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METABOLIC PROPERTIES OF RYE PRODUCTS

Focusing on insulinaemia, glycaemic profile and appetite
regulation in healthy subjects

Liza Rosén

Applied Nutrition and Food Chemistry
Department of Food Technology, Engineering and Nutrition
Faculty of Engineering, Lund University

2011



LUND UNIVERSITY

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“The most exciting phrase to hear in science, the one that heralds the most discoveries, is not 'Eureka!' (I found it!) but 'That's funny'”

Isaac Asimov

“If we knew what it was we were doing, it would not be called research, would it?”

Albert Einstein

Abstract

The prevalence of metabolic disorders, such as type 2 diabetes, cardiovascular diseases and the insulin resistance syndrome (IRS) are increasing worldwide. However, disturbances in the metabolic status can be prevented by changing the daily diet towards more whole grains, vegetables, legumes and dairy products. Also the dietary glycaemic- and insulinaemic indices of foods may play a role. Rye products are interesting in this context as they are usually consumed in wholegrain form and have been demonstrated to induce low insulin responses, with or without a simultaneous lowering of the glycaemic index (GI). The objective of this thesis was to evaluate the possible cause of low postprandial insulin response to rye, and to elucidate potential effects of processing condition, extraction rate and rye variety. Insulin response as well as glycaemic response and course of glycaemia were evaluated in the postprandial phase. Additionally a marker of colonic fermentation was analysed in the postprandial phase (breath hydrogen) and appetite regulating properties were investigated using subjective ratings, analysis of plasma ghrelin, and quantification of voluntary food intake at a subsequent meal.

Wholegrain rye products, ingested as boiled kernels and breads as well as endosperm rye (sifted rye) bread and porridge, induced low insulin responses and also a well regulated course of glycaemia, noted as blood glucose curves with lower incremental peaks, remaining above fasting for a longer time. However, some rye varieties were devoid of benefits on course of glycaemia and insulin economy. Two measurements of the course of glycaemia were introduced, the GP and GP^2 , defined as the duration for incremental postprandial glycaemic response divided by the glucose incremental peak or squared glucose incremental peak, respectively. The GP and GP^2 of the products were correlated to the insulin response, as well as to late subjective satiety, suggesting that they are good predictors of postprandial events. Suggested mechanism for the lowered glycaemic and insulinaemic responses were a high content of viscous fibres, bioactive components, e.g. phenolic acids and a dense food structure, contributing to a lowered digestion and uptake of carbohydrates in the small intestines. Furthermore, rye products

Abstract

induced early colonic fermentation, already in the postprandial phase, possibly explained by the presence of arabinoxylans, fructans, and other dietary fibre compounds of low molecular weight. The increase in colonic fermentation, measured as increase in breath H_2 , correlated with lower late postprandial concentration of FFA and the GP and GP² of the products, suggesting increased glucose tolerance already in the postprandial phase after rye products.

Rye products, in particular boiled rye kernels induced high postprandial subjective satiety and promoted satiety also at a subsequent voluntary meal. The rye kernel breakfast lowered the voluntary energy intake at a second meal with 16%. The mechanism behind this satiating effect of rye was suggested to be the high content of dietary fibres (DF) and high water content introducing a bulking effect. Also, high content of viscous and fermentable DF can lower gastric emptying rate. The low postprandial glycaemia and insulinaemia seen with several rye products appears to contribute to a lowered rebound of the hunger peptide ghrelin prior to the second meal.

Rye products made from whole kernels and wholegrain- and endosperm rye bread induced lower postprandial insulinaemia and glycaemia than wholegrain- and endosperm rye porridges, while porridges and whole kernels induced higher subjective satiety. Boiled rye kernels also suppressed the *desire to eat* to a greater extent than boiled wheat kernels in the later postprandial phase (tAUC 210–270 min). Several components are suggested to contribute to the observed lowered insulinaemia, well regulated glycaemia and improved satiating effects of certain rye products.

Populärvetenskaplig sammanfattning

Förekomsten av välfärdssjukdomar såsom diabetes och hjärt-kärlsjukdomar ökar kraftigt och det finns därför ett stort behov av preventiva åtgärder. Ett förstadium till dessa allvarliga sjukdomar är insulinresistens syndromet (IRS), även känt som det metabola syndromet. IRS utgör ett kluster av flera metabola störningar såsom bukfetma, högt blodtryck, förhöjda blodfetter och förhöjt blodsocker. IRS kan förebyggas genom lämplig kost och livsmedel innehållande fullkorn respektive livsmedel som gynnar ett lågt blodsocker och insulinsvar efter måltid (långt GI och II) har visats vara skyddande. Egenskaper hos råg är av intresse i detta sammanhang, då råg ofta äts som fullkorn och ger ett lågt insulinsvar. I avhandlingen har rågprodukter studerats med avseende på insulin och blodsockersvar samt påverkan på mättnad.

Fullkornsrågprodukter, både hela kokta kärnor och bröd samt rågbröd och gröt gjort av vitt rågmjöl har i denna avhandling visats ge ett lågt insulinsvar samt ett välreglerat blodsockersvar, med lägre maximal stigning och med mindre blodsockerfall i den sena fasen efter måltid än motsvarande produkter gjorda på vitt vetemjöl. Det låga insulin- och blodsockersvaret beror troligen på en kombination av en hög andel lösliga fibrer, närvaro av bioaktiva komponenter såsom fenolsyror samt en tät livsmedelsstruktur. Dessa faktorer har förmågan att sänka spjälknings- och/eller upptagningshastigheten för kolhydrater från tarmen och därmed ge ett mer välkontrollerat blodsockersvar efter måltid.

Rågprodukter och då främst kokta rågkärnor, visade sig ge en bra mättnadskänsla, både direkt efter måltid och vid en efterföljande måltid. En frukost bestående av kokta rågkärnor sänkte således energiintaget vid en efterkommande lunchmåltid med i genomsnitt 16 energiprocent. Mekanismen bakom denna effekt tros vara en hög andel mättande fibrer samt de välkontrollerade insulin och glukossvaren som dämpade halten av hungerhormonet ghrelin i den sena fasen efter frukostmåltiden. Kostfiber i rågprodukter var snabbt åtkomliga för tarmfloras bakterier, och ett samband

Populärvetenskaplig sammanfattning

erhölls mellan bildning av en metabolit från denna fermentering (vätgas i utandningsluft) och gynnsamma effekter på blodsockerreglering respektive mättnadskänsla.

Val av rågsort och produkt-processning visade sig påverka blodsockersvar, insulinbehov samt mättnad efter måltid. Produkter baserade på hela kokta kärnor och bröd av fullkorn och vitt rågmjöl gav lägre insulin- och blodglukossvär än rågmjölsgröt, medan rågmjölsgrötarna och de hela kokta kärnorna var mest mättande.

Resultaten antyder att flera komponenter i råg sannolikt bidrar till rågprodukters gynnsamma effekter på blodsockerreglering och mättnad. En mekanism tycks vara kopplad till rågprodukternas innehåll av lättfermenterbara kostfiber.

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List of papers

This thesis is based on the following papers:

- Paper I **Endosperm and whole grain rye breads are characterized by low post-prandial insulin response and a beneficial blood glucose profile**
L. Rosén, L. Silva, U. Andersson, C. Holm, E. Östman and I. Björck
Nutrition Journal, 2009. 8(1): p. 42
- Paper II **Effects of cereal breakfasts on postprandial glucose, appetite regulation and voluntary energy intake at a subsequent standardized lunch; Focusing on rye products**
Rosén, L., E. Östman, and I. Björck
Nutrition Journal 2011. 10(1): p. 7
- Paper III **Postprandial glycaemia, insulinaemia and subjective satiety in healthy subjects following intake of bread products made from five different rye varieties**
L. Rosén, E. Östman, P. Shewry, J. Ward, A. Andersson, V. Piironen, A. M. Lampi, M. Rakszegi, Z. Bedő and I. Björck
Manuscript, 2011
- Paper IV **Postprandial glycaemia, insulinaemia and satiety responses in healthy subjects after wholegrain rye bread made from different rye varieties grown in Sweden**
Rosén, L., E. Östman, and I. Björck
Manuscript, 2011

The author's contribution to the papers

- Paper I The author, L Rosén, was involved in the study design, developed the test products, coordinated the study, was responsible for the analysis of the test products, blood glucose, serum insulin and subjective satiety, evaluated the results and was responsible for writing the manuscript.
- Paper II The author, L Rosén, was involved in the study design, developed the test products, coordinated the study, was responsible for the analysis of the test products and their hydrolysis index as well as for the analysis of serum insulin, plasma ghrelin, breath hydrogen and subjective satiety, evaluated the results and was responsible for writing the manuscript.
- Paper III The author, L Rosén, was involved in the study design, developed the test products, coordinated the study, was responsible for the analysis of available starch in the test products and their hydrolysis index as well as for the analysis of serum insulin and subjective satiety, evaluated the results and was responsible for writing the manuscript.
- Paper IV The author, L Rosén, was involved in the study design, developed the test products, coordinated the study, was responsible for the analysis of subjective satiety, evaluated the results and was responsible for writing the manuscript.

Other publications relevant to the subject of this thesis

Metabolic effects of whole grain wheat and whole grain rye in the C57BL/6J mouse

U. Andersson, L. Rosén, E. Östman, K. Ström, N. Wierup, I. Björck and C. Holm

Nutrition 26(2): 230-239

A low glycaemic diet improves oral glucose tolerance but has no effect on β -cell function in C57BL/6J mice

U. Andersson, L. Rosén, N. Wierup, E. Östman, I. Björck and C. Holm

Diabetes, Obesity and Metabolism 12(11): 976-982

Abbreviations

AR	Alkylrescorcinols
AX	Arabinoxylans
CVD	Cardio-vascular disease
D. Zlote	Dankowskie Zlote rye
DF	Dietary fibre
ERB	Endosperm rye bread
ERB-lac	Endosperm rye bread with lactic acid
ERP	Endosperm rye porridge
FFA	Free fatty acids
H. Loire	Haute Loire pop rye
iAUC	Incremental area under the curve
iPeak	Incremental peak
IRS	Insulin resistance syndrome (the metabolic syndrome)
nAUC	Negative area under the curve
RBB	Rye bran bread
RK	Wholegrain rye kernels
SCFA	Short chain fatty acids
tAUC	Total area under the curve
T2D	Type 2 diabetes
WGRB	Wholegrain rye bread
WGRB-lac	Wholegrain rye bread with lactic acid
WGRP	Wholegrain rye porridge
WK	Wholegrain wheat kernels
WWB	White wheat bread
WWB ^{+MG}	White wheat bread with monoglycerides
WWP	White wheat porridge

Introduction

Metabolic disorders such as cardiovascular disease, type 2 diabetes and the insulin resistance syndrome (IRS) are increasing worldwide and preventive strategies are urgently needed. The IRS includes metabolic disturbances such as abdominal obesity, elevated triglyceride levels, reduced HDL cholesterol, hypertension and elevated plasma glucose (IDF 2005) and subjects with IRS have increased risk of developing type 2 diabetes and cardio-vascular diseases.

The IRS can be prevented by choice of diet, and, in observational studies, whole grains have been demonstrated to protect against IRS, type 2 diabetes and cardio-vascular diseases as well as weight gain and obesity (Anderson et al. 2000, Fung et al. 2002, McKeown et al. 2004, Lutsey et al. 2007). Mechanisms behind this protection have been suggested to originate from presence of dietary fibre and related components, for example antioxidants, micronutrients and antinutrients, with potential to e.g. increase insulin sensitivity, lower blood lipids and protect against overweight (Fardet 2010). Also, low glycaemic and insulinaemic foods have been shown to have health promoting properties (Salmerón et al. 1997, Salmerón et al. 1997, Liu et al. 2000, Schulze et al. 2004). The importance of the postprandial state is increasingly acknowledged and low postprandial glucose and insulin responses can protect against oxidative stress, sub-clinical inflammation and insulin resistance (Del Prato et al. 1994, Ceriello 2000). In this context, rye products are of interest as they are often consumed as whole grain and have been shown to induce a low postprandial insulinaemia (Leinonen et al. 1999, Juntunen et al. 2003, Björck et al. 2007).

Background

The insulin resistance syndrome

The insulin resistance syndrome (IRS), also known as the metabolic syndrome, is a cluster of metabolic disorders including abdominal obesity, elevated triglyceride levels, reduced HDL cholesterol, hypertension and elevated plasma glucose. The IRS increases the risk of developing cardio-vascular diseases (CVD) and type 2 diabetes (T2D) (IDF 2005), as well as polycystic ovary syndrome and non-alcoholic fatty liver disease (Einhorn et al. 2003). A patient who exhibits abdominal obesity plus two or more of the mentioned disturbances is considered to have IRS according to the International Diabetes Federation's definition (IDF 2005). The incidence of IRS is steadily increasing worldwide. According to the IDF definition, approximately 16% of 50 year old women and 26% of 50 year old men, from the Gothenburg region in Sweden suffer from IRS (Welin et al. 2008). In the U.S approximately 39% are estimated to suffer from IRS (Ford 2005). Alarming, the prevalence of IRS is also increasing in children and young adults (Weiss et al. 2004).

Abdominal obesity is considered to be a central risk marker for the IRS. Excess fat located in the central parts of the body are mainly as visceral adipose tissue, contributing to elevated levels of free fatty acids (FFA) with the ability to induce hepatic and peripheral insulin resistance (Boden et al. 1994). Insulin resistance increases the risk of glucose intolerance and T2D. The elevated blood glucose concentrations in these conditions will promote oxidative stress and contribute to atherosclerosis and increased risk of CVD (Giugliano et al. 1995, Ceriello 2000). Visceral adipose tissue is also an endocrine organ, secreting inflammatory cytokines such as TNF- α and IL-6. With increased visceral fat mass, the exaggerated production of cytokines can induce chronic low-grade inflammation, and increased abdominal obesity has been linked to higher risk of CVD and T2D (Rexrode et al. 1998, Carr et al. 2004, Meisinger et al. 2006). Figure 1 illustrates the suggested relation between increased visceral adipose tissue and the pathophysiology of the metabolic syndrome.

Background

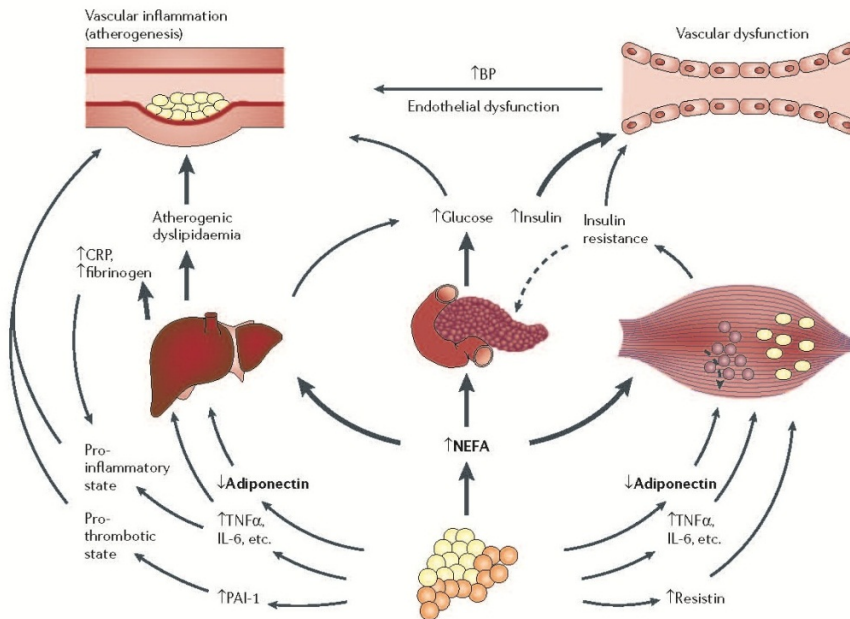


Figure 1. The pathophysiology of the metabolic syndrome. Reprinted by permission from Macmillan Publishers Ltd (Grundy 2006).

More recently, it has been suggested that imbalances in the gut microbiota play a crucial role in the development of low-grade inflammation (Cani et al. 2008, Round et al. 2009). The balance between beneficial microorganisms and potential pathogens in the gut is delicate, and dietary factors may affect this balance. Certain indigestible carbohydrates, prebiotics, can selectively stimulate growth of specific bacteria in the colon (Gibson et al. 1995, Roberfroid 2007), with potential benefits on the hosts health and well-being. Among known prebiotics are the indigestible but easily fermentable oligo-fructans, which stimulates growth of bifidobacteria, thereby reducing the proportion of potential pathogens in the gut (Roberfroid et al. 2010).

The IDF recommends that the primary intervention for patients with diagnosed IRS are moderate caloric restriction, moderate increase in physical activity and change in dietary composition (IDF 2005). These interventions can also prevent the IRS, and protective dietary compositions are discussed in more detail below.

Preventing the insulin resistance syndrome through diet

A ‘western diet’ rich in for example red meat, fried food, refined grains and saturated fats and low in vegetables, dietary fibre, dairy products and fruits, increases the risk of IRS (Esmailzadeh et al. 2007). Observational studies have further indicated that a diet rich in whole grains protects against IRS, T2D and CVD (Anderson et al. 2000, Fung et al. 2002, McKeown et al. 2004, Lutsey et al. 2007) and also facilitates weight regulation (Koh-Banerjee et al. 2003, Liu et al. 2003, O’Neil et al. 2010). This has led to an interest in elucidating mechanisms for potential disease preventive effects of whole grain.

WHOLE GRAIN DEFINITION AND MECHANISMS

The definition of whole grain by US Whole Grains Council is “*Whole grains or foods made from them contain all the essential parts and naturally-occurring nutrients of the entire grain seed. If the grain has been processed (e.g., cracked, crushed, rolled, extruded, and/or cooked), the food product should deliver approximately the same rich balance of nutrients that are found in the original grain seed.*” (2004). This definition is synonymous to that adopted within a recently terminated EU-project, Healthgrain (2010).

Whole grains are important sources of dietary fibre (DF). The latter was recently defined by the Codex Alimentarius (2009) as “*carbohydrate polymers with ten or more monomeric units, which are not hydrolysed by the endogenous enzymes in the small intestine of humans. The polymers should be naturally occurring in food as consumed, obtained from food raw material or synthetic carbohydrates, shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities*”. According to the European Commission, indigestible oligosaccharides with a degree of polymerisation of 3-10 monosaccharide units should also be included in the fibre definition (European Commission 2008). Cereal DF varies in physico-chemical properties such as viscosity, solubility and other features of potential influence on physiological parameters. DF can be fermented by bacteria in the colon and the degree of fermentation varies with type of fibre, with insoluble DF generally being more resistant to bacterial fermentation than soluble DF. The fermentation results in production of several components, among which the short chain fatty acids (SCFA) are suggested to lower blood glucose concentrations by reducing gluconeogenesis

Background

and increasing glycolysis (Roberfroid et al. 1998, Verbrugghe et al. 2009); and to have a beneficial effect on appetite regulation (Ropert et al. 1996, Cani et al. 2004, Nilsson et al. 2008). Also hydrogen (H₂) is formed and the H₂ exhaled through the lungs has been shown to be a sensitive indicator of increased carbohydrate fermentation in colon (Rumessen 1992).

Several health promoting bioactive components are being associated with DF in cereals, for example micronutrients such as minerals, vitamins, folates, choline and betaine, antioxidants such as tocotrienols, tocopherols, alkylresorcinols and phenolic acids and also lignans and sterols (Slavin et al. 1999, Slavin et al. 2001, Fardet 2010) However, most of these components are present in the bran and germ of the cereal kernels and will partly be lost when milling flour with lower extraction rates.

Protective effects of whole grain diets have been observed in intervention studies with improvements in insulin sensitivity (Pereira et al. 2002, Rave et al. 2007), decreased LDL-cholesterol (Keenan et al. 2002, Karmally et al. 2005, Maki et al. 2010), reduced waist circumference (Maki et al. 2010), and lowered blood pressure (Keenan et al. 2002, Tighe et al. 2010). However, effects on individual risk parameters are not consistent in different interventions. It is likely that the outcome of the mentioned studies may be affected by background diets, and the type of whole grain cereal being investigated.

WHOLE GRAINS AND GLYCAEMIA

Another potentially important quality characteristic of whole grain diets is the Glycaemic Index (GI) feature (Jenkins et al. 1986, Slavin et al. 1999, Fardet 2010). Regular consumption of foods with a high glycaemic index (GI), resulting in frequent high postprandial glycaemic episodes, may contribute to oxidative stress and sub-clinical inflammation (Ceriello 2000). In response to raised blood glucose levels, pancreatic β -cells release insulin, thereby facilitating glucose uptake in muscle cells and adipose tissue. High GI foods are thus often characterised by a high insulin index (II). Hyperinsulinaemia is known to contribute to decreased insulin sensitivity (Del Prato et al. 1994). Indeed, observational studies have demonstrated that diets with low GI and II protect against T2D and CVD (Salmerón et al. 1997, Salmerón et al. 1997, Liu et al. 2000, Schulze et al. 2004). Some information is also available from

intervention studies in subjects with impaired glucose tolerance or T2D. Consequently, low GI diets have been shown to contribute to improved blood glucose control in these subjects. However, results from interventions studies with low GI vs. high GI diets in healthy subjects are non-conclusive, in terms of impact on risk parameters in relation to CVD and diabetes (Riccardi et al. 2008). The inconsistency in these results may emanate from the experimental design, but also the background diet may influence the outcome on risk markers. Furthermore, as discussed by Riccardi et al. (2008), potential beneficial metabolic effects may become weaker in healthy subjects since they have a better regulated glycaemic response even after high GI meals.

Although a high content of intrinsic DF in cereal products is not a prerequisite for a low GI, certain whole grain products such as pumpernickel bread, sour-dough bread and cereal based rice analogues do have low GI features. Also of interest in this context are observations with rye, indicating that whole grain rye products display low postprandial insulin responses, with or without a simultaneous lowering of the GI (Leinonen et al. 1999, Juntunen et al. 2003, Björck et al. 2007).

Rye

HEALTH EFFECTS OF RYE

Health protective properties of rye products have been seen in several interventions. In a study in non-insulin dependent T2D, Hagander et al. (1987) demonstrated a lowering of the postprandial glucose response following whole grain rye bread compared to a white wheat bread. However, the postprandial insulin responses were similar, suggesting a more efficient insulin action following the rye bread. In another study, Östman et al (2006) found that exchanging the bread of young women with impaired glucose tolerance for low GI bread rich in rye during a 3 week dietary intervention improved insulin economy as estimated from an intravenous glucose challenge. Rye based diets have also been shown to increase early postprandial insulin compared to white wheat or oat bread and potato diets (Juntunen et al. 2003, Laaksonen et al. 2005). Wholegrain rye bread was shown to improve bowel function and to lower the concentration of compounds that are putative cancer risk markers in comparison with white wheat bread (Gråsten et al. (2000).

Background

Also benefits on blood lipids have been reported. Leinonen et al. (2000) demonstrated that a wholegrain rye bread diet reduced serum total and LDL-cholesterol in men compared to a refined wheat bread diet. However, fasting glucose and insulin responses remained unaffected in that study. Furthermore, rye is a rich source of lignans, a diphenolic compound, structurally similar to oestrogen (Hallmans et al. 2003). In mammals, the gut microbiota transforms most plant lignans into mammalian lignans, i.e. enterolactone and enterdiol, suggested to play a role in prevention of certain forms of cancer (Hultén et al. 2002). A wholegrain rye bread diet induced higher plasma enterolactone concentrations compared with a wholegrain wheat or a refined wheat bread diet in overweight middle-aged men (McIntosh et al. 2003). In that study, both the high-fibre rye and high-fibre wheat diet improved postprandial plasma and insulin responses at a standardised breakfast compared to that following refined wheat consumption.

Consequently, the metabolic properties of cereal diets may differ depending on the type of cereal. Based on the above discussion of lowered postprandial insulin responses, improved insulin economy and first-phase insulin sensitivity following rye products, rye may possess advantageous health effects. The potential mechanisms behind the acute effects of rye and the observed longer term implication on disease risk markers are relevant topics for research. Such studies may add to the understanding of the whole grain benefits seen in observational studies.

COMPOSITION

Rye (*Secale cereale*) is a cereal grown primarily in the northern parts of Europe and can grow on poor soils and in cold weather. It is a common cereal in breads and other products on the north European markets. Rye is rich in dietary fibre, especially arabinoxylans (AX) and fructans (Rakha et al. 2010). AX can be soluble (water extractable AX) or insoluble (water un-extractable AX) depending on the substituents on the (1→4)-linked β -D-xylopyranose backbone (Vinkx et al. 1996). Other types of DF in rye are oligosaccharides (Henry et al. 1989), cellulose, beta-glucans, Klason lignin, resistant starch and arabinogalactan (Rakha et al. 2010). When including fructans, 100% wholegrain rye crisp breads typically contain 18-20 DW% DF (Rakha et al. 2010). A high content of soluble AX with high molecular weight contributes to improved baking quality, due to their ability to increase viscosity and retain

water in the dough (Girhammar et al. 1992, Weipert 1997). Rye breads are usually made with sourdough fermentation to enhance baking performance and sensory quality (Weipert 1997). Rye is usually consumed as whole grain, with the exception of Sweden and Norway, where an extraction rate of 80% is common. Flours with 80% extraction rate contain substantial amounts of the fibre-associated bioactive compounds such as vitamins, minerals, antioxidants and micronutrients, due to difficulties in separating the bran from the inner parts (endosperm) of rye during milling (Weipert 1997). Thus, besides insoluble fibre from the bran, an “endosperm” (sifted) rye bread can contain as much as 80% phenolic compounds, 60% tocopherols and 25% tocotrienols of the amounts in wholegrain rye (Michalska et al. 2007).

