Whole-grain Rye Foods: Effects on Appetite and Metabolism

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Abstract

Overweight, obesity and associated diet-related chronic diseases are increasing worldwide. Diet constitutes a modifiable risk factor for these conditions. Whole-grain consumption has been associated with health benefits, e.g. decreased risk of weight gain, diabetes and cardiovascular disease. Furthermore, whole-grain rye foods, rich in dietary fibres, have upon consumption shown increased satiety and lower postprandial glycaemia and insulinaemia compared with refined wheat.

The overall aim of the present thesis was to investigate how appetite and postprandial glycaemia and insulinaemia were affected by consumption of whole-grain rye porridge and crisp bread compared with isocaloric refined wheat bread, served as part of complete breakfasts. Effects of replacing part of the rye in whole-grain rye porridge with inulin and wheat gluten were evaluated on appetite, food intake, gut fermentation, postprandial glucose, insulin and GLP-1 during 8 h after intake. The effect of daily consumption of whole-grain rye porridge for three weeks was evaluated on appetite, food intake, gut fermentation and gut passage time during 8-12 h after intake. Moreover, the impact of unfermented and yeast-fermented whole-grain rye crisp bread on appetite, postprandial glucose and insulin responses was evaluated during 4 h after intake.

Whole-grain rye porridge reduced appetite during 4 h after intake compared with refined wheat bread, even after three weeks of daily consumption. The satiating effect of whole-grain rye porridge did not increase with added inulin and wheat gluten. The extensive gut fermentation of whole-grain rye porridge 4-8 h after intake was related to a lower second meal glucose response, but not to appetite, and no differences were observed in postprandial insulin and GLP-1 responses compared with refined wheat bread. Whole-grain rye crisp bread reduced appetite and postprandial insulin response during 4 h after intake compared with refined wheat crisp bread, with no differences in postprandial glucose, and the effect was larger for unfermented whole-grain rye crisp bread.

To conclude, whole-grain rye porridge and crisp bread have beneficial effects on appetite regulation and metabolic responses. Including whole-grain rye foods as part of a healthy diet may contribute to appetite control and decrease the risk of diet-related chronic diseases.

Keywords: satiety, hunger, desire to eat, hydrogen, SCFA, gut peptides

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Dedication

To my family

"...appetite turns common food into the fare of kings.”
Laurel Lea

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Lee I, Lin S, Webb DL, Hellström PM, Risérus U, Landberg R. Effects of Whole-Grain Rye Porridge with Added Inulin & Wheat Gluten on Appetite, Gut Fermentation and Postprandial Glucose Metabolism: A Randomized Cross-Over Breakfast Study (submitted).

II Isaksson H, Tillander I, Andersson R, Olsson J, Fredriksson H, Webb DL, Åman P. Whole grain rye breakfast - sustained satiety during three weeks of regular consumption. Physiology & Behaviour. 2012;105(3):877–884.

III Johansson DP, Lee I, Risérus U, Langton M, Landberg R. Effects of Unfermented and Fermented Whole Grain Rye Crisp Breads, Served as Part of a Standardized Breakfast, on Appetite and Postprandial Glucose and Insulin Responses: A Randomized Cross-over Trial. *PLoS One*. 2015;10(3):e0122241.

The contribution of Isabella Lee to the papers included in this thesis was as follows:

I Designed and planned the study in collaboration with the supervisors, developed the test products, performed the study, evaluated the results and had primary responsibility for writing and revising the paper.

II Participated in the design of the study in collaboration with the supervisors, planned and performed the study, developed the test products, developed the food registration booklets and analysed food intake, and contributed to revision of the paper.

III Participated in the design of the study in collaboration with the supervisors, planned and performed the study, and contributed to revision of the paper.

Abbreviations

CCK	cholecystokinin
GI	glycaemic index
GIP	glucose-dependent insulinotropic polypeptide
GL	glycaemic load
GLP-1	glucagon-like peptide-1
GP	glycaemic profile
h	hours
H_2	hydrogen
II	insulinaemic index
OEA	oleoylethanoamine
PPAR-α	peroxisome-proliferator-activated receptor-alpha
PYY	peptide YY
SCFA	short-chain fatty acids
VAS	visual analogue scale
w	weeks

1 Introduction

One of the major public health problems world-wide is the dramatic increase in overweight and obesity among adults and children (WHO 2014). It is estimated that among the world's adult population, 39% are overweight and 13% are obese, and 42 million children under the age of five are overweight or obese (WHO 2015).

Overweight and obesity are associated with metabolic disturbances such as impaired glucose and lipid metabolism, insulin resistance and raised blood pressure that greatly increase the risk of diet-related chronic diseases, such as diabetes, cardiovascular disease and certain cancers (Martin-Rodriguez et al. 2015; Pi-Sunyer 2002; WHO 2005). The alarming increase in overweight and obesity world-wide, together with the enormous economic burden of chronic diseases (WHO 2014), calls for new preventive strategies to halt or reverse the trajectory.

An increase in weight is caused by an imbalance between energy intake (food consumption) and energy expenditure (physical activity), and research indicates that excess food consumption makes a larger contribution to the increased risk of weight gain than physical inactivity (Vandevijvere et al. 2015). In today's obesogenic society there is an abundance of cheap, energy-dense food products that are easily overconsumed thereby causing negative metabolic effects (Van Kleef et al. 2012; WHO 2005).

Foods play an important role in the prevention of chronic diseases and their risk factors. Epidemiological studies have consistently shown inverse associations between whole-grain food intake and type 2 diabetes, cardiovascular disease, certain cancers and all-cause mortality (Aune et al. 2011; Johnsen et al. 2015; Ye et al. 2012; Wu et al. 2015). Whole grain intake has also been associated with improved weight management in epidemiological studies, but results from intervention studies are less clear (Pol et al. 2013). The causality of the observed associations has not been established. Among

whole-grains commonly consumed, whole-grain rye contains the highest amounts of dietary fibre (Aman 2010). Dietary fibres are believed to exert beneficial effect on weight management and chronic diseases through appetite reduction, attenuated glucose response and decreased insulin resistance (Smith & Tucker 2012).

In this context, an increased understanding of the physiological processes by which food products, based on whole-grain rye in particular, influence appetite and metabolism could facilitate the development of food products with increased satiating capacities and favourable metabolic effects that limit food intake and decrease the risk of diet-related chronic diseases (Bellisle & Blundell 2013; WHO 2005).

1.1 The Satiety Cascade

The Satiety Cascade (Figure 1), developed by Blundell, Rogers and Hill in the late 1980s and continuously modified according to new knowledge, offers a theoretical framework for assessing the physiological processes involved in appetite regulation (Bellisle & Blundell 2013; Blundell et al. 2010). According to the Satiety Cascade theory, a sequence of sensory, cognitive, postingestive and postabsorptive signals is generated in the mouth, stomach, intestine, pancreas and fat tissue when food is consumed that affects appetite centres in the brain via neuronal and humoral pathways (Bellisle & Blundell 2013; Blundell et al. 2010; Cummings & Overduin 2007).

Two terms are widely used when describing these events: *satiation*, which refers to the processes that result in termination of an eating occasion, thus affecting the amount of food consumed within a meal, and *satiety,* which refers to processes that reduce hunger, increase fullness and inhibit the motivation to eat between two eating occasions, thereby affecting meal frequency (Bellisle & Blundell 2013; Blundell et al. 2010; Livingstone et al. 2000). Together, satiation and satiety determine the *satiating capacity* of a food product, i.e. a food product's ability to inhibit the motivation to eat and reduce food intake (Bellisle & Blundell 2013; Livingstone et al. 2000).

According to Blundell et al. (2010), *hunger* can be described as the conscious urge to eat with physical sensations, e.g. stomach emptiness, while *fullness* is described as the conscious sensation of being completely filled, e.g. having a full stomach (Blundell et al. 2010; Blundell 2012). Although there is currently no agreed definition (Blundell et al. 2010), the term *appetite* is often used when describing all the processes that together influence eating (Blundell 2012).

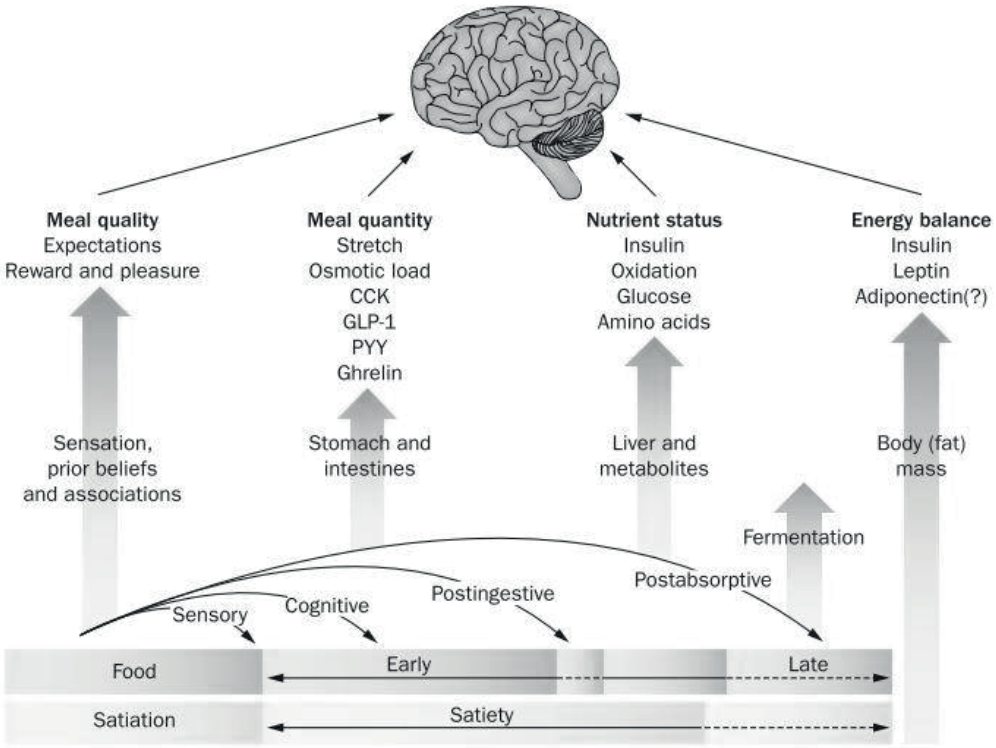

Figure 1. The Satiety Cascade conceptualises underlying psychological and physiological processes involved in satiation and satiety (Blundell 2010). Permission obtained from the Nature Publishing Group ©.

