ($p = 0.072$). The HG group had lower epididymal (−32%, $p = 0.004$), perirenal (−39%, $p = 0.002$), omental (−23%, $p = 0.018$), and inguinal (−45%, $p = 0.012$) fat pad mass compared with LG group (Table 2). The adiposity

Table 1. Correlation between β-glucan concentration and peripheral and central parameters

	β-Glucan concentration	
	R	*p*
Average energy intake, 6 wk	−0.800	<0.001
Energy intake during satiety test		
0.5 h	−0.353	0.032
1 h	−0.387	0.018
2 h	−0.562	<0.001
4 h	−0.622	<0.001
Body weight gain	−0.417	0.010
Visceral fat	−0.593	<0.001
Adiposity index	−0.536	0.001
Plasma $PYY_{3–36}$	0.506	0.002
Arc NPY mRNA	−0.711	0.003

Table 2. Metabolic parameters of obese mice after the low, medium, or high-oat β-glucan diets

	β-Glucan			LC	*F*(3,49)	*p-Value*
	LG	MG	HG			
Energy intake (kcal/24 h)	16.34±0.29[a]	15.41±0.32[a]	13.31±0.21[b]	15.27±0.18[a]	25.524	<0.001
Fat pads (g)						
Epididymal	1.39±0.07[a]	1.16±0.09[ab]	0.94±0.07[b]	1.06±0.10[b]	4.789	0.005
Perirenal	0.72±0.05[a]	0.52±0.04[ab]	0.44±0.06[b]	0.55±0.04[ab]	5.320	0.003
Omental	0.71±0.03[a]	0.54±0.04[ab]	0.55±0.04[b]	0.52±0.03[b]	5.630	0.002
Subscapular	0.30±0.03[ab]	0.24±0.03[ab]	0.23±0.02[b]	0.26±0.02[ab]	1.802	0.160
Inguinal	1.21±0.12[a]	0.93±0.13[ab]	0.66±0.10[b]	0.85±0.09[ab]	3.707	0.018
Adiposity index	7.42±0.03[a]	6.01±0.03[b]	5.50±0.03[b]	5.86±0.03[b]	6.696	0.001
Plasma PYY_{3-36} (pmol/L)	24.55±0.80[a]	26.12±1.16[a]	36.11±3.97[b]	26.46±1.33[a]	5.687	0.002

Values were means±SEM; LG, MG, and HG were the low (0.7%), medium (3.5%), and high (7%) β-glucan diets. LC, lab chow diet. Adiposity index: visceral fat/final body weight × 100. Means in row without a common letter are significantly different, $p<0.05$.

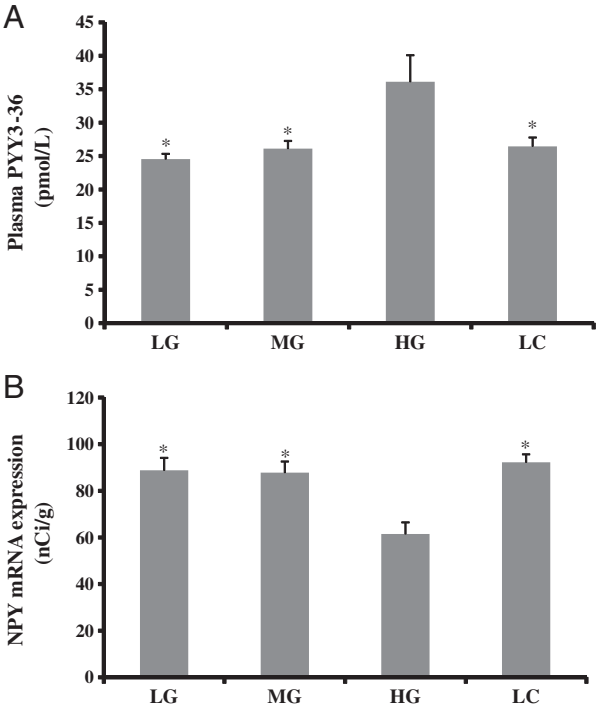

Figure 1. Effect of oat β-glucan on plasma PYY3-36 (A) and hypothalamic NPY mRNA expression. (A) Plasma PYY3-36. (B) NPY mRNA expression in the Arc of obese mice after 6 wk of dietary intervention. LG, MG, and HG were low, medium, and high β-glucan (0.7, 3.5, and 7.0%) diets. LC: lab chow diet. *$p<0.05$ versus HG.

index (visceral fat/final body weight) of the HG-fed mice was lower than the LG-fed mice (−26%, $p = 0.002$).