Regulation of postprandial blood glucose and insulin

The glycaemic and insulinaemic indices

The glycaemic index (GI) was introduced in 1981 by Jenkins and co-workers (Jenkins et al. 1981) as a measure of the blood glucose response after a meal rich in carbohydrates. The GI is defined as the incremental area under the 2 h blood glucose curve after a test product, expressed as a percentage of the corresponding area after an equi-carbohydrate reference product taken by the same subject (Figure 2). Reference products used are a white wheat bread (WWB) or a glucose solution. WWB typically receives a GI of around 70 when tested against a glucose solution, thereby giving a conversion factor of 0.7 between the scales.

Similar to the GI, an insulinaemic index (II) can be calculated as a measure of the insulin response after intake of a particular product. The II is calculated by dividing the incremental area under the 2 h insulin curve after a test product by the corresponding area following the reference product. For most starch-rich products, the GI and II are of the same magnitude (Figure 3).

Background

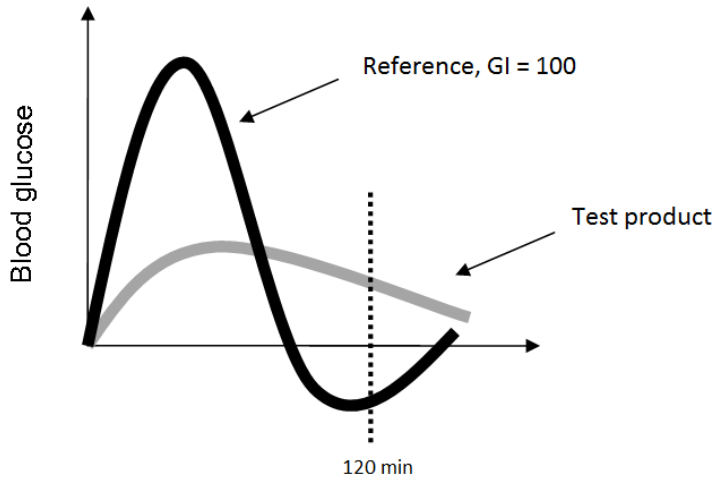


Figure 2. The GI of a test products is calculated by dividing the area under the blood glucose curve following the test product divided by the area under the curve following a reference products (glucose or white wheat bread) taken by the same subject. The two test meals should contribute with the same amount of available starch, usually 50 g.

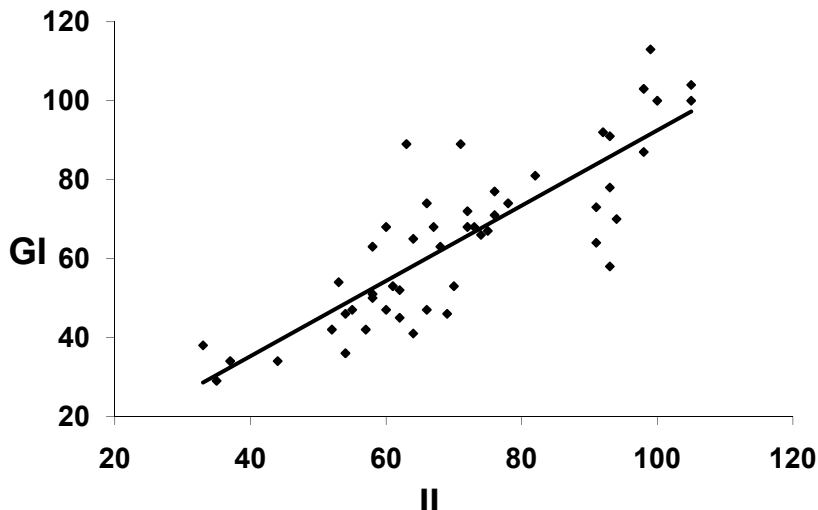


Figure 3. Correlation between GI and II after meals. Reprinted by permission from Cambridge Journals, (Björck et al. 2000).

The utility of the GI concept has been extensively debated. However, the importance of the postprandial metabolism is increasingly being acknowledged. Indeed, the postprandial phase has been demonstrated to be

highly important when evaluating risk of disease related to the IRS (Brand-Miller et al. 2007, Lavi et al. 2009) and the blood glucose level 2 h after a glucose load has been demonstrated to be a powerful risk predictor of CVD (Ceriello 2004). As indicated below (in “The second meal effect”), low GI whole grain foods rich in fermentable carbohydrates may be particularly beneficial in that they may lower glycaemic excursions and insulin demand over several consecutive meals (Nilsson et al. 2008).

FACTORS AFFECTING POSTPRANDIAL GLUCOSE RESPONSE

Several factors affect the postprandial blood glucose response. Basically, hindrance of carbohydrate digestion and absorption in the small intestine will lead to a slower and lower rise of the postprandial blood glucose and give a lower GI value. Factors promoting a lowered digestion and absorption include well organised food structures that prevent amylases from hydrolysing the starch. These structures can be either botanical, such as in whole cereal kernels (Liljeberg et al. 1992), or be physically induced. These structures can be either botanical, such as in whole cereal kernels (Liljeberg et al. 1992), or physically induced. An example of the latter is pasta, where the starch is entrapped in the wheat protein network (Colonna et al. 1990, Granfeldt et al. 1990).

Also, high starch crystallinity will lower the GI of foods. Starch that is present in a crystalline form is more inaccessible to amylases and will thereby obstruct digestion. Consequently, gelatinised starch will have higher GI than native starch packed in granules (Björck et al. 2000). Starch can also become crystalline by retrogradation. Retrograded starch ranges from completely resistant to enzymatic hydrolysis (resistant starch, RS) to slowly digested starch. Thus, a product with a high intrinsic content of RS is characterised by a lower GI, due to its high content of slowly digestible starch (Björck et al. 2000). Retrogradation of starch can be achieved by temperature cycling (Leeman et al. 2005).

The type of starch also affects starch crystallinity. Amylopectin has a branched structure, with more access points for amylase compared to the linear amylose. Amylose will also more easily undergo retrogradation, contributing to a lowering of enzyme availability, with potential effects also on postprandial glycaemia. (Åkerberg et al. 1998). However, the amylase proportion in most common starches is about 25%.

Background

A high content of viscous (DF) has been demonstrated to lower the glycaemic response. Indeed, Östman et al (2006) showed that the fluidity of in vitro digestas of barley breads containing various amounts of β -glucan was strongly correlated with the GI of the breads. The proposed mechanisms behind the reduced metabolic response are delayed gastric emptying and delayed absorption of glucose from the small intestine (Jenkins et al. 1978). Accordingly, Torsdottir et al. (1991) has demonstrated that 5 g of sodium alginate reduced postprandial glycaemia and lowered gastric emptying rate in 7 non-insulin dependent diabetic men.

The presence of organic acids of the type formed upon for example sourdough fermentation or pickling of vegetables can also lower the GI of foods. Lactic acid has been shown to induce protein-starch interactions when present during baking of bread, thereby lowering the degree of starch gelatinisation, and hence enzyme availability (Östman et al. 2002). On the other hand, propionic and acetic acids have been shown to lower the rate of gastric emptying, thereby delaying the rate of starch delivery to the small intestine (Liljeberg et al. 1995, Liljeberg et al. 1998).

Anti-nutrients such as phytic acid have been shown to reduce the postprandial glycaemia, possibly by interacting with the amylases (Thompson et al. 1987). Other dietary compounds such as certain polyphenols lower the GI, and the suggested mechanism could be starch-polyphenol interactions (Thompson et al. 1984). Lectins have also been demonstrated to lower the rate of starch digestion, possibly in the luminal stage (Rea et al. 1985). Some of these compounds are currently being referred to as dietary fibre co-passengers with potential bioactivity (Fardet 2010).

Different low molecular weight carbohydrates have different GI, increasing in the following order: fructose<lactose<sucrose<glucose, yielding differences in GI of food products depending on the nature and proportions of these sugars (Foster-Powell et al. 2002).

Certain amino acids in foods have the ability to lower the glycaemic response by increasing insulin secretion (Nilsson et al. 2007). Some amino acids present in whey have been shown to stimulate the incretin hormone gastric inhibitory polypeptide (GIP), in both healthy and type 2 diabetic subjects, thereby

increasing postprandial insulinaemia (Nilsson et al. 2004, Frid et al. 2005). GIP is predominately secreted from the upper small intestine and acts as an insulin secretagogue in response to various orally ingested nutrients (Vilsbøll et al. 2004).

The second meal effect

In response to a drop in postprandial glycaemia, glucagon is released from the pancreatic α -cells promoting glycogenolysis in the liver. Furthermore, lipolysis is triggered causing release of triglycerides and FFA. High concentrations of FFA induce hepatic and peripheral insulin resistance in healthy individuals and diabetics, leading to reduced insulin mediated glucose uptake (Boden et al. 1994, Boden et al. 1995). FFA also increases the blood glucose concentration by increasing hepatic glucose output. The increase of FFA in the post-absorptive state can influence glucose tolerance already at the subsequent meal; Jenkins et al. demonstrated in 1982 that the postprandial blood glucose response was significantly lower after a standardised lunch served 4 hours after a low GI lentil breakfast, as compared to the post-lunch response following a high GI wholemeal bread breakfast. This “second meal” effect was suggested to depend on lower FFA concentration after the low GI breakfast (Jenkins et al. 1982).

Second meal benefits in a 10 h perspective, from a late evening meal to breakfast or from breakfast to dinner, have been observed following a low GI meal rich in indigestible carbohydrates. Nilsson et al. (2006, 2008) reported improved glucose tolerance at a standardised breakfast served in the morning after barley kernel evening meals compared with white wheat bread evening meal. It was demonstrated that the indigestible carbohydrates induced colonic fermentation, and that the barley kernel evening meals induced significantly higher concentrations of breath hydrogen and lower concentrations of FFA prior to the standardised breakfast compared with the white wheat reference bread. The level of colonic fermentation and FFA prior to the breakfast meals was negatively respectively positively correlated to the postprandial glycaemia after the breakfast (Nilsson et al. 2008).

Importance of the course of glycaemia

Parallel to the GI concept, the course of glycaemia is also of importance for the post-absorptive phase. Liljeberg et al. (2000) reported that spaghetti products, characterised by a low glycaemic incremental peak and a maintained net-increment in the very late post-meal phase, was efficient in improving glycaemia and insulinaemia at a lunch-meal ingested 4h after breakfast. Additionally, the pasta breakfast contributed to reduced serum triglyceride (TG) levels just prior to the lunch, and in the post-meal phase after lunch. Similarly, Wolever et al. (1995) have demonstrated that a prolonged digestive phase suppressed FFA in the late postprandial phase and increased glucose tolerance at a subsequent meal. This indicated that not only the early glycaemic excursion following a meal, but also the ability of a food to maintain a prolonged course of glycaemia may be advantageous from a metabolic point of view. Along those lines, in a study comparing nibbling vs. gorging regimens, nibbling was most favourable with respect to insulin requirements and blood lipids (Jenkins et al. 1989), in support of benefits with a lente administration of carbohydrates, i.e. a low glycaemic response of long duration. Furthermore, avoidance of hypoglycaemia in the late postprandial phase has been demonstrated to promote subjective satiety (Haber et al. 1977), also supporting the benefits of a low but prolonged course of postprandial glycaemia.

Until recently, few studies have addressed the course of glycaemia. As it appears to be of interest both for second meal glucose tolerance and appetite regulation, more investigations are needed, along with functional quantitative measurements of the glycaemic profile.

Objective

The general objective of the present thesis was to elucidate mechanisms for the comparatively low acute insulin demand reported for rye products in healthy humans.

More specifically the aims were to:

- Investigate postprandial glucose and insulin responses after rye products and to introduce measures of the postprandial course of glycaemia.
- Investigate to what extent acute glycaemic and insulinaemic responses were affected by choice of rye variety or processing conditions, including extraction rate.
- Investigate appetite-regulating properties of rye products in the acute postprandial phase, as well as at a subsequent “second meal”.
- Investigate to what extent colonic fermentation of indigestible carbohydrates in rye, as estimated from measurement of breath hydrogen excretion, affected glucose metabolism and appetite regulation.

Materials and Methods

Test Products

Bread products

The white wheat bread (WWB^{+MG}) used as a reference in papers I and III was made in a bread baking machine. The recipe and baking procedure are described in paper I. In papers II and IV, the same recipe and baking procedure was used for the WWB reference, with the exception that this bread did not contain 2% monoglycerides (flour basis, Aromatic, Stockholm, Sweden). The white wheat flour was obtained from Kungsörnen AB (Järna, Sweden) in all four papers. Dry yeast used in all breads was obtained by Jästbolaget AB (Sollentuna, Sweden).

The sifted rye (called endosperm in this theses) and wholegrain rye breads in papers I and IV contained 25% white wheat flour (flour basis), while those in papers II and III consisted of pure rye. The rye bran bread in paper I consisted of 35% rye bran and 65% white wheat flour. The rye doughs in the four papers contained 1.1-1.7% dry yeast and 0.9% NaCl (flour basis). The rye breads containing lactic acid had a concentration of 1.4-1.5% acid (flour basis). All rye breads were baked in a similar way; the dough was mixed in a mixing bowl (6-10 min), followed by proofing at room temperature for 30-40 minutes. The dough was divided into pieces of 1 kg each (750 g in paper III) and placed in a bread making tin, followed by a second proofing for 30-60 min at 38°C, 85% humidity. The rye breads in paper I were proofed for the second time at room temperature. In paper I, the rye breads were baked at 250 °C for 40 min. In papers II-IV, baking was performed initially at 250 °C with 3 sec of steam. The temperature was then immediately lowered to 200°C and the breads were baked for 35-40 min. The recipes and full baking procedures are described in papers I-IV. The endosperm and wholegrain rye flour in papers I-II were commercial blends provided by Lantmännen R&D (Järna, Sweden). The rye bran was provided by Lantmännen R&D (Järna, Sweden) and was milled to

Materials and Methods

pass through a 0.8 mm screen (Laboratory Mill 12, Perten, Huddinge, Sweden) prior to baking. In paper III the wholegrain rye flours were blends of harvests from four different sites in Europe (Martonvásár, Hungary; Woolpit, UK; Choryn, Poland and Clermont Ferrand, France). In paper IV, the commercial whole grain rye blend and the Vicello rye was provided by Lilla Harrie mills (Kävlinge, Sweden) and the Evolo, Picasso and Kaskelott rye were provided by Lantmännen SW Seed AB (Svalöv, Sweden)

The WWB was left to cool for 1 hour and the rye breads for 18-24 hours under cover. The crust was then removed and the breads were sliced and wrapped in aluminium foil in portions sizes, put into plastic bags and stored in a freezer (-18° C) until use. The day before the experiment, the breads were taken from the freezer and were thawed at ambient temperature, still wrapped in aluminium foil and in the plastic bag.

Flour based porridges and boiled kernels

The porridges in paper I were cooked in a microwave oven at 680 W for 3 min. The porridges were freshly prepared each experimental day and were left to cool under aluminium foil for 15 min before serving. The rye porridges contained 25% white wheat flour (flour basis). All three porridges contained 0.5-0.6% NaCl. The recipes of the porridges are described in paper I. The wholegrain wheat kernels (WK) and rye kernels (RK) used in paper II were prepared on the morning of the experiment. The kernels were boiled with 0.5% NaCl for 35-40 min. All water was absorbed by the kernels. The exact proportions between kernels and water are described in paper II.

Chemical analyses of test products

STARCH

Available starch in test products used in papers I, III and IV was analysed according to Björck et al. (1992). In paper II, total and resistant starch (RS) were analysed in the breakfast products according to Björck et al. (1992) and Åkerberg et al. (1998). The available starch was calculated by subtracting RS from total starch.

Total and available starch were analysed on air dried and milled products while resistant starch (RS) was measured on the products themselves.

DIETARY FIBRES

Insoluble and soluble DF were determined with a gravimetric, enzymatic method described by Asp et al. (1983) on air dried and milled breakfast products in all studies.

PROTEIN

In papers I and II, protein content was determined by Kjeldahl analysis (Kjeltec Auto 1030 Analyser, Tecator, Höganäs, Sweden). In papers III-IV, protein content was analysed using an elemental analyser (FlashEA 1112, Thermo Fisher Scientific Inc., Waltham, MA, USA). Protein content was analysed on air dried and milled products in all four studies.

FAT

In paper I, fat was determined according to Lange (1984) on air dried and milled samples with the exception that petroleum ether BP 60-80° C was used instead of petroleum ether BP 40-60° C and 10 ml of each ether was used instead of 15 in the second and third washing step. In paper II, fat content in the products was calculated using data from endosperm and wholegrain rye and wheat flours from Lantmännen.

BIOACTIVE COMPOUNDS

In paper III, analyses of alkylresorcinols (AR), folate, tocopherols, sterols, betaine, choline and phenolic compounds were performed on freeze dried and milled breads. Alkylresorcinols were analysed according to Andersson et al. (2008). Folate and sterols were analysed according to Piironen et al. (2002, 2008). Tocopherols were analysed according to Lampi et al. (2008). Free phenolic compounds were analysed according to Li et al. (2008). Betaine and choline were analysed according to Howarth et al. (2008).

HI

The rate of starch hydrolysis (HI) was determined using an in vitro procedure based on chewing (Granfeldt et al. 1992), with WWB as a reference.

Meal studies

Test subjects

All subjects in papers I-IV were healthy non-smoking volunteers aged 21-37 years (25.5 ± 0.5 ; mean \pm SEM) with normal body mass indices (22.4 ± 0.2 kg/m²; mean \pm SEM) and without drug therapy. Both men and women participated in the four studies (a total of 31 men and 25 women). All subjects had normal fasting blood glucose concentrations. All subjects gave their informed consent and were aware of the possibility to withdraw from the study at any time they desired. Approval of the studies was obtained from the regional ethical review board in Lund, Sweden

Study designs

The products were provided as breakfasts in random order with approximately 1 week between each test. The subjects were instructed to eat a standardised evening meal (21:00-22:00) prior to the test, consisting of a few slices of white wheat bread. No eating or drinking except for small amounts of water was then allowed until the start of the test. The subjects were also told to avoid alcohol and excessive physical exercise the day before each test, but otherwise maintain their regular life style throughout the entire study. The subjects arrived at the laboratory at 07.45 on the test day. A peripheral venous catheter (BD Venflon, Becton Dickinson, Helsingborg, Sweden) was inserted into an antecubital vein. Thereafter the test products, contributing with 50 g of available starch (40 g in paper I), were served with 250 ml of tap water. The test subjects finished the breads and porridges within 14 minutes and the kernels in paper II within 25 minutes. In paper II, the subjects were served 250 ml of tea, coffee or water without any sweeteners or milk products two hours after the breakfast meal. The chosen beverage remained consistent for each individual on all seven visits. Two small sub studies were performed in addition to those in papers I-IV. In the first, 12 subjects from paper III were served WWB with and without monoglycerides at two separate occasions. Plasma glucose and serum insulin were samples and analysed as described in paper III. Also, in 14 of the subjects from paper IV, plasma glucose was analysed in venous blood drawn into tubes containing EDTA, at the same time

as the capillary plasma glucose analysis was performed, and using the same analysis method as for capillary blood.

AD LIBITUM LUNCH BUFFET, PAPER II

In paper II, the test subjects were served an *ad libitum* lunch buffet, composed of meatballs (ICA Handlarnas AB, Solna, Sweden), pasta (Kungsörnen AB, Järna, Sweden), ketchup (Procordia Food AB, Eslöv, Sweden) and cucumbers. The cucumbers were fresh, and were peeled and sliced prior to serving to ensure homogeneity. The meatballs were heated in a microwave oven while the pasta was boiled for 8 min in salted water. One tablespoon of rapeseed oil (Di Luca & Di Luca AB, Stockholm, Sweden) was added to the pasta after boiling. The subjects were instructed to eat the amount needed to reach comfortable satiation. On the subsequent visits they were to eat until they reached the same degree of satiation as on their first occasion. The amount of water served to the *ad libitum* buffet lunch was determined on the subject's first visit, and kept constant on the following visits. The subjects had to finish their lunch within 30 min, before the next blood sampling occasion at 300 min after commencing breakfast. The weight of the different food items ingested was registered individually to allow calculation of the energy intake at the buffet lunch meal. The energy content of the foods in the lunch buffet was obtained from the manufacturer of the products, and that of the cucumber from food tables (Swedish National Food Administration).

Physiological parameters

BLOOD/PLASMA GLUCOSE

Blood glucose was analysed in capillary blood in all four studies. Prior to serving the breakfast test meals, a fasting blood glucose sample was taken. In paper I, blood glucose was then analysed at 7.5, 15, 30, 45, 70, 95, 120 and 180 min after the start of the meal. In papers II-IV, samples were taken at 15, 30, 45, 60, 90, 120 and 180 min. In paper IV, blood glucose was also analysed at 150 min after commencing breakfast and in paper II also at 240 and 270 min. In paper I, blood glucose concentrations were determined in capillary whole blood using a B-glucose analyser (mod no. 120401, Hemocue, Ängelholm, Sweden). In papers II-IV a plasma glucose analyser (Glucose 201+, Hemocue, Ängelholm) was used.

Materials and Methods

SERUM INSULIN

Serum insulin was analysed in venous blood in all four studies. A fasting sample was taken prior to the breakfast test meals. The time points for serum insulin sampling in the four studies were the same as for blood glucose sampling. The serum insulin measurement was performed on an integrated immunoassay analyser (CODA Open Microplate System; Bio-rad Laboratories, Hercules, CA, USA) by using an enzyme immunoassay kit (Mercodia AB, Uppsala, Sweden).

SUBJECTIVE SATIETY

In paper I the subjects rated their feeling of hunger and satiety on a bipolar subjective rating scale graded from -10, representing extreme hunger, to + 10, representing extreme satiety. The feeling of satiety was rated before the meal (0 min) and at 15, 30, 45, 70, 95, 120 and 180 min after commencing breakfast. In papers II-IV, the subjects were asked to fill in their subjective *feeling of fullness*, *hunger* and *desire to eat*, respectively, using a 100 mm Visual Analogue Scale (VAS). The reason for changing the scale was two-fold: the VAS is more commonly used (Blundell et al. 2010) and it was believed that the possibility of investigating the three sensations separately could provide better relations to the hunger hormone ghrelin. In paper II, subjective appetite ratings were performed prior to serving the test breakfast meal every 30 min throughout the experimental day (390 min) and also at 15 and 315 min. In papers II and IV, subjective rating of satiety was performed prior to serving the breakfast test meals and at the same times as blood glucose and serum insulin were measured.

PLASMA GHRELIN

Total plasma ghrelin was analysed in papers I and II. In paper I, samples for ghrelin analysis were taken prior to serving the test breakfast meals and at the same times as samples for blood glucose and serum insulin. In paper II, ghrelin was analysed prior to the test breakfast meal and at 60, 90, 120, 270, 330, 360 and 390 min. In paper II, the plasma was collected into tubes containing 500 KIU aprotinin (Bayer HealthCare AG, Leverkusen, Germany) per ml of whole blood. Plasma ghrelin was determined with a commercially available radioimmunoassay kit (Linco Research Inc., St. Charles, MO, USA).

BREATH HYDROGEN, SERUM FFA AND SERUM ADIPONECTIN

Breath hydrogen excretion (H_2) was measured in paper II using an EC 60 gastrolyzer (Bedfont EC60 Gastrolyzer, Rochester, England). H_2 was measured prior to the test breakfast meal and then each 30 min throughout the test day (390 min). Serum FFA was analysed in paper II prior to the test breakfast meal and at 180 and 270 min using an enzymatic colometric method (NEFA C, ACS-ACOD method, WAKO CHEMICALS gmbH, Germany). Serum adiponectin was analysed in paper II prior to the test breakfast meal and at 180 and 270 min using an enzyme immunoassay kit (Mercodia AB, Uppsala, Sweden).

Calculations and statistical methods

Data is expressed as means \pm SEM. The total, net incremental and negative areas under the curves (tAUC, iAUC, nAUC) were calculated for each subject and test meal, using the trapezoid model. The glycaemic index (GI) and insulinaemic index (II) were calculated using the iAUC (0-120 min) for p-glucose and s-insulin, respectively, with WWB as a reference (FAO/WHO 1997). Incremental glucose and insulin peaks (iPeak) were calculated as maximum postprandial increase from baseline. Also the difference between the highest and the lowest measured blood/plasma glucose concentration (glucose amplitude) was calculated for each subject and meal. In addition, the course of glycaemia was analysed by calculating a glycaemic profile (GP): the time (min) during which the blood glucose remained above fasting concentration was divided by the glucose iPeak (mM) for each subject and test meal (Graph Pad Prism, version 4 and 5, Graph Pad Software, San Diego, CA, USA). In the cases where the glycaemic concentration remained above fasting for the entire 180 min (270 min in paper II), the duration value was set to 180 min (270 min in paper II). GP² was calculated in the same way as GP, but the duration was divided by the squared glucose iPeak. Relative changes (%) from fasting concentration to the nadir and to the concentration at 180 min after commencing breakfast were calculated for plasma ghrelin (paper I). Hydrolysis index was calculated from the 180 min AUC for in vitro starch hydrolysis, in a similar way of calculating GI and II values, using WWB as a reference (Granfeldt et al. 1992) (papers I-III).

Materials and Methods

Time x treatment interactions were analysed using a mixed model (PROC MIXED in SAS release 8, SAS Institute Inc., Cary, NC) with repeated measures and an autoregressive covariance structure. Subjects were modelled as a random variable in all papers and in papers II-IV and corresponding baseline (fasting values) values were modelled as covariate.

The data in paper I was analysed with a mixed model analysis of variance (ANOVA). In papers II-IV, the data was analysed using a mixed model analysis of covariance (ANCOVA) with subject as a random variable and corresponding baseline (fasting values) as a covariate. The reason for changing from ANOVA to ANCOVA analysis between papers I and II was that it is generally considered appropriate to include baseline measurements in the evaluation of results in randomized clinical trials (Pocock et al. 2002, Senn 2006, Van Breukelen 2006, Blundell et al. 2010).

