The effect of barley on glucose regulation, appetite and colonic fermantation in middle aged subjects

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THE EFFECT OF BARLEY ON GLUCOSE REGULATION, APPETITE AND COLONIC FERMENTATION IN MIDDLE AGED SUBJECTS

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Abstract

Use of wholegrain foods has shown importance in terms of prevention of obesity and related comorbidities. Positive metabolic effects of wholegrain barley have been investigated with this regard. In the same line of research, this study aims to investigate the effects of barley kernel bread on glucose regulation, appetite and colonic fermentation in middle aged subjects. A randomized control trial with 41 healthy subjects was performed, whereof, 29 are included in this thesis. Each participant consumed two different test products: white wheat bread (WWB) and barley kernel bread (BKB) for 3 days in random order with a washout period of 3 weeks in-between. The last test product portion in each intervention period was consumed in the evening prior to the experimental day. On the experimental day, after 11 hours of fasting overnight, plasma and serum samples, blood glucose, breath hydrogen excretion and subjective appetite were measured at fasting and at several time points up to 180 minutes after the standardized breakfast. There was significant difference in glucose iAUC (0-120 min, $p< 0.001$), mean breath hydrogen excretion (0-180 min, $p<0.01$) and fasting values for satiety and desire to eat ($p<0.05$) depending on intervention products. The results show that barley kernel bread has positive effects on blood glucose regulation and appetite sensations. A possible mechanism for these results probably emanate from colonic fermentation of the dietary fiber in barley, indicated by increased breath hydrogen concentration after intake of BKB compared with after the WWB.

Preface

This project titled “The effect of barley on glucose regulation, appetite and colonic fermentation on middle aged subjects” was carried out in Food for health science centre, Lund with the guidance of Jonna Sandberg and Anne Nilsson. With this project, I have learnt a lot about intricacies in research, clinical studies and good clinical practice. I would like to express my sincere gratitude to Anne Nilsson for the guidance and suggestions during this thesis. I would like to thank Jonna who had taught me and helped me from the very first day until the end with utmost dedication. Jonna has also taught me a lot more than science and been as a great friend, thank you for that! Thank you to Yvonne Granfeldt to be my examiner and evaluate my thesis.

I’m happy and grateful to have acquainted with other members of the Food for health centre and their innumerable memories during fika and lunch breaks. A special thanks to Elin Östman and Lesli Hingstrup Larsen whose passion for creating better foods for better health has inspired me to work towards nutrition.

Thanks to all my friends in Sweden and India for always being there and making great memories. Finally, I would like to thank my beloved parents and my sister for always believing and supporting me.

Jananee Muralidharan

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1. Introduction

Balance is an important force of nature which when lost creates collapse of the system. Accordingly, when it comes to human body, the imbalance of energy intake and expenditure may induce obesity or underweight. Obesity is a global epidemic viewed and dealt from social, economic, physiological and psychological perspectives. Obesity can result in lowered quality of life since obesity is associated with conditions such as diabetes and cardiovascular risks. In addition, from an economic perspective, the healthcare costs due to obesity related problems in Sweden sum up to 547 million Euros per year (Müller-Riemenschneider, Reinhold, Berghöfer, & Willich, 2008). Hence, preventive actions are much needed.

Balanced consumption of nutrients and also a healthy lifestyle in other respects are recommended to prevent this epidemic. Other than genetic factors, the diet is regarded as the most important factors affecting the risk of being obese, thus, also plays a significant role in prevention and weight management. A relationship between high intake of dietary fiber (DF) and reduced risk factors for cardiovascular diseases (McKeown, Meigs, Liu, Wilson, & Jacques, 2002) and type 2 diabetes (Schulze et al., 2007) has previously been shown.

Recommendations on amount of wholegrain intake have varied over the years and geographically. Consequently, in Australia, 6-12 servings (a serving equates to two slices of bread; one cup of cooked rice, pasta or noodles; one cup of porridge; one cup cereal flakes; or half a cup of muesli) per day is recommended (Fardet, 2010). Nordic nutrition recommendation suggests a high fiber intake of >3 g/1000 KJ, or 25-35 g / day for adult persons (Becker et al., 2013). The differences in serving recommendation could be due to differences in definition of wholegrain products and also depending on local diets and socio economic factors which varies geographically. In the US and the UK, wholegrain food must contain ≥51% of wholegrain ingredients by wet weight, whilst in Sweden and Denmark, it is ≥50% wholegrain ingredients by dry basis (Lang & Jebb, 2003) (Frølich, Åman, & Tetens, 2013). The differences in definition also impacts on interpreting the results from different international studies. However, even though there is ambiguity in definitions, it is of most importance that the inclusion of wholegrain in diet has been promoted.

Bread being one of the major staple food in Scandinavia, it is considered as an important vehicle for delivery of wholegrain (Kyrø et al., 2012). Grains such as barley, rye and oats

have been receiving attention because of their high dietary fiber content and positive health effects, see section 2.3.1 & 2.3.2. Barley and oat grains have particularly high β-glucan content that could range from 2.5-11.3% (Osuji, 2015). β-glucans has shown cholesterol lowering effects (Behall, Scholfield, & Hallfrisch, 2004) and glucose lowering properties (Tosh, 2013).

2. Background

2.1 Wholegrain and dietary fiber

The cereal grain, which contains all three parts: germ, endosperm and the bran, is called a wholegrain (Fardet, 2010). The wholegrain is rich in fibers, starch, proteins, micronutrient and phytonutrient (Lang & Jebb, 2003). Figure 1 shows wholegrain structure of wheat including major nutrient composition found in each part of the grain.

