

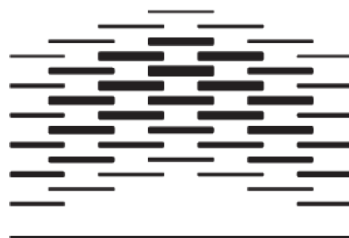
**MASTER THESIS**

Public Health Nutrition

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Intake of beta-glucan changes satiety and markers of gut microbiota in healthy, normal weight individuals

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## Abstract

**Background:** Obesity is one of today's major public health issues, and understanding the mechanisms that control human appetite has gained increased attention. Certain dietary fibres, such as beta-glucan, has been shown to stimulate gut-derived peptides involved in the regulation of appetite, and gut microbiota has been hypothesised to be involved in this regulation. In the current project, we aimed to investigate whether consumption of cereals with various content of beta-glucan affect satiety measures, and if the effects may be related to changes in the gut microbiota.

**Methods:** Fourteen healthy men and women were enrolled in this fixed order cross-over intervention study. The subjects consumed cereals containing different amounts of beta-glucan (0.5 g (control), 3.5 g and 8 g), for three consecutive days as an evening meal. After consuming the meal on day one and day three, the subjects filled out a visual analogue scale (VAS). Circulating gut peptides (PYY and GLP-2) and short chain fatty acids (SCFAs) were measured the following day after each of the three interventions (day 4). Breath H<sub>2</sub> was measured as an indirect measurement of colonic fermentation. Gut peptides, SCFAs and breath H<sub>2</sub> were measured fasting and after an oral glucose tolerance test (OGTT).

**Results:** A three-day intervention with 8 g beta-glucan increased the fasting levels of PYY ( $P=0.001$ ) compared with the control meal, and fasting levels of the SCFA butyrate ( $P=0.041$ ) compared with the 3.5 g meal. 8 g of beta-glucan also increased PYY after the OGTT when compared with the control ( $P=0.010$ ) and the 3.5 g meal ( $P=0.004$ ), and a positive correlation between butyrate and PYY were observed ( $r=0.430$ ,  $P=0.004$ ). GLP-2 did not change after any of the interventions. Subjective satiety sensation was significantly decreased after day one with the 8 g meal compared with day one with the control meal ( $P=0.011$ ). In addition, subjective satiety sensation was increased from day one to day three after intervention with the 8 g meal ( $P=0.08$ ). Furthermore, fasting breath H<sub>2</sub> concentrations increased after intake of the 8 g meal compared with the control meal ( $P=0.048$ ).

**Conclusion:** The present study demonstrates increased concentrations of objective and subjective markers for appetite (PYY and VAS) after intake of beta-glucan in healthy normal weight subjects. Furthermore, the changes in satiety may be mediated through colonic fermentation of beta-glucan, illustrated by the elevated fasting H<sub>2</sub> excretion and fasting butyrate. Although more studies are needed to clarify the relationship between fibre, gut microbiota and satiety, these findings suggest anti-obesogenic potential of foods rich in beta-glucan.

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## Abbreviations

μCRP	Micro C-reactive protein
AMPK	AMP-activated protein kinase
AUC	Area under the curve
BMI	Body mass index
C2	Acetate
C3	Prpoionate
C4	Butyrate
CCK	Cholecystokinin
CNS	Central nervous system
CRF	Case report form
CRP	C-reactive protein
CVD	Cardiovascular disease
DA	Dalton
ENS	Enteric nervous system
FFAR2	Free fatty acid receptor 2
FFAR3	Free fatty acid receptor 3
FFQ	Food frequency questionnaire
GI	Gastro-intestinal
GLP-1	Glucagon-Like Peptide-1
GLP-2	Glucagon-Like Peptide-2
H <sub>2</sub>	Hydrogen
HiOA	Oslo and Akershus University College
IQR	Interquartile range
LDL	Low Density Lipoprotein
MW	Molecular weight
NCD	Non-communicable diseases
NSP	Non-starch polysaccharide
OGTT	Oral glucose tolerance test
PYY	Peptid YY

PPM	Parts per million
PVN	Paraventricular nucleus of hypothalamus
REK	Regional Committees for Medical and Health Research Ethics Sør-Øst
SCFA	Short chain fatty acid
SCR	Screening
SD	Standard deviation
T2D	Type 2 diabetes
TG	Triglyceride
UiO	Oslo University College
V	Visit
VAS	Visual analogue scale

## **1.0 Introduction**

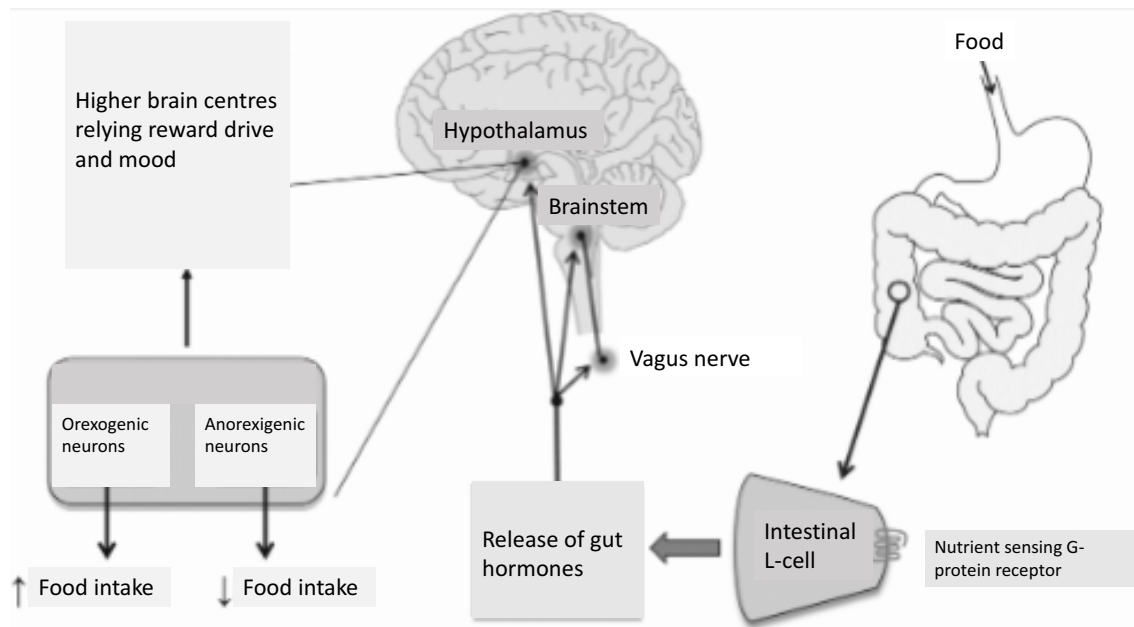
The prevalence of obesity (defined by a body-mass index (BMI)  $\geq 30$  kg/m<sup>2</sup>) have shown a marked increase worldwide over the past four decades (World Health Organization, 2016). In 2016, the number of obese individuals in the world exceeded 650 million, with more than a triple increase since 1975. As a consequence of globalization with new dietary habits and a sedentary lifestyle, the prevalence of obesity is now increasing in both developed and developing countries (Cecchini et al.; Ng et al., 2014). Overweight and obesity are important risk factors for adverse metabolic effects and non-communicable diseases (NCDs), such as cardiovascular diseases (CVDs), cancers and type 2 diabetes (T2D). In fact, obesity and obesity-related disorders are now more common than undernutrition and infectious diseases (World Health Organization, 2016).

The fundamental cause of overweight and obesity is an imbalance between energy intake and energy expenditure (World Health Organization, 2016). Several physiological and environmental factors may influence energy balance, such as ethnic origin, age, fitness, socioeconomic status, food composition and stress (Bellisle, Drewnowski, Anderson, Westerterp-Plantenga, & Martin, 2012). For many modern-day humans, the decision to eat often occurs as a consequence of appetite instead of the need for energy, and the meal initiation becomes non-homeostatic. Appetite occurs based on habit, time of day, social situations, stress or other factors that are not linked to energy needs. Thus, appetite regulation become important elements in the work towards reversing the obesity trend. Several epidemiologic studies have indicated an association between consumption of fiber and reduced body weight, but more research is needed regarding the potential satiety-inducing effects of the different types of dietary fibre (Harland & Garton, 2008; Rebello, O'Neil, & Greenway, 2016).