For voluntary energy intake at lunch and HI in paper II and papers II-IV, respectively, a mixed model analysis of variance (ANOVA) was used with subject as a random variable. Differences between groups were identified using Tukey's multiple comparison tests. In the cases of unevenly distributed residuals (tested with Anderson-Darling test), Box Cox transformation were performed on the data prior to the ANCOVA and ANOVA. MINITAB, release 14-16, (Minitab Inc., State College, PA) was used to calculate ANOVA and ANCOVA.

Correlation analysis was carried out to evaluate the relationships between dependent measures with the use of Spearman's partial correlation coefficients controlling for subjects and in papers II-IV also for corresponding baseline values (two-tailed test, SPSS software, version 17-19; SPSS Inc., Chicago, IL, USA). $p < 0.05$ was considered statistically significant. In paper III, Pearson correlations were also used to evaluate correlations between bioactive compounds and fibre content in the rye breads.

Results and Discussion

Paper I

In paper I, the postprandial metabolic and satiety responses were investigated in rye products, differing in extraction rate (whole grain [100%], endosperm [80%] and bran) and in processing conditions (porridges vs. breads). Also the influence of added lactic acid was investigated in whole grain rye bread, at an enclosure level simulating that obtained during sourdough baking using a homofermentative starter culture. The rye bran was milled and baked into a white wheat bread, consisting of 35% rye bran. The endosperm and wholegrain rye breads were made from 75% Swedish commercial rye blend of unknown composition, and 25% white wheat. A white wheat bread baked with 2% monoglycerides (WWB^{+MG}) was used as reference product. In addition, a white wheat porridge was included in the study. The products were tested in a randomised crossover design in 12 healthy subjects.

The insulin index, II, was lower for all rye products compared to the WWB^{+MG}, ranging from 61 to 73, with the exception of the rye bran bread. Instead, the bran bread induced an II of 128, significantly higher than that of the other rye products. This finding indicated that the insulin-lowering properties seen with rye in the present and in previous studies (Leinonen et al. 1999, Juntunen et al. 2003, Björck et al. 2007) did not appear to be related to the bran fraction, but rather to the endosperm part of rye.

The blood glucose response was also lowered following all rye products in comparison to the WWB^{+MG}, except for the whole grain rye bread and rye bran bread. In order to study the postprandial course of glycaemia, a qualitative measure of the Glycaemic Profile (GP) was introduced. The GP is defined as the duration for incremental postprandial blood glucose response divided by the glucose iPeak. Consequently, a high GP value indicates a prolonged response of low magnitude and a low GP an elevated glucose response of short duration. The endosperm rye bread and the wholegrain rye bread baked with lactic acid yielded higher GPs than the WWB^{+MG}, suggesting a better-regulated

Results and Discussion

postprandial glycaemia following these rye products. Interestingly, the GP values in paper I showed better correlation with the insulin response (calculated as II and insulin iPeak) than did the GI.

The rye porridges were characterised by a higher early insulin response (iAUC 0-30 min) than the corresponding breads. The early insulin response following the endosperm and wholegrain rye porridges was 196 and 187 % of the response following the endosperm and wholegrain rye breads, respectively. For the wholegrain rye breads, the bread baked with lactic acid had a significantly higher GP and lower GI and induced a significantly lower insulin iPeak than the WWB^{+MG}, while the wholegrain rye bread baked without acid was not significantly different from WWB^{+MG} in these aspects. However, no statistically significant difference appeared in the variables stated when comparing whole grain bread with or without the lactic acid addition.

The rye bran bread and the endosperm and wholegrain rye porridges induced a significantly higher subjective satiety, evaluated using a bipolar rating scale from -10, representing extremely hungry to +10, representing extremely full. In contrast, neither the endosperm nor wholegrain rye breads with or without lactic acid induced higher satiety than WWB^{+MG}. The wholegrain rye bread induced 45% of the total subjective satiety response compared to after the wholegrain rye porridge (iAUC 0-180 min, $p < 0.05$). The late postprandial subjective satiety was related to the extent of hypoglycaemia in the late postprandial phase (180 min). A low insulin iPeak and II and a high GP of the products were related both to a milder hypoglycaemia as well as to a milder recovery of the hunger peptide ghrelin in the late postprandial phase (180 min). These findings with the set of rye products from a Swedish commercial blend are noteworthy, and indicate metabolic benefits in addition to lowered insulinaemia.

Paper II

The study described in paper II was designed to investigate whether the low insulin responses seen with rye products, and the corresponding low recovery of the hunger peptide ghrelin in the late postprandial phase, could lower energy intake at a subsequent meal. Contributing to this hypothesis, Erdman et al. (2004) had demonstrated that a high concentration of ghrelin prior to an *ad libitum* meal increased the energy intake. In addition, Östman et al. (unpublished data) had found that boiled rye kernels, but not wheat kernels, increased subjective satiety both after the kernel meal as well as after a standardised second meal. Besides blood sampling for analysis of glucose, insulin, FFA, adiponectin and ghrelin, hydrogen excretion in the breath was measured as a marker of fermentative activity in the gut. Furthermore, subjective satiety was rated using three 100 mm VAS scales instead of the bipolar rating scale used in Paper I, to distinguish *feeling of fullness*, *hunger* and *desire to eat*. The products tested in paper II were wholegrain and endosperm rye breads (100% rye of a commercial Swedish blend of unknown composition, made from the same manufacturer as in paper I but from a different batch) baked with and without lactic acid, boiled wholegrain rye and wheat kernels and a white wheat bread made without added monoglycerides (WWB). The study had a randomized crossover design and involved 10 healthy subjects. At 270 min after the test breakfast, subjects were offered an *ad libitum* buffet, and instructed to eat until pleasantly full. The energy intake of the lunch was recorded.

Also in this work, the insulin responses (II) after the rye products, as well as after the wheat kernels, were significantly lower than after WWB, ranging from 65 to 75. All products but the endosperm rye bread displayed lower GI and glucose iPeaks than WWB. Furthermore, the rye kernels had a significantly higher GP (+83%) than the wheat kernels and WWB, respectively, despite the fact that the rye and wheat kernels induced similar GI values (73 vs. 68).

All test products induced significantly higher subjective *feeling of fullness* compared to WWB in the early postprandial phase (0-60 min). In this phase, *feeling of hunger* and *desire to eat* were significantly higher following the WWB than following the rye and wheat kernels and the endosperm rye bread

Results and Discussion

baked without lactic acid. In the later postprandial phase (210-270 min), rye kernels induced significantly lower *desire to eat* than all other products, including the wheat kernels. Furthermore, the rye kernel breakfast induced a significantly lower (-16%) voluntary energy intake at the subsequent *ad libitum* lunch compared to WWB.

The portion weight, water and indigestible carbohydrate contents were strongly correlated to the early subjective satiety ratings (0-60 min) after the test meals. The lowering of voluntary energy intake at a subsequent lunch was shown to correlate to an increased fermentation, measured as increased breath hydrogen, prior to lunch (120-270 min). The rye kernels and wholegrain rye bread induced significantly higher breath hydrogen prior to lunch (270 min) compared to WWB. In the postprandial phase after lunch, all rye products except the endosperm rye bread baked with lactic acid induced significantly higher breath hydrogen than WWB. No significant effect on breath hydrogen was seen following the wheat kernel breakfast.

Also the postprandial metabolic response to the test products was related to satiety. Consequently, a low insulin response (II and insulin iPeak) correlated to an increased early subjective satiety (0-60 min) and to a lowered energy intake at the subsequent meal. Furthermore, a low concentration of ghrelin prior to the *ad libitum* lunch (270 min) was associated with a lowered subsequent energy intake. A high GP for the test products was related to a lower insulin response (II and insulin iPeak), as noted in paper I, and to a lowered ghrelin response in the late postprandial phase. Furthermore, a high GP for the products correlated with a lower *desire to eat* in the late postprandial phase after breakfast (210-270) min, which in turn was related to a lowered energy intake at the *ad libitum* lunch. Consequently, a high GP appears to beneficially affect appetite regulation, mediated by lowered ghrelin levels.

Paper III

The purpose of paper III was to evaluate potential differences in metabolic and appetite regulation properties of five pure rye varieties, varying in content of bioactive components such as phenolic acids, tocopherols, sterols, alkylresorcinols, folate, betaine and choline. The rye varieties tested were Haute Loire Pop, Rekrut, Amilo, Dankowskie Złote and Nikita rye, which were especially collected from four different sites in Europe and pooled for evaluation within the EU project, Healthgrain. The rye varieties were used to make wholegrain rye breads (100% rye) and tested in a crossover study in 14 healthy subjects. A WWB^{+MG} made with 2% monoglycerides was used as a reference.

The Amilo and Rekrut rye induced insulin responses (IIs) that were significantly lower than that of WWB^{+MG} (72 and 78, respectively). In contrast, Haute Loire Pop, Dankowskie Złote and Nikita rye induced insulin responses similar to that of WWB^{+MG}. The GIs of the rye bread products were not significantly lower than that of WWB^{+MG} and the GP values of all products were similar. These findings indicate that some rye varieties are more beneficial in terms of insulin economy than others. All the rye breads induced higher satiety (0-180 min) than WWB^{+MG}. The Dankowskie-Złote and Rekrut rye also induced a lower *feeling of hunger* than WWB^{+MG}. Furthermore, a high late postprandial (180 min) *feeling of fullness* and a low *desire to eat* correlated with a lower II. The correlation between poor subjective satiety and high insulin responses is in accordance with the findings in Paper I and II.

The phenolic acids caffeic and ferulic acid have previously been shown to obstruct carbohydrate digestion and uptake in rats and rat intestinal brush border membrane vesicles by inhibiting enzymes and by reducing Na⁺ dependent glucose uptake (Welsch et al. 1989, Welsch et al. 1989, Adisakwattana et al. 2009). In the present study, a high amount of caffeic and ferulic acid, and also sinapic and vanillic acid, in the rye breads was related to a low early postprandial glucose and insulin response (tAUC 0-60 min). The causal relationship remains unknown, but the finding suggests that the phenolic constituents of rye might influence glycaemic regulation to rye products.

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Twelve of the subjects from paper III were served a WWB baked without added monoglycerides in addition to the other test meals. The rationale for this was to study the metabolic difference between the two reference products (WWB and WWB+^{MG}) used in this thesis. The WWB+^{MG} induced significantly higher GP values than the WWB, although the difference was small (48.2 vs. 44.1). The details are presented in the Appendix.

Paper IV

Based on the results in Paper 3 which indicated differences in metabolic responses to bread made from different European rye varieties, the potential differences in metabolic properties of bread made from common rye varieties grown in Sweden as well as one Swedish commercial blend of unknown composition were evaluated in paper IV. The commercial blend used in paper IV was provided by a different manufacturer than those in paper I and II. In this randomized crossover study, postprandial glycaemia, insulinaemia and subjective satiety were evaluated in 20 healthy subjects using WWB as a reference product. The pure rye varieties included in paper IV were Vicello, Picasso, Kaskelott, Amilo and Evolo.

The commercial rye blend elicited an II value and an insulin iPeak not significantly different from WWB. Among the other rye breads tested, all but Evolo had significantly lower IIs than WWB (ranging from 74 to 81) and all but Kaskelott induced significantly lower insulin iPeaks than WWB. The Vicello and Picasso rye breads had significantly lower GIs than WWB and all rye breads except Kaskelott and the commercial blend induced significantly lower glucose iPeaks than the WWB. The GP of the breads was not significantly different. In an attempt to further characterise the glycaemic profile, a modified measure, GP², was introduced, to increase the influence of the maximum glycaemia. GP² is, defined as the duration for incremental postprandial blood glucose response divided by the squared blood glucose iPeak. The GP² was significantly higher for the Vicello and Picasso rye breads, compared to WWB. The GP² of the products showed stronger correlations to the II and insulin iPeak, compared with the GI, suggesting that the GP² is a better predictor of the insulin response of the products in the present study.

The rye bread made from the commercial blend induced significantly higher subjective *hunger* and *desire to eat* in the early postprandial phase compared to WWB. The Vicello rye bread induced significantly higher subjective *feeling of fullness* in the early postprandial phase (tAUC 0-60 min) compared to Amilo. Early postprandial satiety was related to a high content of insoluble dietary fibre in the rye breads. A high GP and GP² as well as a low insulin iPeak were related to improved subjective satiety in the late postprandial phase.

In 14 of the 20 subjects in paper IV, a sub-study was performed in order to analyse the glycaemic profile also in venous blood after WWB. The purpose was to investigate possible differences in course of glycaemia in venous vs. capillary blood. The main finding was that the iPeaks were lower and the glycaemic incremental durations were shorter for the venous measurements. This resulted in similar GP values between the blood sampling methods, while GP² values differed more due to a lower glucose iPeak in venous blood measurements. Capillary blood glucose measurements are recommended for analysis of the glycaemic profile, as the lowered glycaemic concentrations after venous blood sampling could diminish the possibility of observing differences between products, both in the later postprandial phase and in maximum glucose measurements. The details are presented in the Appendix.

Postprandial glycaemia and insulinaemia

In papers I-IV, the insulin response following rye products was generally lower than that of WWB. The exceptions were the whole grain rye porridge in paper I, breads made from three of the rye varieties in paper III, and breads made from the commercial rye blend and Evolo in paper IV, which all displayed II values similar to that of WWB. The GI values of the rye products in papers I and II were relatively low, ranging from 64-79, while the GI values in papers III and IV were higher (79-96). Interestingly, several of the rye products in this thesis induced postprandial glycaemic curves with lowered glucose iPeaks and with incremental glycaemia remaining above fasting for a longer time, compared to the WWB (Figure 4). Such products may receive an excessively high estimate of the postprandial glycaemic excursion when using the GI as calculated from the incremental area under the curve, excluding

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values below the fasting level. Since well-regulated postprandial glycaemia with milder hypoglycaemia have been related to improved second meal glucose tolerance (Wolever et al. 1995, Liljeberg et al. 2000) and increased subjective satiety (Haber et al. 1977), a quantified measure of the course of glycaemia was developed including also the duration of net increment above fasting.

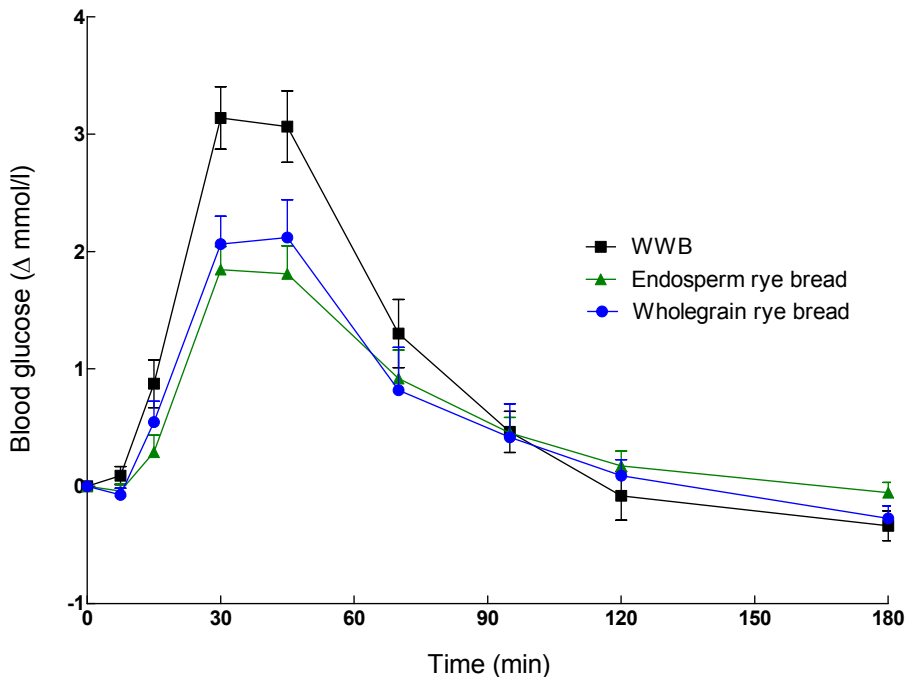


Figure 4. Glycaemic responses following endosperm rye bread, wholegrain rye bread and WWB illustrating different glycaemic profiles in healthy subjects (Paper I).

Estimates of the course of glycaemia

The glycaemic profile, GP, was introduced as a tool to discriminate between differences in blood glucose profiles. The GP is defined as the duration of the incremental postprandial glycaemic response divided by the glucose iPeak, thus rendering a high value for a long and low glycaemic profile.

Similarly, as products eliciting a low but sustained blood glucose curve may receive an unfairly high GI value, a product giving a sustained but high blood

glucose curve would receive a similar GP as a product causing a low but short glycaemic response. In order to compensate for such features, an additional measure of the course of glycaemia was introduced and evaluated. Consequently, GP^2 , defined as the duration of incremental postprandial blood glucose response divided by the squared glucose iPeak, increased the influence of the maximum glycaemia on the overall profile. In Figure 5, four types of glycaemic curves are shown to illustrate the extreme cases of glycaemic profiles.

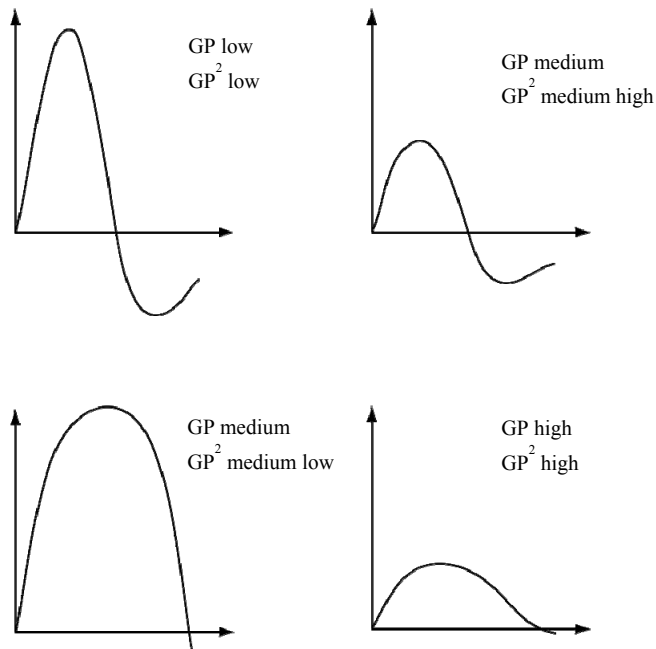


Figure 5. Four types of courses of glycaemia.

The GP^2 values might therefore offer a tool for more sensitive separation of the course of glycaemia in between products displaying variability in glycaemic excursion. In paper IV it was demonstrated that although there were no significant differences in GP between the products, the GP^2 of the Vicello and Picasso rye breads were significantly higher than that of WWB, thereby suggesting a better-regulated glycaemia after these products. In papers I and II, we also see more variation between the test products using the GP^2 measurement compared with GP. Table 1 shows the GP, GP^2 , glucose iPeaks and GI of the products in the four studies. The table also shows the glucose

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amplitude, which is the difference between the highest and lowest glycaemia measured in a subject following a test product. In addition to the glucose iPeak, the glucose amplitude also indicates the level of hypoglycaemia after a test product, thereby indicating the level of postprandial glucose oscillations.

Table 1. GP, GP², GI and glucose amplitude for all products in papers I-IV.

	Meals	GP	GP ²	GI	Glucose amplitude
		min/mM	min/mM ²	%	ΔmM
Paper I	WGRB-lac	74.3±9.7 ^a	45.7±14.9 ^a	74±9.5 ^b	2.48±0.25 ^{cd}
	ERB	69.2±10.1 ^a	44.3±12.6 ^a	64±7.5 ^b	2.41±0.22 ^d
	WGRB	51.0±7.0 ^{ab}	25.2±5.8 ^{ab}	71±9.7 ^{ab}	2.90±0.26 ^{bcd}
	ERP	49.7±6.3 ^{ab}	20.5±3.3 ^{abc}	70±6.3 ^b	2.72±0.13 ^{bcd}
	WGRP	39.7±7.3 ^b	17.1±4.2 ^{bcd}	72±10.2 ^b	3.16±0.27 ^{abc}
	WWB ^{+MG}	37.0±5.6 ^b	12.8±2.8 ^d	100±0.0 ^a	3.79±0.31 ^a
	RBB	35.7±3.4 ^b	12.0±1.9 ^{cd}	87±6.7 ^{ab}	3.67±0.32 ^a
	WWP	35.2±5.0 ^b	12.3±2.2 ^{cd}	77±9.8 ^{ab}	3.42±0.20 ^{ab}
Paper II	RK	94.2±12.6 ^a	47.2±12.0 ^a	73±8.4 ^b	2.60±0.28 ^b
	ERB-Lac	77.7±9.4 ^{ab}	34.8±6.2 ^{ab}	64±9.4 ^b	2.64±0.25 ^b
	WGRB	75.3±13.0 ^{ab}	31.2±8.4 ^{abc}	79±14.1 ^b	2.91±0.23 ^b
	WGRB-Lac	64.7±9.3 ^{ab}	27.6±5.7 ^{abc}	64±7.0 ^b	2.77±0.25 ^b
	ERB	58.9±10.0 ^{ab}	21.9±5.1 ^{bc}	77±7.7 ^{ab}	3.45±0.36 ^{ab}
	WK	50.7±7.0 ^b	20.0±3.7 ^{bc}	68±9.4 ^b	3.28±0.36 ^{ab}
	WWB	49.1±7.3 ^b	15.2±3.6 ^c	100 ^a	4.09±0.41 ^a
Paper III	Amilo	49.5±6.2 ^a	19.4±4.1 ^a	79±5.2 ^a	3.1±0.27 ^a
	WWB ^{+MG}	47.1±3.4 ^a	14.7±1.9 ^a	100±0.0 ^a	3.7±0.31 ^a
	Loire	45.7±3.5 ^a	14.4±1.8 ^a	96±9.7 ^a	3.51±0.23 ^a
	Nikita	42.0±4.5 ^a	13.7±2.6 ^a	91±11.1 ^a	3.53±0.25 ^a
	Rekrut	41.1±3.4 ^a	12.3±1.4 ^a	84±6.9 ^a	3.53±0.18 ^a
	Zlote	40.4±2.7 ^a	11.8±1.4 ^a	96±9.0 ^a	3.72±0.25 ^a
Paper IV	Vicello	59.5±9.5 ^a	31.9±13.4 ^a	79±8.0 ^b	3.26±0.23 ^b
	Amilo	54.2±7.4 ^a	26.9±10.4 ^{abc}	90±8.4 ^{ab}	3.33±0.20 ^b
	Picasso	52.3±4.7 ^a	26.7±7.9 ^{ab}	80±8.4 ^b	3.17±0.24 ^b
	Evolò	52.8±4.6 ^a	19.3±2.6 ^{abc}	92±8.1 ^{ab}	3.45±0.21 ^b
	Commercial	48.2±6.7 ^a	20.0±5.2 ^{bc}	95±8.3 ^{ab}	3.68±0.25 ^{ab}
	Kaskelott	47.9±4.1 ^a	16.6±2.3 ^{abc}	88.4±8.5 ^{ab}	3.51±0.21 ^{ab}
	WWB	41.5±2.8 ^a	12.5±1.5 ^c	100±0.0 ^a	4.11±0.27 ^a

Values are means ± SEM, Products not sharing the same letters were significantly different within each paper, $p < 0.05$ (ANOVA, followed by Tukey's test in paper I, ANCOVA, followed by Tukey's test in papers II-IV). See abbreviation list for abbreviations.

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As seen in Table 1, whole grain rye bread with lactic acid (GI 74) induced significantly higher GP and GP² values than the wholegrain rye porridge (GI 72) in paper I. In paper II, the GP and GP² are significantly higher for rye kernels (GI 73) compared to wheat kernels (GI 68, Figure 6). Despite these differences in GP and GP² values, the test meals induced similar GI values. Previously, pasta has been noted to induce a GI similar to WWB in healthy elderly subjects, despite a markedly lower and prolonged glycaemic profile (Granfeldt et al. 1991). Estimating the GP and GP² in that paper, the pasta received a GP of 120 and a GP² of 80 while the white wheat bread had considerably lower GP and GP² of 41 and 16, respectively.

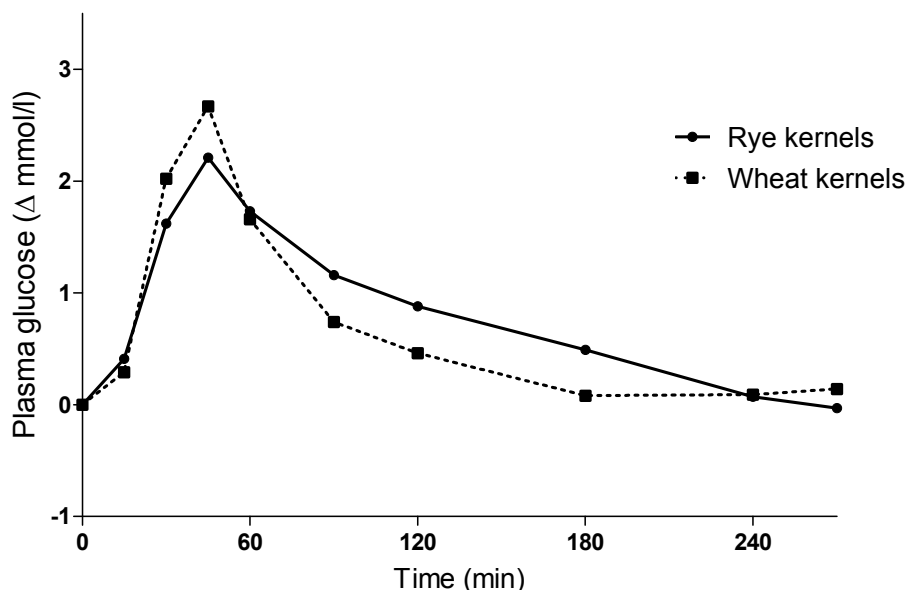


Figure 6. Glycaemic responses following rye kernels and wheat kernels illustrating different glycaemic profiles in healthy subjects (paper II).