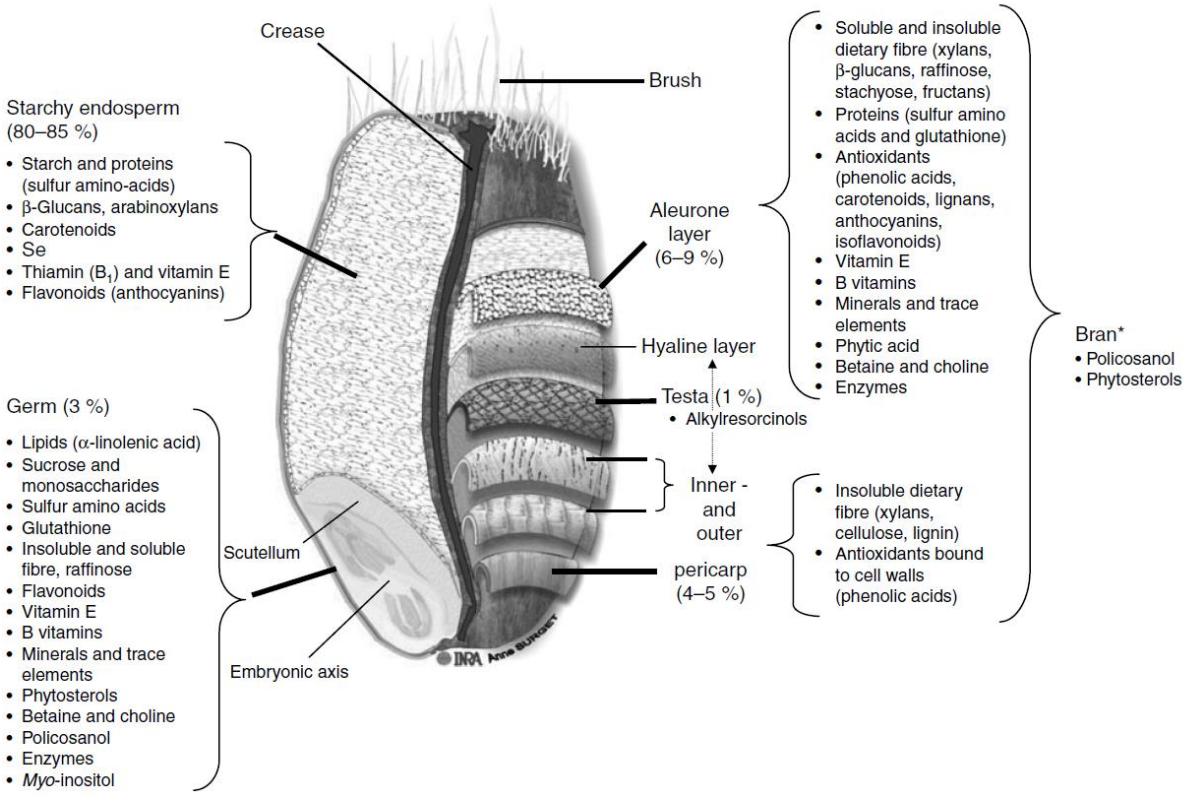

Figure 1: Fractions of wholegrain wheat with its major nutrient composition (Fardet, 2010)

Definition of dietary fiber (DF) according to American Association of Cereal Chemists (AACC) in the year 2000 stated "Dietary fiber is the edible part of plant or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fiber includes polysaccharides, oligosaccharides, lignin, and associated plant substances". The composition of various nutrients also varies based on the type of wholegrain. Protein content in wheat, rye, oats and barley are 11.2, 8.9, 12.7 and 9 %, respectively. Whereas the total non-starch dietary fiber content in oats and barley are around 10% compared to that of 11.4 % in wheat and 14.4 % in rye. Fat is also found in different amounts from oats (7.1%) and wheat, rye, barley (2.1-2.3%)

which could overall show the differences in grain contents (Frølich et al., 2013). Wholegrains are often found in products such as bread, museli, porridge and breakfast cereals. The physio-chemical properties of the final wholegrain product also depend on the milling and other processing treatments (J. L. Slavin, Jacobs, & Marquart, 2000).

Starch is a major form of storage carbohydrates in cereal grain. Its digestion in human system begins in the mouth and continues until large intestine. Starch undergoes an array of chemical reaction to be broken into monosaccharides. Most of starch is digested and absorbed in the small intestine. However, some forms of starch remain un-hydrolysed and are not taken up in the small intestine. These starches are called resistant starches (RS) and are included in the definition of DF (Englyst, Wiggins, & Cummings, 1982). The rest of starch, which undergoes enzymatic reactions and are absorbed by the small intestine, are categorized as available starch.

DF, being important part of wholegrain cereals, plays a key role in mechanisms behind beneficial actions of wholegrain such as regulating bowel movement (Frølich et al., 2013), regulating postprandial blood glucose (Bourdon et al., 1999) and increasing satiety (Burton-Freeman, 2000). Also, the presence of antioxidants, minerals and some proteins may synergistically act towards the health effects of wholegrains (Fardet, 2010). Soluble fibers (e.g. inulin, beta-glucans, wheat dextrins) are generally fermentable by the gut microbes and release metabolites (e.g. short chain fatty acids (SCFA) that are absorbed and give physiological effects. Insoluble fibers (e.g. lignins, cellulose, and some hemicellulose) on the other hand increase the stool weight and helps in laxation of stool (J. Slavin, 2013).

2.2 Colonic fermentation of indigestible carbohydrates

Carbohydrates being one of the important macronutrient, its metabolic pathways are highly studied. As mentioned in section 2.1, fermentation takes place in the large intestine, resulting in metabolites such as hydrogen and carbon dioxide along with SCFA's (Gibson et al., 1990). Some part of this hydrogen is absorbed in the blood and expired via breath. This breath hydrogen expired is taken as a rough indicator for extent of microbial fermentation activity in the gut. There are several mechanisms speculated for the link between metabolism and events in colon. Some of them include production of SCFA, gut peptide secretion etc. and their array of reactions on other molecules (Cani & Delzenne, 2009). Three key gut SCFA that are produced during bacteria fermentation are acetate, propionate and butyrate (Topping &

Clifton, 2001). SCFA aid in inhibiting the growth of pathogenic bacteria by reducing the pH of colonic lumen and helps in increasing bioavailability of some minerals (Dahl & Stewart, 2015). Effects of SCFA also depends on composition and concentration of gut microbiota. And this gut microbial diversity has been observed to be different among individuals. Growing interests have been shown on difference in gut microbiota between healthy and obese individuals also in order to facilitate personalised nutrition.(Sanz, Rastmanesh, & Agostonic, 2013).

2.3 Glucose regulation

In the normal physiological state, our body tries to maintain a constant blood glucose level by balancing the rate of glucose appearance (from food) and rate of glucose disappearance. This is controlled by an array of gluco-regulatory hormones such as insulin (from β cells of pancreas), glucagon (from α cells of pancreas), glucagon like peptide-1 (GLP-1) (from L-cells) and glucose dependent insulinotropic peptide (GIP) (from K cells of intestine).

After consumption of a meal, insulin is responsible for driving glucose into skeletal muscle cells and adipose tissues; when the glucose concentrations reach a peak and fall to, or even below normal blood glucose range, glucagon is released. Glucagon is responsible for hepatic glucose production during the fasting state. In figure 2 below, we can see changes in glucose, insulin and glucagon response with time and also comparison with diabetic subjects.