Before food arrives in the stomach, sensory and cognitive responses emanating from the mouth, nose and eyes act together with physiological responses in the stomach and intestine to control food intake, thereby inducing satiation and early satiety (Chambers et al. 2015). After food has entered the mouth, the oro-sensory stimulation caused by mouth exposure and chewing signals satiation and early satiety (Chambers et al. 2015; Van Kleef et al. 2012).

When food enters the stomach, increased gastric distention and motility inhibit hunger and signal satiation and early satiety (Cummings & Overduin 2007; Näslund & Hellström 2007; Van Kleef et al. 2012). As food is digested, there is an almost instantaneous release of gut peptides, e.g. cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1) and peptide YY (PYY), in response to nutrients reaching the duodenum that affect satiation and satiety. While the satiating peptides are stimulated by meal intake, the gut peptide ghrelin, which is associated with hunger and meal initiation, is inhibited (Bruen et al. 2012). The gut peptides act together to inhibit gastric emptying and limit meal size to

ensure optimal digestion and absorption of nutrients, which further induces satiety in the early postingestive stage (Bruen et al. 2012; Cummings & Overduin 2007).

The gut peptides can also directly induce satiation and satiety via neuronal and hormonal pathways in response to ingested nutrients, which also contributes to enhanced postprandial satiety (Cummings & Overduin 2007; Van Kleef et al. 2012). In addition, in response to ingested nutrients, gut peptides activate a feed-back signal in the distal small intestine, called the ileal brake. The ileal brake inhibits gastric emptying, delays gut passage time and prolongs the sensing and absorption of nutrients, thereby enhancing postingestive satiety (Maljaars et al. 2008).

Furthermore, postabsorptive events such as the oxidation of nutrients, circulating metabolites, hormones and gut fermentation products enhance postprandial, i.e. postmeal, satiety (Van Kleef et al. 2012). The adipose tissue also contributes to food intake regulation by stimulating release of the hormones leptin and insulin, which by interacting with the gut peptide signalling pathways induce satiety (Cummings & Overduin 2007; Van Kleef et al. 2012). This thesis focuses mainly on the postingestive and postabsorptive physiological aspects of appetite regulation.

1.2 Measuring appetite

Much effort has been dedicated to developing uniform methodologies to evaluate and quantify appetite and the underlying mechanistic processes by which food products inhibit the motivation to eat and reduce food intake. This is essential in order to allow comparisons between studies and to substantiate research findings (Blundell 2010; Chapelot 2013).

Short-term (24 h) intervention studies are often conducted to investigate the acute effects of different food products on appetite and metabolic responses. The food products, or meals, are matched with regard to, e.g. energy content, appearance, size and/or macronutrient composition, depending on what is being investigated (Livingstone et al. 2000). Before and after consumption of the test foods, subjective measurements of appetite are repeatedly made at regular time intervals for a fixed period depending on whether early or late responses are being investigated (Blundell et al. 2010; Livingstone et al. 2000). Furthermore, the effects of underlying physiological satiating events on voluntary food intake may be investigated by serving a subsequent *ad libitum* meal or asking participants to self-report their food intake, although the latter method is not recommended due to the risk of, e.g. under-reporting (de Graaf et al. 2004; Livingstone et al. 2000).

The most common way of measuring and quantifying subjective appetite is by visual analogue scale (VAS), which has been shown to be sensitive and reproducible (Mars et al. 2012; Stubbs et al. 2000). The VAS is an easy-to-use measurement tool where participants are asked appetite-related questions (e.g. How hungry are you?) and respond by placing a vertical mark along a horizontal line (100-150 mm) that has extremes anchored at each side (e.g. not at all hungry vs. as hungry as I have ever felt), thereby giving an appetite rating (Livingstone et al. 2000; Stubbs et al. 2000). The ILSI Task Force on Appetite Regulation recommends a set of questions addressing subjective feelings of hunger, fullness, satiety, desire to eat and prospective consumption (Blundell et al. 2010). The VAS can either be completed using pen and paper, or using a validated electronic version (Stubbs et al. 2000; Whybrow et al. 2006).

In addition to VAS, repeated blood sampling can be conducted to investigate contributions of appetite-associated metabolites (Blundell et al. 2010). There has been considerable interest in finding biomarkers for appetite, i.e. physiological measures that can be easily measured with high accuracy and serve as causal factors for subjective appetite. While most of the gut peptides can be used as indicators of appetite in mechanistic explanation models none can be used as a predictor for actual appetite or food intake (de Graaf et al. 2004; Mars et al. 2012).

1.3 Influence of food properties on appetite regulation

From a health perspective, there is great interest in developing food products that enhance satiety, i.e. that reinforce the physiological processes involved in postingestive and postabsorptive appetite signalling, by manipulating different aspects of the food product (Bellisle & Blundell 2013; Chambers et al. 2015; Van Kleef et al. 2012). Appetite is affected by physiochemical aspects of food related to microstructural features, volume and energy density, and type of macronutrient, which in turn influence both the kinetics and extent of digestion and absorption of nutrients (Lundin et al. 2008).

The effect of food microstructure on appetite and metabolic responses involves how the constituent components of food products are altered during processing and how this affects digestion and absorption of nutrients. Whereas, the microstructure of food can be investigated by, e.g. microscopy, determining the effect of microstructure on digestion and absorption in vivo is more difficult and possible interactions are less well understood (Lundin et al. 2008). In vitro models are therefore often developed and applied (Alminger et al. 2012).

A food product with high energy density is in general less satiating and more palatable than a less energy-dense food, which increases the risk of overconsumption (Poppitt 2013; Van Kleef et al. 2012). Energy density is by definition the energy content of the food product divided by its weight (Van Kleef et al. 2012). The macronutrients (protein, fat and carbohydrates) increase the energy density by providing energy, while water, air and dietary fibre decrease the energy density by increasing the volume of the food (Poppitt 2013; Van Kleef et al. 2012).

When food products are consumed in isocaloric mixed meals, a satiety hierarchy among the macronutrients emerges with protein being the most satiating, followed by carbohydrates, and fats the least satiating (Chambers et al. 2015). Macronutrient composition has been identified as a key element to enhanced satiety and reduced food intake, and has been pointed out as more important than palatability and physiochemical features of food such as form and volume (Fromentin et al. 2012).

Protein-induced satiety

The underlying postprandial satiating processes that are induced by protein ingestion are not fully understood, but digested proteins and amino acids are believed to stimulate secretion of satiating gut peptides (Davidenko et al. 2013). Protein is also considered to enhance satiety by increasing substrate oxidation, i.e. diet-induced thermogenesis, and by stimulating gluconeogenesis that improves glucose homeostasis (Veldhorst et al. 2008; Veldhorst et al. 2012). Protein-induced satiety is possibly affected by the amino acid kinetics in the blood, where an elevated concentration of amino acids induces satiety (Veldhorst et al. 2008). More specific, circulating concentrations of certain amino acids, e.g. the branched-chain amino acid leucine, may also directly stimulate appetite centres in the brain or act as neurotransmitter precursors (Davidenko et al. 2013). It has been hypothesised that dietary proteins and peptides interact with the gut microbiota, affecting its composition and the production of fermentation products, which could possibly also affect satiety (Jahan-Mihan et al. 2011).

Although protein is considered the most satiating of the macronutrients, enhancing late postingestive satiety efficiently (Lang et al. 1998; Veldhorst et al. 2008), the role of proteins from different food sources is unclear (Gilbert et al. 2011). Protein from animal sources contains all the essential amino acids and is generally digested more quickly than plant proteins, suggesting a higher satiating capacity (Gilbert et al. 2011; Veldhorst et al. 2008). However, in a study by Lang et al. (1998), no difference in appetite or food intake was observed between animal proteins (egg, casein and gelatine) and plant proteins

(soy, pea and wheat gluten), when consumed in mixed meals. Similarly, a more resent study observed no difference in appetite or food intake between the animal protein whey and the plant proteins soy and wheat gluten consumed as soups (Bowen et al. 2006). Unfortunately, detailed studies on the effects of protein from plant sources on appetite are lacking (Gilbert et al. 2011).

Fat-induced satiety

Dietary fat delays gastric emptying which in turn induces satiation and early satiety. Moreover, fatty acids inhibit gastric emptying by stimulating secretion of satiating gut peptides. Fat intake has also been proposed to stimulate the satiety-inducing ileal brake mechanism since the distal part of the intestine is a site for fatty acid conversion (Hennink & Maljaars 2013; Van Kleef et al. 2012). In addition, consumption of fats, especially polyunsaturated fatty acids, stimulates the release of the satiating lipid messenger oleoylethanoamine (OEA), which via the peroxisome-proliferator-activated receptor-alpha (PPAR-α) inhibits food intake in animals (Hennink & Maljaars 2013; Moussavi et al. 2008). The satiating effect of fats has been suggested to depend on fat quality, which determines the oxidation rate and thereby influences satiety, although the mechanism remains to be revealed (Moussavi et al. 2008).

Carbohydrate-induced satiety

Different appetite responses are observed depending on the type of carbohydrates present in the food product. Available carbohydrates, including simple sugars and starch, are rapidly or slowly degraded by human digestive enzymes (Poppitt 2013). It has been proposed that slowly degraded carbohydrates enhance satiety by producing less fluctuating blood glucose concentrations (Flint et al. 2007), but other physiochemical factors most likely also play a role (Peters et al. 2011). Flint et al. (2007) suggested that it is not the actual glucose kinetics, but rather the secretion of insulin in response to food intake, that induces satiety. Insulin can affect appetite by stimulating appetite centres in the brain, interacting with satiety gut peptides and modulating substrate oxidation in the liver (Flint et al. 2007). Available carbohydrates are also believed to stimulate the ileal brake mechanism (Poppitt 2013). Moreover, fluctuations in stored glycogen have been proposed to affect appetite, with a positive carbohydrate balance signalling satiety. However, the underlying mechanism for this potential neural sensing of energy stores is unknown (Hopkins et al. 2011).