Spearman's correlation analysis was used to investigate the extent to which the suggested estimates of the glycaemic profile, GP and GP², could take into account different features of the glycaemic response compared to GI. More precisely, it was evaluated how these estimates correlated with the early glucose iPeak, the late 120 and 180 min values and the glycaemic amplitude. Spearman's partial correlation coefficients are presented in Figure 7. The absolute values are presented (Absolute r) to simplify comparison.

As can be seen in Figure 7, the GP² showed better correlation than GI to the glucose iPeak, the glucose concentration in the late postprandial phase (180 min) as well as to the glucose amplitude. This indicates that the GP² appears to best depict glucose oscillations, glucose iPeak and late postprandial glycaemia for the products investigated. Within the frame of this thesis, only products rich in starch have been evaluated, and it would be of interest to study the course of glycaemia also following sugars and food containing low molecular weight carbohydrates such as soft drinks, vegetables, fruits, and dairy products.

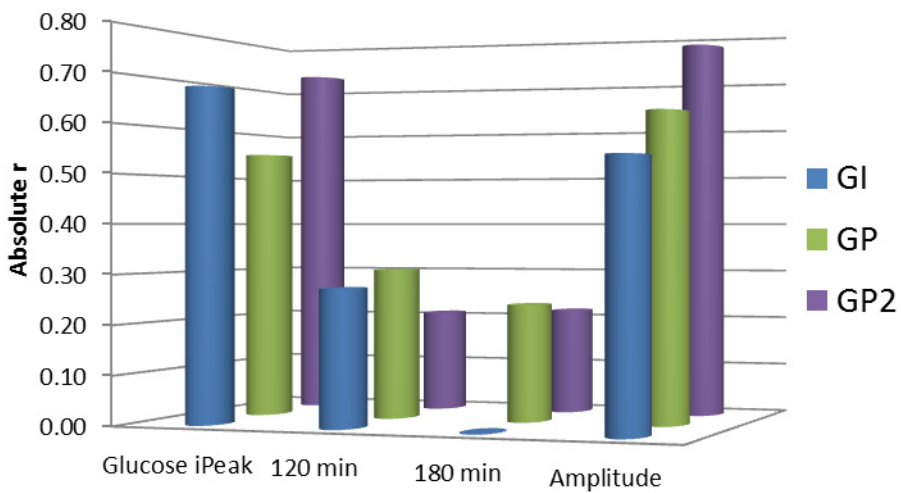


Figure 7. Relationship between the GI, GP and GP² and postprandial glucose iPeak, the concentration of blood/plasma glucose in the late postprandial phase (120 and 180 min) and glucose amplitude. The figure shows the Spearman's correlation coefficient (r), expressed in absolute values to simplify comparison. (Spearman's partial correlation coefficients controlling for subjects and corresponding baseline values, two-tailed test).

In general, postprandial studies of glycaemia are limited to 2 h as this is the standard for GI measurements. Unfortunately, this study duration is too short to determine the GP and GP², as the incremental glycaemia following some products remains above fasting for far longer periods. In papers I, III and IV, the maximum duration possible was 180 min, as this was the study length. Despite the extra hour compared to a standardised GI study, several values had to be set to 180 min, even though the real duration of incremental glycaemia

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could be longer. Even in paper II, some products induced courses of glycaemia remaining above fasting for more than 270 min. By, in reality, shortening the duration of products that induce well-regulated glycaemia to the same duration as the study length, the GP and GP² values of certain high GP/GP² products are lowered. However, the extent to which this lowering of the glycaemic profile influences the results is unknown and needs further attention. Furthermore, studies of longer postprandial durations are needed to more extensively study the very late postprandial course of glycaemia in relation to insulinaemia and associated metabolic features.

In order to study the relationship between measures of glycaemic profile (GP, GP²) and insulin responses (II and insulin iPeak) and compare with correlations between GI and insulin responses, Spearman's partial correlation coefficients were calculated, as presented in Figure 8. To simplify comparison, the absolute values are presented. As seen in the figure, the GP² correlated well with both the II and insulin iPeak, The GP correlates well with the insulin iPeak, while the GI correlates well with the II, indicating that GP² may be a good predictor of both early and late insulin response.

As hyperinsulinaemia can trigger insulin resistance (Del Prato et al. 1994), it could be argued that the insulin iPeak, rather than the overall insulin index expressed as II, is of importance when studying postprandial insulinaemia. Furthermore, the stronger relationship between II and GI values compared to GI and insulin iPeak values may depend on an increase in II values, due to an extended insulin response following a high GP/GP² product.

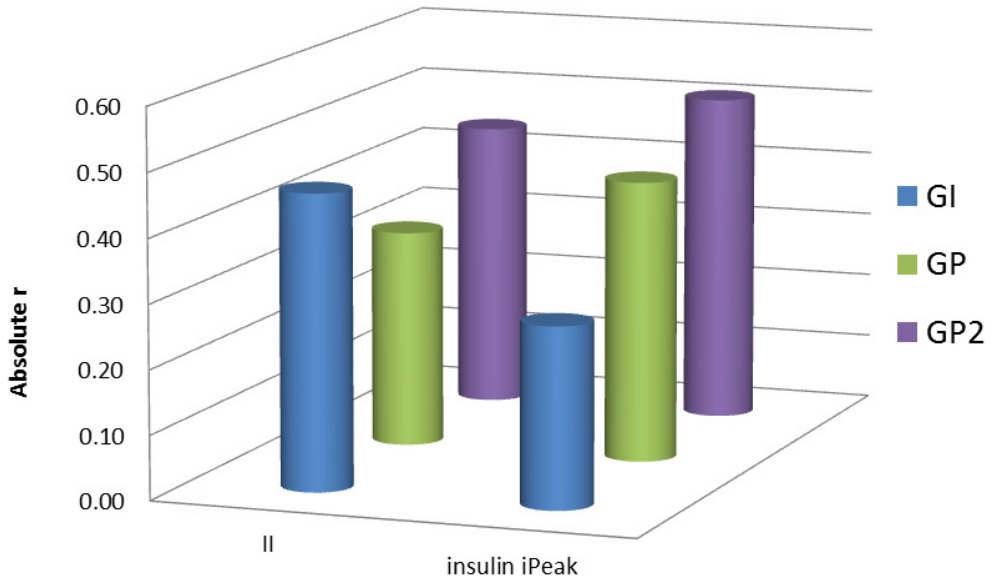


Figure 8. Relationship between the GI, GP and GP² and postprandial insulin responses (II and insulin iPeak). The figure shows the Spearman's correlation coefficient (r), expressed in absolute values to simplify comparison. (Spearman's partial correlation coefficients controlling for subjects and corresponding baseline values, two-tailed test).

Correlations to other parameters were similar for GP and GP², respectively. Consequently, the correlations found in papers I and II between late ghrelin levels and GP of the test meals were also valid with GP². The extent of postprandial hypoglycaemia in paper I also correlated to the GP² of the test products. The exception was that GP² of the products in paper II showed poorer correlations with late satiety. For example, GP² did not correlate significantly with the late postprandial *desire to eat* (tAUC 210-270min), unlike GP. In contrast, GP² and GP showed equally good correlations to late subjective satiety in paper IV.

The GP and GP² calculations therefore provide quantified measures of the course of glycaemia that distinguishes products with similar GI values but with different glycaemic profiles. The GP² measurement has here been demonstrated to be a good predictor of the early and total insulin response (insulin iPeak and II). Furthermore, GP² has been shown to be related to late subjective satiety (paper IV) and late postprandial ghrelin (papers I and II).

GP is possibly a better predictor of late subjective satiety as the duration of the incremental glycaemia is more pronounced in this measure. The GP and GP² could be valuable complements to the GI concept and offer advantages when studying the possible relationship between postprandial glucose metabolism and other metabolically relevant features such as appetite regulation, biomarkers of oxidative stress and post-meal cognitive functions.

Mechanisms behind low postprandial glycaemia and insulinaemia following rye products – upper gut phenomenon

The wholegrain and endosperm rye breads and the boiled rye kernels in paper II induced similar glucose iPeaks and GI values (79, 77 and 73, respectively) whereas the GP tended to be higher for the rye kernels, suggesting a prolonged duration of glycaemia above fasting following the rye kernel product. Also, the insulin responses were similar, with II values of 70, 68 and 60 for the wholegrain and endosperm breads and kernels, respectively. The lowered postprandial glycaemia and insulinaemia following the rye kernels could be explained by a botanically intact structure which protects the starch from amylase digestion (Liljeberg et al. 1992). However, the low insulin response seen also after milled wholegrain and endosperm rye seen in this thesis and the sustained increment in the late postprandial glycaemia noted for several flour based rye products must be explained by other factors.

Previously, a lowered rate of starch hydrolysis has been suggested as a mechanism behind the lowered insulin demand to rye products (Juntunen et al. 2003). The hydrolysis index (HI) was developed about 20 years ago and has been shown to predict the glycaemic response (Granfeldt et al. 1992) for a multitude of starchy foods with the equation “Predicted GI = 6.272+0.912 x HI” (Leeman et al. 2005). In the present thesis (papers I-III), the HI and II were not related when studying all milled rye products (Table 2 and Figure 9). The HI and GI values of the milled rye products showed some relation, however with a large variability, and the predicted GI values calculated from the HI were larger than measured GI values. It could therefore be argued that HI analysis is not a suitable method for predicting the postprandial glycaemia

and insulinaemia in relation to rye products, as potential effects related to e.g. a lowered gastric emptying rate are not included.

Table 2. HI, predicted GI, GI and II values for the products in papers I-III.

	Meals	HI	Predicted GI	GI	II
			%		
Paper I	WWB ^{+MG}	100 ^a	100	100±0 ^a	100±0 ^{ab}
	WWP	85±4 ^{bc}	84	77±10 ^{ab}	87±8 ^{bc}
	ERB	83±2 ^{bc}	82	64±8 ^b	61±8 ^d
	ERP	89±4 ^b	87	70±6 ^b	71±7 ^{cd}
	WGRB	101±3 ^a	98	71±10 ^{ab}	73±8 ^{cd}
	WGRB-lac	94±4 ^{ab}	92	74±10 ^b	71±9 ^{cd}
	WGRP	72±2 ^c	72	72±10 ^b	88±9 ^{bcd}
	RBB	93±2 ^{ab}	91	87±7 ^{ab}	128±16 ^a
Paper II	WWB	100±0 ^a	100	100 ^a	100 ^a
	ERB	87±3 ^b	86	77±8 ^{ab}	68±4 ^b
	ERB-lac	85±2 ^b	83	64±9 ^b	65±7 ^b
	WGRB	87±2 ^b	85	79±14 ^b	70±5 ^b
	WGRB-lac	84±2 ^b	83	64±7 ^b	64±7.0 ^b
	WK	72±3 ^c	72	68±9 ^b	63±9 ^b
	RK	70±5 ^c	70	73±8 ^b	60±7 ^b
paper III	WWB ^{+MG}	100±0 ^{bc}	100	100±0 ^a	100±0 ^a
	Amilo	92 ± 2 ^c	90	79±5 ^a	72±6 ^c
	Nikita	115 ± 4 ^a	111	91±11 ^a	93±9 ^{abc}
	Rekrut	109 ± 4 ^{ab}	105	84±7 ^a	78±8 ^{bc}
	H. Loire	113 ± 5 ^a	109	96±10 ^a	103±12 ^{ab}
	D. Zlote	117 ± 3 ^a	113	96±9 ^a	89±7 ^{abc}

Values are means ± SEM, Products not sharing the same letters were significantly different within each paper, p<0.05 (ANOVA, followed by Tukey's test in paper I, ANCOVA, followed by Tukey's test in papers II-IV). See abbreviation list for abbreviations.

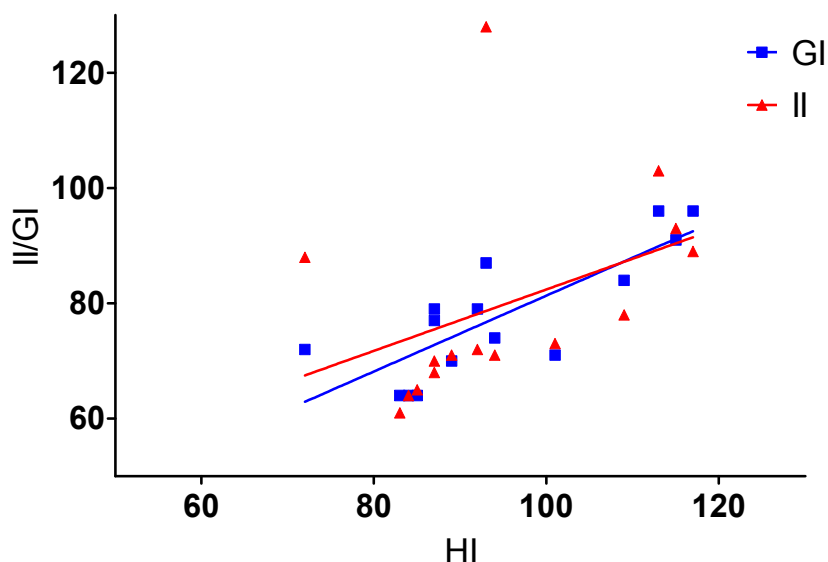


Figure 9. Relationship between HI and GI (red) and between HI and II (blue) for the flour based rye products.

Wholegrain rye flour contains a high amount of soluble dietary fibres. In the present thesis the analysed levels were 5% in wholegrain rye bread (paper II). The analysis method used (Asp et al. 1983) does not include indigestible carbohydrates of low molecular weight, such as oligosaccharides and fructans (Asp 2008). According to previous studies, wholegrain rye breads contain approximately 2.8 DW% soluble arabinoxylans (AX) (Andersson et al. 2009) and 1.9-2.8 DW% fructans (Andersson et al. 2009, Rakha et al. 2010). Rye grains have been demonstrated to contain approximately 1.4 % oligosaccharides (Henry et al. 1989). Among these fibre types, the soluble AX induces high viscosity. Possibly, rye breads and porridges, due to their large proportion of viscous fibres, could cause a delayed gastric emptying (Jenkins et al. 1978, Torsdottir et al. 1991), thus inducing a lower glycaemic and insulinaemic response than predicted by HI. In support of this hypothesis, Hagander et al. (1987) found that after oro-gastric intubation with rye bread and white wheat bread in rats, the gastric content was higher at 15 min after the rye than after the wheat bolus (52.9% vs. 27.8% of intubated contents, respectively). However, Juntunen et al. (2002) did not find any lowering of

gastric emptying rate after rye breads compared to white wheat bread, estimated using paracetamol as marker.

In paper I, the rye breads made from endosperm and wholegrain rye induced significantly lower early postprandial glycaemia (iAUC 0-30 min) than a bread made from white wheat enriched with 35% milled rye bran. The endosperm rye bread also had a significantly higher GP and a lower GI than the rye bran bread. Furthermore, the insulin response, expressed as II and insulin iPeak, was significantly higher following the rye bran bread than the endosperm and wholegrain rye breads. The low postprandial glycaemia and insulinaemia seen with products containing the endosperm part of the rye (endosperm and wholegrain rye bread) as compared to the fibre rich rye bran bread, devoid of the endosperm part of the rye, appears contradictory to the whole grain concept. However, the majority of viscous AX in rye is derived from the cell walls in the endosperm (Delcour et al. 1989). Furthermore, water extracts of endosperm rye breads have been shown to have higher viscosity than that of wholegrain rye and rye bran, possibly due to higher arabinose substitution of the AX in the endosperm (Ragaei et al. 2001, Cyran et al. 2010). Foods rich in viscous dietary fibre might display lower gastric emptying rate and also a slower rate of small intestinal glucose delivery to the blood, hence causing a reduced postprandial glycaemia and insulinaemia (Jenkins et al. 1978, Torsdottir et al. 1991, Östman et al. 2006). There are indications of a lowered gastric emptying rate with rye as compared with wheat from studies in rats (Hagander et al. 1987). Thus, the high content of viscous fibres in rye could contribute to the better regulated glycaemic profiles and lower insulin responses seen with several milled rye products in this thesis. Unfortunately, viscosity measurements were not performed in the present thesis. Liljeberg et al. (1992), demonstrated that a barley bread made with 80% boiled barley kernels displayed GI and II values that were lower than that of a white wheat bread reference (57 and 46), while corresponding indices for bread made from milled normal barley were similar to those of white wheat bread, with a GI of 95 and a II of 87, respectively. Barley breads are also rich in viscous DF (mainly β -glucans), but evidently not sufficiently to cause a blood glucose lowering effect. This may suggest that rye fibre induces higher viscosity than fibre present in milled normal barley or that other factors contribute to the insulin and glucose lowering effect of milled rye breads.

Results and Discussion

Other possible mechanisms behind the reduced insulinaemia and glycaemia with rye products are the presence of bioactive components, and/or a more dense structure in rye products. In paper III, it was demonstrated that a low postprandial glucose and insulin response after rye bread was related to a high content of several phenolic acids such as ferulic and caffeic acid. Both of these phenolic acids have previously been shown to inhibit enzymes, reduce digestion and suppress uptake of carbohydrates in rats and *in vitro* (Welsch et al. 1989, Welsch et al. 1989, Adisakwattana et al. 2009). Furthermore, commercial cultivars of rye have been shown to contain significantly more ferulic acid compared to wheat (Rybka et al. 1993). Whole grain rye is also rich in phytic acid, known to reduce postprandial glycaemia (Thompson et al. 1987). Despite the fact that bioactive components such as phenolic acids are mainly located in the bran, such components may still contribute to the lower postprandial glycaemia and insulinaemia seen with the endosperm rye bread in the present thesis. Consequently, endosperm rye flour usually also contain bran, since separation during milling is difficult (Weipert 1997). Additionally, several other micro-nutrients and bioactive components in rye might affect glycaemia and insulinaemia to rye products. In an extensive review, Fardet (2010) listed a number of bioactive components present in whole grains with potential benefits on glucose control and insulin sensitivity, for instance magnesium, copper, potassium, vitamin B₁ and chiro-inositol. These compounds have not been studied in the present thesis, but are of interest for future investigation.

By looking at the samples, the rye bran bread had a relatively porous structure, similar to that of the WWB reference, while the wholegrain and endosperm rye breads were denser. The wheat gluten included in the rye bran bread is likely to have trapped air bubbles during the yeast fermentation, thereby inducing a porous structure after baking. In contrast, rye proteins form only weak networks and are transformed to water soluble proteins during late phases of fermentation. Instead, the water soluble arabinoxylans play a crucial role for baking properties of rye, being able to bind 4-5 times more water than wheat gluten (Weipert 1997). However, the high water binding ability of rye contributes to sticky dough's and to more compact breads with lower volume. The high postprandial glycaemia and insulinaemia seen after the rye bran bread might therefore be explained by the more porous structure in the case of this bread product, which might facilitate digestion by α -amylases. The dense

rye breads, on the other hand, could be more slowly hydrolysed by human digestive amylases. Using a microscope, Juntunen et al. (2003) demonstrated, that the starch in wholegrain rye, endosperm rye and high fibre wholegrain rye breads showed a continuous phase. Furthermore, the starch in rye breads had partly leaked out from the granules, thereby creating a retrograded coating layer of the grain after cooling, thus inducing a physical hindrance for the digestive amylases.

The endosperm rye bread, despite a lower level of DF thus appear to contribute to less oscillations in postprandial glucose and insulin compared to the DF rich rye bran bread. Compared to for example, wheat endosperm breads, the endosperm rye breads did contain a substantial amount of viscous fibres, and also fibre associated components such as vitamins, minerals and antioxidants, due to the difficulties of separation of bran during milling. From a longer term perspective however, it can be argued that rye breads preferably should be made from whole grain rye, with both the endosperm and bran present to ensure both a high amount of viscous fibres, and the high amount of DF co-passengers and insoluble DF present in rye bran.

The low and sustained postprandial glycaemia and insulinaemia following milled rye products might result from a combination of presence of viscous fibres, bioactive components and a dense food structure, contributing to a lowered digestion and uptake of carbohydrates in the small intestines.

Mechanisms behind low postprandial glycaemia and insulinaemia following rye products – lower gut phenomenon

Already at 270 min after the breakfast meal, rye kernels and wholegrain rye breads induced significantly higher breath hydrogen concentrations than WWB (paper II). In the postprandial phase after lunch, all rye products except the endosperm rye bread with lactic acid induced higher breath hydrogen than WWB. Evidence of early colonic fermentation in the case of rye meals was also seen by Nilsson et al. (2008), who reported increased breath hydrogen following rye kernels in the 240-300 min postprandial phase.

Results and Discussion

Rapid colonic fermentation, as manifested by an early postprandial breath hydrogen excretion following a rye meal, would suggest that some DF in rye products have a short oro-colonic transit time, i.e. they are transported rapidly through the gut. A short intestinal transit time for rye products compared with white wheat is also supported by Gråsten et al. (2000) in healthy subjects. Besides a high content of soluble AX and other soluble DF components analysed in the present thesis, rye breads are also rich in oligosaccharides and fructans, low molecular soluble fibre which has been shown to be easily fermented (Rumessen et al. 1998).

Relations between colonic fermentation and metabolism are a new and interesting area. Low GI test meals rich in fermentable DF and resistant starch have shown to improve glucose tolerance and lower insulin demand at a subsequent meal ingested after 10h (Nilsson et al. 2006, Nilsson et al. 2008). These benefits co-incided with increased plasma levels of the incretin hormone GLP-1 and colonic metabolites such as SCFA, as well as lower plasma FFA levels.

In the present work, the rapid colonic fermentation after rye products appears to effect postprandial glycaemia already in the late postprandial phase. In paper II, a high GP and GP², respectively, was correlated to a higher breath hydrogen excretion in the later postprandial phase after breakfast (tAUC 120-270 min). It could be hypothesised that colonically derived signalling molecules might be delivered to the blood already in the postprandial phase after a meal containing rye products and thereby beneficially influence insulin sensitivity and glycaemic regulation in the late postprandial phase. Supporting this, a high breath hydrogen excretion prior to lunch (270 min) correlated to a lower concentration of FFA at this time point ($r=-0.26$, $p=0.038$, unpublished correlation from paper II). Higher late postprandial concentrations of FFA negatively affecting insulin sensitivity (Boden et al. 1994, Boden et al. 1995) have mainly been attributed to rapid decreases in glucose, triggering lipolysis (Jenkins et al. 1980). However, Tarini et al. (2010) demonstrated a significantly higher increase in SCFA at 4-6 h and a significantly lower concentration of FFA at 4 hours following a high fructose corn syrup (HFCS) drink with added inulin compared to just a HFCS drink in healthy subjects, thereby suggesting involvement of the gut microbiota. An acute decrease in

insulin sensitivity has been demonstrated already in the postprandial phase simultaneously with postprandial lipaemia by Pedrini et al. (2006).

In paper II, the endosperm rye bread baked without lactic acid tended to induce higher breath hydrogen levels than the endosperm rye bread baked with lactic acid in the postprandial phase after lunch (Figure 11). A possible explanation could be that presence of lactic acid during fermentation inhibits enzymes that can hydrolyse DF into low molecular fragments during bread fermentation. In support of this hypothesis, Lappi et al. (2010) demonstrated that wholegrain wheat bread baked with sourdough fermentation contained slightly higher amounts of high molecular water extractable AX than wholegrain wheat bread baked with yeast.

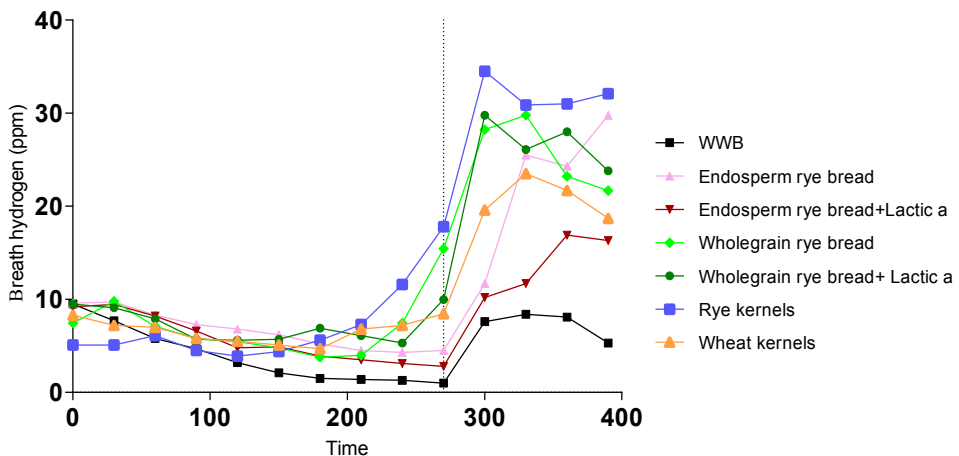


Figure 11. Breath hydrogen responses after the rye and wheat products in paper II. The dotted line at 270 min indicates the serving of a voluntary lunch meal.

The findings in the present thesis suggest that certain indigestible carbohydrates in rye, presumably of lower molecular weight, can have prebiotic properties. It would be of interest to study if such rye components can affect beneficial gut bacteria positively. A shift towards a higher proportion of beneficial bacteria can suppress potential pathogens and thereby reduce the inflammatory status of the gut and also protect against overweight (Sanz et al. 2008, Cani et al. 2009), high-lighting the role of colonic metabolism for the aetiology of the IRS.

Results and Discussion

A high content of rapidly fermentable DF in rye, such as AX, oligosaccharides and fructans were demonstrated to induce elevated metabolites of colonic fermentation in the blood already in the late postprandial phase, thereby reducing risk of hypoglycaemia and beneficially affecting glucose tolerance at a subsequent meal. The marker of colonic fermentation used in the present thesis, breath H_2 , was related to a lowered recovery of FFA in plasma, and also related to a higher GP and GP^2 .