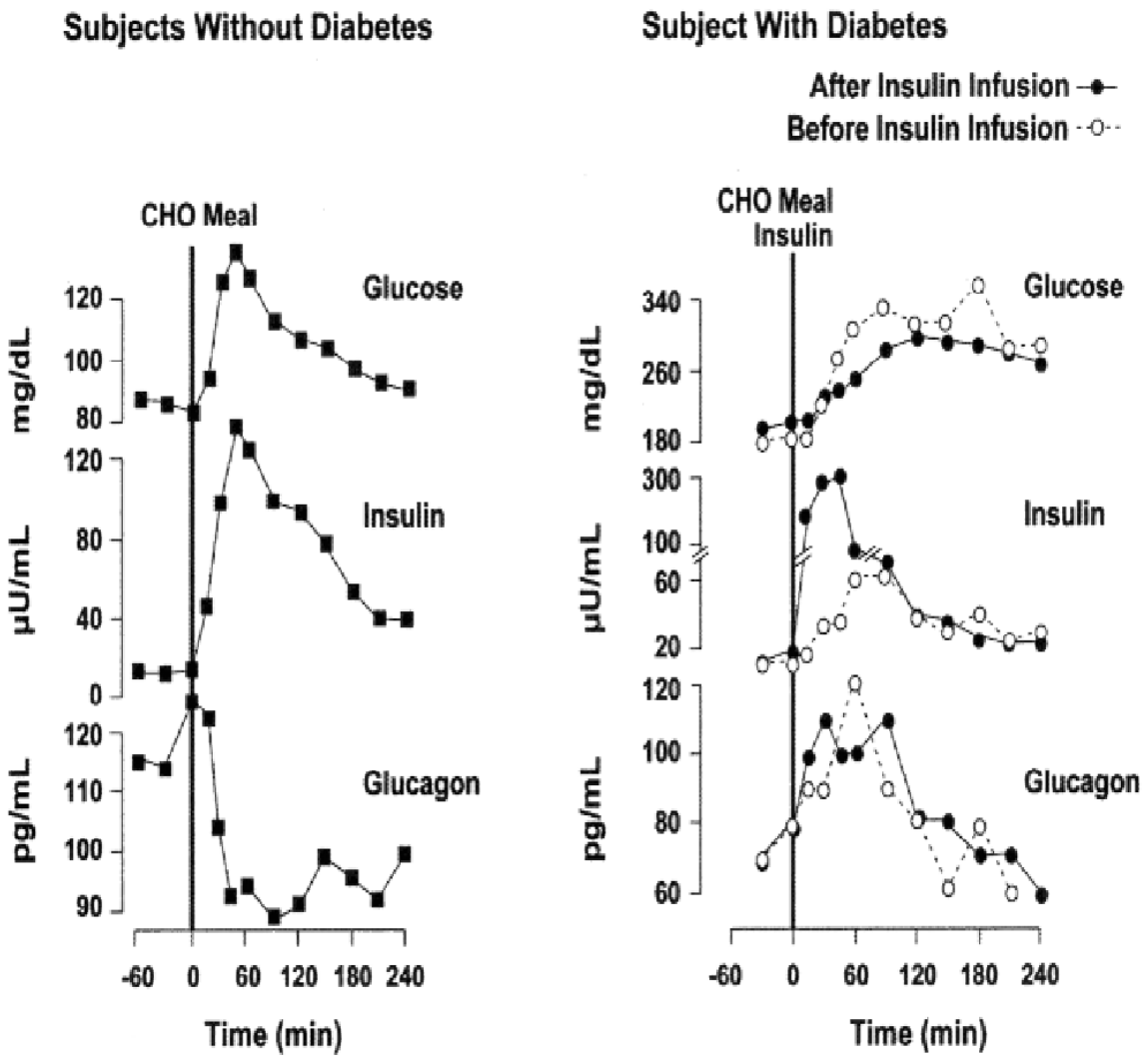

Figure 2: Glucose, insulin and glucagon secretion with respect to time and comparison with Type 2 diabetic and non-diabetic subjects (Aronoff et al., 2004)

We can see the difference in peaks among the non-diabetic and diabetic individuals for glucose, insulin and glucagon. GLP-1 and GIP plays role stimulation of insulin in the post meal state (Aronoff, Berkowitz, Shreiner, & Want, 2004).

Glycemic index (GI) is a way of classifying carbohydrate rich foods based on their effects on blood glucose response. It is one of the highly-used forms of presenting metabolic response to food. Consumption of high GI food, i.e. foods that give rise to high postprandial blood glucose concentrations, for a continued longer term is associated with insulin resistance and the vicious circle of obesity, type 2 diabetes and inflammation (Brand-Miller, Holt, Pawlak, & McMillan, 2002). GI is defined as the ratio of glycemic response of a test food (based on 50g of available carbohydrates) to glycemic response of standard food, usually white wheat

bread or glucose containing same amount of available carbohydrate (Jenkins et al., 2002). This response is related to the rate of release and uptake of glucose to the bloodstream. Higher GI of a particular food has more tendencies for the body to release insulin, which in the long term increase the risk of diabetes type 2. Glucose regulation mechanisms may be divided in acute regulatory mechanisms and semi-long or long term regulation.

2.3.1 Acute effects of cereal products on blood glucose regulation

According to a review by Tosh (2013) it was shown that among the 72 treatments (31 for barley, 41 for oats), 50 treatments showed significant reduction in peak postprandial blood glucose response with oats and barley products compared to the control meal. For treatments with barley, the average β-glucan dose was 4.7±2.5 g and for oats it was 5.4±2.9 g. Also in studies where intact kernels (oats or barley) were used in the test product, there was reduction in average AUC (Area under the curve) compared to processed kernel form (such as ground and broken). One explanation to the lowered glucose response is the presence of fibers, especially viscous fiber, in the test meal since these results in a slower rate of glucose release into the blood due to slower gastric emptying rate, and slower digestion of carbohydrates and absorption of monosaccharides in the small intestine. Viscosity and solubility of the fiber is an important factor altering the effect of wholegrain on health (J. Slavin, 2013). Different cereal grains exert different physiological effects when consumed. In a study comparing different cereals with different GI and different dietary fibers, lowest postprandial blood glucose response during the daylong intervention was obtained for rye and barley kernel breakfast, compared to WWB (Anne C Nilsson, Östman, Granfeldt, & Björck, 2008). Whereas, breakfast consisting of e.g. wheat kernels did not significantly lower the daylong glucose response, compared to WWB. In addition, it was observed that barley kernel based breakfast had significantly lower glucose increments 30 min after standardized lunch, i.e. 4.5 hours after intake, than did whole grain barley flour porridge, WWB+ barley DF and WWB. The rye kernel , barley kernel breakfast induced higher breath hydrogen compared to WWB, after the standardized lunch (4-6 hours after test breakfast); And barley kernel breakfast induced a non-significant lower postprandial glucose and significantly higher breath hydrogen compared to WWB after standardised dinner (9.5-11.5 hours after test breakfast).This result was attributed to the low GI nature of barley and rye kernels probably due to the higher dietary fiber content and also the intact botanical structure of the kernels (Anne C. Nilsson, Östman, Holst, & Björck, 2008). Other than these factors, presence of

compounds such as phenolic acids (such as caffeic, freulic, sinapic acids) in rye grains might also aid in reduction of postprandial (0-60 min) glucose and insulin response (Rosén et al., 2011). As mentioned in section 2.1, DF has potential of reducing postprandial glucose levels. The rate of starch digestion is also seen to be reduced in the presence of some organic acids such as lactic acid, acetic acid in the food matrix. The interactions of acids during starch gelatinization is seen to be an important factor for this effect (Östman, Nilsson, Liljeberg Elmståhl, Molin, & Björck, 2002) (Liljeberg & Björck, 1998).

2.3.2 Second meal effect

Some foods have a tendency to show metabolic effects for a prolonged time (from 4-14 hours after food intake) beyond their acute effects, i.e. after a second- or a third meal. For example, some foods with low GI have ability to improve the glucose tolerance after the next meal consumed after approximately 4h (Wolever, Jenkins, Ocana, Rao, & Collier, 1988). In a longer time perspective, e.g. 10 h after consumption of a meal, probably other factors than the GI of the food is involved. In this case the effect on glucose regulation has been suggested to stem from mechanisms originating from colonic fermentation of DF. For example, in a study involving cereal based evening meal, including different dietary fiber content of barley kernel bread and white bread, intake with varied amount of indigestible carbohydrates but similar GI was observed (Anne C. Nilsson, Östman, Holst, & Björck, 2008). This showed a reduced incremental blood glucose peak (after 11 hours after consumption) for all the kernel based evening meals compared to that of WWB and WWB with added RS. In addition, an inverse relation between the gut hormone GLP-1 and blood glucose response was observed after the subsequent breakfast (11-14 hours later) (Anne C. Nilsson et al., 2008). In the review by Tasyurek, Altunbas, Balci, and Sanlioglu (2014) it has been discussed that GLP-1 have both antidiabetic- and satiety regulatory properties (Tasyurek, Altunbas, Balci, & Sanlioglu, 2014). The relationship between fermentable carbohydrates (observed with breath hydrogen) and second meal effect was also seen in the study mentioned above. This was observed by decreased free fatty acids (FFA) and increased GLP-1 at fasting that could be correlated with gut fermentation. Glucose response was also found to be inversely related to colonic fermentation observed by changes in breath hydrogen excretion. The possible reasons suggested were that production of SCFA, e.g. propionate, by gut bacteria fermentation of indigestible carbohydrate stimulated further reactions in benefiting the glucose metabolism (Anne C. Nilsson et al., 2008).