Dietary fibres, i.e., carbohydrates that are not degraded by human digestive enzymes, have large variability in physiochemical properties such as water solubility, viscosity, bulking and fermentability, that affect appetite and food

intake (Burton-Freeman 2000; Schneeman 1999). It is not clear if there is a difference in satiating capacity between different types of dietary fibres, and physiochemical properties such as particle size may have a larger influence on appetite than the dietary fibre dose (Clark & Slavin 2013). Moreover, dietary-fibre rich foods, such as whole-grains, also contain bioactive compounds that may affect appetite (Wanders et al. 2011).

Dietary fibres can enhance satiety and decrease food intake by affecting several physiological processes related to appetite regulation. Dietary fibres increase chewing and mouth exposure time, especially larger particle sizes (Clark & Slavin 2013; Wanders et al. 2011). The generally large water-holding capacity of dietary fibres decreases energy density and increases gastric distention, especially viscous dietary fibres (Smith & Tucker 2012). Furthermore, dietary fibres delay gastric emptying, decrease digestion rates and delays gut passage time (Wanders et al. 2011). As previously mentioned, the prolonged nutrient sensing in the gut stimulates the release of satiating gut peptides and the ileal brake mechanism (Lyon & Kacinik 2012).

Some dietary fibre types are fermented by the gut microbiota, resulting in the production of short-chain fatty acids (SCFA) and gases such as hydrogen that can be used as an indicator of gut fermentation, since this is the only known source of hydrogen in humans (Rumessen 1992; Schneeman 1999). In comparison with insoluble and bulking dietary fibre, soluble dietary fibres with a high water-holding capacity, e.g. inulin and oligofructose, are rapidly fermented by the gut microflora (Delzenne et al. 2010; Lyon & Kacinik 2012; Schneeman 1999). The produced SCFA are believed to enhance satiety by modifying gut motility and the release of satiating gut peptides, as well as acting directly on appetite centres in the brain, although further research is needed to establish causality (Bruen et al. 2012; Kristensen & Jensen 2011; Lyon & Kacinik 2012).

1.4 Postprandial glycaemia and insulinaemia

As discussed above, the postprandial metabolic response to food intake is influenced by macronutrient composition, physiochemical properties and energy content, which are important determinants of blood glucose kinetics and insulin response (Blaak et al. 2012; Thomas & Pfeiffer 2012). Even with the fine-tuned system for regulating glucose, fluctuating postprandial glucose concentrations are understood to be an important element in the development of insulin resistance (Blaak et al. 2012). Food products that exert lower postprandial glycaemic and insulinaemic responses have been linked to

beneficial effects on chronic disease risk (Blaak et al. 2012; Riccardi et al. 2008).

Blood glucose homeostasis is tightly regulated by several processes involving the gut, liver, pancreas, muscle, adipose tissue and the brain that ensure efficient absorption, metabolism and storage of glucose (Blaak et al. 2012; Thomas & Pfeiffer 2012). Incretins, such as GLP-1, are specific gut peptides that potentiate insulin secretion and inhibits glucagon release, thereby lowering postprandial glycaemia (Blaak et al. 2012; Brubaker & Anini 2003). GLP-1 is released from L-cells localized mainly in the distal part of the gut in response to carbohydrate and fat intake, and protein intake if co-ingested with carbohydrates (Brubaker & Anini 2003; Bruen et al. 2012). The secretion is also stimulated by SCFA that can bind to and increase the number of L-cells in the intestine (Bruen et al. 2012). Furthermore, SCFA can lower glucose levels by affecting glucose production in the liver and lower the glucose response after a subsequent meal, a phenomenon referred to as the second meal effect (Brighenti et al. 2006; Delzenne et al. 2013; Russell et al. 2013). Also, a recent study highlights the role of the gut microbiota as a determinant of interpersonal variation in glycaemic responses (Zeevi et al. 2015). Moreover, research has revealed bile acid signalling to be an important regulator of postprandial glycaemia through effects on incretin secretion and interactions with the gut microbiota (Li & Chiang 2015).

The most important nutrient in the diet that affects postprandial glucose and insulin responses is the type and amount of carbohydrates (Blaak et al. 2012). Available carbohydrates that are rapidly digested increase the blood glucose response, while indigestible dietary fibres slow down the absorption rate of glucose (Riccardi et al. 2008). Moreover, circulating amino acids, especially branched-chain amino acids, stimulate the secretion of insulin from the pancreas, thereby attenuating blood glucose levels by increasing glucose uptake in muscle and adipose tissue (Jahan-Mihan et al. 2011). In contrast, fat influences glycaemia and insulinaemia by delaying gastric emptying and gut passage time (Maljaars et al. 2008; Wolever et al. 1991).

To determine the impact of food on glucose concentrations, the glycaemic index, glycaemic load and, more lately, the glycaemic profile have been introduced (Blaak et al. 2012). The glycaemic index measures the postprandial glucose levels in response to the amount of available carbohydrates present in the food (Jenkins et al. 1981), but does not take into account the amount of protein, fat and dietary fibre in the food. The glycaemic load, on the other hand, takes these factors into consideration as it is a product of the glycaemic index and the content of available carbohydrates in a portion of food (Blaak et al. 2012). The glycaemic profile takes into account the shape of the blood

glucose curve, where a prolonged postprandial glucose response above fasting is suggested to improve insulin sensitivity (Rosén et al. 2009).

Consequently, several factors influence the glycaemic response, such as macronutrient content, food form, processing and microstructure (Wolever et al. 1991), but also the amount of food and meal frequency. Eating several small meals results in prolonged nutrient absorption with less fluctuating glucose levels, thereby lowering glycaemia and insulinaemia (Jenkins & Jenkins 1995; Lundin et al. 2004). Thus, it is important to evaluate the effect on the glycaemic response from intake of test foods as part of a complete meal, as results obtained from single food studies may not be applicable to real-life situations (Riccardi et al. 2008).

1.5 The effects of rye foods on appetite and postprandial metabolism

Whole-grain rye is an important ingredient in soft bread, crisp bread, porridge and breakfast cereal in Northern and Eastern European countries. Whole-grain rye is a rich source of both soluble and insoluble dietary fibre; the β-glucan and fructan content is rapidly fermented, while arabinoxylan is slowly fermented (Aman 2010; Rakha et al. 2011). In comparison with wheat, rye contains more fermentable dietary fibre, and less cellulose (Aman 2010). The physiochemical properties (content, structure and solubility) of the dietary fibres in whole-grain rye are to some extent altered during processing, such as baking, boiling and fermenting (Aman 2010; Rakha et al. 2011). The effect on appetite and metabolic responses from whole-grain rye is most likely mediated by the large content of dietary fibre, but may also be due to bioactive compounds present in the kernel and structural properties (Isaksson et al. 2011; Juntunen et al. 2003; Kamal-Eldin et al. 2009; L. A. Rosén et al. 2011).

The short-term effects of rye foods compared to refined wheat products on appetite and metabolic responses in healthy individuals are summarised in Table 1, wherein a wide range of food products, such as porridge, boiled kernels, crisp bread, soft bread and beverage, are represented. The majority of the food products are made from the whole grain, whereas part of the grain or only bran is used occasionally.

Nine out of ten studies report lower appetite from porridge, boiled kernels, crisp bread and soft breads; four also report enhanced late satiety from intake of porridge and soft bread. Although intake of an evening meal of boiled kernels and soft bread did not lower appetite, lower food intake and higher gut fermentation were observed the next day. Both studies investigating the effect on gut fermentation from intake of boiled kernels and soft bread report higher

gut fermentation. Three out of four studies report lower subsequent food intake from intake of boiled kernels, crisp bread and soft bread.

Twelve studies report effects on postprandial glycaemia and insulinaemia, while two studies report only glucose response. The three studies investigating the effect of porridge and boiled kernels report lower glucose and insulin response. The majority of studies investigate the effect of soft bread and, whereas, some report lower glucose and insulin, others only report lower insulin response. This is possibly because of the rye variety and processing method used in the studies. Bran added to a drink together with refined wheat bread lowered glucose and insulin response. One study examining the effect of crisp bread report no effect on glucose and insulin response. Only two studies report the effect on the gut peptide GLP-1, and there seems to be an effect of both time and food structure.

To conclude, few previous studies have investigated the effect of whole-grain rye porridge on appetite, metabolic responses and gut fermentation in healthy individuals (Nilsson et al. 2008; L. A. Rosén et al. 2011; Rosén et al. 2009). In addition, the effect of whole-grain rye crisp bread on appetite and food intake has only been investigated in two previous studies in healthy individuals (Forsberg et al. 2014; Leinonen et al. 1999), although using different processing methods. Moreover, the importance of gut fermentation for appetite and metabolic responses has not yet been fully elucidated and additional benefits of adding a combination of fermentable dietary fibre and plant protein have not been evaluated.