After 6 wk, the plasma PYY_{3-36} was significantly different among the four groups ($F_{3,49} = 5.687$, $p = 0.002$); higher in the HG group than the LG (+47%, $p = 0.003$), MG (+38%, $p = 0.013$), and LC (+36%, $p = 0.018$) groups (Fig. 1A). A positive correlation was found between the plasma PYY_{3-36} and the amount of dietary β-glucan ($R = 0.506$, $p = 0.002$; Table 1).

NPY mRNA expression in the Arc was significantly different among the four groups ($F_{3,19} = 6.443$, $p = 0.008$); significantly decreased in the HG group compared with LG (−31%, $p = 0.009$) and LC (−33%, $p = 0.002$) groups (Fig. 1B). The HG group had 30% lower Arc NPY mRNA expression than that of the MG group ($p = 0.016$). There was no significant difference in Arc NPY mRNA expression among LG, MG, and LC groups (Supporting Information Fig. 3). There was a strong negative correlation between the Arc NPY mRNA expression and the amount of dietary β-glucan (Table 2).

The mouse model used in this study represents the chronic obesity status with DIO mice fed a high-fat diet for 20 wk [14]. Similarly, in humans, obesity represents chronic overfeeding accompanied by metabolic disorders. Oat β-glucan may lead to a reduction of the rate of macronutrient absorption [15], increasing viscosity of the gastrointestinal contents with delay of gastric emptying, interference with the contact of digestive enzymes with food and disruption of micelle formation. Unabsorbed macronutrients in the colon can in turn affect gut hormone production and influence hypothalamic satiety regulation [16]. This study provides mechanistic evidence that a diet high in oat β-glucan can elevate plasma PYY_{3-36} and increase satiety, which is supportive of the use of β-glucan in obesity treatment.

The present study is the first to show that an oat β-glucan diet can lead to an elevation of plasma PYY_{3-36} concentration in mice and this increase is in a dose-responsive manner. This is consistent with our human studies [9]; however, the use of animals here has allowed investigation of the relevant mRNA expression, clearly identifying the mechanism of action. Previous studies have shown that peripheral injection of total PYY significantly lowers food intake in humans and rodents [4, 17, 18]. Total PYY is cleaved to form PYY_{3-36}, crossing the blood–brain barrier to downregulate Arc NPY. When PYY_{3-36} binds to the Arc Y2 receptor, the NPY expression is decreased [19]. In Y2

knockout mice, the anorectic effect of peripheral PYY injection was diminished [4]. In the present study, oat β-glucan elevated plasma PYY_{3-36} and downregulated hypothalamic Arc NPY mRNA expression, showing that the effect of oat β-glucan on satiety may be mediated by the PYY_{3-36}-NPY axis.

Results of the satiety test in week 6 showed that a tolerance effect was not developed with an oat β-glucan diet, which is encouraging for human applications. The current study also showed significantly lower energy intake with the HG diet together with body weight reduction. This is particularly interesting given that all diets were matched for total fiber content (with slightly lower total fiber in the HG group). While epidemiological studies show links between fiber and weight control, intervention trials with individual fibers show more mixed results. While all fibers may have positive effects (and this is indeed part of the definition of fiber), it may be that different fibers have varied effects on weight control. The current study included high insoluble fiber from wheat to match total fiber between groups, with the fiber in the highest β-glucan group (HG) consisting entirely of oat fiber (both soluble and insoluble). This seems clear evidence that oat β-glucan (and perhaps more generally oat fiber) may have some advantages over other sources.

In summary, a gut-hypothalamic anorexigenic pathway (PYY_{3-36}-NPY) was significantly activated with a high oat β-glucan diet in DIO in mice. The response was in a dose-dependent manner. An oat β-glucan diet showed an increased satiety that appeared to be long-lasting without the development of a tolerance effect. This suggests potential benefits of oat β-glucan in the cachet of dietary tools for combating obesity.