Appetite-regulating properties of rye products

Several of the rye products studied in papers I-IV induced higher subjective satiety than the WWB references. In particular, the rye kernels in paper II influenced appetite both directly in the postprandial phase in terms of higher subjective satiety, as well as at a subsequent meal by lowering voluntary energy intake by 16% after a voluntary lunch compared to WWB. Furthermore, the rye kernels suppressed the *desire to eat* to a greater extent than boiled wheat kernels in the later postprandial phase (tAUC 210–270 min). The low insulin response seen after the endosperm and wholegrain rye products was related to a higher feeling of subjective satiety, both in the early postprandial phase (paper II) and the late phase (papers III-IV). Foods with low insulin demand have previously also been shown to affect subjective satiety beneficially and lower subsequent food intake (Haber et al. 1977, Holt et al. 1992, van Amelsvoort et al. 1992, Holt et al. 1995, Rigaud et al. 1998, Ludwig et al. 1999, Pasman et al. 2003)

It can be hypothesised that low insulin-demanding food may reduce the risk of rapid blood glucose declines and hypoglycaemia, and thereby improve the postprandial glycaemic profile. Indeed, in paper I, a high insulin iPeak, II and also a low GP and GP^2 was related to a greater hypoglycaemia, in turn related to a lower subjective satiety in the later postprandial phase (180 min). To our knowledge, only Haber et al. (1977) have previously demonstrated a direct relationship between hypoglycaemia and subjective hunger. Also in papers II and IV, a high GP and GP^2 respectively correlated with improved subjective satiety in the late postprandial phase. The plasma level of the hunger peptide ghrelin was lowered following the rye and wheat test meals in papers I and II,

reaching a nadir at around 60-70 min. Ghrelin has previously been suggested to be lowered by increasing insulin during euglycaemic clamp (Möhlig et al. 2002, Saad et al. 2002, Flanagan et al. 2003) and meal studies (Blom et al. 2005, Reynolds et al. 2008). However, no relation between plasma levels of ghrelin and the subjective *feeling of satiety* or *hunger* could be found in papers I or II. Interestingly, a low rebound of ghrelin in the late postprandial phase was related to a lower insulin response (II and insulin iPeak) in paper I and to a higher GP and GP² in papers I and II. Additionally, a low late postprandial level of ghrelin was related to a lowered subsequent energy intake in paper II. The relations between low insulin responses, high glycaemic profiles and a lower rebound of ghrelin in the late postprandial phase suggests that well-regulated insulinaemia and glycaemia could counteract oscillations in ghrelin response, thereby affecting hunger prior to a subsequent meal.

A high content of DF contributed to a high early subjective satiety after rye products. In papers II and IV, content of DF+RS and DF, respectively, was positively correlated to subjective *feeling of fullness* and negatively correlated to *feeling of hunger* and *desire to eat* in the early postprandial phase (tAUC 0-60 min). Also in paper I, a high content of insoluble fibres was related to a higher subjective satiety (iAUC 0-70 min, not published). DF lowers the energy density of foods by introducing bulk. Furthermore, soluble DF binds water, thereby contributing further to a satiety inducing bulking effect, demonstrated to induce satiety as well as lowered voluntary energy intake at a subsequent meal (Geliebter 1988, Rolls et al. 1998). In addition, soluble fibres can contribute to an extended absorption time in the small intestine, thereby prolonging exposure to macronutrients, stimulating secretions of satiety peptides such as GLP-1. Consequently, the wholegrain rye porridge in paper I induced significantly higher subjective satiety than the wholegrain rye bread in paper I. The rye porridges contained a substantial amount of water, contributing to a bulking effect and thereby explaining the improved appetite regulation properties compared to the breads.

The increased colonic fermentation after rye products may contribute to a subsequent lower energy intake. Colonic fermentation of indigestible carbohydrates, analysed as breath H₂ excretion in paper II, results in formation of short chain fatty acids (SCFA) (Wong et al. 2006). The latter have shown to lower gastric tone and could thereby induce satiety (Ropert et al. 1996).

Results and Discussion

Furthermore, SCFA and appetite regulating hormones such as GLP-1 have been shown to increase simultaneously after prebiotic supplementation; Cani et al. (2004) reported increased GLP-1, lowered ghrelin secretion and lowered energy intake in rats fed a high fructan diet for 3 week compared to the unsupplemented diet.

Rye products, by virtue of their low insulin responses and high content of soluble and insoluble DF may help delay return of hunger and thereby contribute to lower weight gains in the longer term. Slabber et al. (1994) has shown that a 12 week diet with low insulin producing foods decreased fasting insulin and reduced body weight more than a high insulin producing control diet of similar energy content in obese subjects. An 8 week. intervention with high fibre rye breads in postmenopausal women with elevated serum total cholesterol, increased ribonic acid that correlated positively with tryptophan, a precursor for hunger suppressing serotonin (Lankinen et al. 2010). Rye breads were therefore suggested to mediate positive effects on satiety and weight management (Lankinen et al. 2010). Also, Andersson et al. (2010) has demonstrated that mice fed wholegrain rye had a lower body weight and decreased adiposity compared with mice fed a wholegrain wheat diet for 22 week.

The insoluble and the viscous fibres in rye products can contribute to increased subjective satiety by inducing a bulking effect and by increasing the time of macronutrient exposure in the small intestine and thereby the time for secretion of satiety hormones. The well-regulated insulinaemia and glycaemia generally seen with rye products, might affect late postprandial increase of the hunger peptide ghrelin, and thereby lower subsequent food intake. Furthermore, fermentation of indigestible carbohydrates may slow gastric emptying rate and thereby reduce hunger and voluntary food intake at a subsequent meal.

Influence of processing of rye on metabolic responses

The endosperm and wholegrain rye porridges in paper I induced higher early (iAUC 0-30 min) insulin and glucose responses than their corresponding

endosperm and wholegrain rye breads when compared in a paired analysis. An explanation might be that the starch in the dense rye breads was more slowly hydrolysed by amylases in the small intestine. However, this hypothesis is not supported by the HI analysis of the products, which indicated a slower rate of starch hydrolysis *in vitro* for the wholegrain rye bread (HI = 72) than for the wholegrain rye bread (HI = 101) and a similar response for the endosperm rye bread and porridge (HI = 83 and 89, respectively).

The boiled wholegrain rye kernels induced similar GI and glucose iPeak as the wholegrain rye bread. However, the incremental plasma glucose duration tended to be longer following the rye kernels, seen as a slightly higher GP and GP². The apparent longer release of starch from the rye kernels can be explained by the intact structure, protecting the starch from digestion (Liljeberg et al. 1992).

There were no significant differences between the wholegrain and endosperm rye breads baked with and without lactic acid in papers I and II. However, in paper I, the wholegrain rye bread baked with lactic acid induced a significantly lower insulin iPeak and had a significantly higher GP and lower GI than the WWB^{+MG}, while the bread baked without acid did not differ significantly from WWB^{+MG} in these aspects. The breads in paper I were baked with 25% white wheat flour, and the extra wheat gluten present in the breads might explain this tendency. Consequently, Östman et al. (2002) demonstrated that the lowered bioavailability of starch in bread with lactic acid present during baking could be potentiated following enrichment with wheat gluten.

Commercial rye blends typically consist of several pure rye varieties. In papers III and IV it was demonstrated that the specific choice of rye varieties in commercial blends will affect the metabolic responses to rye products. Several of the pure rye varieties tested induced glycaemic and insulinaemic responses similar to that of WWB. The metabolic response of bread made from the commercial rye blends in paper IV tended to be higher than that following the commercial blend included in the wholegrain rye breads in papers I and II. Furthermore, the endosperm rye bread in paper II did not display as beneficial metabolic responses as the endosperm rye bread in paper I, as judged from the slightly higher GI and lower GP values. All commercial blends were different in terms of growing location, year, provider and varietal composition.

Results and Discussion

Different varieties of rye can contribute with different proportions and types of DF and bioactive components. Also cultivation conditions affect these factors. For example, wet weather has been shown to increase the proportion of water extractable AX and can also cause sprouting with some enzymatic degradation of starch (Weipert 1997). Furthermore, the content of free phenolic acids in rye breads has been shown to correlate positively with temperature and negatively with precipitation during grain development (Shewry et al. 2010). We do not know the type and proportion of rye varieties in the rye blends used in papers I, II and IV. It can however be anticipated that a commercial Swedish rye blend, as that used in the bread products in paper IV contained high amounts of for example Evolo, with a metabolic response similar to that of WWB as judged from the data in Paper IV. Differences in metabolic responses after rye varieties should be taken into account when selecting rye varieties for commercial rye blends and products.

Conclusions and Future Perspectives

The insulin response was lowered following most rye products in comparison with a white wheat bread reference. However, the benefits on insulin demand were dependent on type of process and rye variety, and wholegrain rye porridge and certain rye varieties induced insulin responses similar to that of the reference. Also, white wheat bread enriched with rye bran displayed high insulin responses.

The GI values of the rye products varied from low to similar to that of a white wheat bread reference (GI: 64-96). Several of the rye products induced low but sustained incremental glycaemia which can lead to unfairly high GI values, as calculated from the net incremental area. Therefore, estimates of the course of glycaemia, the Glycaemic Profile (GP and GP²) were introduced, defined as the duration of incremental postprandial glycaemia divided by the glucose iPeak or squared iPeak, respectively. The GP² showed a strong relationship with both early and total insulin responses (insulin iPeak and II), whereas the GI value correlated to the II but only were poorly correlated to the insulin iPeak. Furthermore, high GP and GP² were related to milder hypoglycaemia in the later postprandial phase (nAUC 30-180 min), higher colonic fermentation, higher subjective satiety and lower ghrelin levels in the late postprandial phase, suggesting that these measures of the course of glycaemia could provide a valuable complement to the GI concept. Several of the rye products (kernels, porridges and breads) induced higher subjective satiety after the test meals, and the rye kernels also promoted a higher satiety at a second meal as judged from a reduced voluntary energy intake (-16%).

Suggested mechanisms behind the low and sustained postprandial glycaemia and insulinaemia following rye products are a combination of viscous fibres and bioactive components, such as phenolic acids together with a dense food structure, contributing to a lowered rate of carbohydrate digestion and absorption in the small intestine. Furthermore, fermentation of rye dietary

Conclusions and Future Perspectives

fibre, including soluble AX, fructans and oligosaccharides by the gut microbiota, might increase glucose tolerance already in the later postprandial phase after the rye meal. In support of the fermentation hypothesis, FFA levels at 270 min were lower and measures of the glycaemic profiles were higher following products inducing high colonic fermentation in the later postprandial phase after breakfast. This was especially noteworthy in the case of the boiled rye kernels which also contained fermentable resistant starch.

The appetite regulating properties seen with rye can be explained by a high content of DF and a high water content, which can promote early satiety. Colonic fermentation of DF, together with the low insulin response, well-regulated glycaemia and reduced rebound of ghrelin may contribute to the increased satiety in the later postprandial phase and at a second meal.

It was concluded that wholegrain rye products, made with rye kernels or milled flour, using certain rye varieties appear to lower insulin responses, reduce oscillations in postprandial glycaemia and reduce hunger. Consequently, a diet rich in such rye products could contribute to a lowered risk of obesity and IRS. In this respect, potential differences between rye varieties should be thoroughly investigated, to optimize the health effects of commercial blends.

For future studies, the low molecular DF, including also fructans and oligosaccharides, should be characterised in rye, and their possible prebiotic properties be investigated. Also, the effect of rye breads and other products on metabolic risk markers, colonically derived fermentation metabolites and long term appetite regulation need to be explored in longer-term intervention studies. The Glycaemic Profile concept should be further evaluated, in other types of products and in longer postprandial study durations, to evaluate the utility of the GP and/or GP² as a complement to the GI.

“There are no facts, only interpretations”

Nietzsche

Appendix

Methodological aspects relating to evaluation of postprandial glycaemia and insulinaemia

Choice of baking conditions for white reference bread; impact of monoglycerides

A sub study was carried out in 12 subjects from paper III, comparing metabolic responses after WWB baked with or without monoglycerides. The glucose response after the two WWB varieties is shown in Table 3 and Figure 12. The iAUC 0-120 min for plasma glucose following the WWB^{+MG} was 2.6% larger than following the WWB, although not significant. Also the glucose iPeaks were similar. However, the WWB^{+MG} tended to induce a longer plasma glucose profile, resulting in a GP that was significantly larger (9.3%) than that of the WWB. The GP² of the WWB^{+MG} were 13.3% larger, but this was not significant. This could indicate that in papers II and IV, where a WWB without monoglycerides was used, it could have been slightly easier to detect differences between the reference bread and the test products with regard to these glycaemic parameters. To test this hypothesis, the GP and GP² values were increased by 9.3% and 13.3% respectively for each subject following the WWB in papers II and IV. This increase of the glycaemic measurements did not affect the statistical differences in the results in paper II, while in paper IV the significant difference found between Picasso rye bread and WWB disappeared. However, the significant difference found between WWB and Vicello bread remained.

The postprandial insulin response after the WWB^{+MG} and WWB respectively is shown in Table 3 and Figure 12. The insulin iAUC 0-120 min and insulin iPeak after the WWB^{+MG} was 7.9 and 11.1% respectively, larger than that following WWB but not significantly different. To test the possible outcome if

Appendix

a WWB^{+mg} reference had been used in Papers II and IV, the iAUC and insulin iPeaks following the WWB were increased by 7.9 and 11.1% for each subject. This increase did not affect the statistical conclusions in paper II, but induced a significant difference in the insulin iPeak between WWB and commercial rye and Kaskelott rye (paper IV). The iAUC 0-120 min following Kaskelott rye became significantly lower than that of WWB. Also, the II values of the test products were subsequently lowered by 7.9% when the iAUC was increased for the reference product.

The slightly improved glycaemic profile seen with the WWB^{+MG} could depend on the presence of amylose-lipid complex that could contribute to a slower rate of digestion of part of the starch in the WWB^{+MG} bread (Holm et al. 1983), thereby also inducing a late increment in plasma glucose as manifested by a longer duration of glycaemia. The WWB^{+MG} bread also tended to induce a higher insulin iPeak, which could be explained by the monoglycerides' ability to induce a softer bread crumb with slower retrogradation, thereby facilitating carbohydrate digestion and uptake. It would be interesting to add different types of monoglycerides to rye breads to investigate the influence on GP also for these breads.

In conclusion, adding monoglycerides to the WWB reference tended to increase the GP and GP² values as well as the II and insulin iPeak values. It may therefore be easier to detect the lowered insulin responses following rye breads with a WWB^{+MG} reference, but harder to detect differences in glycaemic profile. Over-all, adding monoglycerides or not to the white wheat bread has a marginal effect on the assessment of postprandial glycaemia and insulinaemia.

Table 3. Postprandial plasma glucose and serum insulin response following a WWB baked with (WWB^{+MG}) and without (WWB) monoglycerides

	WWB ^{+MG}	WWB
Glucose iAUC 0-120 min (mM·min)	217.4±23.9	211.9±22.8
Glucose iPeak (ΔmM)	3.6±0.3	3.6±0.2
GP (min/mm)	48.2±3.6	44.1±2.0*
GP ² (min/(mm) ²)	14.5±1.9	12.8±1.2
Insulin iAUC 0-120 min (nM·min)	16.78±2.18	15.55±2.18
Insulin iPeak	0.30±0.03	0.27±0.03

*Values are means ± SEM. n=12. * indicates significant difference compared to WWB^{+MG}, p < 0.05, ANCOVA.*

Appendix

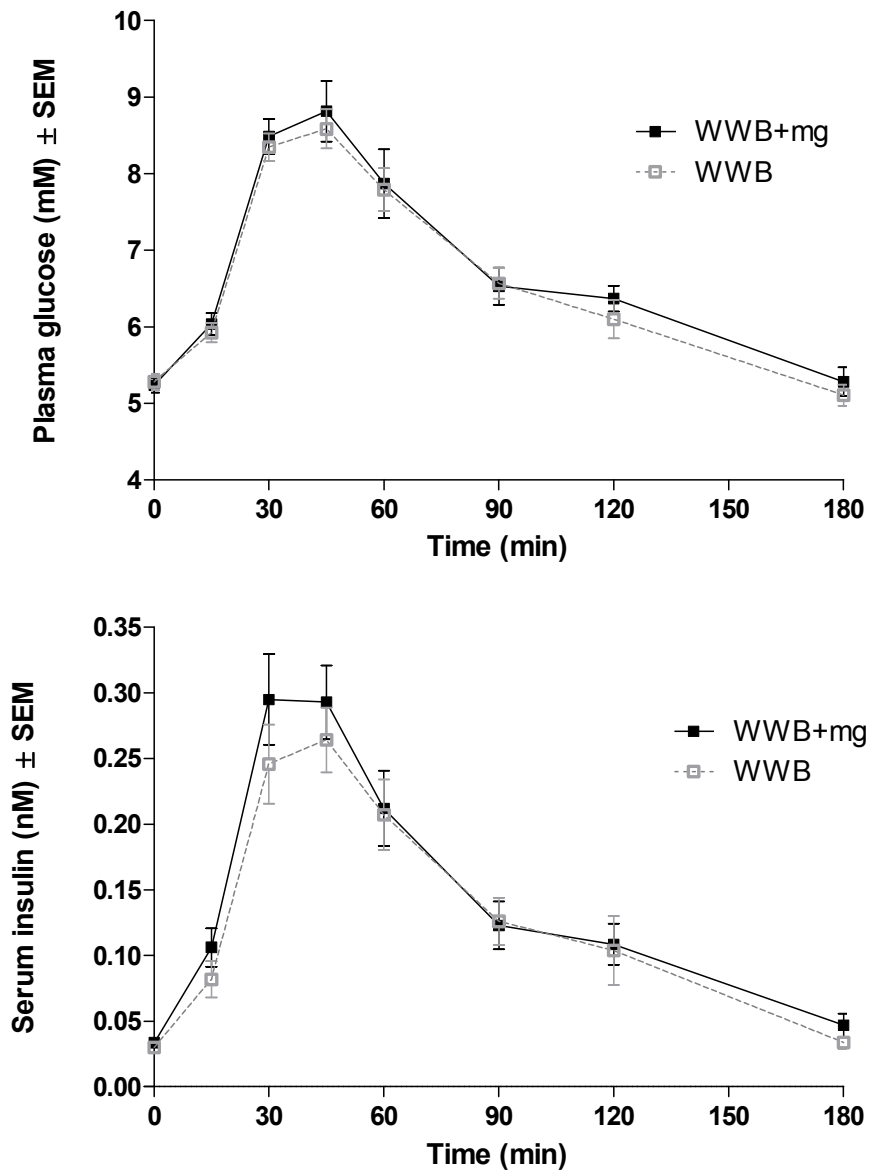


Figure 12. Glucose and insulin responses following WWB with and without added monoglycerides. Values are means \pm SEM, n=12.

Choice of capillary vs. venous blood sampling

In 14 of the subjects in paper IV, a sub-study was performed in order to analyse the glycaemic profile in venous blood after WWB. Studies of postprandial glycaemia are recommended to be performed in capillary blood, as it showed less variation compared with venous blood sampling following the same meal (Wolever et al. 2003, Hätönen et al. 2006). Consequently, it is easier to detect differences in GI values between foods when using capillary blood sampling. However, venous studies allow analysis of a range of additional parameters or biomarkers. Since venous glucose concentrations are lower than in capillary blood, it was of interest to see how the assessment of the glycaemic profile was affected. The venous blood was drawn into EDTA treated tubes and plasma glucose was analysed using a p-glucose analyser from Hemocue of the same model as that used for capillary measurements.

The iAUC following capillary glucose measurements were almost double compared to the measurement in venous blood (Table 4 and Figure 13). In addition, the glucose iPeak were about 45% larger for the capillary measurements. However, the GP calculated from capillary and venous measurements were similar, due to shorter incremental glycaemic durations in the venous measurements. The capillary measurements resulted in GP value that was 2.4% larger. The GP² was 57.5% larger for the venous measurements compared to the capillary, which can be explained by the higher glucose iPeak in capillary blood. Visually, the glucose profiles from the capillary and venous measurement are similar, with a lowering of the entire curve in the case of venous blood measurements. The lowered glycaemic curves after venous blood glucose measurements could conceal information about differences between products in the late postprandial phase, and also diminish the possibility of observing differences between products in maximum glucose measurements. Therefore, capillary blood sampling is recommended for analysis of the glycaemic profile. However, in the present sub-study, only one product was evaluated, and a larger study on the differences in GP and GP² values following capillary and venous blood sampling should be performed in products with a wide range in postprandial glycaemic profiles, to investigate statistical differences between products depending on sampling method.

Appendix

Table 4. Postprandial plasma glucose responses following WWB and a commercial rye bread. Capillary and venous plasma glucose sampling was compared.

	Glucose iAUC 0-120 min mM·min	Glucose iPeak Δ mM	GP min/mM	GP ² min/(mM) ²
WWB-capillary	197.1 \pm 18.3	3.6 \pm 0.3	41.8 \pm 3.6	13.2 \pm 1.8
WWB-venous	109.1 \pm 15.2*	2.5 \pm 0.2*	40.8 \pm 4.9	20.8 \pm 4.6

Values are means \pm SEM, n=14.

* indicates that the venous measurements were significantly different from the capillary measurements, $p < 0.05$, ANCOVA.

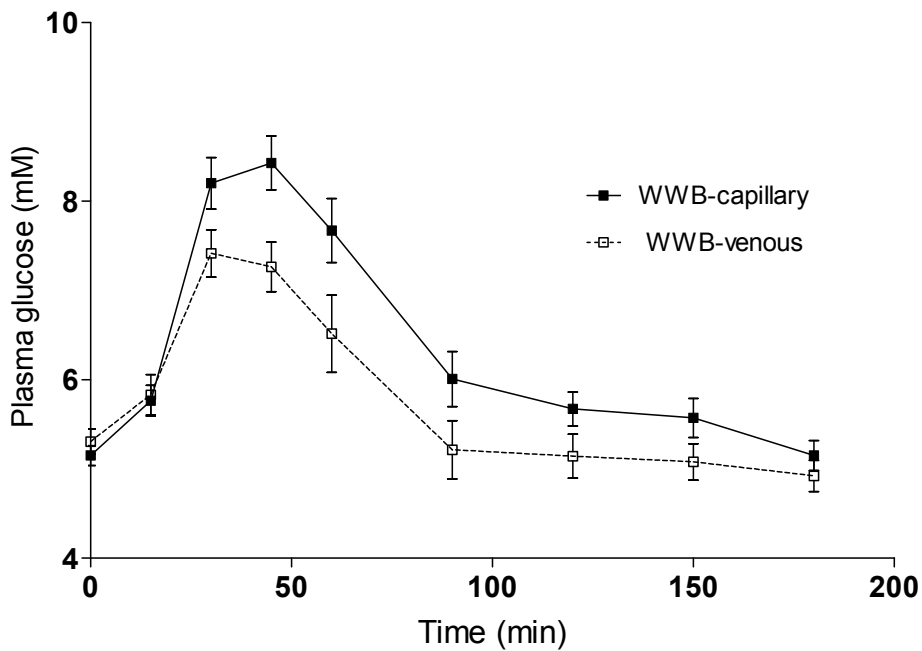


Figure 13. Glucose responses following WWB and commercial rye bread breakfast. Venous and capillary blood samples were compared. Values are means \pm SEM, n=14

Conclusions

A WWB baked with monoglycerides (WWB^{+MG}) induced significantly but marginally higher GP values than a WWB baked without. Furthermore, the II of a WWB^{+MG} was 7.9% higher than that of a WWB. The differences between the two references were small, but should be considered when comparing results from different studies.

The glycaemic responses from capillary blood were significantly higher (as seen from iAUC 0-120 min) compared to venous blood. The glycaemic incremental durations were shorter after venous measurements, resulting in similar GP values between venous and capillary measurement. However, GP² values indicate larger differences between venous and capillary blood, due to the lower glucose iPeaks in venous blood. Capillary blood glucose measurement are recommended for analysis of the glycaemic profile.

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Paper I

Research

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Endosperm and whole grain rye breads are characterized by low post-prandial insulin response and a beneficial blood glucose profile

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Abstract

Background: Rye products have previously been shown to induce comparatively low post-prandial insulin responses; irrespectively of their glycaemic indices (GI). However, the mechanism behind this lowered insulin demand remains unknown. An improved insulin economy might contribute to the benefits seen in epidemiological studies with whole grain diets on metabolic risk factors and weight regulation. The objective of this study was to explore the mechanism for a reduced post-prandial insulin demand with rye products.

Methods: 12 healthy subjects were given flour based rye products made from endosperm, whole grain or bran, produced with different methods (baking, simulated sour-dough baking and boiling) as breakfasts in random order in a cross-over design. White wheat bread (WWB) was used as a reference. Blood glucose, serum insulin, plasma ghrelin and subjective satiety were measured during 180 minutes. To evaluate the course of post-meal glycaemia, a measure of the glycaemic profile (GP) was introduced defined as the duration for the incremental post-prandial blood glucose response divided with the blood glucose incremental peak (min/mM).

Results: The study shows that whole grain rye breads and endosperm rye products induced significantly ($p < 0.05$) lower insulinaemic indices (II's) than WWB. Rye bran bread (RBB) produced significantly higher II compared with all the other rye products. Furthermore, the acute insulin response showed better correlations with the GP than with the GI of the products. The endosperm rye bread and the whole grain rye bread with lactic acid induced a significantly higher GP than RBB, WWB, white wheat- and whole grain rye porridge, respectively. A low insulin incremental peak was associated with less severe late post-prandial hypoglycaemia ($r = 0.38$, $p < 0.001$), and hypoglycaemia was negatively correlated to subjective satiety at 180 min ($r = -0.28$, $p < 0.05$). A low insulin incremental peak was also associated with a milder recovery of plasma ghrelin in the late post-prandial phase (180 min, $r = 0.34$, $p < 0.01$).

Conclusion: Our study shows that endosperm and wholegrain rye products induce low acute insulinaemic responses and improved glycaemic profiles. The results also suggest that the rye products possess beneficial appetite regulating properties. Further studies are needed to identify the unknown property or bioactive component(s) responsible for these beneficial metabolic features of rye.