Table 1 Short-term, cross-over studies on appetite, voluntary food intake, metabolic responses and gut fermentation in healthy men and women

Subjects	*Test and control foods; weight, df and energy content per portion*	*Duration*	*Appetite*	*Ad libitum food intake*	*Glucose*	*Insulin*	*GLP-1*	*Hydrogen & SCFA*	*Reference*
men and women (n = 20)	**Porridge** milled whole rye kernels (66 g; 11.7; 950 kJ)	8 h	Hunger ↓ (0-4 h) Satiety ↑ (0-4; 4-8 h) Desire to eat ↓ (0-4 h)						(Isaksson et al. 2011)
	Porridge whole rye kernels (66 g; 10.7 g; 900 kJ)		Hunger ↓ (0-4; 4-8 h) Satiety ↑ (0-4; 4-8 h) Desire to eat ↓ (0-4; 4-8 h)						
	Control: Bread refined wheat (86 g; 3.4 g; 950 kJ)								
men and women (n = 12)	**Porridge** whole-grain rye flour (51.1 g; 10.1 g; NA)	180 min	Satiety ↑ (0-180 min)		Glucose ↓ (0-120 min) GI ↓	Insulin ↓ (0-120 min)			(Rosén et al. 2009)
	Porridge sifted rye flour (45.4 g; 6.5 g; NA)		Satiety ↑ (0-180 min)		Glucose ↓ (0-120 min) GI ↓	Insulin ↓ (0-120 min) II ↓			
	Control: Bread refined wheat (101.1 g; 1.8 g; NA)								

Table 1 Short-term, cross-over studies on appetite, voluntary food intake, metabolic responses and gut fermentation in healthy men and women

Subjects	*Test and control foods; weight, df and energy content per portion*	*Duration*	*Appetite*	*Ad libitum food intake*	*Glucose*	*Insulin*	*GLP-1*	*Hydrogen & SCFA*	*Reference*
men and women (n = 22)	**Porridge** whole-grain rye flakes (62 g; 9.0; 1683 kJ) with **pasta** whole-grain wheat (100 g; 12.6 g; 2306 kJ) for lunch	8 h Appetite registrations continued after the *ad libitum* meal until bedtime.	Hunger ↓ (0-210; 240-450 min) Satiety ↑ (0-210; 240-450 min) Desire to eat ↓ (0-210; 240-450 min)	No difference 8 h after breakfast					(Isaksson et al. 2008)
	Porridge whole-grain rye flakes (62 g; 9.0; 1683 kJ) with **pasta** refined wheat (94 g; 5.9 g; 2302 kJ) for lunch		Hunger ↓ (0-210; 240-450 min) Satiety ↑ (0-210; 240-450 min) Desire to eat ↓ (0-210; 240-450 min)	No difference 8 h after breakfast					
	Control: Bread refined wheat (71 g; 2.6 g; 1658 kJ) with **pasta** refined wheat (94 g; 5.9 g; 2302 kJ) for lunch								
men and women (n = 12)	**Boiled** whole rye kernels (147 g; 23.1 g; 2475 kJ)	Test food eaten in the evening. Standardized breakfast of refined wheat bread the next day followed 3 h measurements	No difference	Food intake ↓ 3 h after a standardized breakfast				H_2↑ SCFA ↑	(Ibrügger et al. 2014)
	Control: Bread refined wheat (111 g; 7.0 g; 2475 kJ)								

Table 1 Short-term, cross-over studies on appetite, voluntary food intake, metabolic responses and gut fermentation in healthy men and women

Subjects	*Test and control foods; weight, df and energy content per portion*	*Duration*	*Appetite*	*Ad libitum food intake*	*Glucose*	*Insulin*	*GLP-1*	*Hydrogen & SCFA*	*Reference*
men and women (n = 10)	**Boiled** whole rye kernels (106.6 g; 36.4 g; 1066 kJ)	270 min	Hunger ↓ (0-60; 60-120; 120-210; 210-270 min) Fullness ↑ (60-120; 60-120; 120-270 min) Desire to eat ↓ (0-60; 60-120; 120-210; 210-270 min)	Food intake ↓ 270 min after breakfast	Glucose ↓ (0-60 min) GI ↓ GP ↑ Glucose peak ↓	Insulin ↓ (0-60; 60-120; 120-270 min) Insulin peak ↓ II ↓		H_2↑ (120-270; 270-390 min)	(L. A. Rosén et al. 2011)
	Control: Bread refined wheat (124.0 g; 6.0 g; 1015 kJ)								
men and women (n = 12)	**Boiled** whole rye kernels (93.9 g; 17.9 g; NA)	11.5 h Standardized lunch 4 h after breakfast Standardized dinner of refined wheat bread 9.5 h after breakfast			Glucose ↓ (0-120 min after breakfast. lunch and dinner) GI ↓ Glucose peak ↓			H_2↑ (4-6 h)	(Nilsson et al. 2008)
	Control: Bread refined wheat (118.9 g; 5.1 g; NA)								

Table 1 Short-term, cross-over studies on appetite, voluntary food intake, metabolic responses and gut fermentation in healthy men and women

Subjects	*Test and control foods; weight, df and energy content per portion*	*Duration*	*Appetite*	*Ad libitum food intake*	*Glucose*	*Insulin*	*GLP-1*	*Hydrogen & SCFA*	*Reference*
men and women (n = 21)	**Crisp bread** whole-grain rye (80 g; 13 g; 1188 kJ)	4 h	Hunger ↓ (0-240 min) Fullness ↑ (0-240 min) Desire to eat ↓ (0-240 min)	No difference					(Forsberg et al. 2014)
	Control: Bread refined wheat (108 g; 3.8 g; 1180 kJ)								
men and women (n = 20)	**Crisp bread** whole-grain rye (64 g; 10 g; 953 kJ)	4 h	Hunger ↓ (0-240 min) Fullness ↑ (0-240 min) Desire to eat ↓ (0-240 min)	Food intake ↓					(Forsberg et al. 2014)
	Control: Bread refined wheat (86 g; 2.6 g; 936 kJ)								
men and women (n = 20)	**Crisp bread** whole-meal rye (79.4 g; 12.1 g; 994 kJ)	180 min			No difference	No difference			(Leinonen et al. 1999)
	Control: Bread refined wheat (121.1 g; 2.3 g; 1341 kJ)								

Table 1 Short-term, cross-over studies on appetite, voluntary food intake, metabolic responses and gut fermentation in healthy men and women

Subjects	*Test and control foods; weight, df and energy content per portion*	*Duration*	*Appetite*	*Ad libitum food intake*	*Glucose*	*Insulin*	*GLP-1*	*Hydrogen & SCFA*	*Reference*
men and women (n = 12)	**Bread** whole rye kernels (143 g; 18.6 g; 2475 kJ)	Test food eaten in the evening. Standardized breakfast of refined wheat bread the next day followed 3 h measurements	No difference	Food intake ↓ 3 h after a standardized breakfast				H_2↑ SCFA ↑	(Ibrügger et al. 2014)
	Control: Bread refined wheat (111 g; 7.0 g; 2475 kJ)								
men and women (n = 24)	**Bread** milled whole rye kernels (158 g; 13.0 g; 1650 kJ)	8 h	Satiety ↑ (0-4; 4-8 h) Desire to eat ↓ (4-8 h)						(Isaksson et al. 2011)
	Bread whole rye kernels (156 g; 11.0 g; 1600 kJ)		Hunger ↓ (4-8 h) Satiety ↑ (0-4; 4-8 h) Desire to eat ↓ (0-4; 4-8 h)						
	Control: Bread refined wheat (144 g; 5.3 g; 1650 kJ)								

Table 1 Short-term, cross-over studies on appetite, voluntary food intake, metabolic responses and gut fermentation in healthy men and women

Subjects	*Test and control foods; weight, df and energy content per portion*	*Duration*	*Appetite*	*Ad libitum food intake*	*Glucose*	*Insulin*	*GLP-1*	*Hydrogen & SCFA*	*Reference*
men and women (n = 16)	**Bread** rye bran (133 g; 14.5 g; 1090 kJ)	8 h Standardized lunch 3.5 h after breakfast and a banana 5.5 h after breakfast	Hunger ↓ (0-210; 240-450 min) Satiety ↑ (0-210 min) Desire to eat ↓ (0-210; 240-450 min)						(Isaksson et al. 2009)
	Bread intermediate rye fraction (120 g; 6.5 g; 1090 kJ)		Hunger ↓ (0-210; 240-450 min) Satiety ↑ (0-210 min) Desire to eat ↓ (0-210; 240-450 min)						
	Bread sifted rye flour (100 g; 3.5 g; 1090 kJ)		Hunger ↓ (0-210; 240-450 min) Satiety ↑ (0-210 min) Desire to eat ↓ (0-210; 240-450 min)						
	Control: Bread refined wheat (98 g; 1.5 g; 1090 kJ								

Table 1 Short-term, cross-over studies on appetite, voluntary food intake, metabolic responses and gut fermentation in healthy men and women

Subjects	*Test and control foods; weight, df and energy content per portion*	*Duration*	*Appetite*	*Ad libitum food intake*	*Glucose*	*Insulin*	*GLP-1*	*Hydrogen & SCFA*	*Reference*
men and women (n = 19)	**Bread** rye bran (121 g; 9.0 g; 1090 kJ)	8 h Standardized lunch 3.5 h after breakfast and an apple 5.5 h after breakfast	Satiety ↑ (0-210 min)						(Isaksson et al. 2009)
	Bread rye bran (114 g; 6.0 g; 1090 kJ)		Satiety ↑ (0-210 min)						
	Bread intermediate rye fraction (126 g; 8.5 g; 1090 kJ)		No difference						
	Bread intermediate rye fraction (123 g; 6.0 g; 1090 kJ)		Satiety ↑ (0-210 min)						
	Control: Bread refined wheat (98 g; 1.5 g; 1090 kJ								

Table 1 Short-term, cross-over studies on appetite, voluntary food intake, metabolic responses and gut fermentation in healthy men and women

Subjects	*Test and control foods; weight, df and energy content per portion*	*Duration*	*Appetite*	*Ad libitum food intake*	*Glucose*	*Insulin*	*GLP-1*	*Hydrogen & SCFA*	*Reference*
men and women (n = 10)	**Bread** whole-grain rye (163.4 g; 26.3 g; 1078 kJ)	270 min	Hunger ↓ (0-60 min) Fullness ↑ (60-120 min)	No difference	Glucose ↓ (0-60 min) GI ↓ Glucose peak ↓	Insulin peak ↓ II ↓		H_2↑ (270-390 min)	(L. A. Rosén et al. 2011)
	Bread whole-grain rye with lactic acid (158.4 g; 23.6 g; 1069 g)		Fullness ↑ (60-120 min)	No difference	Glucose ↓ (0-60 min) GI ↓ Glucose peak ↓	Insulin ↓ (0-60 min) Insulin peak ↓ II ↓		H_2↑ (270-390 min)	
	Bread sifted rye with lactic acid (133.1 g; 18 g; 1033 kJ)		Hunger ↓ (0-60 min) Fullness ↑ (60-120 min)	No difference	Glucose ↓ (0-60 min) GI ↓ Glucose peak ↓	Insulin ↓ (0-60 min) Insulin peak ↓ II ↓			
	Bread sifted rye (134.8 g; 17.3 g; 1033 kJ)		Fullness ↑ (60-120 min) Desire to eat ↓ (60-120 min)	No difference		Insulin ↓ (0-60; 60-120 min) Insulin peak ↓ II ↓		H_2↑ (270-390 min)	
	Control: Bread refined wheat (124.0 g; 6.0 g; 1015 kJ)								

Table 1 Short-term, cross-over studies on appetite, voluntary food intake, metabolic responses and gut fermentation in healthy men and women