2.4 Appetite regulation

Food consumption and appetite regulation are of paramount importance in human survival and involves complex mechanisms. The mechanisms involve central nervous system (CNS), gastrointestinal tract (GI), and the enteric nervous system. Dysregulation or impairment in the appetite mechanism sometimes leads to overeating or under eating, thus imbalance in the energy intake and expenditure may results in fat accumulation and eventually to obesity (Näslund & Hellström, 2007).

Ghrelin, leptin, cholecystokinin (CCK), peptide tyrosine- tyrosine (PYY), glucagon like peptide-1 (GLP-1), oxyintomodulin (OXM) are some of the studied gastric and intestinal signal hormones for food intake and regulation mechanisms. High fiber diet has an influence on CCK which stimulates hypothalamic center for satiety and regulates pancreatic secretion of insulin (Bourdon et al., 1999).

Ghrelin also called hunger hormone is a secretagouge for growth hormone and a neuropeptide which acts on CNS for creating hunger signals. There is an increase in ghrelin concentration before meals, which goes down during consumption of food and increases before the next meal. Ghrelin is a marker for obesity where the circulating levels of ghrelin is less in obese individuals compared to lean individuals (Näslund & Hellström, 2007).

Leptin is a hormone produced in the adipose. Leptin signals for satiety or the fullness and has shown effects in prolonged time. GLP-1 and PYY are both peptide signals inducing reduction of food intake. They have similar implications by acting on ileal brake which acts as a transit control mechanism during digestion and contribute to delay in gastric emptying and has implications on blood glucose regulation (Strader & Woods, 2005). Both GLP-1 and PYY are expected to increase post meal in normal individuals. SCFA can also stimulate PYY and GLP-1 via free fatty acid receptors (Ffar2 and Ffar3) (Tremaroli & Bäckhed, 2012). Some of the receptors in the endocrine cells are also nutrient specific, which stimulates signals by presence or increase of a particular nutrient (e.g., regulation in release of PYY by response to all three macronutrients, with fat inducing the most release of PYY) (Feinle-Bisset, Patterson, Ghatei, Bloom, & Horowitz, 2005). The vagal nervous system which also plays a role in transmitting satiety signals is reactive to gastric volume, lumen volume, lumen pressure (Näslund & Hellström, 2007; Strader & Woods, 2005). OXM has also shown to reduce ghrelin release in human subjects showing its potential indirect role in appetite control

(Cohen et al., 2003).

In order to relate subjective appetite sensations with appetite signalling hormones (such as ghrelin, GLP-1, PYY), subjective appetite variables can be measured with scales of sensation of hunger or satiety or other appetite factor at each point of time measured. It is measured using Visual Analogue Scale (VAS) which has been in use from as early as 1921 (Aitken, 1969). VAS is generally lines of varying lengths (usually 10 cm) marked with numbers or words anchored to it. The extremes of the lines are marked with highest and lowest sense of a sensation (in this case appetite factors: e.g., I'm not hungry at all / I'm have never been hungrier) (Raben, TAGLIABUE, & Astrup, 1995)

3. Objectives

The overall aim of this thesis is to observe and understand the beneficial effects on glucose and appetite variables of dietary fibers from barley. Hence this thesis will deal with organizing a human study including healthy middle-aged subjects with the purpose to investigate the effects of barley kernel bread (BKB) consumed in the evening, on glucose tolerance and subjective appetite variables the following morning after a standardized breakfast (11-14 hours later). The effects were evaluated in comparison with a white wheat bread (WWB, reference product) and related to gut microbial fermentation activity, determined by breath hydrogen excretion.

4. Methods and materials

This thesis is a part of larger study conducted at Food for Health Science Centre, Lund. 40 participants were registered for the study, but data from only 29 subjects are included in this report due the time limit of the thesis work. Preparation of test products, characterization of test products, preparation for human study and assistance in sampling participants throughout the study was major part of this thesis work. Blood glucose response, breath hydrogen excretion, appetite parameters were analysed and are reported for the 29 randomly chosen subjects in this thesis. Analysis and report of the other physiological test variables included in the extended study (40 subjects) (plasma, serum, faecal samples) were not included in the scope of thesis.

4.1 Subjects for the study

The study design was a randomized, controlled, cross-over study (Figure 1) with 29 participants included in the scope of this thesis report. The inclusion criteria were healthy men and women, non-smoker with no known metabolic disorders or allergic conditions at an age of 50- 70 years (mean±standard deviation (SD): 63.8 ± 6.3 years), BMI of 19-28 kg/m^2 (mean±SD: 24.3 ± 3.2 kg/m^2). Exclusion criteria were subjects with metabolic disorders, gastrointestinal disorders and subjects with hypersensitiveness to any food or illness that could affect the study results.

4.2 Physiological test variables

Blood glucose: This was measured using finger prick capillary blood sampling and analyzed in HemoCue® B-glucose machine. Blood glucose determination was performed at fasting and then at 15, 30, 45, 60, 90, 120, 150, 180 minutes after a standardized breakfast.

Plasma and serum sampling: These samples were collected using cannula placed in the antecubital vein. Sampling tubes were prepared with necessary inhibitors prior to the study. The inhibitor Pefabloc is a protease inhibitor and was used for samples collected for ghrelin analysis. For storage and analyzing GLP-1, GLP-2, OXM and PYY from plasma sample, the tubes were treated with Dipeptidyl peptidase 4 (DPP 4) inhibitors. These test markers are susceptible to proteolytic damage by DPP 4 in human plasma hence the addition of DPP 4 inhibitors is required (Mentlein, 1999). Sampling was done at fasting and then at 15, 30, 45, 60, 90, 120, 150, 180 minutes after a standardized breakfast. Serum samples are allowed to

stand and coagulate for at least 30 minutes (and maximum of 60 minutes) after collection of sample and centrifuged at 4000 rpm for 12 minutes at 3-5^{0}C. The plasma samples are well mixed and centrifuged immediately after blood collection. It is centrifuged at 3000 rpm for 10 minutes at 3-5^{0}C. After centrifugation, the separated plasma and serum were transferred to Eppendorfs and stored at either -40^{0}C or -80^{0}C depending on future biomarker analysis.

Subjective appetite: Subjective appetite sensation was measured using VAS. VAS was given to rank appetite, desire to eat and hunger at different time intervals. The VAS had ranking of 0 to 100 in a 100mm scale ranging from degree of 'none' to 'extreme'. Measurements were requested to be filled at an interval of 0, 15, 30, 45, 60, 90, 120, 150, 180 minutes after the standardized breakfast.

Breath hydrogen: This was measured using a hand held hydrogen monitor (Gastrolyzer® Bedfont Scientific Ltd) to observe the colonic fermentation activity. Sampling was done at fasting, 15, 30, 45, 60, 90, 120, 150, 180 minutes after a standardized breakfast. Participants were directed to hold their breath deeply for 15 seconds and calmly blow through the mouthpiece of the machine.

Body weight: The weight was measured during the experimental day using Bosch E-Nr.PPW3300/01.

Stool sample: Participants were given containers marked with their study ID ('X00') and were instructed to deliver stool sample on the day of study. This was collected and stored at -80^{0}C in order not to lose its microbiological activity.