Background

Whole grain products have been shown to protect against type 2 diabetes and CVD [1-7] and also to facilitate weight regulation [2,6,8,9]. Dietary fibre and other potentially bioactive compounds e.g. antioxidants, vitamins and minerals present in whole grains have been shown to contribute to the protective properties of whole grains foods [10-14]. However, the underlying mechanisms remain unclear. Foods with low glycaemic indices (GI) have also been demonstrated to protect against type 2 diabetes and CVD [1,15-18]. One mechanism behind the protective effect of low GI foods in relation to CVD may be the avoidance of frequent and elevated blood glucose excursions, which are associated with oxidative stress and inflammation [19]. Furthermore, a low GI is generally accompanied by a low acute insulin response. Consequently, hyperinsulinaemia lasting for 48-72 hours under physiologic euglycaemic conditions has been reported to decrease insulin sensitivity in healthy subjects [20]. In a recent dietary intervention in subjects suffering from the metabolic syndrome, it was shown that foods causing low acute insulinaemia may be less prone to promote sub-clinical inflammation [21], a feature commonly associated with insulin resistance [22,23].

Based on the above, whole grain foods characterized by a low GI/low insulinaemia are of particular interest as type 2 diabetes and CVD preventing foods. Previous studies have demonstrated that whole grain rye products display low insulinaemic index (II), regardless of their GI [24-26]. This anomaly between the GI and II that is sometimes present in whole grain rye products has not yet been explained and may indicate improved insulin economy. One suggested mechanism for the lowered acute insulinaemia with whole grain rye bread products include the structural features of rye bread; leading to obstructed amylolysis and a lowered rate of glucose delivery to the blood [25]. However, a lowered rate of glucose delivery caused by such a mechanism is likely to affect also the glycaemia to rye bread, and the insulin saving properties of certain whole grain rye products remain obscure.

The aim of the present study was to investigate the potential improvement of insulin economy following rye products, with a particular focus on evaluating the influence of different fractions of rye. For this purpose, rye products were produced from different parts of the rye grain (endosperm, whole grain and bran), and processed with different methods (baking vs. boiling). Since rye bread is commonly produced by use of sour-dough fermentation, the effect of lactic acid addition was also evaluated. The products were studied as to their effects on post-prandial glucose and insulin response in healthy subjects using white wheat bread as reference. In parallel, subjective satiety and total ghrelin responses were studied in the post-

prandial phase. Additionally, the rate of in vitro starch hydrolysis was determined in order to evaluate the possibility of obstructed amylolysis.

Methods

Test subjects

Twelve healthy non smoking volunteers (9 men and 3 women) aged 25.3 ± 0.8 y with normal body mass indices: 23.1 ± 0.6 kg/m², and without drug therapy participated in the study. All subjects had normal fasting blood glucose concentrations (4.6 ± 0.03 mM). The subjects were recruited in August 2005 and the study was performed from September to December 2005. All test subjects gave their informed consent and were aware of the possibility of withdrawing from the study at any time they desired. Approval of the study was obtained by the Ethics Committee in Lund, Sweden.

Test meals

Four rye breads, two rye porridges, one white wheat (endosperm) porridge and white wheat (endosperm) bread (WWB, reference product) were included in the study. Whole grain rye flour, endosperm rye flour and rye bran from commercial blends were provided by Lantmännen R&D (Järna, Sweden) and commercial white wheat flour was obtained from Kungsörnen AB (Järna, Sweden). The rye bran was milled to pass through a 0.8 mm screen (Laboratory Mill 12, Perten, Huddinge, Sweden). Dry yeast was obtained from Jästbolaget AB (Sollentuna, Sweden) and lactic acid (88-92% extra pur) was obtained from Riedel-de Haën (Morris Township, NJ, USA). Monoglycerides were obtained from Aromatic (Stockholm, Sweden).

Breads

The ingredients of the breads are shown in Table 1. WWB was made in a bread machine (BM 3983, Severin, Sundern, Germany) using a program for white bread: The dough was mixed for 30 minutes and was proofed for 130 min, with 10 seconds short stirring every 39, 31 and 60 min. Baking was then performed for 55 minutes.

Four types of rye breads were made: Endosperm rye bread (ERB), whole grain rye bread (WGRB), whole grain rye bread with lactic acid (WGRB-lac) containing 18 mmol lactic acid/100 g flour and rye bran bread (RBB). All rye breads were made using a uniform method: The dough was mixed in a mixing bowl for 6 min and was proofed in room temperature for 30 min. The dough was divided into pieces of 1 kg each and placed in a bread making tin, followed by a second proofing for 60 min in room temperature. Baking was performed at 250°C for 40 min.

The WWB was left to cool for 1 hour and the rye breads for 18 hours under a cloth. Thereafter, the crust was removed

Table 1: Bread ingredients.

WWB	ERB	WGRB	WGRB-lac	RBB
360 g water	950 g water	1020 g water	995 g water	1100 g water
540 g white wheat flour	348 g white wheat flour	348 g white wheat flour	348 g white wheat flour	905 g white wheat flour
4.8 g dry yeast	1044 g endosperm rye flour	1044 g whole grain rye flour	1044 g whole grain rye flour	487 g rye bran flour
4.8 g NaCl	24 g dry yeast	24 g dry yeast	24 g dry yeast	24 g dry yeast
12 g monoglycerides	12 g NaCl	12 g NaCl	12 g NaCl	12 g NaCl
			25 g lactic acid	

The breads were divided into portions contributing with 40 g of available starch.

and the breads were sliced and wrapped in aluminium foil in portions sizes, put into plastic bags and stored in a freezer (-18°C) until use. The day before the experiment, the breads were taken from the freezer and were thawed at ambient temperature, still wrapped in aluminium foil and in the plastic bag.

Porridges

Three types of porridges were cooked: white wheat porridge (WWP), endosperm rye porridge (ERP) and whole grain rye porridge (WGRP). The ingredients of the porridges are shown in Table 2. All porridges were cooked in a microwave oven (MM 140-1, Elektro Helios AB, Stockholm, Sweden) at 680 W for 3 min. The porridges were freshly prepared each experimental day and were left to cool under aluminium foil for 15 min before serving.

Chemical analysis of the test products

Prior to all analyses, except for the determination of starch hydrolysis, the samples were dried and milled to pass through a 0.5 mm screen (Cyclotec, Tecator, Höganäs, Sweden).

The available starch content of the products was determined according to Holm et al. [27]. Insoluble and soluble fibres were determined with a gravimetric, enzymatic method described by Asp et al. [28]. Fat was determined according to Lange [29] with the exceptions that petroleum ether BP 60-80°C was used instead of petroleum ether BP 40-60°C. 10 ml of each ether was used instead of 15 in the second and third washing step. Protein content was determined by Kjeldahl analysis (Kjeltec Auto 1030 Analyser, Tecator, Höganäs, Sweden). The rate of starch hydrolysis (HI) was determined using an in vitro procedure based on chewing [30]. WWB was used as a reference

in the HI analysis. The nutritional compositions and the HI values of the products are presented in Table 3.

Study design

The products were provided as breakfasts on 8 different occasions in random order with approximately 1 wk between each test. The subjects were instructed to eat a standardized meal in the evening (21.00-22.00) prior to the test, consisting of a few slices of white wheat bread. They were instructed to avoid eating and drinking anything but small amounts of water until the start of the test. In addition, they were told to avoid alcohol and excessive physical exercise the day before each test. The subjects arrived at the laboratory at 07.45 on the test day. A peripheral venous catheter (BD Venflon, Becton Dickinson, Helsingborg, Sweden) was inserted into an antecubital vein to be used for blood sampling and fasting blood samples were taken prior to the meal. All test products contributed with 40 g of available starch and were served with 250 ml of tap water. The test subjects were instructed to finish the test product and water within 12 min. The subjects feeling of hunger and satiety was rated on a bipolar subjective rating scale graded from -10, representing extreme hunger, to + 10, representing extreme satiety. The feeling of hunger/satiety was rated before the meal (0 min) and at 15, 30, 45, 70, 95, 120 and 180 min after commencing breakfast. The test subjects were not allowed any further water or any caffeinated drinks during the test.

Blood sampling and analysis

Both capillary and venous blood samples were taken at 0, 7.5, 15, 30, 45, 70, 95, 120 and 180 min after the start of the meal for analysis of blood glucose, serum insulin and plasma ghrelin. Blood glucose concentrations were determined in capillary whole blood using a B-glucose analyzer (mod no. 120401, Hemocue, Ängelholm, Sweden).

Table 2: Porridge ingredients.

WWP	ERP	WGRP
231.6 g water	182 g water	204.5 g water
57.9 g white wheat flour	15.2 g white wheat flour	17.0 g white wheat flour
0.5 g NaCl	45.4 g endosperm rye flour	51.1 g whole grain rye flour
	0.5 g NaCl	0.6 g NaCl

The porridges contributed with 40 g of available starch.

Table 3: Composition and HI of the test meals.

Meals	Weight	Available starch	Protein	Fat	Insoluble fibres	Soluble fibres	Total fibres	HI
					<i>g/serving</i>			
WWB	101.1	40.0	6.2	1.5	1.0	0.8	1.8	100 ^a
WWP	273.3	38.2	5.7	1.1	2.1	0.5	2.6	85 ± 4.4 ^{bc}
ERB	106.2	40.0	5.2	1.3	4.2	2.5	6.7	83 ± 1.8 ^{bc}
ERP	227.6	37.7	4.6	1.3	4.9	1.7	6.5	89 ± 3.6 ^b
WGRB	123.4	40.0	6.5	1.9	6.8	2.8	9.6	101 ± 3.1 ^a
WGRB-lac	122.6	40.0	6.3	2.0	7.4	2.9	10.2	94 ± 4.4 ^{ab}
WGRP	258.2	38.6	5.4	1.7	7.9	2.2	10.1	72 ± 2.4 ^c
RBB	141.7	40.0	9.7	2.6	10.3	2.0	12.3	93 ± 2.3 ^{ab}

HI values are means ± SEM, n = 6. Products not sharing the same letters were significantly different, $p < 0.05$ (ANOVA, followed by Tukey's test).

Serum and plasma (EDTA) were left in room temperature for approximately 1 h before being centrifuged for 11 min ($1800 \times g$, 20°C). Serum were frozen at -20°C and plasma where frozen at -40°C until analysis. The serum insulin measurement was performed on an integrated immunoassay analyzer (CODA Open Microplate System; Bio-rad Laboratories, Hercules, CA, USA) by using an enzyme immunoassay kit (Mercodia AB, Uppsala, Sweden). Plasma ghrelin (total) were determined with a commercially available radioimmunoassay kit (Linco research inc, St. Charles, MO, USA).

Calculations and statistical methods

Data are expressed as means ± SEM. The incremental areas under the curve (iAUC) for blood glucose, serum insulin, subjective satiety and in vitro rate of starch hydrolysis as well as the negative area under the curve for glucose (neg AUC), were calculated using the trapezoid model. The glycaemic and insulinaemic indexes (GI and II) were calculated from the 120 min incremental post-prandial area for blood glucose and serum insulin by using WWB as a reference (GI and II = 100). In addition, the course of glycaemia was analyzed by calculation a glycaemic profile (GP); The time (min) during which the blood glucose was above fasting concentration was divided with the incremental peak value (mM) of blood glucose for each subject and test meal (Graph Pad Prism, version 4.03, Graph Pad Software, San Diego, CA, USA). In the cases where the blood glucose concentration remained above fasting for the entire 180 min, the duration value was set to 180 min. A GP index was calculated from the GP by using WWB as a reference (GP Index = 100). HI was calculated from the 180 min incremental area for starch hydrolysis in vitro by using WWB as a reference. Relative changes (%) from fasting concentration to the nadir and to the concentration at 180 min after commencing breakfast were calculated for plasma ghrelin.

The data were analyzed with a general linear model (ANOVA) followed by Tukey's multiple comparison test

(MINITAB, release 14.13, Minitab Inc, State College, PA). In the cases of unevenly distributed residuals (tested with Anderson-Darling and considered unevenly distributed when $p < 0.05$), Box Cox transformation were performed on the data prior to the ANOVA.

Significant difference between the products at different time points where evaluated using a mixed model (PROC MIXED in SAS release 8.01, SAS Institute Inc, Cary, NC) with repeated measures and an autoregressive covariance structure. When significant interactions between treatment and time were found, Tukey's multiple comparison test were performed for each time point (MINITAB, release 14.13, Minitab Inc).

Correlation analysis was conducted to evaluate the relation among dependent measures with the use of Spearman's partial coefficients controlling for subjects (two-tailed test), (SPSS software, version 16.0; SPSS Inc, Chicago, IL, USA).

Due to problems drawing capillary blood samples from one subject, the blood glucose statistics was analyzed with $n = 11$. One subject failed to ingest the WGRP meal according to instructions and data from this product for that subject was therefore excluded from the statistical analysis.

Results

Blood glucose responses

The endosperm products ERB and ERP, as well as the whole grain products WGRB-lac and WGRP induced significantly lower incremental areas (iAUC 0-120 min) than WWB, with glycaemic indices (GI's) of 64, 70, 74 and 72, respectively (Table 4, Figure 1A, B). In the case of the ERB, WGRB and WGRB-lac, the early incremental blood glucose area (iAUC 0-30) was significantly reduced compared with WWB, WWP and RBB, respectively. When comparing the endosperm products ERP and ERB, a significantly larger incremental area was obtained with the

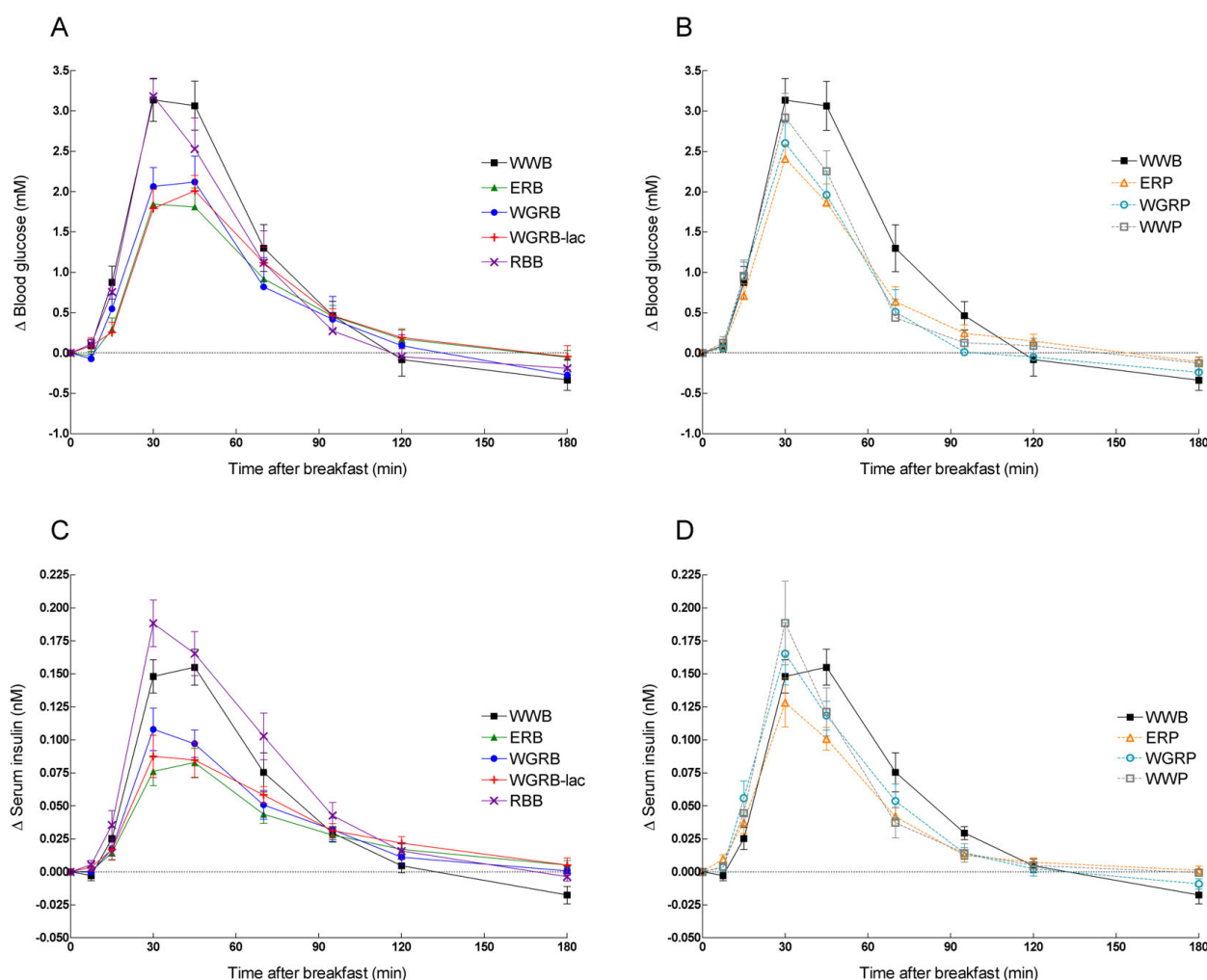


Figure 1

Incremental change (Δ) in blood glucose in breads (A) and porridges (B). Incremental change (Δ) in serum insulin in breads (C) and porridges (D). WWB is visible in all graphs. Values are means \pm SEM, $n = 11$ for blood glucose and $n = 12$ for serum insulin (WGRP: $n = 10$ resp. 11). Significant treatment effect and time \times treatment interactions were found, $p < 0.0001$ for both blood glucose and serum insulin (PROC MIXED in SAS).

porridge ERP in the early post-prandial phase (iAUC 0-30 min) (+51%) (Paired analysis ANOVA, $p < 0.05$, data not shown). Similarly, when comparing the whole grain products, WGRP and WGRB, respectively, the 30 min incremental area (iAUC 0-30 min) was 43% larger following the porridge (Paired analysis ANOVA, $p < 0.05$, data not shown).

The glycaemic profile (GP, min/mM) was significantly higher for ERB and WGRB-lac compared with WWB, RBB, WWP and WGRP.

Significant differences in blood glucose were observed at specific time points (time \times treatment $p < 0.0001$). RBB and WWB induced significantly higher glucose response

than the non-supplemented rye products at 30 min (data not shown).

Serum insulin responses

With the exception of RBB, all rye breads, and the ERP, induced significantly lower incremental insulin areas (iAUC 0-120 min) than WWB, resulting in insulinaemic Indices (II's) ranging from 61 to 73 (Table 5, Figure 1C, D). Instead, RBB showed an II of 128, with a significantly larger incremental area (iAUC 0-120 min) than all other rye products and the WWP. The insulin areas at 30 min (iAUC 0-30) were significantly smaller with the ERB, WGRB and WGRB-lac than with the enriched rye product RBB, and the porridges WGRP and WWP. Both rye porridges, ERP and WGRP, induced significantly higher insu-

Table 4: Blood glucose responses after the test meals

Meals	GP	GPI	Glucose iAUC (0-30 min)	Glucose iAUC (0-120 min)	GI
	min/mM	%	min·mM	min·mM	%
WWB	37.0 ± 5.6 ^b	100 ± 0.0 ^b	34.6 ± 4.1 ^a	167.5 ± 17.8 ^a	100 ± 0.0 ^a
WWP	35.2 ± 5.0 ^b	107 ± 15.6 ^b	34.2 ± 4.7 ^a	119.0 ± 13.0 ^{ab}	77 ± 9.8 ^{ab}
ERB	69.2 ± 10.1 ^a	200 ± 24.9 ^a	17.0 ± 3.2 ^c	104.0 ± 15.9 ^b	64 ± 7.5 ^b
ERP	49.7 ± 6.3 ^{ab}	145 ± 18.6 ^{ab}	25.7 ± 3.0 ^{abc}	103.1 ± 7.6 ^b	70 ± 6.3 ^b
WGRB	51.0 ± 7.0 ^{ab}	142 ± 11.5 ^{ab}	22.1 ± 3.6 ^{bc}	118.9 ± 21.8 ^{ab}	71 ± 9.7 ^{ab}
WGRB-lac	74.3 ± 9.7 ^a	226 ± 32.9 ^a	17.9 ± 3.1 ^c	113.6 ± 11.0 ^b	74 ± 9.5 ^b
WGRP	39.7 ± 7.3 ^b	111 ± 17.7 ^b	31.5 ± 3.8 ^{ab}	110.0 ± 14.4 ^b	72 ± 10.2 ^b
RBB	35.7 ± 3.4 ^b	113 ± 17.7 ^b	33.5 ± 3.0 ^a	147.2 ± 23.1 ^{ab}	87 ± 6.7 ^{ab}

Values are means ± SEM, n = 11 (WGRP: n = 10). Products not sharing the same letters were significantly different, p < 0.05 (ANOVA, followed by Tukey's test).

lin responses (iAUC 0-30 min) than the corresponding bread products, amounting to +96% and +87%, respectively. RBB and WWP induced significantly higher insulin incremental peaks compared to the ERB, WGRB and WGRB-lac

Significant differences in serum insulin were observed at specific time points (time × treatment p < 0.0001). RBB induced significantly higher insulin response than all other rye products at 45 and 70 min (data not shown).

Ghrelin responses

Plasma ghrelin levels after all products decreased to a nadir, occurring at 64.9 ± 3.1 min in the post-prandial phase. (Table 6, Figure 2A, B). The ghrelin levels following WWB, ERP, WWP and RBB all rose significantly above the fasting level at 180 min, with the WWB and WWP causing a higher relative increase in ghrelin from fasting level to 180 min than WGRB-lac.

Subjective satiety

WGRP and ERP induced significantly higher subjective satiety (expressed as iAUC from 0 to 180 min) than WWB and WGRB, with the iAUC for WGRP being 123% larger

than that of WGRB (Table 6, Figure 2C, D). RBB induced a higher feeling of subjective satiety (iAUC) than WWB at 0-180 min.

In vitro rate of starch hydrolysis

All porridges and the ERB were characterized by a lower hydrolysis index (HI) than WWB and WGRB. In addition, WGRP showed a lower HI than WGRB-lac, RBB and ERP (Table 1).

Correlations

Correlations between II, insulin incremental peak, GI, GP, hypoglycaemia, ghrelin and subjective satiety are presented in Table 7. The hydrolysis index (HI) was neither correlated with the GI, II, GP or with the hypoglycaemia. The subjective satiety (iAUC 0-180 min) was not correlated with the protein, caloric, starch, fat, water or fibre contents of the test meals.

Discussion

In the present study, the endosperm products and whole grain rye breads induced significantly lower II's than WWB, which is in agreement with previous findings [24-26]. In contrast to the other rye breads in the study, the

Table 5: Serum insulin responses after the test meals

Meals	Insulin incremental peak	Insulin iAUC (0-30 min)	Insulin iAUC (0-120 min)	II
	nM	min·nM	min·nM	%
WWB	0.168 ± 0.011 ^{ab}	1.42 ± 0.15 ^{ab}	8.35 ± 0.50 ^{ab}	100 ± 0.0 ^{ab}
WWP	0.201 ± 0.029 ^a	1.96 ± 0.32 ^a	7.19 ± 0.66 ^{bc}	87 ± 7.8 ^{bc}
ERB	0.089 ± 0.011 ^d	0.76 ± 0.12 ^c	4.99 ± 0.57 ^d	61 ± 8.1 ^d
ERP	0.131 ± 0.018 ^{bc}	1.49 ± 0.24 ^{ab}	5.77 ± 0.55 ^{cd}	71 ± 6.9 ^{cd}
WGRB	0.124 ± 0.013 ^{bcd}	1.03 ± 0.16 ^{bc}	6.06 ± 0.59 ^{cd}	73 ± 7.5 ^{cd}
WGRB-lac	0.103 ± 0.014 ^{cd}	0.91 ± 0.20 ^{bc}	5.98 ± 0.70 ^{cd}	71 ± 8.9 ^{cd}
WGRP	0.177 ± 0.019 ^{ab}	1.93 ± 0.31 ^a	7.31 ± 0.69 ^{bcd}	88 ± 8.7 ^{bcd}
RBB	0.202 ± 0.016 ^a	1.87 ± 0.22 ^a	10.45 ± 1.06 ^a	128 ± 15.9 ^a

Values are means ± SEM, n = 12 (WGRP: n = 11). Products not sharing the same letters were significantly different, p < 0.05 (ANOVA, followed by Tukey's test).