Subjects	*Test and control foods; weight, df and energy content per portion*	*Duration*	*Appetite*	*Ad libitum food intake*	*Glucose*	*Insulin*	*GLP-1*	*Hydrogen & SCFA*	*Reference*
men and women (n = 20)	**Bread** 75 % whole-grain rye (157.6 g; 13.9 g; NA) Commercial	180 min	Hunger ↓ (0-60 min) Desire to eat ↓ (0-60 min)		No difference	No difference			(L. A. H. Rosén, Östman & Björck 2011)
	Bread 75 % whole-grain rye (154.1 g; 13.8 g; NA) Kaskelott		No difference		Glucose ↓ (0-60 min)	No difference			
	Bread 75 % whole-grain rye (153.9 g; 13.4 g; NA) Picasso		No difference		Glucose ↓ (0-60 min) Glucose peak ↓ GI ↓ GP^2 ↑	Insulin ↓ (0-60 min) Insulin peak ↓			
	Bread 75 % whole-grain rye (148.9 g; 13.1 g; NA) Vicello		No difference		Glucose ↓ (0-60 min) Glucose peak ↓ GI ↓ GP^2 ↑	Insulin ↓ (0-60 min) Insulin ↓ (0-180 min) Insulin peak ↓			
	Bread 75 % whole- grain rye (154.9 g; 12.6 g; NA) Amilo		No difference		Glucose ↓ (0-60 min) Glucose peak ↓	Insulin ↓ (0-60 min) Insulin peak ↓			
	Bread 75 % whole-grain rye (152.6 g; 12.3 g; NA) Evolo		Hunger ↓ (0-60 min and 0-180 min) Fullness ↑ (60-120 min)		Glucose peak ↓	Insulin ↓ (0-60 min) Insulin peak ↓			
	Control: Bread refined wheat (125.9 g; 2.8 g; NA)								

Table 1 Short-term, cross-over studies on appetite, voluntary food intake, metabolic responses and gut fermentation in healthy men and women

Subjects	*Test and control foods; weight, df and energy content per portion*	*Duration*	*Appetite*	*Ad libitum food intake*	*Glucose*	*Insulin*	*GLP-1*	*Hydrogen & SCFA*	*Reference*
men and women (n = 14)	**Bread** whole-grain rye (171.0 g; 17.48 g; NA) Rekrut	180 min	Hunger ↓ (0-60; 0-180 min) Fullness ↑ (0-60; 60-120; 120-180; 0-180 min)		Glucose ↓ (60-120 min)	Insulin ↓ (60-120 min) Insulin ↓ (0-180 min) II ↓			(L. A. H. Rosén, Östman, Shewry, et al. 2011)
	Bread whole-grain rye (171.9 g; 16.06 g; NA) Amilo		Fullness ↑ (120-180; 0-180 min)		No difference	Insulin ↓ (0-60 min) Insulin ↓ (60-120 min) Insulin ↓ (0-180 min) Insulin peak ↓ II ↓			
	Bread whole-grain rye (175.8 g; 15.54 g; NA) H.Loire		Hunger ↓ (60-120 min) Fullness ↑ (0-180 min)		No difference	No difference			
	Bread whole-grain rye (168.8 g; 14.96 g; NA) Nikita		Fullness ↑ (60-120; 120-180; 0-180 min)		No difference	No difference			
	Bread whole-grain rye (165.3 g; 12.93 g; NA) D.Zlote		Hunger ↓ (0-60; 60-120; 120-180; 0-180 min) Fullness ↑ (0-60; 60-120; 120-180; 0-180 min)		No difference	No difference			
	Control: Bread refined wheat (122.7 g; 3.43 g; NA)								

Table 1 Short-term, cross-over studies on appetite, voluntary food intake, metabolic responses and gut fermentation in healthy men and women

Subjects	*Test and control foods; weight, df and energy content per portion*	*Duration*	*Appetite*	*Ad libitum food intake*	*Glucose*	*Insulin*	*GLP-1*	*Hydrogen & SCFA*	*Reference*
men and women (*n* = 12)	**Bread** rye bran (141.7 g; 12.3 g; NA)	180 min	Satiety ↑ (0-180 min)		No difference	No difference			(Rosén et al. 2009)
	Bread whole-grain rye with lactic acid (122.6 g; 10.2 g; NA)		No difference		Glucose ↓ (0-30; 0-120 min) GI ↓ GP ↑ GPI ↑	Insulin ↓ (0-120 min) Insulin peak ↑ II ↓			
	Bread whole-grain rye (123.4 g; 9.6 g; NA)		No difference		Glucose ↓ (0-30 min)	Insulin ↓ (0-120 min) II ↓			
	Bread sifted rye (106.2 g; 6.7 g; NA)		No difference		Glucose ↓ (0-30; 0-120 min) GI ↓ GP ↑ GPI ↑	Insulin ↓ (0-30; 0-120 min) Insulin peak ↑ II ↓			
	Control: Bread refined wheat (101.1 g; 1.8 g; NA)								

Table 1 Short-term, cross-over studies on appetite, voluntary food intake, metabolic responses and gut fermentation in healthy men and women

Subjects	*Test and control foods; weight, df and energy content per portion*	*Duration*	*Appetite*	*Ad libitum food intake*	*Glucose*	*Insulin*	*GLP-1*	*Hydrogen & SCFA*	*Reference*
women (n = 19)	**Bread** whole-meal rye (142.7 g; 15.2 g; 1295 kJ)	180 min			No difference	Insulin ↓			(Moazzami et al. 2014)
	Bread refined rye (111.9 g; 6.1 g; 1056 g)				No difference	Insulin ↓			
	Control: Bread refined wheat (105.5 g; 2.7 g; 1177 kJ)								
men and women (n = 16)	**Bread** sifted rye (110.6 g; 7.6 g; 961.3 kJ)	4 h			Glucose ↑ (at 90 min) as refined wheat bread response dropped below fasting	Insulin ↓ (at 30, 45 and 60 min) Insulin peak ↓ Insulin ↓ (0-240 min)			(Bondia-Pons et al. 2011)
	Control: Bread refined wheat (105.9 g; 3.7 g; 1216.38 kJ)								
men and women (n =10)	**Bread** whole-meal rye (150 g; 3.75 g; 390 kJ)	90 min			No difference				(Hlebowicz et al. 2009)
	Control: Bread refined wheat (150 g; 0 g; 435 kJ)								

Table 1 Short-term, cross-over studies on appetite, voluntary food intake, metabolic responses and gut fermentation in healthy men and women

Subjects	*Test and control foods; weight, df and energy content per portion*	*Duration*	*Appetite*	*Ad libitum food intake*	*Glucose*	*Insulin*	*GLP-1*	*Hydrogen & SCFA*	*Reference*
women (n = 19)	**Bread** whole-meal rye with rye bran (199.4 g; 29.0 g; 1486 kJ)	180 min			Glucose ↑ (at 180 min) as refined wheat bread response dropped below fasting	Insulin ↓ (at 0, 45, 60 and 90 min) Insulin ↑ (at 180 min) Insulin peak ↓	GLP-1 ↑ (at 150 and 180 min)		(Juntunen et al. 2003)
	Bread whole-meal rye (142.7 g; 15.2 g; 1295 kJ)				Glucose ↑ (at 150 and 180 min) as refined wheat bread response dropped below fasting	Insulin ↓ (at 45, 60 and 90 min) Insulin ↓ (0-180 min) Insulin peak ↓			
	Bread sifted rye (111.9 g; 6.1 g; 1056 kJ)				Glucose ↑ (at 150 and 180 min) as refined wheat bread response dropped below fasting	Insulin ↓ (at 30, 45, 60 and 90 min) Insulin ↑ (at 180 min) Insulin ↓ (0-180 min) Insulin peak ↓			
	Control: Bread refined wheat (105.5 g; 2.7 g; 1177 kJ)								

Table 1 Short-term, cross-over studies on appetite, voluntary food intake, metabolic responses and gut fermentation in healthy men and women

Subjects	*Test and control foods; weight, df and energy content per portion*	*Duration*	*Appetite*	*Ad libitum food intake*	*Glucose*	*Insulin*	*GLP-1*	*Hydrogen & SCFA*	*Reference*
men and women (n = 20)	**Bread** 60 % whole rye kernels (135 g; 12.8 g; 1084 kJ)	180 min			No difference	Insulin ↓ (at 30, 45, 60, 90, 120 and 150 min) Insulin ↓ (0-180 min) Insulin peak ↓	GLP-1 ↓ (at 30, 45 and 60 min) GLP-1 peak ↓		(Juntunen et al. 2002)
	Control: Refined wheat bread (112.4 g; 3.1 g; 1117 kJ)								
men and women (n = 20)	**Bread** whole rye kernels (148.4 g; 13.5 g; 1173 kJ)	180 min			No difference	Insulin ↓ (at 45, 60, 90, 120 and 150 min) Insulin ↓ (0-180 min) Insulin peak ↓			(Leinonen et al. 1999)
	Bread whole-meal rye (98.2 g; 10.1 g; 974 kJ)				No difference	No difference			
	Control: Bread refined wheat (121.1 g; 2.3 g; 1341 kJ)								
(n = 10)	**Bread** 80 % rye kernels (NA)	120 min			Glucose ↓	Insulin ↓			(Liljeberg et al. 1992)
	Control: Bread refined wheat (NA)								

Table 1 Short-term, cross-over studies on appetite, voluntary food intake, metabolic responses and gut fermentation in healthy men and women

Subjects	*Test and control foods; weight, df and energy content per portion*	*Duration*	*Appetite*	*Ad libitum food intake*	*Glucose*	*Insulin*	*GLP-1*	*Hydrogen & SCFA*	*Reference*
men and women (n = 8)	**Bread** pumpernickel (160 g; 9 g; 1558 kJ)	Glucose 180 min Insulin 120 min			Glucose ↓ (45 and 70 min) Glucose ↓ (0-180 min) GI ↓	Insulin ↓ (0, 45 and 95 min) Insulin ↓ (0-120 min) II ↓			(Liljeberg & Björck 1994)
	Control: Bread refined wheat (116 g; 2.8 g; 1552 kJ)								
men and women (n = 13)	**Beverage** with rye bran (30.6 g; 12.0 g; 1695 kJ) served with refined wheat bread (51.5 g)	180 min			Glucose ↓ (0-120 min) Glucose peak ↓	No difference			(Ulmius et al. 2009)
	Control: Beverage with no added fibre (1641 kJ) served with refined wheat bread (67.7 g)								

↑ higher; ↓ lower; df, dietary fibre; GI, glycaemic index; GLP-1, glucagon-like peptide-1; GP, glycaemic product; GPI, glycaemic profile index; h, hours; H_2, hydrogen; II, insulinaemic index; SCFA, short-chain fatty acids

2 Aims

The overall aim of this thesis was to investigate the effects of whole-grain rye foods served as part of a complete breakfast on appetite and some metabolic responses. An additional aim was to evaluate the role of plant protein, gut fermentation, regular consumption and food processing on these responses.