4.3 Experimental design

Participants consumed WWB and BKB for three days in two separate intervention periods, separated with a three week washout period. Thus, the study design enabled that each participant serves as his/her own control. The order of test products was randomly chosen. Participants were requested not to consume probiotics or antibiotics for at least four weeks before the study and during the study. Participants were asked to come at least 5 days before the start of experimental day to collect the product and were requested to freeze the samples as soon as possible to avoid defrosting. Each participant received a bag of products with 9 separate packs per product (3 portions a day for 3 days). Portions were consumed at approximately 8.00, 14.00 and 21.00, respectively. In the first two days each portion

contained 127.86 g of product BKB or 86.33 g of WWB which added up to 100g of available carbohydrate each day from the products (total of 384 g BKB or 259 g WWB). On the third day 100g of available carbohydrate was divided as 25g for morning and afternoon portions and 50g for the evening portion. During the intervention periods the participants were encouraged to standardize their meal pattern and to avoid alcohol, excessive physical exercise or foods rich in DF. Otherwise participants were encouraged to maintain their regular diet and record it in their food records questionnaire. They were given instructions on defrosting of breads and also were instructed about time for eating the breads. As shown in the figure 3, after the evening meal at 21.00 on the third day participants were required to fast overnight and until they arrive to the experimental unit the following morning.

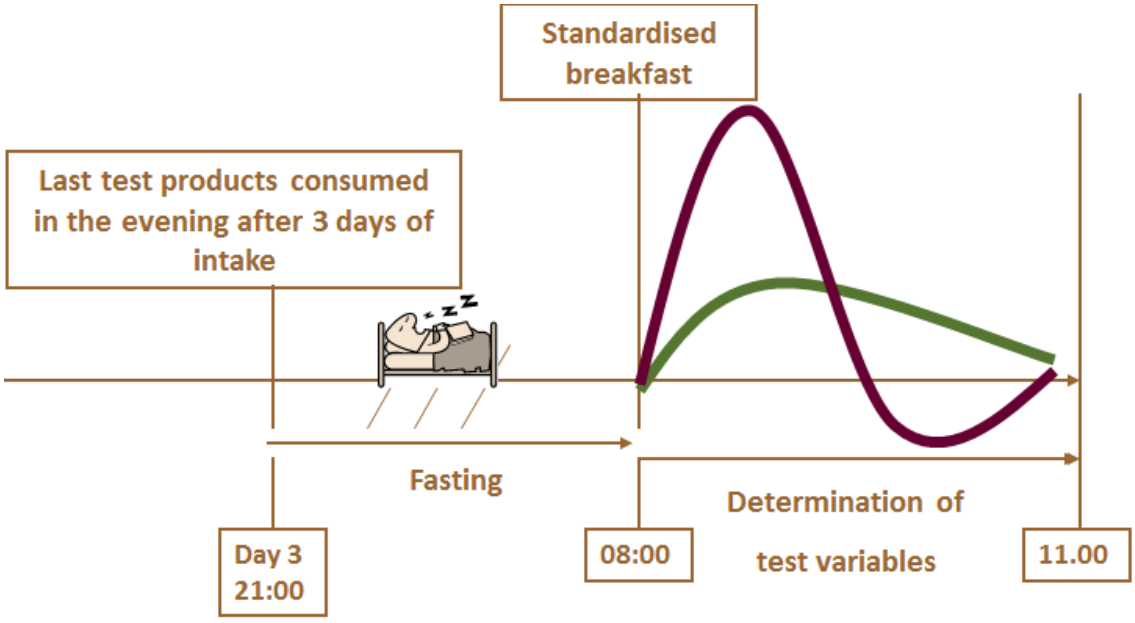

Figure 3: Experimental design

4.4 Experimental days

At the experimental day the test persons arrived in the morning around 7.15-7.30 am. Food records were collected and they were instructed how to use the breath hydrogen machine and how to fill out the VAS for subjective appetite registration. After a cannula was placed intravenously, the participants were allowed to relax for ten minutes and during this time they could fill out the forms and perform the breath hydrogen test before the blood pressure were measured. Fasting blood samples were then collected. They were then given 129.50 g standardized WWB, corresponding to 50 g available carbohydrate with 250 ml of tap water,

and were instructed to consume the breakfast within 10 to 12 minutes. After the breakfast blood samples were collected at an interval of 15, 30, 45, 60, 90, 120, 150, 180 minutes. At each of this time intervals also subjective appetite sensation and breath hydrogen was measured using hydrogen monitor (Gastrolyzer® Bedfont Scientific Ltd).

4.5 Evening test and reference meals, standardized breakfast

WWB was baked using home baking machine Severin model number 3893 according to procedure followed from (Sandberg, Björck, & Nilsson, 2016). The bread was made from 540g white wheat flour, 360g water, 4.8 g of yeast and 4.8 g of NaCl. Dry ingredients were weighed and mixed separately and slowly added to the warm water in the bowl placed inside the baking machine. Standardized breakfast included WWB that was similar to the reference bread.

BKB was made of 595g barley kernels, 105g wheat flour, 6g yeast and 5g salt. Barley kernels were cooked with 520g of water, cooled and mixed with other ingredients in the mixing machine. The dough was allowed to proof twice for 30 and 35 minutes respectively. The bread was baked for 60-70 minutes until the core temperature of the bread reached 96^0C. Then it was cooled for two hours wrapped in towel, later the bread was left in a plastic bag overnight and sliced in the next morning. The slices were weighed, wrapped in aluminium foil, and stored at -20^0C. The portion sizes are shown in Table 1.

Table 1: Portion sizes of WWB and BKB on Day 1, 2, 3 (based on available carbohydrates 100 g/day)

Product	**Day 1 & 2**	**Day 3**
WWB		
Breakfast:	86.3 g	64.5 g
Lunch:	86.3 g	64.5 g
Dinner:	86.3 g	129.5 g
BKB		
Breakfast:	127.86 g	96 g
Lunch:	127.86 g	96 g
Dinner:	127.86 g	192 g

4.6 Characterization of product

For the test, reference and standardized breakfast products total starch, resistance starch, soluble and insoluble fibers were determined. Total starch was determined by following I. M. Björck & M. A. Siljeström, 1992, resistance starch by following Åkerberg, Liljeberg, Granfeldt, Drews, & Björck, 1998 and dietary fiber by Asp, Johansson, Hallmer, & Siljestroem, 1983.

4.6.1 Analysis of total starch and available starch

In this protocol, we have analyzed total starch content. In this method 4 M KOH is added while determining the total starch content in order to disperse the resistant starch structures and hydrolyze it. By analyzing total starch and resistant starch (see below), we calculate the amount of available starch in the samples. This could be written in simple terms as:

Total starch – Resistance starch = Available starch

Initial step of pH adjustment was done by adding 10 ml of phosphate buffer to 500mg of powdered cereal sample. To this sample 4 M KOH is added and incubated in room temperature for 30 minutes with stirring once in 10 minutes. After 30 minutes, 5 ml of 5M HCl is added and adjusted to pH 6. Then Termamyl was added to cleave the 1-4 alpha amylosidic linkages in amylose and amylopectin. This mixture was allowed to react in boiling water bath for 20 minutes with a stirring of 2-3 times in between. After adjusting the dilution and pH the next enzyme amyloglucosidase was added. This mixture was incubated at 60^0C for 30 minutes and stirred at regular intervals. Amyloglucosidase sequentially releases glucose from the non-reducing ends of long starch molecules by breaking alpha 1, 4 and alpha 1, 6 linkages. A glucose oxidase peroxidase system was created in this mixture by addition of Glox to the samples. This system has a coupled enzymatic reaction in order to determine the glucose content. The absorbance in the final mixture is measured in the spectrophotometer at 450nm (I. M. E. Björck & M. A. Siljeström, 1992).

4.6.2 Resistant starch analysis

Resistant starch analysis involves a methodology for 3 days.

Day 1:

Each participant receives weighed amount of sample bread (containing 1g total starch). They are instructed to chew the sample 15 times within 15 seconds and to spit into the beaker.