The authors thank A/Professor K. Russell (Department of Applied Statistics, University of Wollongong) for his suggestions regarding the statistical analysis. This study was supported by an ARC-Linkage grant of Australia.

The authors have declared no conflict of interest.

References

[1] Karra, E., Chandarana, K., Batterham, R. L., The role of peptide YY in appetite regulation and obesity. *J. Physiol.* 2009, *587*, 19–25.

[2] Boey, D., Lin, S., Enriquez, R. F., Lee, N. J. et al., PYY transgenic mice are protected against diet-induced and genetic obesity. *Neuropeptides* 2008, *42*, 19–30.

[3] Konturek, S. J., Konturek, J. W., Pawlik, T., Brzozowski, T., Brain-gut axis and its role in the control of food intake. *J. Physiol. Pharmacol.* 2004, *55*, 137–154.

[4] Batterham, R. L., Cowley, M. A., Small, C. J., Herzog, H. et al., Gut hormone PYY(3-36) physiologically inhibits food intake. *Nature* 2002, *418*, 650–654.

[5] Batterham, R. L., Ffytche, D. H., Rosenthal, J. M., Zelaya, F. O. et al., PYY modulation of cortical and hypothalamic brain areas predicts feeding behaviour in humans. *Nature* 2007, *450*, 106–109.

[6] Batterham, R. L., Cohen, M. A., Ellis, S. M., Le Roux, C. W. et al., Inhibition of food intake in obese subjects by peptide YY3-36. *New Eng. J. Med.* 2003, *349*, 941–948.

[7] Le Roux, C. W., Batterham, R. L., Aylwin, S. J. B., Patterson, M. et al., Attenuated peptide YY release in obese subjects is associated with reduced satiety. *Endocrinology* 2005, *147*, 3–8.

[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] Beck, E. J., Tapsell, L. C., Batterham, M. J., Tosh, S. M., Huang, X. F., Increases in peptide Y-Y levels following oat β-glucan ingestion are dose-dependent in overweight adults. *Nutr. Res.* 2009, *29*, 705–709.

[10] Beck, E. J., Tosh, S. M., Batterham, M. J., Tapsell, L. C., Huang, X. F., Oat β-glucan increases postprandial cholecystokinin levels, decreases insulin response and extends subjective satiety in overweight subjects. *Mol. Nutr. Food Res.* 2009, *53*, 1343–1351.

[11] Aleixandre, A., Miguel, M., Dietary fiber in the prevention and treatment of metabolic syndrome: a review. *Crit. Rev. Food Sci. Nutr.* 2008, *48*, 905–912.

[12] Greenway, F., O'Neil, C. E., Stewart, L., Rood, J. et al., Fourteen weeks of treatment with Viscofiber increased fasting levels of glucagon-like peptide-1 and peptide-YY. *J. Med. Food* 2007, *10*, 720–724.

[13] Hariri, N., Thibault, L., High-fat diet-induced obesity in animal models. *Nutr. Res. Rev.* 2010, *230*, 270–299.

[14] Levin, B. E., Dunn-Meynell, A. A., Chronic exercise lowers the defended body weight gain and adiposity in diet-induced obese rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 2004, *286*, R771–R778.

[15] Battilana, P., Ornstein, K., Minehira, K., Schwarz, J. M. et al., Mechanisms of action of beta-glucan in postprandial glucose metabolism in healthy men. *Eur. J. Clin. Nutr.* 2001, *55*, 327–333.

[16] 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.

[17] Vrang, N., Madsen, A. N., Tang-Christensen, M., Hansen, G., Larsen, P. J., PYY(3-36) reduces food intake and body weight and improves insulin sensitivity in rodent models of diet-induced obesity. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 2006, *291*, R367–R375.

[18] le Roux, C. W., Bloom, S. R., Peptide, Y. Y., Appetite and food intake. *Proc. Nutr. Soc.* 2005, *64*, 213–216.

[19] Chen, C. H., Stephens, R. L., Rogers, R. C., PYY and NPY: control of gastric motility via action on Y1 and Y2 receptors in the DVC. *Neurogastroenterol. Motil.* 1997, *9*, 109–116.