Specific aims were to evaluate:

- The effect of replacing part of the rye in whole-grain rye porridge with rapidly fermented dietary fibre (inulin) and plant protein (wheat gluten), compared with refined wheat bread, on: appetite, gut fermentation, GLP-1, postprandial glycaemia and insulinaemia during 8 h after consumption, and voluntary food intake (Paper I).

- The effect of regular intake of whole-grain rye porridge, compared with refined wheat bread, for three weeks, on: appetite, gut fermentation and gut passage time during 8-12 h after consumption, and voluntary food intake (Paper II).

- The effect of unfermented and yeast-fermented whole-grain rye crisp bread, compared with refined yeast-fermented refined wheat crisp bread, on: appetite and postprandial glycaemia and insulinaemia during 4 h after consumption (Paper III).

3 Materials and methods

Only a brief summary of the materials and methods used in the studies is provided in this section. More detailed descriptions can be found in Papers I-III.

3.1 Test products

The investigated test products were served in the morning together with additional breakfast foods, in order to simulate a realistic breakfast situation. The breakfast meals were standardised with regard to portion size, and the energy content of the breakfasts corresponded to national recommendations for adults (National Food Agency Sweden 2005). The portion size, composition and energy content of the whole-grain rye foods and the corresponding refined wheat control foods that were investigated are summarised in Table 2.

In Paper I, five whole-grain rye porridges were compared against each-other, and with an isocaloric refined wheat bread, as this is a common type of breakfast bread. Two whole-grain rye porridges contained 55 g and 40 g of rye flakes, respectively. Whereas three contained 40 g rye flakes with 15 g of a combination of inulin (Orafti®GR inulin, purity 90%; Beneo GmbH, Mannheim, Germany) and wheat gluten (Vital Wheat Gluten, purity 77%; Arrowhead Mills Inc., Hereford, TX, USA). Additional breakfast foods included 100 g milk 1.5 %, 25 g raspberry jam and 150 g coffee/tea. Margarine 40% was added to make breakfast meals isocaloric.

In Paper II, whole-grain rye porridge was compared with an isocaloric refined wheat bread during three weeks of daily consumption. Additional breakfast foods included 200 g milk 1.5%, 25 g raspberry jam and 150 g coffee/tea. Margarine 40% was added to make breakfast meals isocaloric.

In Paper III, unfermented whole-grain rye crisp bread and yeast-fermented whole-grain rye crisp bread were compared against each-other, and with

isocaloric yeast-fermented refined wheat crisp bread, to investigate the effect of process-induced changes in food structure. Additional breakfast foods included 100 g orange juice, 20 g cheese and 150 g coffee/tea. Margarine 40% was added to make breakfast meals isocaloric.

A lunch meal was included 4 h after breakfast in the whole-grain rye porridge studies (Papers I and II). In Paper I, the lunch meal size was standardised in terms of portion size, while in Paper II the lunch meal size was based on participants individual energy needs.

Table 2 *Whole-grain rye foods investigated in Papers I-III*

Test product (per portion)	Serving[1] (g)	Energy[1] (kJ)	CHO[1] (g)	DF[1] (g)	Pro[1] (g)	Fat[1] (g)
Paper I						
Porridge: 40 g rye flakes with 9 g inulin and 3 g gluten (54 g)	486	1187	42	15.5	10	5
Porridge: 40 g rye flakes with 6 g inulin and 6 g gluten (55g)	485	1185	42	12.6	13	4
Porridge: 40 g rye flakes with 3 g inulin and 9 g gluten (55 g)	483	1192	42	10.4	16	4
Porridge: 55 g rye flakes	534	1205	50	9.7	8	4
Porridge: 40 g rye flakes	482	1191	41	7.1	7	9
Control: Soft refined wheat bread (55 g)	342	1158	39	3.4	10	8
Paper II						
Porridge: 55 g rye flakes	590	1354	42	10.8	13	8
Control: Soft refined wheat bread (74 g)	309	1321	44	3.2	14	10
Paper III						
Crisp bread rye, unfermented (58.5 g)	194	1538	46	12.0	11	13
Crisp bread rye, yeast-fermented (60 g)	195	1561	47	10.9	11	13
Control: Crisp bread wheat, yeast-fermented (52 g)	187	1561	44	3.6	12	16

[1]Including additional breakfast foods. CHO, carbohydrates. DF, dietary fibre. Pro, protein.

Chemical characterisation

In all studies, the whole-grain rye food products and corresponding refined wheat control food products were analysed in duplicate or triplicate for their concentrations of fat, protein, carbohydrates and total dietary fibre including fructan. Manufacturer's data were used for all additional food products. Standard food energy conversion factors were used to calculate energy content of the foods: protein and available carbohydrates 17 kJ/g; fat 37 kJ/g; and dietary fibre 8 kJ/g.

Microscopy

In Paper III, the whole-grain rye crisp breads and refined wheat crisp bread control were embedded, sliced (Leica EM UC6, Leica, Austria) and stained (Lugol's solution) before being examined under a Nikon Eclipse Ni-U microscope.

3.2 Study design

All studies were conducted as randomised, single-blinded, cross-over intervention trials on healthy men and women. A within-subject, repeated measures design was chosen, as it takes into consideration the fact that the subjective feelings of appetite, and the interpretation and answer to the questions asked, are highly individual (Stubbs et al. 2000). Participants were recruited in Uppsala, Sweden, and screened to ensure healthy participants, which included measurements of anthropometrics and biomarkers in fasting blood samples. Written informed consent was obtained from all study participants and the studies were approved by the Regional Ethical Review Board, Uppsala.

Before each study visit, participants were instructed regarding dietary intake and physical activity level in an effort to standardise conditions. In the two studies measuring gut fermentation, participants were instructed to consume a diet low in dietary fibre during the whole day prior to visit days (Paper I) or to consume a dinner low in dietary fibre on the evening before each study visit (Paper II).

On each study visit, participants arrived fasted and consumed the test products with additional food products, after which they remained at the clinic for appetite measurements and blood sampling (Papers I-III). In Paper II, participants continued registering appetite after they had left the facilities until bedtime and additional breakfast products were provided for home consumption. The two short-term studies included one study visit per test product (Papers I and III), whereas the study on repeated consumption included

three study visits during each of the two three-week study periods (Paper II). All studies included a wash-out period to avoid carry-over effects from the previous treatment: ≥ 5 days (Papers I and III) and 3-4 weeks (Paper II).

3.3 Measurements

3.3.1 Subjective appetite

In all studies, subjective appetite was assessed by an electronic VAS (Palm z22; Palm Inc., Sunnyvale, CA, USA). Participants rated their appetite by answering questions on hunger, fullness and desire to eat by making a tick along the unipolar scale presented on the touchpad screen, which was translated by the computer to a number between 0 and 100 (Figure 2). The same question was asked, in Swedish, but was described by fullness in Papers I and III, and by satiety in Paper II. The questions were repeatedly asked in sequence every 30 min, starting before breakfast and continuing during 4 h (Paper III), 8 h (Paper I) and 12 h (Paper II) after breakfast. In the short-term studies (Papers I and III), appetite was assessed once for each test food and control, whereas in the study on regular consumption, appetite was assessed on three separate occasions during each of the two three-week study periods (Paper II).

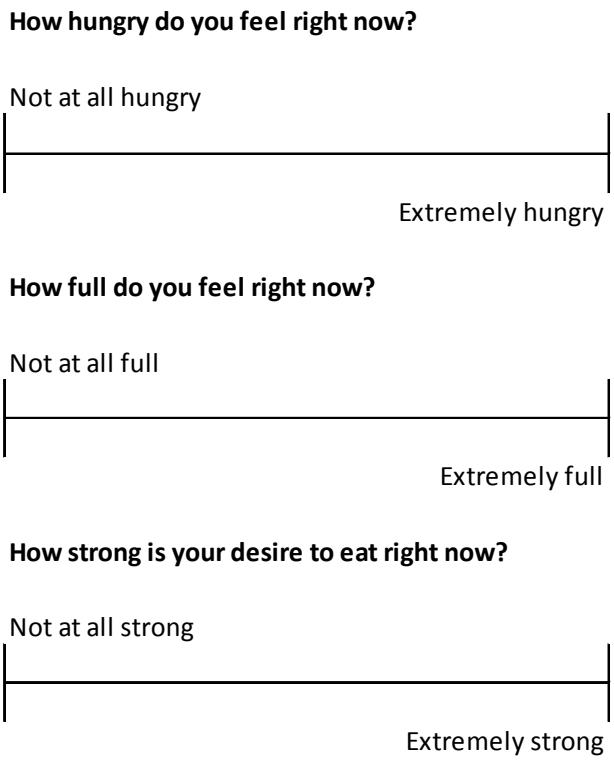

Figure 2.
Unipolar scales used for measuring subjective appetite in Papers I-III.

3.3.2 Voluntary food intake

Both studies on whole-grain rye porridge (Papers I and II) included measurements of energy intake. An *ad libitum* meal was served 8 h after breakfast consumption in Paper I, while participants were asked to self-report their food intake using 3-day-weighed food diaries in Paper II.

3.3.3 Gut fermentation and gut passage time

Both studies on whole-grain rye porridge had an extended measurement period, and included measurements of hydrogen excreted in breath. In Paper I, hydrogen and methane were analysed using a breath hydrogen and methane analyser (QuinTron BreathTracker DP; QuinTron Instrument Company, Inc. Milwaukee, WI, USA). In Paper II, hydrogen was analysed using a portable breath hydrogen analyser (MicroH2 Meter, Micro Medical Ltd., Rochester, UK). The studies measured breath hydrogen at -30, +30, +90, +150, +210, +270, +330, +390, +450, and +480 min (Papers I and II).