### **1.1 Satiety signals and gut peptides**

During recent years, the biological system contributing to the control of appetite is becoming better understood (Blundell et al., 2010). This system involves brain processes, such as sensory signals through the hypothalamus in the central nervous system (CNS), and the enteric nervous system (ENS) located within the wall of the gastro-intestinal (GI) tract (Furness, Callaghan, Rivera, & Cho, 2014). There is a bidirectional communication between the two systems,

connecting emotional and cognitive centres of the brain with peripheral intestinal functions (Carabotti, Scirocco, Maselli, & Severi, 2015). Termination of a meal due to the sensation of fullness is related to stomach distention and satiety signals from the CNS (de Graaf, Blom, Smeets, Stafleu, & Hendriks, 2004). Of particular interest are gut peptides released by the GI tract during a meal. As a response, a decrease in orexigenic signals and increase in anorexigenic signals from hypothalamus occurs, and will either enhance or reduce food intake (Sam, Troke, Tan, & Bewick, 2012). The gut peptides mediate inhibitory feedback mechanisms on intestinal transit, which leads to prolonged gastric distention and increased satiety between meals (Lin, Zhao, Wang, & Wong, 1996). This interaction is referred to as the gut-brain axis, and is illustrated in fig. 1.



**Fig.1.** Gut-brain axis: regulation of food intake. After digestion, nutrients activate G-protein coupled receptors, such as intestinal L-cells. This stimulates the release of gut hormones, which may influence food intake by the vagus nerve, brainstem and hypothalamus. Peripheral signals from neuronal populations of the hypothalamus, the orexigenic or the anorexigenic neurons alter the drive to eat. Further connections between the hypothalamus and higher brain centres may exist, and control the pleasure aspects of food ingestion.

PVN; paraventricular nucleus of hypothalamus (Modified by Sam et al., 2012).

Signals influencing food intake and energy expenditure can be separated into two categories, long- and short-term signals. The long-term signals are activated by the chemoreception of nutrients, and includes the anorexigenic adiposity signals insulin and leptin. Insulin is an endocrine hormone synthesized in the beta-cells of pancreas (Woods, Seeley, Baskin, & Schwartz, 2003). Secretion starts right after meal consumption as blood glucose levels rise, promoting glucose uptake in cells and storage as glycogen in liver (Suzuki, Simpson, Minnion, Shillito, & Bloom, 2010). Once entering the brain, insulin suppresses food intake. Leptin is an adipocyte hormone with less direct connection to meals than insulin (Teff & Townsend, 2004; Woods & D'Alessio, 2008). Its key role is regulation of body weight and body fat mass, by inhibiting hunger (Izadi, Saraf-Bank, & Azadbakht, 2014). Levels of leptin increase with increased body fat mass, and decrease when adipose mass is lost (Maffei et al., 1995). However, many individuals with diet-induced obesity have developed leptin resistance, characterized by elevated levels of circulating leptin and decreased leptin sensitivity (Pan, Guo, & Su, 2014).

The short-term signals are required to prevent over-eating, and is activated by both psychological factors as taste and smell, mechanical factors like gastric distension, and release of gut peptides (Plata-Salaman, 1991). These signals lead to the two states satiation and satiety generated during the meal. Satiation comprises the signals leading to sensation of fullness that contributes to termination of the meal. Satiety comprises the continuation of the interval until hunger or the urge to eat appears again. (Woods, Seeley, Porte, & Schwartz, 1998). Several gut peptides secreted from the GI tract are involved in the control of food intake (Moran, 2009). The different release-patterns of the gut peptides provides an indication of its specific role in the appetite control. Ghrelin is a gastric peptide stimulating food intake, with suppressed levels in response to food intake. In contrast, the duodenal peptide cholecystokinin (CCK) inhibits eating, and is released in response to food intake. Pancreatic glucagon and amylin also works in a similar way, contributing to meal termination. The lower gut peptides, glucagon-like peptide 1 and 2 (GLP-1 and GLP-2) and peptide YY (PYY), are released more slowly in response to food intake, and remain elevated several hours after a meal (Moran, 2009). In this project, we focused on the short-term satiety signals GLP-2 and PYY.

GLP-1 and GLP-2 are both derived from proglucagon (Halford & Harrold, 2012). GLP-1 is known to enhance insulin secretion, as well as inhibit gastric motility, secretion, and gastric emptying (Flint, Raben, Astrup, & Holst, 1998; Giralt & Vergara, 1999). GLP-2 acts as a receptor to stimulate intestinal mucosal growth and participate as a regulator of gut barrier function (Woods & D'Alessio, 2008). GLP-2 also function as a regulator of glucose homeostasis in the GI tract, by promoting intestinal glucose absorption (Guan et al., 2003). GLP-1 and GLP-2 are co-encoded within the proglucagon gene, and are secreted in a 1:1 ratio by the L-cells, primarily in distal ileum and colon (Drucker & Nauck, 2006; Lim et al., 2009). Secretion is stimulated by ingestion of glucose, fatty acids and dietary fibre (Brubaker, 2006). Because of the parallel secretion, secretion mechanisms behind GLP-1 and GLP-2 are considered equivalent (Lim et al., 2009).

Like the proglucagon-derived peptides, PYY is also synthesized and secreted by L-cells in distal ileum and colon (Halford & Harrold, 2012). PYY is released into the circulation in response to fat, protein and carbohydrate, and is best known as an anorectic peptide, which contribute to reduce food intake and weight gain (Batterham et al., 2002; Halford & Harrold, 2012; Small & Bloom, 2004). This has been demonstrated in several studies conducted in both rodents and humans, in which PYY reduced food intake (Batterham et al., 2002; Guo et al., 2006; Halatchev, Ellacott, Fan, & Cone, 2004). PYY has also been shown to delay gastric emptying, inhibit intestinal motility and increase absorption of fluids and electrolytes (Boey et al., 2006).

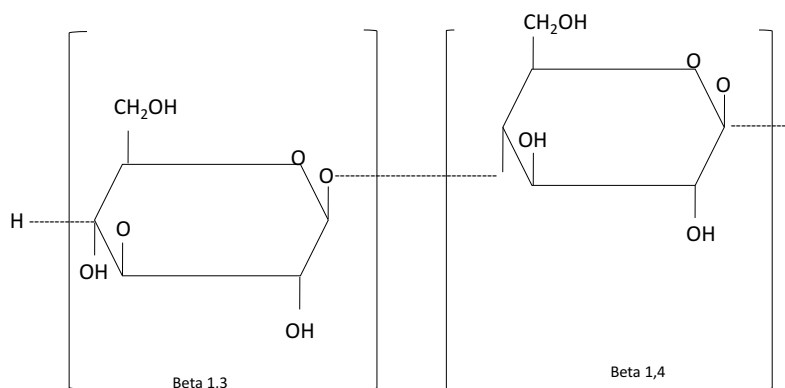
To provide insight into motivational determinants of eating behaviour, measurements of subjective appetite sensations is useful (Halford & Harrold, 2012). Appetite has many dimensions, both the physical impact of food, as well as satisfaction, cravings or desire for specific foods. Out of these, the most used in research are hunger, fullness, prospective consumption and desire to eat (de Graaf et al., 2004). These subjective states of appetite can be rated in several ways, but the most common is the visual analogue scale (VAS), developed by Rogers and Blundell in the late 1970s (Parker, 2004; Rogers & Blundell, 1979). The standard VAS is most often composed of lines with words describing the extremes at each end, whereas the subjects make a mark across the line corresponding to their sensations (Flint, Raben, Blundell, & Astrup, 2000). In other research areas, VAS scores have been well studied, and is

regarded as the gold standard in pain research. However, subjective sensation of appetite with VAS after intake fibre have shown conflicting results (Beck, Tosh, Batterham, Tapsell, & Huang, 2009; Cohen et al., 2013; Wanders, Mars, Borgonjen-van den Berg, de Graaf, & Feskens, 2014).

## 1.2 Dietary fibre

Dietary fibre is defined as nondigestible carbohydrates, resistant to human digestive enzymes (Jalili, Wildman, & Medeiros, 2000). This includes non-starch polysaccharides (NSP), such as beta-glucan (Anderson et al., 2009). A diet rich in fibre is associated with several positive health effects, such as reduced blood glucose levels, lowering total and LDL cholesterol levels, as well as contributing to weight loss (Dhingra, Michael, Rajput, & Patil, 2012; Francelino Andrade et al., 2014; J. L. Slavin, 2005).