After, saliva is collected in the mouth for 1 minute and spit into the beaker. Participants then rinse their mouth with 5ml of water and spit it out in the beaker with the sample. Finally, saliva is collected once more and spit in the beaker. While mixing the sample, 1 ml pepsin is added. The pH is adjusted to 1.5 by adding HCl and left to incubate at 37^0C for 30 minutes and stirred at 10 minutes interval. After the incubation the samples are adjusted to pH 5.0 with NaOH. Once the pH is adjusted, 125µl mineral solution, 125µl pancreatin solution, 400µl amyloglucosidase solution, 100µl isopropanol is added. A magnet is added to each beaker and water is added until the lower mark of the beaker. This is covered with parafilm and incubated in a hot water bath at 37^0C for 16 hours and stirring of 100 rpm.

Day 2:

Filtration was carried out in the filter device (Tecator, Fibertec System E, 1023 Filtration Module). Two bottles of warm 95% ethanol is prepared before the experiment. This warm ethanol is added to the beaker from overnight incubation and allowed to precipitate for 1 hour. The magnets from the beakers are carefully removed and rinsed with ethanol. One sample is treated at a time and poured into the funnel which is placed on top of the crucible in the filtration module. The samples are bubbled through and let to sink, once the bubbling has stopped filtration is started and the funnel and crucible are well rinsed. The same is followed with all the samples. After the filtration, the filter cake (crucible+celite), is rinsed with 95% ethanol and then 99% ethanol. The crucible and celite is left to stand in room temperature for two hours and kept at oven (105^0C) for overnight drying. Then the filtrates collected in the filtrate collection bottles are taken. These are transferred to a 500 ml flask and filled with water until the mark. This is mixed thoroughly as water and ethanol needs to be well mixed. 2.2ml of this filtrate solution is transferred to a 100 ml flask and water is added until the mark. This flask is kept closed with parafilm to avoid evaporation. 1 ml of sample from the flask is taken in duplicates in test tubes and 4ml of GLOX is added to all samples and they are incubated for one hour at room temperature and well mixed before analyzing in spectrophotometer. Glucose standards are prepared simultaneously and analyzed in spectrophotometer at 450nm.

Day 3:

The filter cakes are taken out of oven and kept at desiccator for one hour in room temperature. This is weighed and noted. The filter cakes are ground and analyzed for total

starch content as described above.

4.6.3 Dietary fiber analysis

This method uses the enzymatic breakdown of complex carbohydrates, proteins and the solubility property of some fibers in water. Filtration was carried out in the filter device (Tecator, Fibertec System E, 1023 Filtration Module). Insoluble fibers are separated first by glass filter crucibles. Soluble fibers are obtained from the precipitation of filtrate and in a second filtration it is separated with crucibles. The enzymes used include pepsin- (100mg/ml) and pancreatin suspension (50mg/ml) to mimic the human digestive enzymes. Initial step involves taking 1g finely ground sample in 500 ml beaker and suitable pH of 7 is adjusted by adding 25ml of 0.1N sodium phosphate buffer. When the solution is completely mixed, 40 µl Termamyl is added and the solution is incubated in a boiling water bath for 20 minutes with regular stirring. pH is adjusted to 1.5 in order to add pepsin-suspension and incubated at 40^0C for 60 minutes. For the next enzyme, pH is adjusted to a near neutral pH of 6.8±0.1 by addition of 1 N NaOH. To this mixture 1 ml pancreatin suspension is added and again incubated at 40^0C for 60 minutes. In the final step of enzyme incubation, 4-5ml of 0.5 N HCl is added in order to bring the solution close to isoelectric point of most proteins and avoids protein precipitation during fiber precipitation.

The content of the beakers are transferred to the filtration device. Insoluble fiber was first separated using Millipore water, 95% ethanol and 99% ethanol. The filtrate was used to obtain soluble fiber. Filtrate was precipitated with warm ethanol (95%) for an hour and then filtered using 78%, 95% and 99% ethanol. The crucibles from the filtering device were dried overnight at 105^0C and were cooled down the next morning by placing in the desiccator. The crucibles were weighed after they were cooled. The DF content was determined gravimetrical by subtracting celite weight, protein, ash and RS content from total weight of filter residue.

4.7 Statistical analysis:

Collected data from the study concerning blood glucose and subjective appetite was put into the program GraphPad Prism to obtain visualization of the data and to obtain values for the area under the curve (AUC), used for the statistical calculations. The program GraphPad was also used to visualize the hydrogen concentration data but mean values were obtained for statistical calculations instead of AUC. Both fasting values and incremental AUC (iAUC) for blood glucose and AUC for subjective appetite variables from VAS scale was statistically

analyzed by Minitab software. For breath hydrogen values, a weighted mean was calculated, i.e. by first calculating one mean per hour and then produce an overall mean. This was calculated in order to evaluate the mean based on equal time intervals since the number of time points within each hour differentiated. Additionally, the fasting and mean values for hydrogen were also statistically analyzed using Minitab. A two way ANOVA with "block design" (each person in separate blocks) was performed to evaluate if there were significant differences between the BKB and WWB with respect to affect blood glucose, subjective appetite variables and breath hydrogen values. Values of $P<0.05$ was considered as significant throughout the results.

5. Results

5.1 Chemical analysis of reference and test products

Results from the analysis in bread products of total starch content, available starch content, RS and DF (soluble and insoluble) are shown in table 2 below.

Table 2: Amount of available starch, resistant starch and dietary fibers in test and reference bread (as % dry matter)

	BKB	**WWB**
Total starch %	65.5	76.1
Available starch %	57.2	74.1
Resistant starch %	8.4	2.0
Insoluble Dietary fiber %	8.4	3.9
Soluble dietary fiber %	4.0	2.0
Total Non-starch dietary fiber %	12.4	5.9
Daily portion sizes g (based on 100g of available carbohydrate)	384	259
Resistant starch in g/day	14.7	2.7
Soluble dietary fiber g/day	7.0	2.7
Insoluble dietary fiber g/day	14.7	5.3
Total dietary fiber in g/day	36.4	10.7
Energy content (Kcal/100g)*	228.7	212

*Based on ingredient quantities

5.2 Blood glucose concentrations

The mean fasting blood glucose concentration for BKB was 5.50±0.1 mmol/L and for reference product was 5.4±0.1 mmol/L. No significant differences were seen in fasting glucose concentrations ($P>0.05$). The blood glucose response vs time can be seen in Figure 4 and Table 3. Since we do not see a significant difference in fasting values of blood glucose, the iAUC values were used to see the overall change in response. The 0-180 minutes iAUC after the standardized breakfast, following the evening meal with BKB was significantly less than that after the WWB evening meal (p-value = 0.001).

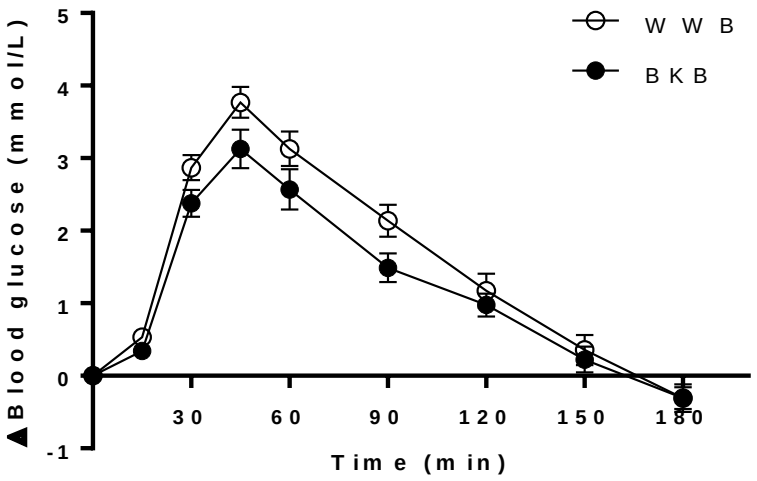

Figure 4: Blood glucose response for test and reference bread after an overnight fasting (0 min), after standard breakfast until 180 min

5.3 Breath hydrogen concentrations

The means for hydrogen levels at fasting in the morning and 0-180 min after the standardized breakfast were significantly higher after consuming BKB the previous evening than after consuming an evening meal of WWB ($p=0.001$ and $p<0.001$, respectively). Hydrogen mean values are presented in Table 3. Figure 5 shows the difference in breath hydrogen levels (ppm) vs time.