In Paper II, gut passage time was measured by the salicylazosulphapyridine/ sulphapyridine method in the middle of each study period and in a subset of participants. It is an indirect method where sulphapyridine, which is released during fermentation of salicylazosulphapyridine in the proximal colon, can be measured in blood (Kennedy et al. 1979). Blood samples were taken at time points 0 (before breakfast), +120, +240, +300, +360, +420 and +480 min (Paper II).

3.3.4 Glucose, insulin and GLP-1

Paper I and III included analysis of glucose and insulin in EDTA plasma samples. Glucose was analysed with Architect *c*16000 (Abbott Laboratories, Chicago, IL, USA) and insulin was analysed with Cobas® C8000 e602 (Roche Diagnostics GmbH, Mannheim, Germany). The coefficients of variation were <4% and <3%, respectively. Paper I also included measurements of GLP-1, which were taken at the same time as glucose and insulin. To prevent the degradation of GLP-1, a protease inhibitor cocktail was added to the test tube after blood sampling. GLP-1 was analysed in EDTA plasma samples with the Multi Species GLP-1 Total ELISA kit (EZGLP1T-36K; EMD Millipore, Billerica, MA, USA). The intra- and interassay coefficients of variation were <5% and <12%, respectively. Blood samples were taken at time points -15, +15, +35, +65, +95, +125, +185, +230, +275, +305, +365, and +470 min (Paper I) and -15, +15, +35, +65, +95, +125, +185 and +230 min (Paper III).

3.4 Statistical analysis

In Papers I and III, ANCOVA was used to evaluate differences in response variables between breakfast meals using PROC MIXED in SAS (Version 9.3; SAS Institute Inc., Cary, NC, USA). *P*-values were adjusted according to Bonferroni in order to reduce the risk of type I error. In Paper II, ANOVA followed by Tukey's test was used to evaluate comparisons between the two periods on response variables using Minitab (version 16, LEAD technologies, Inc., USA). Appetite responses were evaluated using repeated measures (Papers I-III) and area under the curve (Papers I and III). Results were presented as least square means with standard error and considered statistically significant when $P<0.05$.

No formal power calculations were conducted for the appetite (Papers I-III) and metabolism studies (Paper I and III), since estimates of required number of subjects under similar conditions are available in the literature. Eighteen participants were sufficient to detect a 10% difference in appetite ratings in different conditions, and glucose ratings, in a paired design with a power of 80% and a level of significance at $P<0.05$ (Brouns et al. 2005; Flint et al. 2000).

4 Results and discussion

4.1 Appetite

Appetite response up to 4 h after consumption

Both studies on whole-grain rye porridge consumed as part of complete breakfasts showed reduced appetite during 4 h after intake compared with refined wheat bread (Papers I and II).

Paper I included estimates of effect size and showed that a larger portion of whole-grain rye porridge (55 g) elicited 20% lower hunger and 28% higher fullness compared with refined wheat bread during 4 h after intake. However, no difference was observed from intake of the small whole-grain rye porridge (40 g) compared with refined wheat bread. The results indicate that >40 g rye per portion porridge is needed to see an effect on appetite.

Replacing part of the rye in whole-grain rye porridge with a combination of the rapidly fermented dietary fibre inulin and the plant protein wheat gluten reduced appetite in a similar way as observed for plain whole-grain rye porridge compared with refined wheat bread (Paper I). Although, in the literature, macronutrient composition has been identified both as a key element to enhanced satiety and reduced food intake, and also more important than physiochemical features (Fromentin et al. 2012), no difference was observed from adding inulin and wheat gluten to the porridges.

There may be different explanations for the lack of effect on appetite. Previous studies have shown similar appetite responses from intake of wheat gluten as from other plant and animal proteins (Bowen et al. 2006; Lang et al. 1998). However, our data do not support adding wheat gluten as a way to increase the satiating capacity of a food product. Although wheat gluten is known to be digested quickly (Gilbert et al. 2011), co-ingestion of carbohydrates and fat with protein may decrease the satiating effect of protein (Lang et al. 1998). The satiating effect is more likely attributable to the added and native dietary fibre of the whole-grain rye porridges.

Consumption of unfermented and yeast-fermented whole-grain rye crisp bread, served as part of a complete breakfast, elicited 11 and 12% lower hunger, respectively, and 16% higher fullness compared with yeast-fermented refined wheat crisp bread control during 4 h after intake (Paper III). A previous study on yeast-fermented whole-grain crisp bread compared with refined wheat soft bread observed a larger effect on appetite, which is most likely due to the different type of wheat bread control used (Forsberg et al. 2014). However, no effect of food processing on appetite response was observed when comparing the effects from unfermented and yeast-fermented whole-grain rye crisp breads. Despite apparent differences in dietary fibre composition and microstructure (Figure 3), the variations in composition might have been too small for any differences in appetite response to occur (Isaksson et al. 2011).

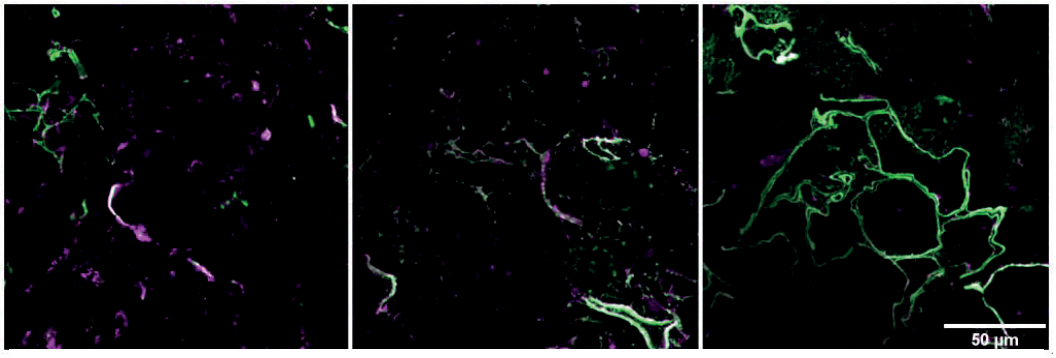

Figure 3. Micrographs of the three crisp breads investigated in Paper III. Left: Yeast-fermented refined wheat crisp bread. Centre: Yeast-fermented whole-grain rye crisp bread. Right: Unfermented whole-grain rye crisp bread. Arabinoxylan is coloured magenta, β-glucan is coloured green and white indicates overlapping structures.

Appetite response 4-8 h after consumption

Neither of the studies on whole-grain rye porridge, served as part of a complete breakfast, showed sustained satiety 4-8 h after intake compared with refined wheat bread breakfast (Papers I and II). Although an effect on whole-day appetite (0-8 h) was apparent in Paper I, the effect was not evident on separate analysis of appetite ratings in the afternoon (4-8 h).

Sustained satiety up to 8 h after intake has been observed in previous studies on whole-grain rye porridge as part of a complete breakfast (Isaksson et al. 2008; Isaksson et al. 2011). However, those studies used a larger portion of rye (>60 g), and the smaller portion size (55 g) employed in Papers I and II could offer a possible explanation for the discrepancy in results. Another explanation could be that inclusion of a rather large lunch meal 4 h after

breakfast in Papers I and II could have obscured possible differences in appetite response during the 4 h after lunch. In Paper I, the lunch meal was a standardised portion of 400 g, while in Paper II the lunch meal size was standardised to individual energy requirements with 400 g being the smallest portion administered. One of the previous studies cited (Isaksson et al. 2008) included a standardised lunch meal 4 h after breakfast of a much smaller size (100 g) and differences in appetite were apparent during the 4 h after lunch in that study.

In Papers I and II, gut fermentation, as indicated by breath hydrogen concentration, increased 4 h after intake of the whole-grain rye porridge breakfast. In Paper I, breath hydrogen increased in a dose-dependent manner in response to increased dietary fibre content of the products. The increased gut fermentation was however not accompanied by differences in appetite in the afternoon (Paper I). Taken together, our results do not support that gut fermentation of inulin might contribute to increased satiety as has been proposed in previous studies. Daily consumption of 16 g inulin during two weeks have in a previous study shown to lower appetite, increase gut fermentation and increase GLP-1 levels (Cani et al. 2009). A recent review on inulin-type fructan concludes that regular intake of inulin-type fructan may facilitate weight loss, although not because of increased satiety but possibly through modulation of the gut microbiota (Liber & Szajewska 2013).

Measurements of gut passage time by the salicylazosulphapyridine/ sulphapyridine method in Paper II showed that the digesta reached the proximal colon 5 h after intake of the whole-grain rye porridge and refined wheat bread breakfast, irrespective of breakfast type. However, according to the breath hydrogen method also used in Paper II, gut fermentation begins at 4 h after intake. Since the same participants were used for both measurements, the discrepancy could be due to the different methods used, where salicylazosulphapyridine/sulphapyridine travels faster than the fibre-rich digesta to the proximal colon since it is water soluble and most likely emptied with the liquid phase. Since the whole-grain rye porridge and the refined wheat bread arrived in the proximal colon at the same time, and with no difference in appetite response between the breakfasts at that time, it is difficult to either confirm or reject the proposal that gut passage time affects satiety.

Paper I included measurements of the gut peptide GLP-1 and no difference was observed between whole-grain rye porridge and refined wheat bread. The lack of difference in gut passage time between whole-grain rye porridge and refined wheat bread in Paper II could offer an explanation for the lack of difference in GLP-1 response. However, the potency of the satiety gut peptides produced by food intake is smaller than when administered exogenously at

supra-physiological concentrations (Mars et al. 2012) which implies that they can be overridden by external stimuli in the environment (Bruen et al. 2012). Furthermore, the co-ingestion of fat and additional breakfast products could have obscured the effects, as GLP-1 release is stimulated by both glucose and fat (Gibbons et al. 2013).

Appetite response from regular consumption

The study on regular consumption of whole-grain rye porridge, as part of a complete breakfast, compared with refined wheat bread breakfast showed that the effect of whole-grain rye on satiety observed in short-term/acute studies persists after three weeks of daily consumption (Paper II). Paper II only measured breath hydrogen once during each study period and it is therefore not possible to tell if regular consumption would modulate the gut microflora to produce more fermentation products and gases, which possibly could have an effect on the sustained satiety.