Some of these effects is due to physical properties, such as the gel forming capability of soluble fibres in the gut (Weickert & Pfeiffer, 2008). Fibre is classified as soluble or insoluble, based upon its properties of water solubility. When mixed with liquids, as through digestion, the soluble fibre forms a viscous solution. The viscosity contributes to gastric distention and delay in gastric emptying, leading to enhanced sensation of satiety due to a greater volume in the stomach. A type of soluble fiber with high viscosity is beta-glucan found in cereals, mainly oat and barley. Beta-glucan is composed of glucose molecules in long linear glucose polymers with mixed-linkage beta-bonds ( $\beta$  (1,3),  $\beta$  (1,4)) (Fig. 2) (El Khoury, Cuda, Luhovyy, & Anderson, 2012).



**Fig. 2.** Cereal beta glucan. A linear polysaccharide of beta D-glucose consisting of 1 beta-3 linkage for every three or four beta-4 linkages.

The properties of beta glucan, such as hydration, solubility and viscosity are determined by the mixed linkages. The viscosity and solubility of beta-glucan depends on the molecular size and number. The molecular weight (MW) reported for beta-glucan in cereals range from 50,000 to 3 million dalton (Da) (Lyly, 2004). Increased molecular weight will increase the viscosity, which leads to delayed gastric emptying, slower digestion and absorption of glucose, and increased satiety sensations. The latter have been shown in several studies comparing barley-based food with food with no dietary fibres (Kaplan & Greenwood, 2002; Nilsson, Johansson-Boll, & Bjorck, 2015; Schroeder, Gallaher, Arndt, & Marquart, 2009).

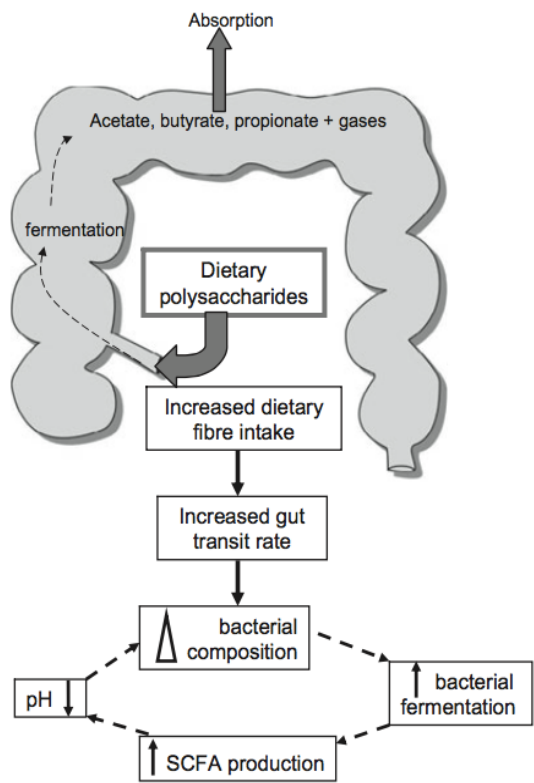
In addition to the postprandial effect on appetite, it has been hypothesised that fibre affect satiety through gut microbiota. One hypothesis is that the bacterial fermentation produces short-chain fatty acids (SCFAs), which in turn influences the release of satiety inducing gut peptides as well as gastric motility.

### **1.3 Gut microbiota, fermentation and SCFA**

The GI tract contains an enormous number of microorganisms, known as the microbiota (Beck, Tosh, et al., 2009; Suzuki et al., 2010). The gut microbiota enables us with several important functions, such as digestion and absorption in the GI tract (Bhat & Kapila, 2017). The gut microbiota also synthesizes several vitamins, such as vitamin K and certain B vitamins, including biotin, folates, riboflavin, thiamine and cobalamin (Yatsunencko et al., 2012). In addition, the gut microbiota ferments indigestible dietary fibre in our diet. The fermentation give rise to several metabolites, in which SCFAs are the major end products. The SCFAs are saturated aliphatic organic acids consisting of one to six carbons (G. den Besten, van Eunen, et al., 2013). The main SCFAs are acetate (C2), propionate (C3) and butyrate (C4) (J. Slavin, 2013). Butyrate is predominantly utilized as an energy source by the colonic epithelium, whereas propionate is primarily a precursor for intestinal gluconeogenesis (G. den Besten, Lange, et al., 2013). Acetate has shown to increase energy expenditure in liver and muscle by activating the AMP-activated protein kinase (AMPK) (Gijs den Besten et al., 2015). The SCFA receptors, free fatty acid receptors (FFA2 and FFA3), are connected to the L-cells in the intestines, where it stimulates the release of the gut peptides PYY, GLP-1 and GLP-2 (Psichas et al., 2015).



In addition to SCFAs, the fermentation of nutrients by the microbiota also yields hydrogen (H<sub>2</sub>), which may function as a marker for colonic fermentation. The H<sub>2</sub> is transported to the alveoli after diffusion into the bloodstream and can be detected through expired breath (Fig. 3) (Levitt 1969).



**Fig. 3.** The pathway of the dietary fibres and polysaccharides in the large intestine, and the effects on gut transit and bacterial fermentation that may occur. Solid arrows indicate a direct effect, and stapled arrows indicates interlinked effects (Adapted from K.P. Scott et al, 2008).

## **2.0 Aim of the study**

The present master thesis is a part of a project where the outcome was to investigate intake of beta-glucan and postprandial regulation of blood glucose and satiety regulation in healthy individuals. The aim of this master thesis was to explore the role of beta-glucan in appetite regulation, and to investigate if the effect is related to increased fermentation in the gut.

The objectives were:

1. To investigate the effect on the circulating gut hormones PYY and GLP-2 after a short intervention with different amounts of beta glucan followed by a glucose challenge
2. To measure subjective satiety and appetite after consuming different amount of beta-glucan, measured by visual analogue scale (VAS)
3. To investigate the effect on the colonic fermentation after intake of different amounts of beta glucan, measured through the fermentation products hydrogen (H<sub>2</sub>) excretion and SCFAs

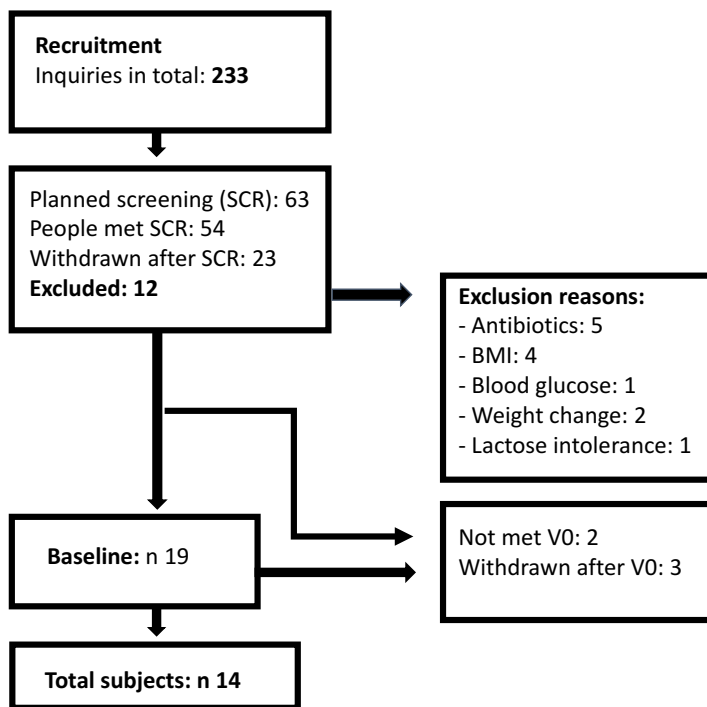
## 3.0 Materials and method

### 3.1 Subjects

Healthy men and women between the age of 18-65 years, with a normal BMI (18.5-27 kg/m<sup>2</sup>) and without any metabolic disorders or food allergies were encouraged to voluntarily participate in the study. The subjects were recruited among students and employees at the Oslo and Akershus University College (HiOA) through e-mail, information in classes and through Facebook.

Exclusion criteria were chronic metabolic diseases, bowel diseases, food allergies, pregnancy /lactation, smoking and excessive alcohol consumption (>40 g/day). Subjects with fasting blood glucose  $\geq 6.1$  mmol/L, CRP > 10 mg/L and BMI <18.5 and >27 kg/m<sup>2</sup> were also excluded. Planned weight reduction and or  $\pm 5\%$  weight change the last 3 months, use of antibiotics  $\leq 3$  months ago and during the time of the study, giving blood the last 2 months before start of the study and/or during the study and hormone treatment (except birth control) were not permitted.