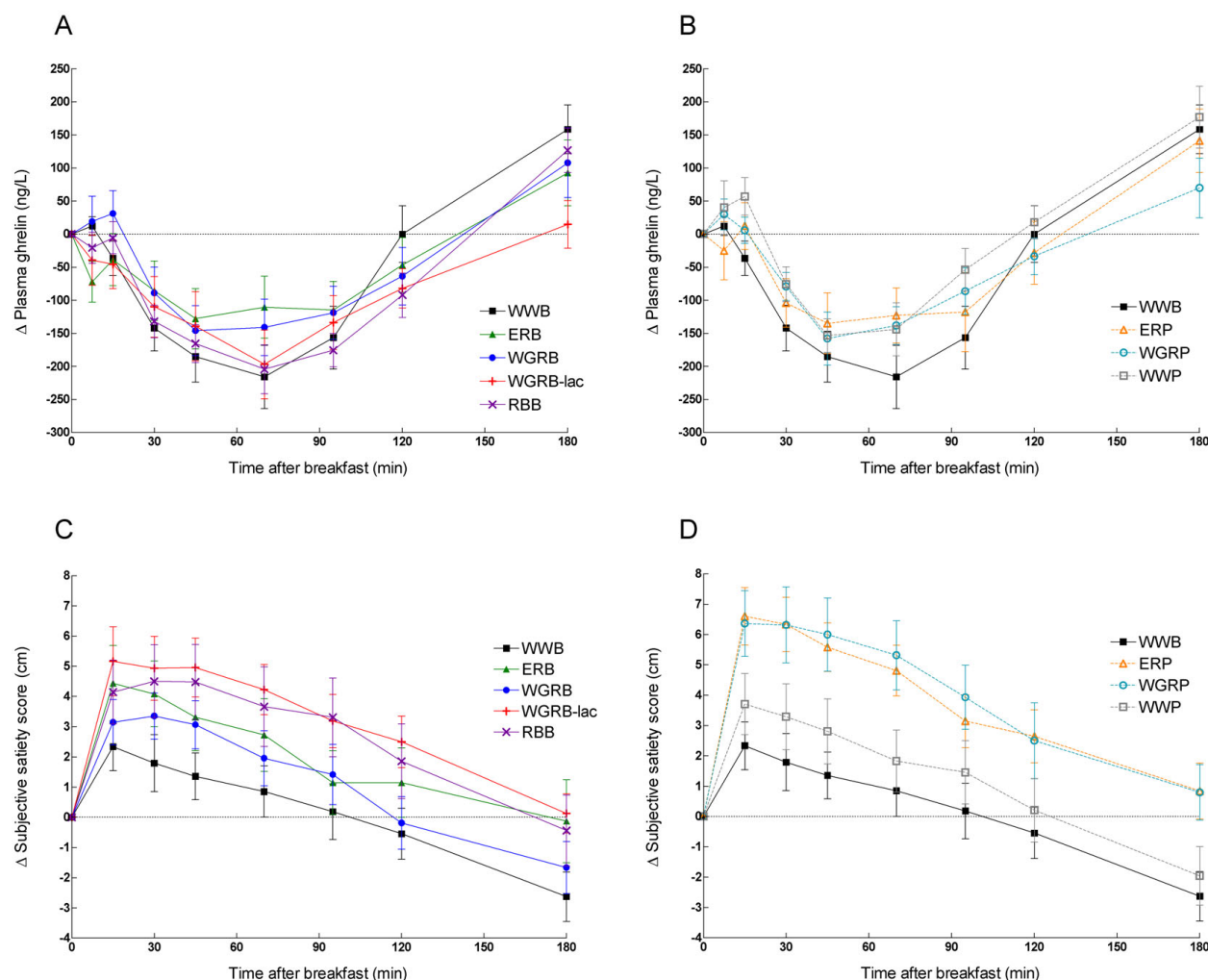


Figure 2

Incremental change (Δ) in plasma ghrelin in breads (A) and porridges (B). Incremental change (Δ) in subjective satiety scores in breads (C) and porridges (D). WWB is visible in all graphs. Values are means \pm SEM, $n = 12$ (WGRP: $n = 11$). A significant treatment effect was found ($p < 0.0001$), but no significant time \times treatment interaction, $p = 0.67$ for ghrelin and $p = 0.97$ for subjective feeling of satiety (PROC MIXED in SAS)

RBB i.e. white wheat flour enriched with rye bran (35 wt %) showed a significantly higher II compared to all other rye products.

To explain the low post-prandial insulin response of the endosperm and whole grain rye products, the course of glycaemia was analyzed. The rye products tended to induce blood glucose curves that remained above fasting for a longer time, with a lower glucose peak and a less pronounced late hypoglycaemia. It could be hypothesized that the inconsistency between GI and II, reported for some rye products, is caused by this low but prolonged net increment in post-prandial blood glucose response, resulting in an improved insulin economy, but maintain-

ing a high GI as calculated from the 120 min area. In order to quantify the profile of the blood glucose curve, the glycaemic profile (GP) was introduced, defined as the duration for incremental post-prandial blood glucose response divided with the blood glucose incremental peak. Thus, a high GP is indicative of a facilitated post-prandial glycaemic regulation, with a lower glucose peak and a less pronounced hypoglycaemia. As judged from their higher GP's, it could be argued that ERB and WGRB-lac are characterized by a more beneficial glucose regulation than RBB, WWB, WWP and WGRP, respectively.

In the present study, the II showed a stronger correlation with GP than with the GI of the products. Furthermore,

Table 6: Plasma ghrelin responses and subjective satiety responses after the test meals

Meals	Ghrelin relative decrease (from fasting to nadir)	Ghrelin relative increase (0 to 180 min)	Subjective satiety iAUC (0-180 min)
	%	%	min·cm
WWB	-20.5 ± 2.4 ^{a*}	17.1 ± 4.6 ^{a*}	220 ± 71 ^c
WWP	-17.2 ± 1.9 ^{a*}	15.5 ± 4.7 ^{a*}	371 ± 78 ^{abc}
ERB	-16.7 ± 2.9 ^{a*}	8.0 ± 4.3 ^{ab}	465 ± 146 ^{abc}
ERP	-16.9 ± 3.6 ^{a*}	14.4 ± 5.2 ^{ab*}	716 ± 109 ^a
WGRB	-16.9 ± 2.8 ^{a*}	11.1 ± 5.4 ^{ab}	321 ± 99 ^{bc}
WGRB-lac	-18.1 ± 3.5 ^{a*}	1.3 ± 3.4 ^b	570 ± 126 ^{abc}
WGRP	-18.3 ± 2.1 ^{a*}	7.0 ± 4.0 ^{ab}	718 ± 162 ^a
RBB	-20.3 ± 1.8 ^{a*}	12.2 ± 4.0 ^{ab*}	587 ± 154 ^{ab}

Values are means ± SEM, n = 12 (WGRP: n = 11). Products not sharing the same letters were significantly different, $p < 0.05$ (ANOVA, followed by Tukey's test). * indicates significant difference from fasting concentration ($p < 0.05$, ANOVA, followed by Tukey's test).

the insulin incremental peak was negatively correlated to the GP but showed no correlation with the GI. This indicates that the GP is a better predictor, than the GI, of the acute insulin response of rye products.

We suggest that the GP is a useful tool for evaluation of post-prandial glycaemia to carbohydrate foods in general. Granfeldt et al. [31] noted that although the time course for the post-prandial glycaemia were considerably different with pasta and white wheat bread in healthy elderly subjects; the GI's remained similar due to enduring incremental blood glucose response in the late phase with pasta. If calculating GP values from estimated data in that study; GP's of 120 and 41 were estimated for pasta and bread, respectively. The high GP pasta meal significantly

improved glucose tolerance at a standardized "second-meal", ingested after 4 h, compared with the low GP white wheat bread reference [32]. Moreover, when studying a range of cereal breakfasts in healthy subjects it was found that the blood glucose level 4 h after commencing the test breakfast was negatively correlated to the blood glucose incremental peak at a following standardized lunch ($r = -0.29$, $p = 0.043$) [33]. These results suggest that products characterized by high GP's are more prone to induce benefits on second-meal glucose tolerance.

The beneficial glycaemic profile and low post-prandial insulin response seen with endosperm rye bread does not rule out a dietary fibre-related mechanism. In contrast to WWB (1.8 g DF/100 g bread), the endosperm rye bread

Table 7: Correlations between blood glucose, serum insulin, plasma ghrelin and subjective satiety responses following the test meals

	GP	II	Insulin Incremental peak	GI	Hypoglycaemia neg AUC (30-180 min)
Subjective satiety 180 min (delta from fasting)	NS	NS	NS	NS	-0.28*
Ghrelin relative increase (0 to 180 min)	-0.40 ***	0.33 **	0.34 **	NS	0.26*
Hypoglycaemia neg AUC [†] (30-180 min)	-0.43 ***	0.22 *	0.38 ***	-0.39 ***	
GI	NS	0.35 **	NS		
Insulin incremental peak	-0.64 ***	0.72 ***			
II	-0.48 ***				

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, NS = non significant. (Spearman's partial coefficients controlling for subjects (two-tailed test)).

(ERB) are rich in soluble fibres (6.7 g DF/100 g bread of which 2.5 g was soluble and 4.2 g insoluble fibres). However, since the more fibre rich RBB appeared to be devoid of acute metabolic benefits, it could be suggested that some unknown property or component(s) present in the endosperm, but not the bran fraction of rye, affects the course of glycaemia and lowers the insulin demand. This is a new observation showing that the benefits of rye products on insulin demand cannot be mimicked by adding rye bran to a white wheat background. The results also show the importance of maintaining all parts of the whole grain, including the endosperm, in rye products. The improved insulin economy seen with endosperm and whole grain rye breads could possibly be due to an improvement in insulin sensitivity or by an increase in first phase insulin release, previously seen with rye breads [34,35].

Whole grain and endosperm rye porridges induced significantly higher early insulin and glucose responses (0-30 min) compared with the corresponding bread products. Also the insulin incremental peak was higher for the porridges; ERP inducing a 48% higher peak than ERB and WGRP a 42% higher insulin peak than WGRB. Thus, in the present study, the type of processing affects the glycaemic and hormonal responses to flour based rye products, in favour of bread making.

In a study by Juntunen et al [25] it was suggested that a lowered post-prandial insulin response to rye products could be explained by a mechanically firmer structure, leading to obstructed amylolysis and a slower rate of glucose delivery. The hydrolysis index (HI), calculated with WWB as reference, was lower than that of WWB for some, but not all whole grain and endosperm rye products in the present study. HI was not correlated with GI, GP or II. Hence, in the present work, the high GP and low II of rye products are not explained by obstructed amylolysis.

A connection between low II features of foods and increased post-meal satiety has been shown in several studies comparing equicarbohydrate food portions [36-39]. In the present study, the insulin incremental peak was related to the extent of hypoglycaemia (neg AUC 30-180); a higher insulin surge being related to a more pronounced dip in blood glucose below fasting level ($r = 0.38$, $p < 0.01$). The extent of hypoglycaemia was in turn negatively correlated to the feeling of subjective satiety at 180 min ($r = -0.28$, $p < 0.05$). As a possible measure of appetite regulation, we studied total plasma ghrelin in the post-prandial phase. In the present study, a high insulin incremental peak was related to a more potent recovery of ghrelin in the later post-prandial phase ($r = 0.34$, $p < 0.01$). Also, previous studies, both clamp [40-42] and meal studies [43,44], has found that total ghrelin concen-

trations were influenced by insulin. High levels of total ghrelin at 4 h after a preload has been demonstrated to increase voluntary energy intake at a subsequent meal [45]. Thus, it can be hypothesized that a whole grain- or endosperm rye bread breakfast, causing low acute insulin response might reduce hunger in the late post-prandial phase and possibly lower energy intake at a subsequent meal compared with a high II breakfast such as WWB. Semi-acute and longer term studies are needed to verify this hypothesis.

Conclusion

Endosperm rye products and whole grain rye breads induced significantly lower II's than white wheat bread (WWB). In addition, these products induced low and prolonged glucose profiles i.e. high GP's, in the post-prandial phase. The rye bran bread, devoid of the endosperm part of the rye grain, induced GI, GP and II similar to that of a WWB. The finding that the presently introduced GP better predict the insulin response of rye products in the acute post-prandial phase, compared with the GI, is important. It is put forward that the GP could be exploited when evaluating post-prandial glycaemia of food products. The results also indicate that a higher acute insulin response was associated with more prominent late hypoglycaemia, feeling of hunger and an increase in plasma ghrelin, respectively. Thus, low II rye breakfast products may improve appetite regulation. The latter warrants further investigations which are currently under way.

List of abbreviations

BMI: body mass index; CVD: cardiovascular diseases; ERB: endosperm rye bread; ERP: endosperm rye porridge; GI: glycaemic index; GP: Glycaemic profile; HI: hydrolysis index; iAUC: incremental area under the curve; II: insulinaemic index; neg AUC: negative area under the curve; RBB: rye bran bread; WGRB: whole grain rye bread; WGRB-lac: whole grain rye bread made with lactic acid; WGRP: whole grain porridge; WWB: white wheat bread; WWP: white wheat porridge.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

LAHR coordinated the study and was responsible for the study design, the collection and analysis of the data, statistical analysis and for writing the paper. EMÖ was involved in the study design, interpretation of data and in writing the paper. IMEB was the guarantor for the founding of the study and was involved in the study design, interpretation of data and writing of the paper. LOBS was involved in the analysis and statistical analysis of total ghrelin. UKA and CH was involved in the study design.

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Paper II

RESEARCH

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Effects of cereal breakfasts on postprandial glucose, appetite regulation and voluntary energy intake at a subsequent standardized lunch; focusing on rye products

Liza AH Rosén*, Elin M Östman, Inger ME Björck

Abstract

Background: Rye products have been demonstrated to lower the acute insulin demand, induce a low and prolonged blood glucose response (high Glycemic Profile, GP) and reduce subclinical inflammation. These products may therefore contribute to a lowered risk of obesity, type 2 diabetes and cardio vascular disease. The objective of the present paper was to evaluate the mechanism for a reduced postprandial insulin demand with rye products, and to explore possible appetite regulating properties.

Methods: 10 healthy subjects were served breakfast meals (50 g of available starch) with endosperm- or whole grain rye breads, with and without lactic acid, boiled whole grain rye- (RK) or wheat (WK) kernels, or white wheat bread reference (WWB) in random order in a cross-over design. Plasma concentrations of glucose, ghrelin, serum insulin, free fatty acids, adiponectin, breath hydrogen excretion (H_2), and subjective satiety was evaluated during the postprandial phase. 270 min after the breakfast, an ad lib lunch buffet was served and the voluntary energy intake (EI) was registered.

Results: All rye products and WK induced lower insulinemic indices (II) than WWB. A lower incremental insulin peak following breakfast correlated with a lower EI at lunch ($r = 0.38$). A low II was related to improved satiety in the early postprandial phase (fullness AUC 0-60 min, $r = -0.36$). RK induced a higher GP compared to WWB and WK. A higher GP was related to a lowered *desire to eat* before lunch (AUC 210-270) and to a lower concentration of ghrelin in the late postprandial phase after breakfast (270 min), $r = -0.29$ and -0.29 , which in turn was related to a lower voluntary EI ($r = 0.43$ and 0.33). The RK breakfast improved satiety in the early postprandial phase (0-60 min) compared to WWB, and induced a lower EI at lunch (-16%). A high content of indigestible carbohydrates in the breakfast products was related to improved satiety (0-60 min, $r = 0.68$ for fullness), and a higher breath H_2 in the late postprandial phase (120-270 and 270-390 min, $r = 0.46$ and 0.70). High H_2 (AUC 120-270 min) also correlated with lower EI ($r = -0.34$).

Conclusions: Rye products, rich in indigestible carbohydrates, induce colonic fermentation already post the breakfast meal, and lowers acute insulin responses. A high excretion of breath H_2 also correlated with a higher GP. Especially, rye kernels induced a high GP which was associated with a 16% lowering of energy intake at a subsequent lunch meal. The bulking effect of rye fiber, colonically derived fermentation metabolites, a high GP and a low insulin response possibly all contributes to the benefits on glucose- and appetite regulation seen in an acute and semi-acute perspective.

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Background

The prevalence of obesity and type 2 diabetes (T2D) is increasing globally and preventive strategies are urgently needed. Whole grain foods have been shown to facilitate weight regulation and to lower the risk of T2D, cardiovascular disease (CVD) and the metabolic syndrome [1-8]. The protective mechanism may be related to the presence of dietary fiber or other potentially bioactive components [9,10]. In addition, some whole grain products are characterized by a low glycemia and insulinemia, properties also known to protect against T2D and CVD [3,11-14]. Products made from rye (*Secale cereale*) have previously been shown to induce a low insulin response [15-18] which may counteract development of insulin resistance [19]. Furthermore, evidence of a facilitated glycemic regulation has been found with rye products in healthy subjects, with lower incremental peaks, avoidance of hypoglycemia, and glucose excursions remaining above fasting for a longer time i.e. a higher glycemic profile (GP) [18]. The mechanisms behind the beneficial glycemic and insulinemic responses of rye products are, however, not known.

Avoidance of frequent and elevated blood glucose excursions is protective against oxidative stress and sub-clinical inflammation [20]. Furthermore, several studies have shown that carbohydrate foods causing low postprandial insulin responses induce higher satiety and a lower voluntary food intake at a subsequent meal, as compared with foods inducing high acute insulinemia [21-25]. In light of the low acute insulin responses commonly induced by rye products, such products could be anticipated to possess appetite regulating properties. In support of this, meal studies with rye products in healthy adults demonstrated that a low insulin response was associated with less accentuated late postprandial hypoglycemia, lowered sense of hunger, and lowered late postprandial increase of plasma ghrelin [18]. These findings indicate that further insights regarding the metabolic- and appetite regulating properties of rye products might add to the knowledge regarding mechanisms for health benefits of whole grains.

Since rye bread products are frequently processed using sour-dough fermentation, the potential effect of organic acids also deserve attention in this context. Lactic acid addition to bread has previously been reported to lower glycemic response [26].

In the present study, the metabolic and appetite regulating properties of rye breads made from endosperm or wholegrain rye flour, were investigated in healthy subjects, using white wheat bread as reference (WWB). The effect of adding lactic acid to the rye breads was also studied. In addition, test products made by boiling of kernels from rye and wheat, respectively, were included.

The test products were provided as breakfasts and postprandial plasma were analyzed for measures of glucose metabolism (glucose, serum insulin, FFA, adiponectin), and appetite regulating hormones (ghrelin). Additionally markers of colonic fermentation were measured (breath hydrogen excretion). Finally, subjective satiety was evaluated in the postprandial phase. At 270 min after the breakfast, an *ad libitum* buffet lunch meal was served and the energy intake was registered.

Methods

Test subjects

Ten healthy non-smoking volunteers (5 men and 5 women) aged 26.0 ± 1.1 y with normal body mass indices (22.6 ± 0.4 kg/m²) and without drug therapy participated in the study. All subjects had normal fasting plasma glucose concentrations (5.5 ± 0.1 mmol/L). The subjects were recruited in March 2007 and the study was performed from April to June 2007. All subjects gave their informed consent and were aware of the possibility of withdrawing from the study at any time they desired. Approval of the study was obtained from the regional ethical review board in Lund, Sweden (reference number 109/2007)

Breakfast products

Four rye breads, boiled rye and wheat kernels and a white wheat bread reference were included in the study. Whole grain rye flour, kernels and endosperm rye flour (commercial blends) were provided by Lantmännen R&D (Järna, Sweden). Commercial white wheat flour was obtained from Kungsörnen AB (Järna, Sweden). Whole wheat kernels (Tiger) were provided by BFEL (Germany). Dry yeast was obtained from Jästbolaget AB (Sollentuna, Sweden) and lactic acid (88-92% extra pur) from Riedel-de Haën (Morris Township, NJ, USA).

WWB

The white wheat bread (WWB) was made from 540 g of white wheat flour, 360 g water, 4.8 g dry yeast, 4.8 g NaCl and baked in a bread baking machine (BM 3983, Severin, Sundern, Germany) using a program for white bread.

ERB

Endosperm rye bread (ERB) was made from 5000 g endosperm rye flour, 3413 g water, 84 g dry yeast and 43 g NaCl (containing 5 mg KI/100 g). The dough was mixed for 8 minutes and proofed at room temperature for 30 minutes. It was divided into pieces of 1000 g each and placed in baking tins. The dough was subjected to a second proofing (38°C, 85 % humidity) during 30 minutes for the endosperm rye breads and 45 minutes for the whole grain rye breads. Baking was performed initially at 250°C with 3 sec of steam. The temperature was then immediately lowered to 200°C and the breads were baked for 40 min.

ERB-lac

Endosperm rye bread with lactic acid (ERB-lac) was made from 5000 g endosperm rye flour, 3322 g water, 90 g lactic acid, 84 g dry yeast and 43 g NaCl (containing 5 mg KI/100 g). The bread was made using the same method as for ERB.

WGRB

Whole grain rye bread (WGRB) was made from 5000 g coarse whole grain rye flour, 3661 g water, 84 g dry yeast and 43 g NaCl (containing 5 mg KI/100 g). The bread was made using the same method as for ERB but was baked for 45 min.

WGRB-lac

Whole grain rye bread with lactic acid (WGRB-lac) was made from 5000 g coarse whole grain rye flour, 3574 g water, 90 g lactic acid, 84 g dry yeast and 43 g NaCl (containing 5 mg KI/100 g). The bread was made using the same method as for WGRB.

The WWB was left to cool for 1 hour and the rye breads for 21 hours under cover. Thereafter, the crust was removed and the breads were sliced and wrapped in aluminum foil in portions sizes, put into plastic bags and stored in a freezer (-20°C) until use. The day before the experiment, the breads were taken from the freezer and were thawed over night at ambient temperature, still wrapped in aluminum foil and in the plastic bag.

RK and WK

The wholegrain wheat kernels (WK) and rye kernels (RK) were prepared on the day of the experiment. 97.1 g of whole wheat kernels and 0.5 g NaCl were boiled in 156.4 g water for 40 minutes. 106.6 g whole rye kernels and 0.5 g NaCl were boiled in 189.5 g water for 35 minutes. All water was absorbed by the kernels.

Composition of the lunch buffet

An *ad libitum* lunch buffet was served at 270 min after breakfast to measure voluntary food intake. The buffet meal was a common Swedish lunch meal and was composed of meatballs (ICA Handlarnas AB, Solna, Sweden), pasta (Kungsörnen AB, Järna, Sweden), ketchup (Procordia Food AB, Eslöv, Sweden) and cucumbers. The cucumbers were fresh, and were peeled and sliced prior to serving to ensure homogeneity. Meatballs (240 g) were heated in a microwave oven (MS 2334B, LG, LG Electronics Inc., Seoul, Korea) for 4 min at 850 W. The pasta (325 g dry weight) was boiled for 8 min in 3 liters of water with 2 teaspoons (13 g) NaCl. One tablespoon of rapeseed oil (Di Luca & Di Luca AB, Stockholm, Sweden) was added to the pasta after boiling.

Chemical analysis of the breakfast products

Prior to analysis of the total starch, fiber and protein content, the breakfast products were air dried and milled

(1.0 mm screen, Cyclotec, Tecator, Höganäs, Sweden). Measurements of resistant starch (RS) was performed on products as is. Total and resistant starch was analyzed according to Björck et al. [27] and Åkerberg et al. [28]. The available starch was calculated by subtracting RS from total starch. Insoluble and soluble dietary fiber were determined with a gravimetric, enzymatic method described by Asp et al. [29]. Protein content was determined by Kjeldahl analysis (Kjeltec Auto 1030 Analyser, Tecator, Höganäs, Sweden). Fat content in the products was calculated using data from endosperm and whole-grain rye and wheat flours from Lantmännen. Energy content of the test meals were calculated using fat, carbohydrate and protein contents of the meals (17 kJ per gram of protein and available carbohydrate and 37 kJ per gram fat). The rate of starch hydrolysis (HI) was determined using an in vitro procedure based on chewing [30], with WWB as a reference. The nutritional composition and HI of the products are shown in Table 1.

Study design

The test and reference products were provided as breakfasts, on 7 different occasions, in random order separated by approximately 1 wk. The subjects were instructed to eat a standardized evening meal (9:00-10:00 P.M) prior to the test, consisting of a few slices of white wheat bread. No eating or drinking except for small amounts of water was then allowed until the start of the test. The subjects were also told to avoid alcohol and excessive physical exercise the day before each test, and otherwise as far possible maintain their regular life style throughout the entire study. The subjects arrived at the laboratory at 07.45 a.m. on the test day. A peripheral venous catheter (BD Venflon, Becton Dickinson, Helsingborg, Sweden) was inserted into an antecubital vein.

Fasting blood samples were taken prior to the breakfast meal at time 0. Thereafter the test meals, contributing with 50 g of available starch, were served with 250 ml of tap water. The test subjects finished the breads within 14 minutes and the kernels within 25 minutes. At 120 min after the breakfast, the test subjects were served 250 ml of tea, coffee or water without any sweeteners or milk products. The chosen beverage remained consistent for each individual at all 7 visits.

At 270 min after commencing the breakfast meals, the subjects were provided the lunch buffet and were instructed to eat the amount needed to reach comfortable satiation. At the following visits they should eat until they reached the same degree of satiation as on their first occasion. On their first visit, the subjects could drink as much water as they desired, and the same amount of water was then served at the following 6 visits. The subjects had to finish their lunch within 30 min, before the next blood sampling occasion at 300 min

Table 1 Composition of the breakfast products

Meals	Portion size	Available starch	Total starch	Resistant starch	Protein	Soluble fibers	Indigestible carbohydrates	Water content	Energy content	HI
					g/portion					
								kJ		
								%		
WWB	124.0	50.0	51.1	1.1	7.3	0.5	4.4	59.0	1 015	100 ± 0 ^a
ERB	134.8	50.0	51.4	1.4	6.1	4.0	11.9	64.3	1 033	87 ± 3 ^b
ERB-lac	133.1	50.0	51.7	1.7	6.0	4.5	11.8	62.0	1 033	85 ± 2 ^b
WGRB	163.4	50.0	52.5	2.5	8.3	4.0	19.8	79.7	1 078	87 ± 2 ^b
WGRB-lac	158.4	50.0	51.8	1.8	7.9	4.1	17.7	76.7	1 069	84 ± 2 ^b
RK	226.6	48.4	55.9	7.5	9.2	3.7	25.2	131.9	1 066	70 ± 5 ^c
WK	171.9	50.3	57.8	7.5	12.2	1.5	19.8	84.4	1 170	72 ± 3 ^c

Indigestible carbohydrate content consists of soluble fibers, insoluble fibers and resistant starch in the breakfast products. n = 2 (total starch and proteins), n = 3 (fiber content), n = 6 (RK, n = 4, resistant starch), n = 6 (HI). Products not sharing the same letter are significantly different, p < 0.05 (ANOVA, followed by Tukey's test).

after commencing breakfast. The weight of the different food items ingested was registered individually to allow calculation of the energy intake at the buffet lunch meal. The energy content of the foods in the lunch buffet was obtained from the manufacturer of the products, and that of the cucumber from food tables (Swedish National Food Administration).

Physiological parameters

Capillary blood samples were taken for analysis of plasma glucose (p-glucose). Venous blood samples were drawn for the analysis of serum insulin, serum free fatty acids (s-FFA), s-adiponectin and p-ghrelin. Breath hydrogen excretion (H₂) was measured as a marker of colonic fermentation, using an EC 60 gastrolyzer (Bedfont EC60 Gastrolyzer, Rochester, England). In addition, the subjects were asked to fill in their subjective *feeling of fullness*, *hunger* and *desire to eat*, respectively, using a 100 mm Visual Analogue Scale (VAS).