Voluntary energy intake

Voluntary food intake was measured in Papers I and II, and the results showed no reduced food intake from consuming whole-grain rye porridge, as part of a complete breakfast, compared with refined wheat bread, in an acute setting or from regular consumption.

In Paper I, voluntary food intake was measured by providing an *ad libitum* dinner 8 h after breakfast, i.e. 4 h after a standardised lunch. The lunch meal may have obscured possible differences in appetite response in the afternoon, but most likely also attenuated differences in subsequent voluntary food intake. Similarly, Isaksson et al. (2008) did not observe decreased energy intake at an *ad libitum* dinner 8 h after intake of whole-grain rye porridge for breakfast compared with refined wheat bread. In contrast, boiled rye kernels for breakfast reduced appetite compared with refined wheat bread and reduced voluntary food intake 4.5 h after breakfast (L. A. Rosén et al. 2011). No study to date has provided whole-grain rye porridge as part of a complete breakfast and measured *ad libitum* food intake 4 h later. Moreover, in Paper II, voluntary food intake was derived from self-reported food records, which are known to suffer from inaccuracies such as underreporting, and direct measurement of food intake might have given a different result.

Taken together, the results imply that energy intake was not reduced, although satiety was sustained, by intake of whole-grain rye porridge. However, neither of the studies measured voluntary food intake as a primary end-point and the power to detect a significant difference with the number of subjects included in Papers I and II may have been too small.

4.2 Postprandial metabolism

Glucose, insulin and GLP-1 responses up to 4 h after consumption

Whole-grain rye porridge with or without addition of inulin and wheat gluten, served as part of a complete breakfast, produced similar postprandial glucose, insulin and GLP-1 responses as a refined wheat bread breakfast during 4 h after intake (Paper I).

Delayed gut passage time could potentially have prolonged glucose absorption (Maljaars et al. 2008), but no difference was observed in gut passage time between whole-grain rye porridge and refined wheat bread in the study on regular consumption of whole-grain rye porridge (Paper II). Furthermore, the higher glucose response observed from intake of the larger plain whole-grain rye porridge was most likely because it contained 20% more available carbohydrates than the other test products (Paper I).

Previous studies on whole-grain rye porridge and boiled rye kernels have shown lower glucose response with a corresponding reduction in insulin response 2 h after intake (L. A. Rosén et al. 2011; Rosén et al. 2009), or only lower glucose response from consumption of boiled rye kernels (Nilsson et al. 2008). However, in those studies the test products were not served with additional food products. A mixed meal consists of several food products with different physiochemical features (Delzenne et al. 2010), thereby affecting metabolic responses differently compared with a single item meal. The discrepancy in result from our and other studies with porridge or boiled kernels may highlight the importance of evaluating food products in a realistic setting before drawing conclusions on possible health effects.

Consumption of unfermented and yeast-fermented whole-grain rye crisp bread, served as part of a complete breakfast lowered the insulin response, without a corresponding reduction in glucose, compared with a yeast-fermented refined wheat crisp bread breakfast during 4 h after intake (Paper III). Furthermore, the unfermented whole-grain rye crispbread also lowered the insulin response compared with the yeast-fermented whole-grain rye crisp bread (Paper III). The effect of only lowering insulin response, without a corresponding change in glucose, is a common feature of soft rye breads (L. A. H. Rosén, Östman, Shewry, et al. 2011; Moazzami et al. 2014; Juntunen et al. 2003; Juntunen et al. 2002; Leinonen et al. 1999). It is believed to be due to differences in glucose kinetics related to the microstructure of the breads, and not the dietary fibre content (Eelderink et al. 2015; Juntunen et al. 2003). The higher content of branched-chain amino acids in the refined wheat crisp bread compared with the whole-grain rye crisp breads could have contributed to the increased insulin response. Hypothetically, the higher insulin concentrations

from refined wheat crisp bread intake, together with a faster glucose absorption and uptake of glucose in the tissues, could have resulted in a similar glucose response from intake of whole-grain rye crisp bread and refined wheat crisp bread (Eelderink et al. 2015).

Glucose, insulin and GLP-1 responses 4-8 after consumption

In Paper I, whole-grain rye porridge with or without addition of inulin and wheat gluten, served as part of a complete breakfast, lowered postprandial glucose response after intake of a lunch meal 4 h after breakfast compared with a refined wheat bread breakfast (Figure 4). This so-called second meal effect on glucose response from consumption of whole-grain rye porridge is most likely a result of the SCFA produced. The large and significant increase in breath hydrogen from 4 h after intake of the whole-grain rye porridges indicates extensive gut fermentation. Measuring SCFA in future studies could give valuable insights into the mechanism behind the second meal effect. However, no differences in insulin and GLP-1 responses were observed between the whole-grain rye porridges with or without addition of inulin and wheat gluten and the refined wheat bread, nor when comparing the porridges against each other.

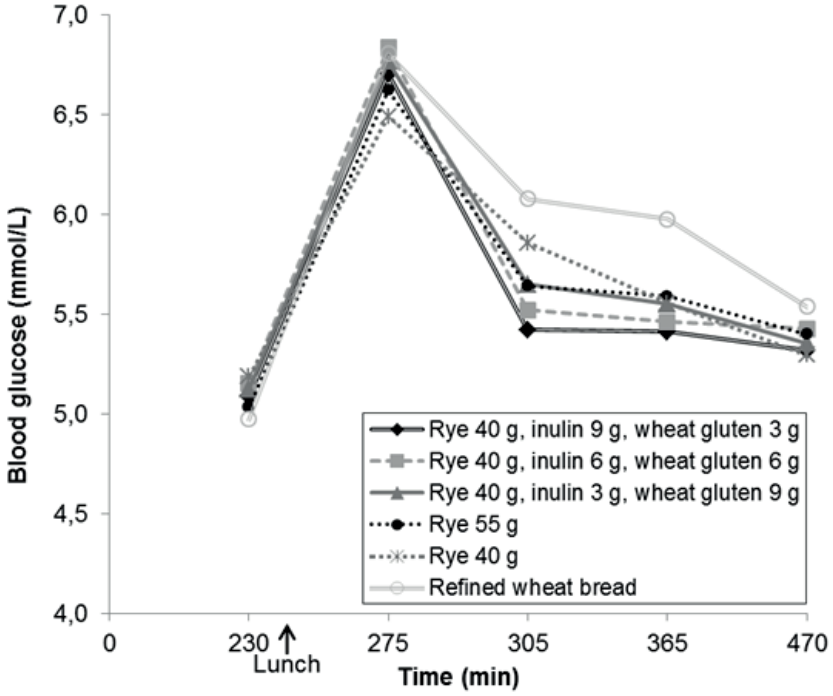

Figure 4 Glucose responses after a standardized lunch meal from intake of whole-grain rye porridge, with or without addition of inulin and wheat gluten, compared with refined wheat bread as part of a complete breakfast. Adapted from Paper I.

4.3 Conclusions

Whole-grain rye porridge, served as part of a complete breakfast, had a large satiating capacity and consistently reduced appetite up to 4 h after intake compared with refined wheat bread, an effect which was sustained after three weeks of regular consumption. Replacing part of the rye with the rapidly fermented dietary fibre inulin and plant protein wheat gluten did not increase the satiating effect of whole-grain rye porridge. Whole-grain rye porridge elicited similar glycaemic and insulinaemic responses to refined wheat bread up to 4 h after intake. Compared with refined wheat bread, whole-grain rye initiated extensive gut fermentation 4-8 h after intake and attenuated blood glucose response after a second meal 4 h after intake, without a corresponding reduction in insulin and GLP-1 responses.

Whole-grain rye crisp bread served as part of a complete breakfast had a large satiating capacity and reduced appetite and insulin response up to 4 h after intake compared with refined wheat crisp bread, without a corresponding reduction in glucose response. An effect of food processing on insulin response was evident since consumption of the unfermented whole-grain rye crisp bread lowered insulin response compared with yeast-fermented whole-grain rye crisp bread and yeast-fermented refined wheat crisp bread up to 4 h after intake.

In summary, whole-grain rye foods reduced appetite and affected some metabolic responses, which may in the long run contribute to weight management and reduce the risk of diet-related chronic diseases.

5 Main findings

- Whole-grain rye porridge and whole-grain crisp bread, served as part of a complete breakfast, reduced appetite compared with refined wheat bread breakfast during 4 h after intake.

- The effect on appetite of whole-grain rye porridge breakfast was dependent on portion size.

- Replacing part of the rye with inulin and wheat gluten in whole-grain rye porridge did not enhance satiety compared with plain whole-grain rye porridge.

- Regular consumption of whole-grain rye porridge breakfast during three weeks resulted in sustained satiety compared with refined wheat bread breakfast.

- Gut fermentation increased 4-8 h after consumption of whole-grain rye porridge, but did not contribute to differences in appetite response.

- Whole-grain rye porridge breakfast lowered the second-meal glucose response, without a corresponding reduction in insulin and GLP-1 responses, compared with refined wheat bread breakfast 4-8 h after consumption.

- Whole-grain rye crisp bread breakfast lowered the insulin response, without a corresponding reduction in glucose response, compared with refined wheat crisp bread breakfast during 4 h after intake.

- The insulin response was lower for unfermented whole-grain rye crisp bread compared with yeast-fermented whole-grain rye crisp bread, where the dietary fibres were more intact.

6 Future perspectives

Future research on the effect of whole-grain rye foods on appetite, metabolism and human health could address the following:

- To measure *ad libitum* food intake 4 h after consumption of whole-grain rye porridge breakfast to validate that consistent findings of reduced appetite also leads to lower energy intake.

- To measure SCFA concentrations in blood and faeces to see if the extensive gut fermentation observed from whole-grain rye porridge also increases the production of SCFA.

- To investigate if the second meal effect on glucose response observed from consumption of whole-grain rye porridge is related to specific SCFA or SCFA profiles.

- To explore the effect of whole-grain rye foods on gut fermentation, bile acids and metabolic responses.

- To explore the effect of daily consumption of several whole-grain rye foods on appetite, metabolism, gut microbiota and weight-loss.

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