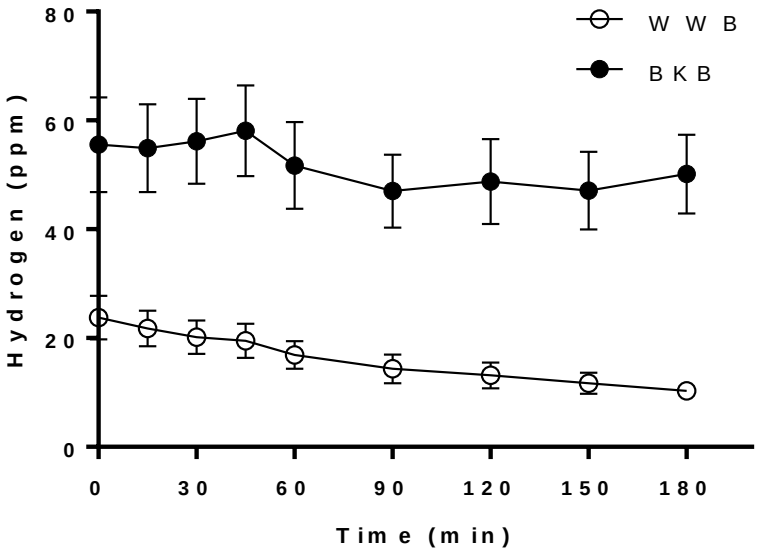

Figure 5: Breath hydrogen levels for test and reference bread after an overnight fasting (0 min) and after standard breakfast until 180 min

5.4 Appetite parameters (Satiety, hunger and desire to eat):

Fasting satiety levels after consuming BKB was significantly higher compared with after consuming WWB ($p<0.05$). Similar result was seen for fasting ($p<0.05$) and AUC of desire to eat ($p=0.05$), with desire to eat being significantly lower after the BKB evening meal. No significant difference was seen for hunger sensations but there were tendencies in breakfast postprandial iAUC (0-180 minutes) towards a lowered hunger sensations after the barley evening meal ($P=0.086$). The changes in appetite factors with respect to time could be seen in Figure 6, 7 and 8.

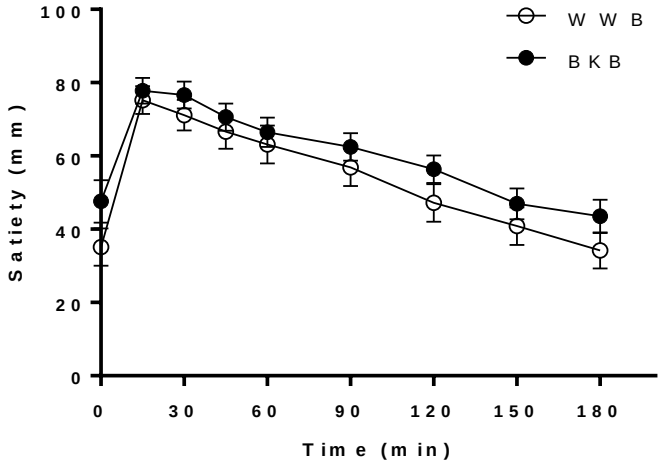

Figure 6: Satiety ratings (mean values) measured by VAS after an overnight fasting (0 min) and after standard breakfast until 180 min

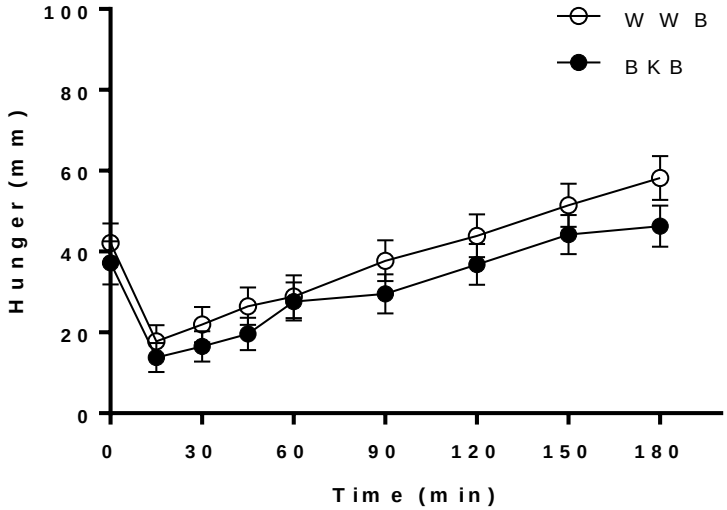

Figure 7: Hunger ratings (mean values) measured by VAS after an overnight fasting (0 min) and after standard breakfast until 180 min

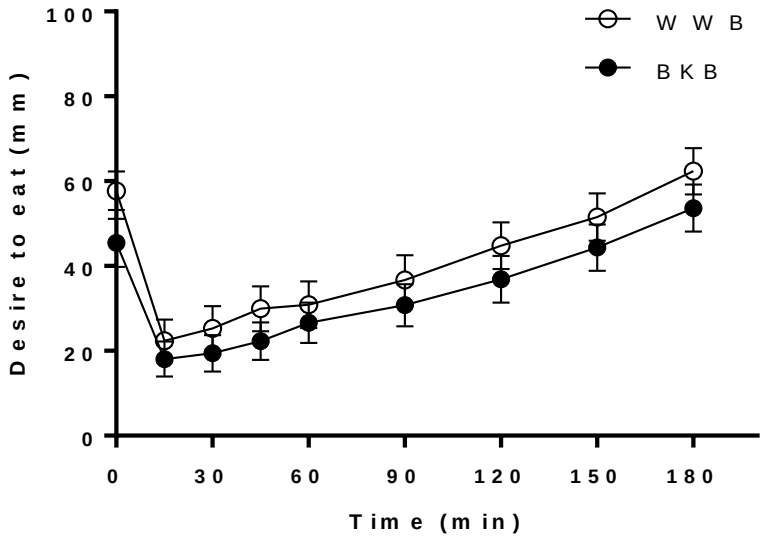

Figure 8: Desire to eat (mean values) measured by VAS after an overnight fasting (0 min) and after standard breakfast until 180 min

Table 3: Fasting glucose, glucose AUC, breath hydrogen levels and subjective appetite ratings (satiety, hunger and desire to eat) values

Test parameters	Test and reference product	
	BKB	WWB
	Mean±SEM	Mean±SEM
Fasting glucose (mmol/L)	5.5±0.1	5.4±0.1
Glucose iAUC (0-180 min) (mmol·min/L)	229.7±19.7**	296.0±22.9
Fasting breath Hydrogen (ppm)	55.6±8.7**	23.8±4.0
Hydrogen weighted mean (0-180 min) (ppm)	50.9±7.3***	14.9±2.2
Fasting satiety (mm)	47.6±5.8*	35.1±5.1
Satiety iAUC (0-180 min) (mm·min)	10915±6	9663±822
Fasting hunger (mm)	37.2±5.3	42.1±4.8
Hunger iAUC (0-180 min) (mm·min)	5618±768	6866±831
Fasting desire to eat (mm)	46.8±5.5*	57.9±4.5
Desire to eat iAUC (0-180 min) (mm·min)	6037±819*	7598±909

Significantly different from WWB: *P ≤ 0.05, ** P< 0.01, ***P<0.001

6. Discussion

This study was a randomized cross over study in order to investigate the effect of evening barley based meal on appetite, glucose response and gut microbial fermentation activity. Among 41 participants only 29 participants' data were analyzed and discussed within this thesis. The results from this study could allow us to understand more about effects of colonic fermentation of DF from barley, perspective of longer effects of fermentation (second meal effect) on glucose metabolism and appetite regulation.