The recruitment and intervention were performed during two periods, the first from September – December 2016, and the second from January – March 2017. After recruitment, 54 people met at screening (SCR) whereas 23 people chose to withdraw due to personal reasons, and 12 people were excluded. Reasons for exclusion were use of antibiotics, BMI over 27 kg/m<sup>2</sup> or under 18 kg/m<sup>2</sup>, fasting blood glucose exceeding 6,1 mmol/l, weight change  $\pm 5\%$  within a 3-month period, and lactose intolerance. Five subjects dropped out of the study after randomization, leaving a total of 14 subjects completing the intervention (Fig.4).



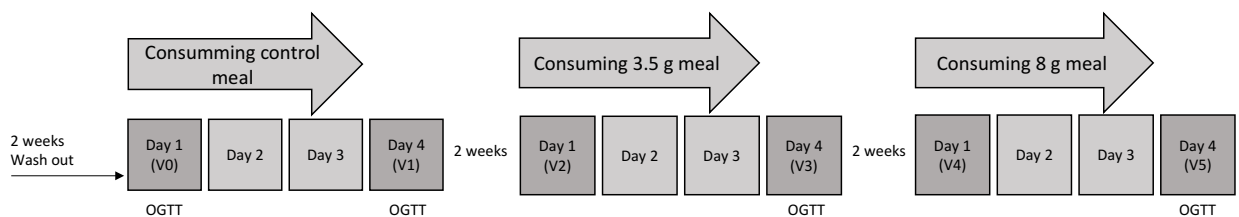
**Fig. 4.** Flowchart depicting the steps throughout the recruitment process. SCR, screening; BMI, Body Mass Index; V0, first visit.

Before the first visit, the subjects filled out a food frequency questionnaire (FFQ) to measure their usual dietary intake. Two weeks prior to study start and throughout the study period, the subjects had to refrain from whole grain (except the test meals given in the study), probiotics, and dietary supplements. Except from this, the subjects were asked to live as normal. Approval of the study was given by Regional Committees for Medical and Health Research Ethics (REK) in Oslo. Written informed consent was obtain from all participants.

### **3.2 Postprandial study design**

The intervention was carried out as a fixed order cross-over design. In total, the study lasted nine weeks, consisting of three test weeks, with the first counting as the control. The test weeks were separated by two weeks, and two weeks of wash out prior to study start (Fig. 5). During the study period, the subjects met at campus Kjeller six times in total, two visits every test week. The meals were given at campus and consumed at home as an evening meal for three consecutive days. The three different test meals consisted of 100 g cereals, containing 0.5 g, 3.5 g and 8 g beta-glucan

(hereafter referred to as control, 3.5 g and 8 g meal) derived from oat and barley. All subjects started with the control meal the first test week, followed by the 3.5 g meal the second test week and the 8 g meal the third test week. The fourth day, the participants returned to campus and went through a postprandial oral glucose tolerance test (OGTT). The OGTT contained 75 gram glucose (82 g D(+)-Glucose monohydrate) solved in 100 ml of water. At every visit, the subjects met fasting (the last 12 hours), and were also instructed not to drink alcohol and refrain from hard physical activity. Fasting blood samples were collected every visit, as well as fasting H<sub>2</sub> breath and registration of anthropometric measurements. Postprandial blood samples were collected after OGTT. The first week counting for the control measurements, the participants went through the OGTT with following venous blood samples both the first and the fourth day. The remaining two test weeks they only did this the fourth day.



**Fig. 5.** Schematic view of the study design. OGTT, oral glucose tolerance test; V, visit

Information about the participants regarding date of visit, health status, changes according to way of living, physical state and medications were documented each visit in a case report form (CRF).

### 3.3 Evening test meals

The meals were delivered by Mills AS and stored at campus at 4°C, before they were handed out to the subjects. The 8 g meal was a mix with the commercially product Betavivo “Havrehjerter” and the 3.5 g meal. The proportion was 60/40 (60 g/40 g) 3.5 g meal/Betavivo. The cereals were consumed with 2 dl semi skimmed milk at home as an evening meal. Analysis of total fibre and beta-glucan were performed by Nofima, while analysis of macronutrients was performed by Eurofins, Norway.

### **3.4 Measurements of appetite**

A visual analogue scale (VAS) as described by Flint *et al* (Flint et al., 2000), was used to measure the subjective appetite perception of the subjects. The VAS was handed out with the meal, and consisted of three questions based on the variables hunger, satiety and desire to eat. The subjects drew a vertical mark on a 100mm horizontal line from left to right to score their ratings. Marks towards the right end indicated strong sensation of hunger, satiety or desire to eat, while the left end indicated the opposite sensation. The scale had to be filled out just before they started eating (t0), and 15, 30, 60, 90, 120 and 150 minutes after termination of the meal. To determine the VAS score, the distance from the left border of the line to the vertical mark was measured as a numeric response (millimetres) with a ruler.

### **3.4 Biological measurements and analysis**

Fasting breath H<sub>2</sub> was registered in expired breath (Bedfont EC60 Gastrolyzer; Bedfont) as an indicator of colonic fermentation. The subjects were told to inhale and hold their breath for a pre-set 15 seconds count down, before they were told to slowly blow into the mouthpiece of the monitor until they had emptied their lungs completely. The results were reported in parts per million (ppm).

Venous blood samples were determined at fasting, 30, 60 and 120 minutes after the OGTT. Serum and plasma were separated by centrifugation, whereas serum was stored at 4°C at campus before they were sent to Fürst medical laboratory within 24 hours. Venous blood samples determining serum (s-)insulin and Triglycerides (TG) as well as plasma (p-) GLP-2 and p-PPY was performed at fasting 30, 60 and 120 minutes after the OGTT. SCFA obtained from plasma was determined at fasting.

Bioimpedance testing was preformed using Tanita 418 MA, measuring BMI, weight and anthropometric measures. The test was performed fasting every visit.

Spot samples of morning urine and faecal samples were collected at every visit. The faeces samples were taken before baseline and from the first defecation after the last test meal. The urine samples were stored at -80 °C, and the faecal samples were stored at -20 °C until analysis. These

samples will be used in future perspective regarding analysis of metabolic changes and gut microbiota, and will therefore not be discussed in the present master thesis.

### **3.5 Statistical methods and power calculation**

To evaluate VAS, PYY, GLP-2, SCFAs and H<sub>2</sub>, the results from each subject were plotted into Microsoft® Excel. The area under the curve (AUC) was calculated for VAS, PYY and GLP-2 from each subject using the trapezoidal rule ( $A = \frac{y^1+y^2}{2} * (x_2 - x_1)$ ). All statistical analyses were performed in IBM SPSS statistic (version 23.0), after processing the data in Microsoft® Excel for Mac (version 15.33). Statistical figures were performed with the use of GraphPad Prism 7 for Mac OS X (version 7.0c).

Differences within the test variables was assessed with the non-parametric test Friedmans ANOVA, and if statistical significance, the Wilcoxon Signed Rank was performed. Spearman's Rank Order Correlation was used to investigate the relationship between SCFA, H<sub>2</sub> and PYY. Data are presented as median values with interquartile range.

In the case of missing data in VAS scores from an entire test day, this was removed from the data set, hence only VAS fully completed were used in the analysis. Missing data points from only a single time sequence was included by calculating the average from the entire group at this exact time point. In this intervention, VAS from 3 subjects were withdrawn due to missing measurements, hence the total number of VAS analysed was 11.

Power calculations were based on the primary outcome of the study, which was changes in blood glucose 0-180 min after OGTT. Previous postprandial studies with similar design included 15-20 healthy subjects, in a study investigating changes in blood glucose after consuming different amounts of barley as an evening meal (Nilsson, Ostman, Knudsen, Holst, & Bjorck, 2010). With a strength of 80% and an acceptance of 5% of type 1 error, the total number of subjects required was set to approximately 13-17 subjects in our study. The significance level was set to 5 %  $P \leq 0,05$

## 4.0 Results

### 4.1 Characteristics of subjects

Fourteen subjects completed the study, 2 men and 12 women with a median age of 28 (24.0 - 38.3). The subjects were healthy with median body mass index (BMI) at 22.2 kg/m<sup>2</sup> (20.8 - 24.2). Fasting values of glucose, insulin, cholesterol and triglycerides (TG) at baseline are presented in table 1.

**Table 1.** Baseline characteristics

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Gender	
Male	2
Female	12
Age (y)	28 (24.0 - 38.3)
BMI (kg/m <sup>2</sup> )	22.2 (20.8 - 24.2)
Blood glucose (mmol/L)	5.1 (4.8 - 5.7)
Insulin (pmol/L)	64.5 (58.8 - 83.8)
TG (mmol/L)	0.74 (0.65 - 0.87)
μCRP (mg/L)	0.4 (0.3 - 0.8)
Cholesterol (mmol/L)	4.4 (3.8 - 4.5)

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BMI, Body Mass Index; TG, Triglyceride; μCRP, micro C-Reactive Protein.  
Values are presented as median and interquartile range (IQR).

### 4.2 Analysis of test meals

The meals were analysed for content of total macronutrients including total fibre, inulin, available starch, and soluble and insoluble non-starch polysaccharides (NSP). Molecular weight (MW) of the beta- glucan in the three different meals was also measured. Total energy (kcal) did not differ substantially between the three meals, with 351 kcal in the control meal, 360 kcal in the 3.5 g meal and 347 kcal in the 8 g meal. The content of macronutrients differed slightly, with the 8 g meal containing more protein and fat (13 g and 4.4 g respectively) than both the 3.5 g meal (9.7 g and 3.8 g respectively) and the control meal (8.7 g and 2.2 g respectively). The content of carbohydrates was higher in the control meal (69.8 g) than in the 3.5 g meal (65.5 g) and the 8 g



meal (54.6 g). The 8 g meal had the highest level of total fibre (20,1g ± 0,6) and beta-glucan, while the content of inulin was highest in the control meal (8,4% ± 0,3). Available starch did not differ substantially between the different meals: 58.3 g (control meal), 54.6 g (3.5 g meal) and 50g (8 g meal). A high molecular weight (MW) of beta-glucan was measured in both 3.5g meal (890080 Da) ± 26163) and 8g meal (1049400 Da) ± 12728). The content of beta-glucan in the control meal was too low to calculate the MW. The amount of beta-glucan in the test meals were estimated to contain 0.5 g, 3.5 g and 8 g, and are therefore referred to as these amounts, and not the analysed amounts. The composition of the test meals is presented in table 2.

**Table 2.** Energy and nutritional composition of the evening test meals

	<b>Control meal</b> (± SD)	<b>3.5g meal</b> (± SD)	<b>8g meal</b> (± SD)
Energy (kcal)	351	360	347
Fat (g)	2,2	3.8	4.4
Protein (g)	8.7	9.7	13.2
Carbohydrates (g)	69.8	65.5	54.6
Total amount of fibre (g)	15.8 (± 0.3)	18.5 (± 0.2)	20.1 (± 0.6)
Total NSP (%)	7.4 (± 0.3)	11.8 (± 0.2)	15.7 (± 0.6)
Beta-glucan (%)	0.7 (± 0.02)	3.5 (± 0.08)	7.1 (± 0.03)
Soluble NSP (%)	2.3 (± 0.3)	6.5 (± 0.2)	9.3 (± 0.2)
Inulin (%)	8.4 (± 0.3)	6.7 (± 0.1)	4.4 (± 0.1)
Total amount of soluble fibre (%) <sup>1</sup>	10.7 (± 0.3)	13.2 (± 0.2)	13.7 (± 0.2)
Total amount of available starch (%)	58.3 (± 1.6)	54.6 (± 2.5)	50 (± 2.2)
Beta-glucan MW (Da)	Not detectable	890080 (± 26163)	1049400 (± 12728)

<sup>1</sup>the amount of NSP, soluble NSP; and inulin combined

Values per 100 g cereal, dry matter. Values are presented as mean ± SD.

NSP, Non-starch Polysaccharide; MW, molecular weight; Da, Dalton.

### 4.3 Gut peptides

The morning after the three-days intervention with the test meals, the gut peptide PYY and GLP-2 were measured in venous blood samples at fasting, 30, 60 and 120 minutes after the OGTT. Compared with the control meal, a significant increase was observed in fasting PYY levels after intervention with the 3.5 g meal ( $P=0.022$ ) and the 8 g meal ( $P=0.001$ ) corresponding to an increase of 16% and 43% respectively. A significant increase corresponding to 23% was also detected in the 8 g meal compared to the 3.5 g meal ( $P=0.04$ ). There were no significant changes in fasting GLP-2 between the test meals. The fasting values of PYY and GLP-2 are shown in table 3.

**Table 3.** Fasting levels of PYY and GLP-2 after intake of control, 3.5 g and 8 g meal for three days (n=14)

Evening test meal	Fasting PYY (ng/ml) Median (IQR)	Fasting GLP-2 (ng/ml) Median (IQR)
Control meal	125 (102 – 147)	1.2 (1.1-1.6)
3.5 g beta-glucan	145 (130 – 169)*	2.0 (1.3-2.4)
8 g beta-glucan	179 (133 – 226)*#	1.8 (1.1-2.0)

\* Significant increase from the control meal:  $P<0.05$

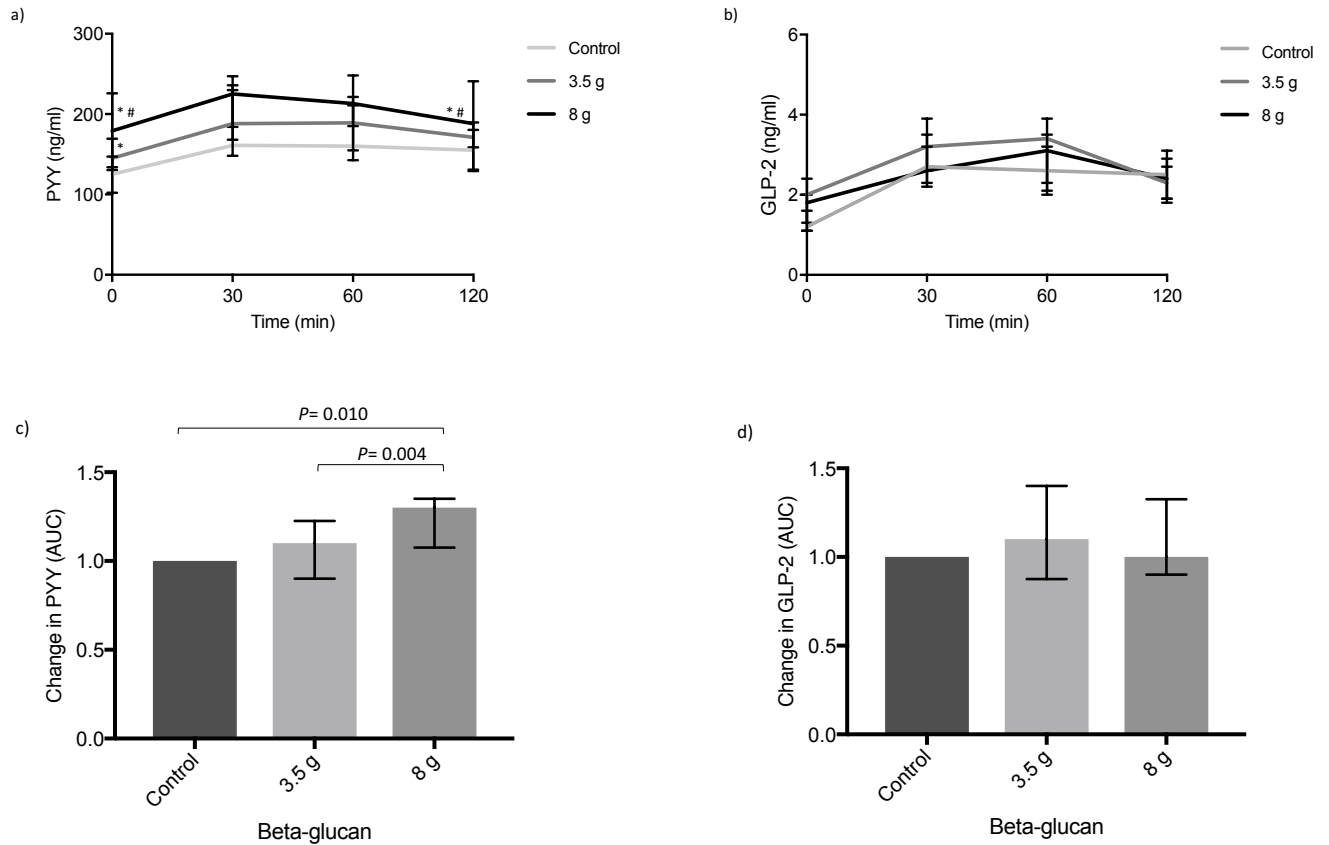
# Significant increase from 3.5 g meal:  $P=0.04$

Values are presented as median and interquartile ranges (IQR).