From the chemical analysis of the BKB and WWB we can see that total- and available starch content in WWB was greater than BKB seen from table 2. This could mainly be accounted to that BKB had higher amount of DF components. There is difference in composition of nutrients in different grains, and starch and protein matrix differs between each type of grain. Therefore, during processing some grains are left with highly broken starch structures and some with intact structures (Herrera-Saldana, Huber, & Poore, 1990). The intact botanical structure of the barley kernels, and also the presence of strong aleuronic layer in the wholegrain makes it difficult for enzymes to access the starch, which increase the content of RS. This type of physically inaccessible starch is referred to as RS1. In addition to non-starch DF also the RS is fermented by the gut bacteria and give rise to increased SCFA. Also, the non-starch DF was higher in BKB compared with WWB. β-glucan is a soluble fiber found in comparatively higher amounts in wholegrain barley (4.2 % β-glucan oven dry basis) than in wholegrain wheat (0.63% β-glucan oven dry basis) (Henry, 1987). These soluble fibers are highly fermentable and used for producing SCFA's. Using refined wheat flour in our study accounts to lesser RS1 content as there is a mechanical breaking of botanical structures. However, this does not apply much in the case of barley, as we use kernels where the structure is preserved and the mechanical degradation is less.

The blood glucose response to the standardized breakfast following the evening meal with BKB was lower compared to after WWB evening meal. One important aspect of the observed results on blood glucose response is that, frequent hyperglycemic responses could result in increasing risk of type 2 diabetes. One explanation for reduced glucose response several hours after consumption of BKB could be the effect of metabolites produced from colonic fermentation. This suggestion is supported by the higher concentrations of breath hydrogen after the BKB compared to after WWB at the time of the breakfast. This result is also

consistent with previous finding of consumption of evening barley meal (A. Nilsson, Granfeldt, Ostman, Preston, & Bjorck, 2006), (Johansson, Nilsson, Ostman, & Bjorck, 2013). It has also previously been shown that there is a negative correlation between breath level hydrogen and blood glucose iAUC following a subsequent breakfast in the morning after consumption of a barley kernels based evening meal (Anne C Nilsson et al., 2008). Figure 6 shows an overview of schematic mechanism of SCFA's effect on glucose metabolism.

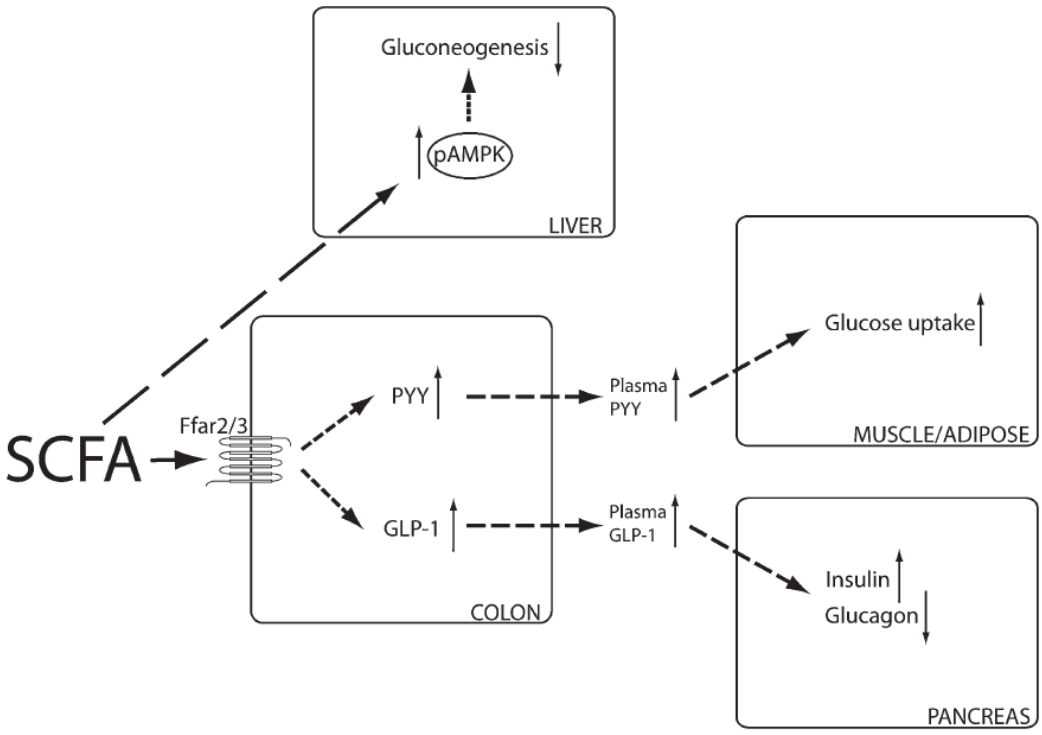

Figure 9: Schematic overview of SCFA's effect on glucose metabolism (Den Besten et al., 2013)

In this study, results showed that, compared to WWB, BKB has a beneficial effect on appetite sensations (increased feeling of satiety and lowered the desire to eat and hunger (trend for hunger)) 11 -14 hours after intake. The time elapsed between intake of BKB and the observed effects, and the pronounced increase in breath hydrogen concentrations, suggests that the effects on appetite variables could be due to fermentation of DF in BKB and increased production of SCFA. However, within this thesis work we did not analyse the SCFA concentrations but the increased hydrogen concentration indicates a higher fermentation activity after BKB. A possible increase in SCFA could result in increased release of satiety induced hormones such as GLP-1and PYY which partly could explain the improved appetite sensations. Although, this is just a hypothesis since these test variables have not been

analysed in this work but BKB has previously shown to increase PYY and GLP-1 10-16 hours after intake (Johansson, 2013).

The study design included that the participants consumed the test products at home and registered their food intake which did not allow control of compliance. However, by observing the breath hydrogen measurements an approximate indication of fiber intake was given and the higher hydrogen results after BKB compared to WWB indicate that the instructions regarding intake of test products were followed. In this study, the BKB showed positive effects on glucose response and subjective appetite sensations after only 3 days of consumption. Thus, for future studies it would be interesting to observe the effects of continuous BKB intake on cardiometabolic risk markers in e.g. a long-term study.

7. Conclusion

In this thesis in comparison to the WWB we could see beneficial effects in healthy people with consumption of evening barley kernel meal on appetite factors and glucose response after the following breakfast. The beneficial metabolic effects were accompanied by an increased gut microbial fermentation activity, as determined by increased breath H_2 excretion, pointing at underlying mechanisms emanating from colonic fermentation of the barley DF. The beneficial metabolic effects of barley kernel meal could therefore be a useful food item in the prevention of obesity and type 2 diabetes. Hence, including barley as well as protecting the biological structure by using intact kernel should be kept in mind while developing future wholegrain products.

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