PYY, peptide YY; GLP-2, Glucagon-like peptide-2

After intervention with the 8 g meal, a significant increase in PYY values was also seen 120 minutes after the OGTT compared with both the control meal ( $P=0.013$ ) and the 3.5 g meal ( $P=0.002$ ). GLP-2 did not show any significant difference in the postprandial response after the OGTT. The postprandial values of PYY and GLP-2 are illustrated in fig. 6a and b.

PYY AUC and GLP-2 AUC were calculated, and the 3.5 g meal and the 8 g meal were related to the control meal AUC. A significant increase in PYY AUC was observed after consumption of the 8 g meal compared to both the control meal ( $P=0.010$ ), and the 3.5 g meal ( $P=0.004$ ). The changes in GLP-2 AUC was not significant different between the meals. The changes in PYY AUC and GLP-2 AUC are illustrated in fig. 6c and d.



**Fig. 6.** (a) Postprandial (0-120 minutes) PYY levels and (b) GLP-2 levels after OGTT the following morning after consuming the different test meals for three consecutive days. Plasma concentrations of relative values of (c) PYY AUC and (d) GLP-2.

\* Significant increase from control:  $P < 0.05$

# Significant increase from 3.5 g meal:  $P = 0.04$

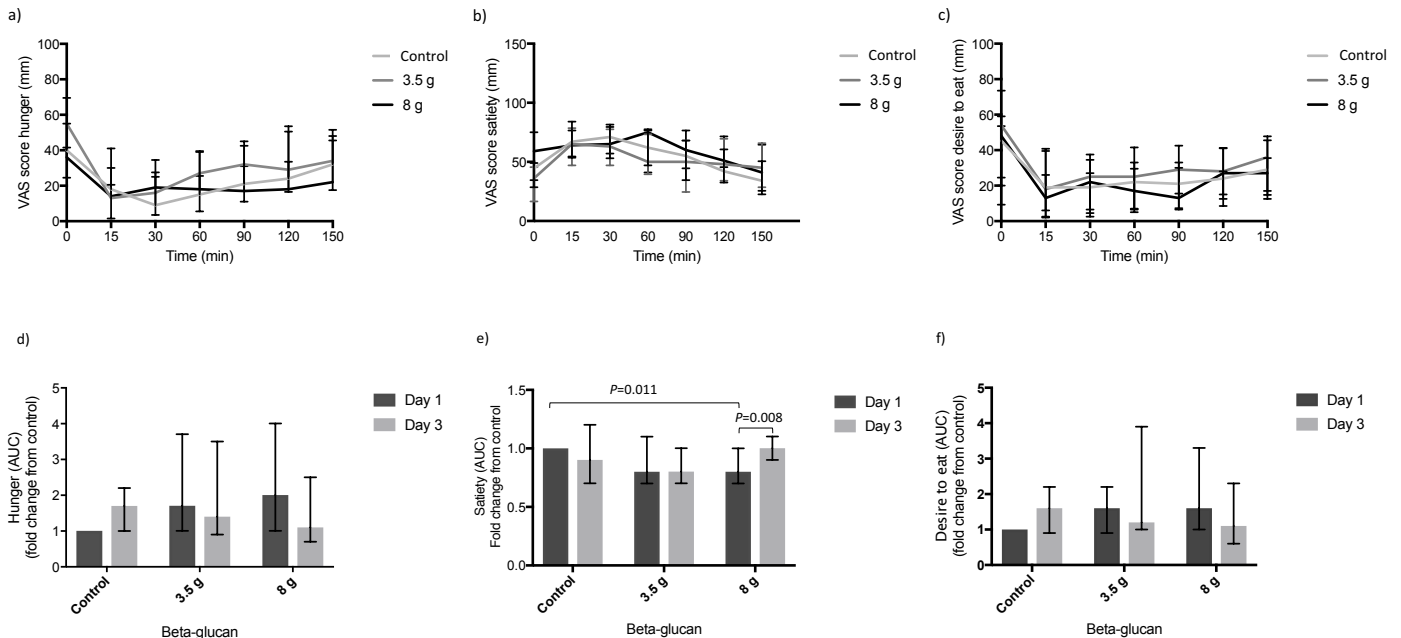
Values are presented as median and interquartile range (IQR).  $N = 14$

OGTT, oral glucose tolerance test; PYY, peptide YY; GLP-2, Glucagon-like peptide-2; AUC, area under curve

#### 4.4 Subjective ratings of appetite

The subjects registered their subjective sensation of appetite by filling out a self-reported VAS at day one and day three of the interventions. This was performed immediately before consuming the test meal and 15, 30, 60, 90, 120 and 150 minutes after consumption. When comparing the different time points, no significant differences was observed between the test meals (Fig. 7a, b and c).

AUC values for hunger, satiety and desire to eat was calculated and related to the control meal at day one. A decrease in satiety was observed after intake of the 8 g meal on day one, compared with the control meal, day one ( $p=0.011$ ). Additionally, a significant increase in satiety from day one to day three after the intervention with the 8g meal ( $P=0.008$ ) was detected. The AUC values for hunger, satiety and desire to eat are presented in fig. 7d, e and f.



**Fig. 7.** The line chart represents (a) Subjective ratings of hunger, (b) satiety and (c) desire to eat, as measured by VAS at fasting and during 150 min after eating the test meal day three of each intervention. The bar graphs show the scores as AUC for (d) hunger, (e) satiety and (f) desire to eat as measured by VAS day one and day three of each intervention. The AUC data are presented relative to the control meal. Values are expressed as median with interquartile range (IQR). N=11. VAS, visual analogue scale; AUC; area under curve.

## 4.6 Breath H<sub>2</sub> and SCFA

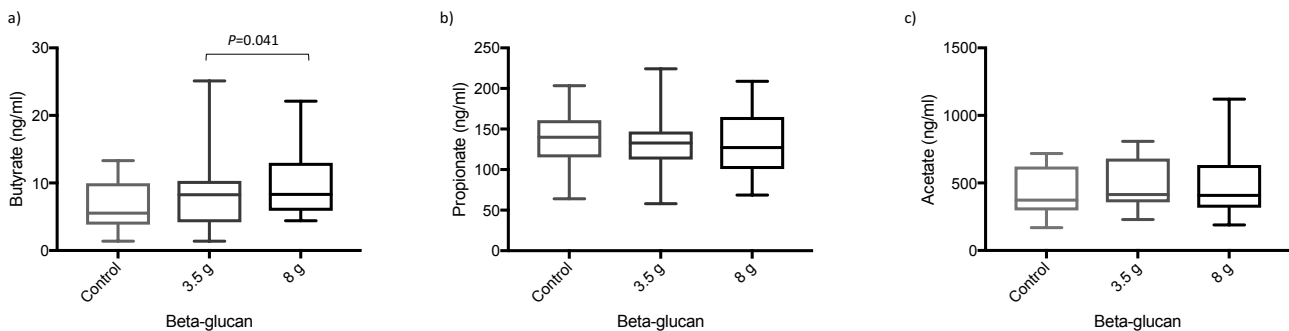
After consuming beta-glucan for three consecutive days, fasting breath H<sub>2</sub> excretion was determined as an indirect measure of colonic fermentation. Intervention with the 8 g meal showed a significant increase in fasting breath H<sub>2</sub> level compared with the 3.5 g meal ( $P=0.048$ ), corresponding to a 57% increase. There was no significant difference between the control meal and the 3.5 g meal or the 8 g meal. The values of fasting breath H<sub>2</sub> are shown in table 4.

**Table 4.** Fasting breath H<sub>2</sub> after intake of control, 3.5 g meal and 8 g meal for three days (n=14)

Evening test meal	Fasting breath H <sub>2</sub> (ppm) Median (25 – 75 percentile)
Control meal	15.0 (8.5 - 45.0)
3.5 g meal	17.0 (12.0 - 30.0)
8 g meal	23.5 (13.5 - 69.8)*

\* Significant increase from consuming the 3.5 g meal,  $P=0.048$   
H<sub>2</sub>, Hydrogen; ppm, parts per million

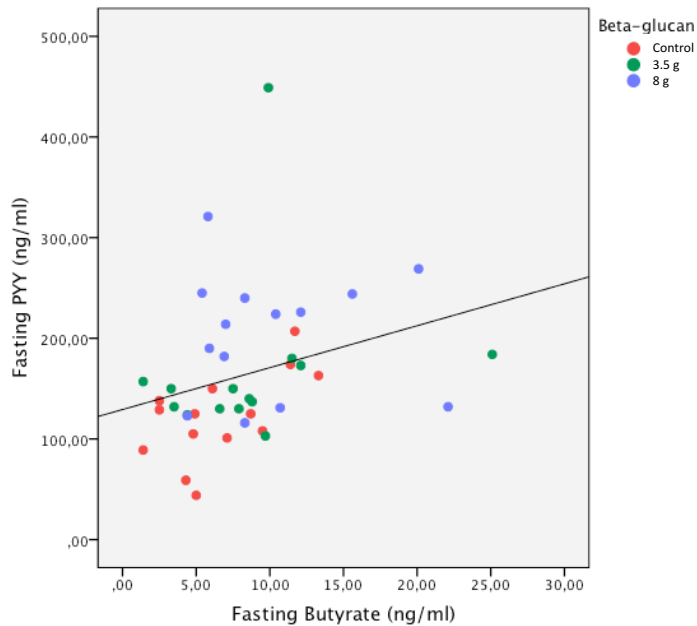
Fasting values of the SCFAs (acetate, propionate and butyrate) were measured in plasma after the interventions. All meals increased the levels of butyrate and acetate compared with baseline ( $P<0.05$ ) (data not shown), but no significant differences were detected when compared with the control meal. A significant increase in butyrate was seen after the 8 g meal compared with the 3.5 g meal ( $P=0.041$ ). No significant differences were detected in propionate between the different interventions. Fasting values of butyrate, acetate and propionate are presented in fig. 8.



**Fig. 8.** Fasting plasma concentrations of the SCFAs (a) butyrate, (b) propionate and (c) acetate. Values are expressed as median and interquartile range (IQR). N=14.

#### 4.7 Relationship between markers of gut fermentation and gut peptides

The SCFAs are suggested to affect the release of the appetite suppressing gut peptide PYY, due to stimulation of the L-cells via the receptor FFA2 (Psichas et al., 2015). To study the relationship between the SCFAs and PYY, correlation analyses were performed. Fasting butyrate positively correlated with fasting PYY ( $r=0.430$ ,  $P=0.004$ ), as shown in fig. 9.



**Fig. 9.** Correlation analyses of fasting butyrate and fasting PYY. The different colours represent the three different interventions with the control, 3.5 g and 8 g beta-glucan. PYY, peptide YY

## 5.0 Discussion

In this fixed order cross-over intervention study we investigated the effect of different amounts of beta-glucan on perceived appetite sensations, combined with biological markers for appetite after a glucose challenge in healthy individuals. The present study demonstrates that a short-term intervention with the highest dose of beta-glucan leads to increased levels of the gut peptide PYY. The increase was observed both fasting and after a glucose challenge. Subjective measures of appetite show a significant increase in satiety from day one to day three after intake of 8g beta-glucan. Furthermore, fasting SCFA butyrate and fasting breath H<sub>2</sub> concentrations were significantly increased after intake of 8 g beta-glucan compared with 3.5 g beta-glucan, indicating an increased colonic fermentative activity after consumption of the highest amount of beta-glucan for three days.

Postprandial secretion of PYY and other anorexigenic peptides have been shown to increase after consuming fiber (Beck, Tapsell, Batterham, Tosh, & Huang, 2009; Beck, Tosh, et al., 2009). Previous findings demonstrate that high viscosity producing beta-glucan increase postprandial PYY concentrations in healthy individuals, when compared with fiber with low viscosity (Juvonen et al., 2009). The same effect was observed in a study conducted in overweight subjects, consuming three cereals of varying concentration of beta-glucan (between 2.2 and 5.5g) (Beck, Tapsell, et al., 2009). They found that the highest dose of beta-glucan resulted in increased levels of fasting PYY, which is in accordance with our findings. In addition to the increased levels of fasting PYY, we also demonstrate that beta-glucan increased the level of PYY after OGTT. This indicates that beta-glucan may affect the PYY levels in a longer term, and not only directly after intake, as also shown by others (Moran, 2009). Other mechanisms than the viscous postprandial effects of beta-glucan are probably involved in the regulation of the gut peptide (Beck, Tapsell, et al., 2009).

Nilsson *et al.* (2015) also reported an increase in fasting PYY levels after a three-days intake of barley-kernel based bread as an evening meal compared with white wheat bread. The increase was suggested to occur as a result of increased activation of L-cells (Nilsson et al., 2015). In rats, this was demonstrated after supplementation with oligofructose (P. D. Cani, Hoste, Guiot, &

Delzenne, 2007). Similar results were also observed in an *in vitro* experiment, using human intestinal tissue or endoscopic biopsy samples to observe L-cell development (Petersen et al., 2014). Stimulation of intestinal tissue with the SCFAs acetate, propionate and butyrate resulted in almost doubled number of L-cells in the organoids within 48 hours. The increase in L-cell number was accompanied by increased GLP-1 levels, supporting the theory that dietary fibers and SCFAs as a fermentation product increase L-cell numbers and anorexigenic peptides. In our study, we demonstrated that an increased amount of beta-glucan led to increased fasting levels of the SCFA butyrate. Fasting butyrate also correlated positively with fasting PYY. The relationship between butyrate and PYY have earlier been demonstrated by Larraufie *et al.* By using a cellular model of human L-cells, they revealed that stimulation with butyrate increased PYY expression (Larraufie, Doré, Lapaque, & Blottière, 2017). Increased butyrate concentrations have previously been observed in both human faeces and portal vein, as well as in the peripheral blood of pigs after diets containing rye (Bach Knudsen, Serena, Canibe, & Juntunen, 2003; Bach Knudsen, Serena, Kjaer, Jorgensen, & Engberg, 2005; McIntosh, Noakes, Royle, & Foster, 2003). It is therefore tempting to speculate that the increase in PYY after intake of 8 g beta-glucan in our study is caused by an increase in butyrate and is related to increased number of L-cells.

Elevated microbial fermentation may also be reflected by alterations in breath H<sub>2</sub> excretions as an indirect measure after consuming fibers such as beta-glucan (Behall, Scholfield, van der Sluijs, & Hallfrisch, 1998). In the present study, the 8 g meal resulted in a significantly elevated breath H<sub>2</sub> level compared with the 3.5 g meal. This is in accordance with a previous study demonstrating an increased breath H<sub>2</sub> after consuming barley-kernel based bread compared to white wheat bread (Nilsson et al., 2015). Behall *et al* investigated breath H<sub>2</sub> expiration after two different doses of beta-glucan (Behall et al., 1998). The subjects consumed 1g beta-glucan/100 g carbohydrate (1%) followed by 10 g beta-glucan /100g carbohydrate (10%) for three days, with an OGTT after the two periods. Breath H<sub>2</sub> expiration was significantly elevated after both doses, with greater rise after the highest dose. Another study investigated H<sub>2</sub> breath production after consuming different types of dietary fibers for nine days each, compared with a fiber-free diet (Fernandes, Vogt, & Wolever, 2011). Consumption of dietary fibres resulted in an elevated H<sub>2</sub> breath excretion. This may explain the relatively small difference in fasting H<sub>2</sub> between the control and the 3.5g meal in our study, as inulin was used as a substitute for beta-glucan in the control meal. Inulin is, like



beta-glucan, fermented in the large intestine by the bacteria, which lead to increased production of SCFAs and H<sub>2</sub> (Cummings & Stephen, 1980). Furthermore, the present study was not a fully controlled dietary intervention study, and the subjects were able to eat relatively freely except the test meals and the dietary restrictions given. Other carbohydrates, such as vegetables and sugar alcohols, as well as proteins, may also undergo intestinal fermentation and yield H<sub>2</sub> (Rumessen & Gudmand-Hoyer, 1998; Windey, De Preter, & Verbeke, 2012).

Despite the association between soluble fibres and health benefits, the effect on subjective measures of satiety appears to be inconclusive (El Khoury et al., 2012). However, soluble fibres such as beta-glucan are more strongly associated with reduced appetite than meals low in fibre (Dikeman & Fahey, 2006). The efficacy of beta-glucan on satiety depends on different factors, with dose as one of the proposed determinants (Kim, Behall, Vinyard, & Conway, 2006). The present study revealed a lower feeling of satiety the first day after consuming the highest dose of beta-glucan compared with the control. Further, a significant increase from day one to day three after intervention with the 8 g meal was detected. Previous studies using beta-glucan to investigate the effect on satiety, have shown inconsistent results (Rytter et al., 1996; Saltzman et al., 2001). One trial found that 2 g beta-glucan from barley served in cereals had no acute effect on satiety (Kim et al., 2006). They concluded that to achieve satiety control, more beta-glucan was needed. In comparison, a study investigating the satiety inducing effect of 4 g beta-glucan as oat-based cereal, showed no effect on satiety (Hlebowicz, Darwiche, Bjorgell, & Almer, 2008), whereas Beck *et. al* concluded that between 4 and 6 g beta-glucan would be the optimal dose to affect satiety (Beck, Tapsell, et al., 2009). Another study demonstrated improved ratings of hunger after doses of 2.2 g of beta-glucan (Beck, Tosh, et al., 2009), while Barone *et al* demonstrated increased fullness and satiety, as well as reduced energy intake the rest of the day after providing 3g of oat beta-glucan in a breakfast beverage (Barone Lumaga, Azzali, Fogliano, Scalfi, & Vitaglione, 2012). A previous systematic review reported that short-term fiber intervention did not impact food intake, and were not associated with satiety responses (Clark & Slavin, 2013). One could therefore argue that it is not only the amount of beta-glucan that is important for the satiety enhancing effect, but also its physical effect such as viscosity may be of importance.

The physical effect of beta-glucan is largely determined by the molecular size and solubility (Burkus & Temelli, 2003). The MW of beta-glucan can decrease and degrade during isolation, purification and extraction procedures, resulting in reduced viscous effects (Maheshwari, Sowrirajan, & Joseph, 2017). This might be an explaining factor of the various results in previous findings. In the present study, the values of the MW in both the 3.5 g and the 8 g meals were high. The effect on satiety were increased at day three compared to day one after intake of 8 g beta-glucan. On the other hand, a decrease in satiety was observed after consuming the 8 g meal day one compared with the control meal day one, indicating that other factors may also play a part regarding subjective appetite sensations.

In the present study, we examined the subjective sensation of hunger, satiety and desire to eat by VAS. The main advantages of using VAS in assessing appetite sensations are its non-invasive nature and the ease of administering. Nevertheless, some challenges exist. First, assuming that a mark of a certain value on the rating (i.e. 30 mm) will predict half the rating of the doubled value (60 mm) will only be based on speculations. Second, the subjects may also be reluctant to use the extremes of the scale, preferring the mid sections. Similarly, others may only record the extremes. In the present study, we did not measure palatability response as a sensory evaluation on the test meals. Palatability and satiety have shown to have opposite effects on food intake, in which palatability increases appetite and satiety limits consumption (Drewnowski, 1998a). To measure palatability, perceived pleasantness of a type of food, intent to eat and amount of food consumed is registered (Drewnowski, 1998b). Overall appetite control may also be influenced by visual and oligofactory input, and socio-economic factors (Cooper et al., 2011; Kozimor, Chang, & Cooper, 2013). This was not taken into consideration in our study.

The test meals in the present study were similar in the content of calories, although content of macronutrients differed slightly. The 8 g meal contained the highest amount of protein and fat, which previously have been reported to lead to increased levels of PYY and satiety (Lomenick, Melguizo, Mitchell, Summar, & Anderson, 2009). In the present study, there was no control of the subject's general food intake except the test meals we provided. In addition, the meal prior to the test meal was not standardized and may have varied in size and composition and therefor also affect the subjective sensation of appetite. There must be taken into consideration that the general

diet, and the content of macronutrients, may differ between the subjects. This could be a possible factor affecting the results. However, the significant increase of PYY, butyrate and H<sub>2</sub> breath in the 8 g meal compared with the 3.5 g meal after the three-days intervention, indicates that the subjects followed the instructions regarding the test meal. Moreover, another concern to be addressed is the type of control used. In the control meal in the present study, the beta-glucan was replaced with the soluble fiber inulin, which also has been hypothesised to affect appetite. A cross-over placebo-controlled pilot study, found that treatment with inulin increased satiety, reduced hunger and prospective food consumption in a following meal (P. D. Cani, Joly, Horsmans, & Delzenne, 2006). This is further supported by similar experiments conducted in rats (P. D. Cani, Dewever, & Delzenne, 2004; Patrice D. Cani, Neyrinck, Maton, & Delzenne, 2005). Another study conducted in healthy humans demonstrated reduced food intake in women after intake of a high dose of inulin, but increased food intake in men (Hess, Birkett, Thomas, & Slavin, 2011). A systematic review previously evaluated effects of inulin supplementation, and stated that the effect on acute satiety were inconclusive (Liber & Szajewska, 2013).

A particular strength with our study is the single blinded cross over design, making the intervention evaluated within the same subject, and hence the between-subjects variability is reduced (Maclure, 1991). In addition, by keeping a washout period of two weeks between each intervention, we attempted to avoid a carry-over effect, to ensure that there were no overlap of effects between interventions (Louis, Lavori, Bailar, & Polansky, 1984). The fact that the subjects were not informed of what amount of beta-glucan they got at each intervention, reduced the risk of systematic bias. The cross-over design also makes it possible to obtain equal statistical power with the limited number of subjects.

The present study has certain limitations. One potential limitation is the small number of subjects in our study. Three subjects were excluded from the VAS calculations, due to missing values. It is possible that the results regarding VAS would be different if the number of participants were higher. Another possible limitation is that the subjects both consumed the test meal and filled out the VAS scores at home, without allowing for control of compliance. VAS scores for both day one and day three of the intervention were handed in by the subjects day four, making it possible to compare their own answers at home within the same intervention period. This may have

affected their score the third day. Regarding test subjects, an uneven gender balance was present. Only two males compared with twelve females were included in the study. However, re-calculating VAS scores without men did not affect the outcome.

## **6.0 Conclusion**

The present study demonstrates increased satiety (PYY and VAS) after a short-term intake of beta-glucan. The effect on PYY was also evident after a glucose challenge. The altered levels of PYY may be mediated through colonic fermentation of beta-glucan, illustrated by the elevated fasting H<sub>2</sub> excretion and fasting butyrate 12 hours after the test meal. In addition, a positive relationship between butyrate and PYY support the results, indicating a possible causal relationship between production of SCFA and secretion of gut peptides. Although more studies are needed to clarify the relationship between fibre, gut microbiota and satiety, these findings suggest anti-obesogenic potential of foods rich in beta-glucan.

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## Appendix: Visual Analogue Scale (VAS)



### Visuell Analog Skala

I *Beta-glukan og blodsukker*-studien ønsker vi å registrere sult og metthet ved bruk av en Visuell analog skala.

Vi ber deg derfor besvare et skjema med 3 spørsmål ved å sette en horisontal strek på linja som er beskrivende for hvordan du har det i forhold til hvert spørsmål. Du skal besvare skjemaet mandag og onsdag kveld i forbindelse med kveldsmåltidene du har fått utdelt. Du får utdelt 2 x 7 like skjemaer som skal besvares til bestemte tider. Tidspunktene for besvarelse er 0 min (rett før måltidet), 15 min, 30 min, 60 min, 90 min, 120 min og 150 min etter at måltidet er ferdig spist. Hvert av skjemaene har markert tidspunktet for besvarelse. Måltidet skal være ferdig spist innen kl. 20.00 på kvelden. Vi foreslår at du spiser kveldsmåltidet ca. kl. 19.30. Da rekker du å spise opp måltidet innen kl. 20.00, og du har tid til å besvare siste gang innen du legger deg. Det er viktig at forrige måltid på dagen må være spist minimum 2 timer i forkant av kveldsmåltidet. De dagene (mandag og onsdag) du skal fylle ut skjemaet, er det viktig at du har like lang tid mellom kveldsmåltidet og forrige måltid. Dette gjelder gjennom hele studien.

Dersom du skulle få problemer med å besvare ett av tidspunktene er det likevel viktig at du besvarer de resterende tidspunktene.

**Vi ønsker også at du besvarer følgende spørsmål rett før kveldsmåltidet:**

Når spiste du forrige måltid?

Hva spiste du ved forrige måltid?

**Besvart klokken:**



### Visuell Analog Skala

1) Hvor sulten er du nå?

Ikke sulten i det hele tatt

---

veldig sulten

2) Hvor mett føler du deg nå?

Ikke mett i det hele tatt

---

veldig mett

3) Hvor lyst har du på mat nå?

Ikke lyst på mat i det hele tatt

---

veldig lyst på mat