Glucose and insulin were measured at 0, 15, 30, 45, 60, 90, 120, 180, 240 and 270 min. FFA and adiponectin were measured at 0, 180 and 270 min. Ghrelin was measured at 0, 60, 90, 120, 270, 330, 360 and 390 min. Subjective appetite ratings were performed every 30 min throughout the experimental day and also at 15 and 315 min. H₂ was measured every 30 min.

After sampling, serum and plasma (EDTA) tubes were left in ice bath for approximately 1 h before being centrifuged for 11 min (1800 × g, 4°C). Serum and plasma were thereafter immediately separated and the samples were frozen at -20°C (serum) or -40°C (plasma) until analysis. Plasma for ghrelin analysis was sampled into tubes containing 500 KIU aprotinin (Bayer HealthCare AG, Leverkusen, Germany) per ml of whole blood.

Glucose was analyzed using a p-glucose analyzer (Glucose 201+, Hemocue, Ängelholm). The s-insulin analysis was performed on an integrated immunoassay analyzer (CODA Open Microplate System; Bio-rad Laboratories,

Hercules, CA, USA) using an enzyme immunoassay kit (Mercodia AB, Uppsala, Sweden). S-FFA were analyzed using an enzymatic colorimetric method (NEFA C, ACS-ACOD method, WAKO CHEMICALS gmbH, Germany). S-adiponectin was analyzed using an enzyme immunoassay kit (Mercodia AB, Uppsala, Sweden), and p-ghrelin with a radioimmunoassay kit (Linco research Inc., St. Charles, MO, USA).

Calculations and statistical methods

One subject was excluded from the analysis of data from the WGRB breakfast due to having a cold at that particular test day. The data for WGRB is therefore analyzed with n = 9. Data are expressed as means ± SEM.

The total area under curve (AUC) was calculated for each subject and test meal, using the trapezoid model. The glycemic index (GI) is defined as the incremental positive area under the blood glucose curve after a test product, expressed as a percentage of the corresponding area after an equi-carbohydrate reference product taken by the same subject [31]. The insulinemic index (II) is calculated from the corresponding insulin areas. Thus, GI and II were calculated using the net incremental AUC (0-120 min), with WWB as a reference. Incremental peaks for glucose and insulin were calculated as maximum postprandial increase from baseline.

The glycemic profile (GP) defined as the duration of the glucose curve divided with the incremental glucose peak was calculated [18], with the modification that in cases where the glucose remained above fasting for the entire 270 min before lunch, the duration value was set to 270 min.

Hydrolysis index were calculated from the 180 min AUC for in vitro starch hydrolysis, in a similar way of calculating GI and II values, using WWB as a reference [30].

Time × treatment interactions for glucose, insulin, ghrelin, satiety, breath hydrogen, FFA and adiponectin responses were analyzed using a mixed model (PROC

MIXED in SAS release 8.01, SAS Institute Inc., Cary, NC) with repeated measures and an autoregressive covariance structure. Subjects were modeled as a random variable and corresponding baseline (fasting values) value were modeled as covariate. The data were analyzed using a mixed model analysis of covariance (ANCOVA) with subject as a random variable and corresponding baseline (fasting values) as a covariate. For voluntary energy intake at lunch and HI, a mixed model analysis of variance (ANOVA) was used with subject as a random variable. Differences between groups were identified using Tukey's multiple comparison tests. (MINITAB, release 16, Minitab Inc., State College, PA). In the cases of unevenly distributed residuals (tested with Anderson-Darling test), Box Cox transformation were performed on the data prior to the ANCOVA and ANOVA. Correlation analysis was conducted to evaluate the relation among dependent measures with the use of Spearman's partial correlation coefficients controlling for subjects and corresponding baseline values (two-tailed test), (SPSS software, version 19; SPSS Inc., Chicago, IL, USA). $p < 0.05$ was considered statistically significant.

Results

Starch hydrolysis (HI)

The rye products and WK displayed a lower rate of starch hydrolysis, expressed as HI, than did WWB (Table 1). Furthermore, RK and WK displayed lower HI's than the rye breads.

Glucose responses following breakfast

All products except the ERB displayed lower glycemic indices (GI's) than WWB, with GI's ranging from 64 to 79 (Table 2). WK and all rye products, except ERB, induced lower early glucose responses than WWB (AUC 0-60 min, Figure 1). The incremental glucose peak was lowered following all products in comparison to WWB, with the exceptions of ERB. The glycemic profile (GP, $\text{min} \cdot \text{mmol}^{-1} \cdot \text{L}^{-1}$) was higher for RK than for WWB and WK. Generally, rye products induced higher GP's than

wheat products (mean GP 74 for rye and 50 for wheat). No time x treatment interaction was found (0-270 min).

Insulin responses following breakfast

All breakfast products induced lower insulimemic indices than WWB ranging from 60 for the kernel based products to 75 for the whole grain rye bread (Table 2). In the early postprandial phase all products except WGRB induced lower insulin responses than WWB (AUC 0-60 min, Figure 1). All products induced lower incremental insulin peak values than WWB. No time x treatment interaction was found (0-270 min).

H₂ excretion following breakfast and buffet lunch

Prior to lunch, RK induced higher H₂ excretion than WWB (AUC 120-270 min, Figure 2). All products except ERB-lac and WK induced higher increment in H₂ than WWB in the postprandial phase following the buffet lunch (AUC 270-390 min). Furthermore, RK, WGRB and WGRB-lac generated higher H₂ than ERB-lac following the buffet lunch (AUC 270-390 min).

Significant differences in H₂ were observed at specific time points (time x treatment $p = 0.0016$ for the 0-270 min interval). At 240 min, WGRB induced higher H₂ excretion than WWB and ERB and at 270 min; RK and WGRB induced higher H₂ excretion than WWB and ERB-lac. No significant time x treatment interaction was found for the interval 270-390 min.

Subjective appetite ratings following breakfast and buffet lunch

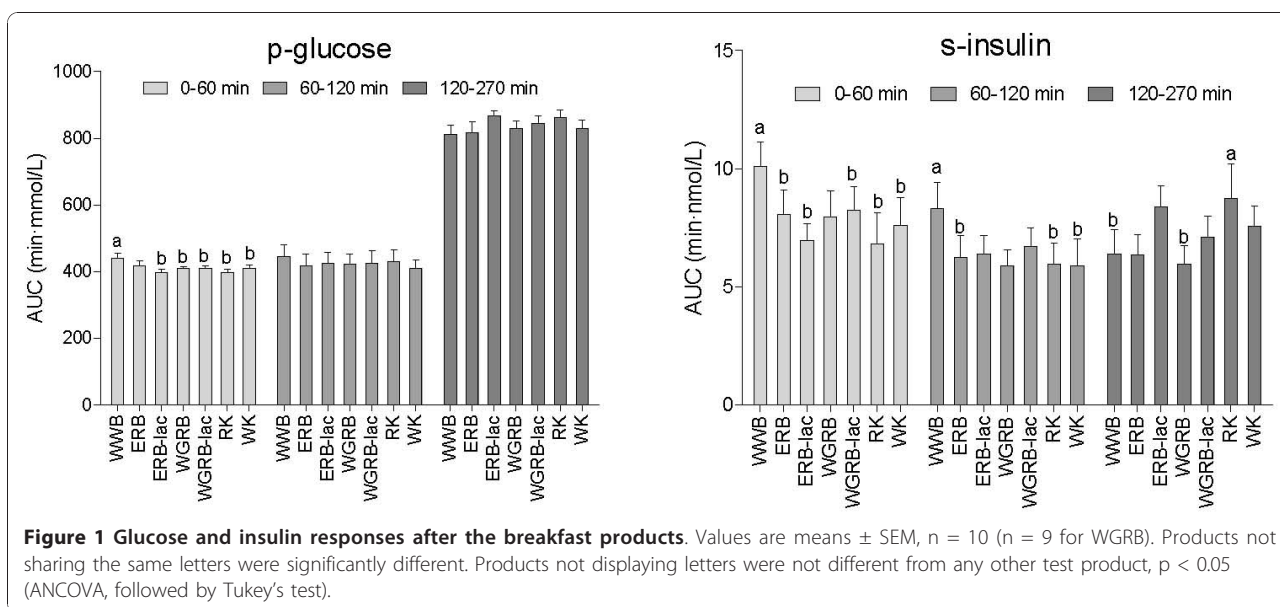
All test products induced a higher *feeling of fullness* and all but ERB-Lac, WGRB-lac and WGRB induced less *desire to eat* than WWB in the early postprandial phase after breakfast (AUC 0-60 min, Figure 3). During this phase, the RK breakfast also induced higher *feeling of fullness* than did WGRB-lac and ERB-lac.

Feeling of hunger was higher following the WWB compared to after RK, WK, ERB and WGRB in the

Table 2 Glucose and insulin responses and after the breakfast products

Meals	GI	Incremental glucose peak	GP	Incremental insulin peak	II
	%	$\Delta \text{mmol/L}$	$\text{min} \cdot \text{mmol}^{-1} \cdot \text{L}^{-1}$	$\Delta \text{nmol/L}$	%
WWB	100 ^a	3.9 ± 0.4^a	49 ± 7^b	0.250 ± 0.029^a	100 ^a
ERB	77 ± 8^{ab}	3.2 ± 0.3^{ab}	59 ± 10^{ab}	0.177 ± 0.026^b	68 ± 4^b
ERB-Lac	64 ± 9^b	2.5 ± 0.3^b	78 ± 9^{ab}	0.152 ± 0.017^b	65 ± 7^b
WGRB	79 ± 14^b	2.7 ± 0.2^b	75 ± 13^{ab}	0.180 ± 0.026^b	70 ± 5^b
WGRB-Lac	64 ± 7^b	2.6 ± 0.2^b	65 ± 9^{ab}	0.180 ± 0.025^b	75 ± 8^b
RK	73 ± 8^b	2.5 ± 0.3^b	94 ± 13^a	0.140 ± 0.027^b	60 ± 7^b
WK	68 ± 9^b	3.0 ± 0.4^b	51 ± 7^b	0.173 ± 0.029^b	63 ± 9^b

Values are means \pm SEM, $n = 10$ ($n = 9$ for WGRB). Products not sharing the same letter are significantly different, $p < 0.05$ (ANCOVA, followed by Tukey's test).



early postprandial phase (0-60 min). Furthermore, WGRB-lac and ERB-lac induced higher feeling of hunger than RK breakfast in this interval. In the later postprandial phase (120-210 min), the test subjects felt hungrier and had a larger desire to eat following the WWB and WGRB breakfasts compared to following the RK breakfast. Furthermore, ERB-lac and WK caused a larger desire to eat compared to RK in this phase. In the hour prior to lunch (210-270 min), hunger was higher following all test products but ERB and WK compared

to after the RK breakfast while desire to eat was higher for all breakfast, compared to after RK.

There were no differences in *feeling of fullness*, *hunger* or *desire to eat* after the ad lib lunch following the breakfast meals (AUC 300-390), indicating that subjects succeeded in eating to the same degree of satiation, as was intended in the experimental design. No time \times treatment effect was found for *feeling of fullness*, *feeling of hunger* or *desire to eat* in the intervals 0-270 or 300-390.

Voluntary lunch intake

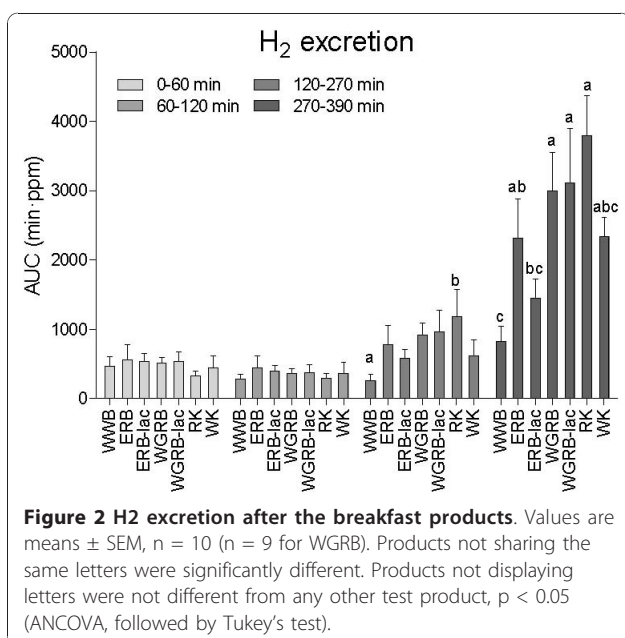
To reach the same degree of satiation, subjects decreased their energy intake (EI) at lunch after the RK breakfast compared with the corresponding intake after the WWB breakfast with 16% (Figure 3). Also the cumulative EI over the breakfast- and lunch meals was lower on the RK breakfast test day, compared to the WWB breakfast test day.

Ghrelin responses following breakfast and buffet lunch

For ghrelin, WGRB-lac induced lower ghrelin AUC in the postprandial period 120-270 min compared to WK. No differences were found between AUC following the different products, in the interval 270-390 min. No time \times treatment interaction was found in the interval 0-270 or 270-390 min. (Figure 4).

Free fatty acids

RK induced lower concentrations of FFA than WWB in the later postprandial phase (AUC 180-270 min, Figure 4). A time \times treatment interaction was found in the interval



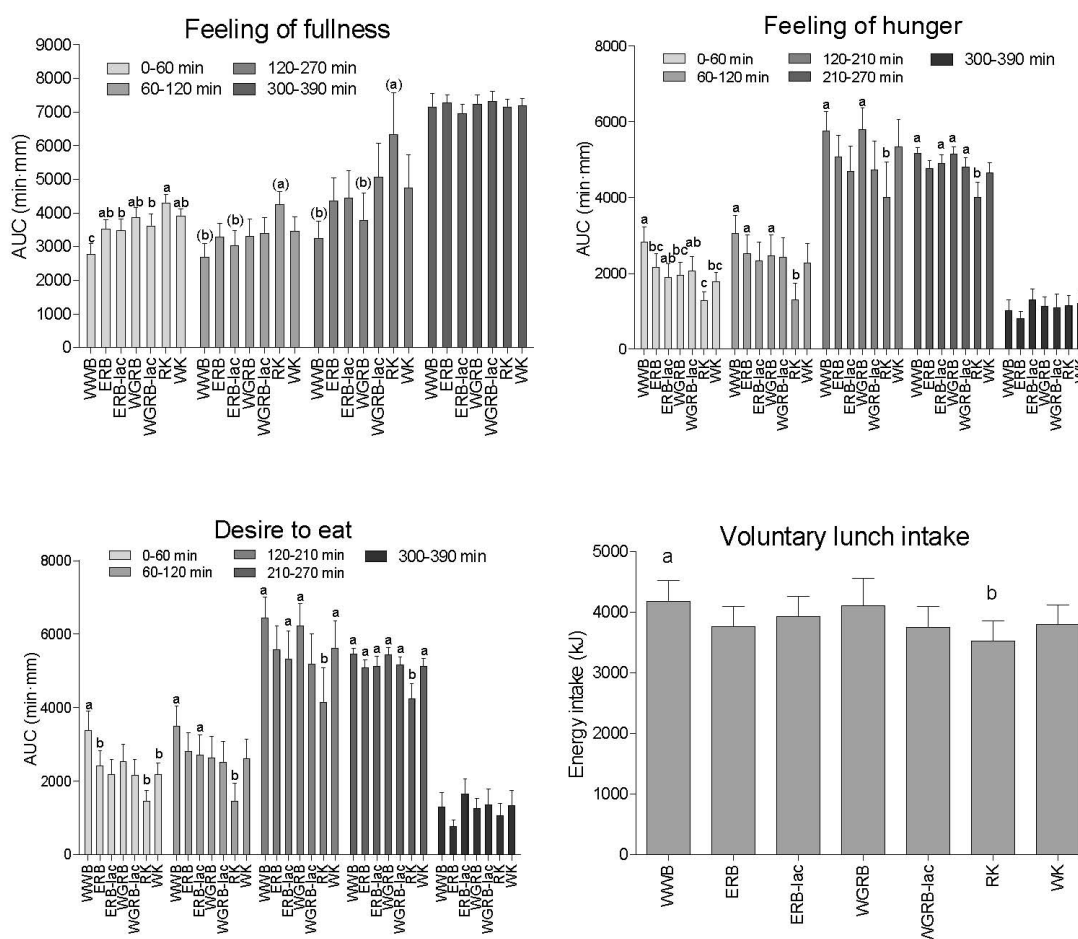


Figure 3 Subjective satiety and voluntary energy intake at lunch after the cereal test breakfasts. Values are means \pm SEM, $n = 10$ ($n = 9$ for WGRB). Products not sharing the same letters were significantly different. Products not displaying letters were not different from any other test product, $p < 0.05$ (ANCOVA, followed by Tukey's test). (.) indicates that the ANCOVA did not have normal distributed residuals, $p < 0.05$. Voluntary lunch intake was analyzed with ANOVA followed by Tukey's test.

0-270 min ($p < 0.001$) and the WWB induced higher FFA at 270 min compared to WK and RK, respectively.

Adiponectin following breakfast

For adiponectin (0-270 min), no change over time, and no time \times treatment interaction was found. Nor was there any difference in AUC 0-270 min or 180-270 min between the products (Figure 4).

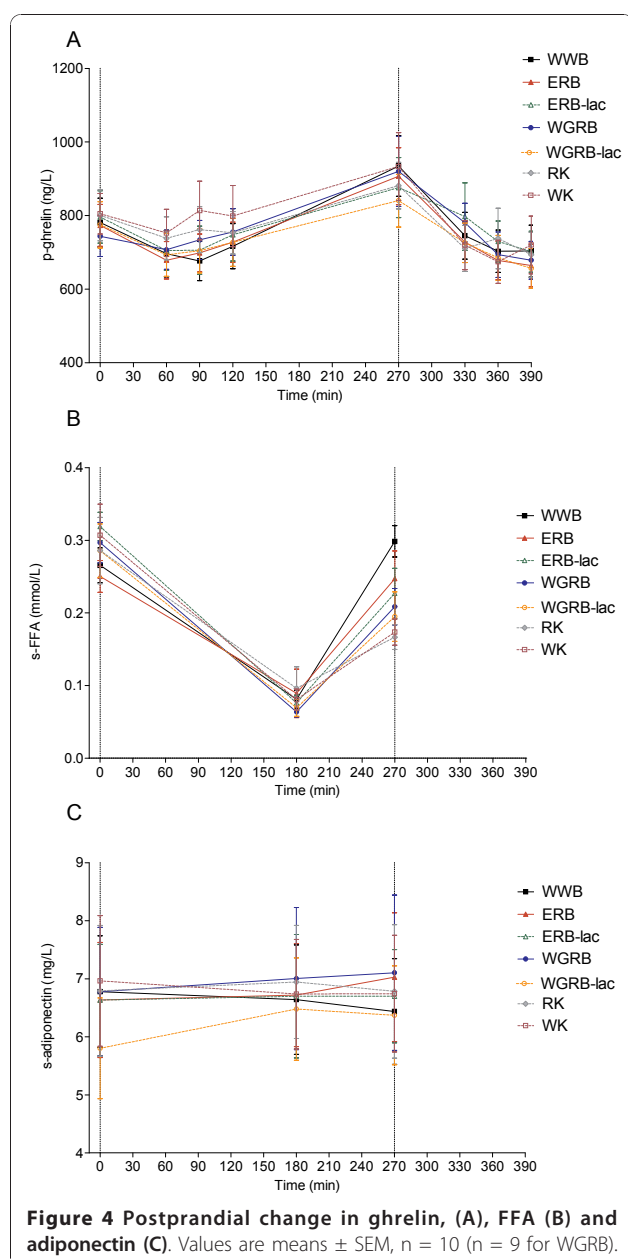
Correlations between parameters

Correlations between appetite ratings, physiological parameters and product properties are presented in Tables 3 and 4. A low II, incremental insulin peak, and GI, respectively, was related to a higher *feeling of fullness*, lower *feeling of hunger* and lower *desire to eat* the first 60 min after breakfast. A high GP was related to a lower *desire to eat*, both in the early and late postprandial phase (0-60 and 210-270 min). Low late desire to

eat (AUC 210-270 min) was also related to a lower EI at the subsequent lunch. A high GP was related to a lower concentration of ghrelin prior to lunch (270 min), in turn correlated with a lower EI. Furthermore, a higher content of indigestible carbohydrates, as well as high water content and larger portion sizes correlated with increased *feeling of fullness* and a lowered *feeling of hunger* and *desire to eat* (0-60 min) as well as lowered EI at lunch. High content of indigestible carbohydrates correlated with a high H_2 excretion (120-270 min), which, in turn, was related to a lower EI at lunch. A high H_2 excretion (90-270 min) correlated to a higher GP. GP and GI did not correlate. HI for the rye breads was not related to GI, II or GP.

Discussion

In the present study, we confirm previous findings of a low insulin response and a high Glycemic Profile



following rye products. Furthermore, we demonstrate that rye products beneficially affect both early and late appetite regulation, making them an interesting food component in weight management. The RK breakfast, in particular, improved appetite regulation and increased satiety acutely and at a subsequent meal, as judged from a lower energy intake. Consequently, compared with WWB, RK induced improved satiety ratings in the early postprandial phase after breakfast, and substantially lowered the voluntary energy intake (-16%) at a subsequent lunch. As a result of this the cumulative energy intake (breakfast + lunch) was lower on the RK day compared to the WWB test day.

Part of the early satiating effect of RK may be explained by portion size. Despite being similar to the other test products regarding energy content, the RK breakfast had the largest volume of the test products. The difference in size of the test products can be explained by the fact that RK was the test product containing the highest amount of indigestible carbohydrates (dietary fiber + resistant starch), contributing to a higher water-binding capacity during processing (cooking). Improved early (0-60 min) satiety ratings after the test meals correlated well with a high content of indigestible carbohydrates, a large portion size and a high water content in the products. The satiating effect of bulk-inducing indigestible carbohydrates and water has previously been demonstrated by Rolls et al. [32] who showed that the amount of food eaten, but not the energy content of the foods affect satiety in healthy men. Also, Geliebter demonstrated in 1988 [33] that larger distension of the stomach reduced voluntary food intake in both lean and obese subjects.

A low voluntary energy intake at lunch could be explained by increased colonic fermentation; Breath H_2 has been shown to be a sensitive indicator of increased carbohydrate fermentation in colon [34] and a high breath H_2 prior to lunch (120-270 min) was related to a following lowered voluntary energy. Rye products tended to increase breath H_2 prior to lunch, significantly so following the RK and WGRB breakfasts. After lunch, all rye products except the ERB-lac induced higher H_2 excretions than WWB. All rye products had a high content of soluble fibers, probably explaining the early fermentation. Colonic fermentation of indigestible carbohydrates yields SCFA [35], which may promote feeling of satiety through a relaxation of the gastric tone and a slower gastric motility [36]. In support of the fermentation hypothesis; Cani et al [37] has demonstrated lowered energy intake, increased GLP-1 secretion and lowered ghrelin secretion in rats fed a high fructan diet. Furthermore, Nilsson et al. [38] demonstrated that an evening meal consisting of bread made from barley kernels, rich in dietary fiber and resistant starch, increased subjective satiety and reduced gastric emptying rate at a subsequent standardized breakfast in healthy subjects. The benefits on satiety were assigned to colonic fermentation and stimulation of GLP-1. Taken together, these findings demonstrate the potential of colonic fermentation as a modulator of satiety.

Improved satiety ratings in the early postprandial phase after breakfast and a lowered voluntary energy intake at lunch were also associated with a low insulin response (II and incremental insulin peak) following the breakfast test meals. All rye products and the WK were characterized by a lower II than WWB. In a review by de Graaf et al. [39], insulin was suggested to be a poor

Table 3 Correlations between appetite ratings, physiological parameters and product properties

	<i>feeling of fullness</i> (AUC 0-60 min)	<i>feeling of hunger</i> (AUC 0-60 min)	<i>desire to eat</i> (AUC 0-60 min)	<i>desire to eat</i> (AUC 210-270 min)	Energy intake at lunch (kJ)
	<i>r</i>				
<i>Desire to eat</i> (AUC 210-270 min)	-0.17, <i>p</i> = 0.18	0.11, <i>p</i> = 0.39	0.21, <i>p</i> = 0.095		0.43, <i>p</i> < 0.001
Energy intake at lunch (kJ)	-0.25, <i>p</i> = 0.042	0.18, <i>p</i> = 0.15	0.31, <i>p</i> = 0.013	0.43, <i>p</i> < 0.001	
II	-0.36, <i>p</i> = 0.004	0.37, <i>p</i> = 0.003	0.42, <i>p</i> = 0.001	0.17, <i>p</i> = 0.18	0.30, <i>p</i> = 0.013
Incremental insulin peak	-0.38, <i>p</i> = 0.002	0.36, <i>p</i> = 0.003	0.44, <i>p</i> < 0.001	0.22, <i>p</i> = 0.082	0.40, <i>p</i> = 0.001
GI	-0.48, <i>p</i> < 0.001	0.44, <i>p</i> < 0.001	0.39, <i>p</i> = 0.002	0.04, <i>p</i> = 0.74	0.02, <i>p</i> = 0.87
GP	0.25, <i>p</i> = 0.050	-0.23, <i>p</i> = 0.067	-0.30, <i>p</i> = 0.016	-0.29, <i>p</i> = 0.020	-0.22, <i>p</i> = 0.078
Indigestible carbohydrate (g)	0.68, <i>p</i> < 0.001	-0.48, <i>p</i> < 0.001	-0.56, <i>p</i> < 0.001	-0.25, <i>p</i> = 0.045	-0.34, <i>p</i> = 0.005
Water content (g)	0.65, <i>p</i> < 0.001	-0.48, <i>p</i> < 0.001	-0.55, <i>p</i> < 0.001	-0.29, <i>p</i> = 0.017	-0.36, <i>p</i> = 0.002
Portion size (g)	0.65, <i>p</i> < 0.001	-0.48, <i>p</i> < 0.001	-0.55, <i>p</i> < 0.001	-0.29, <i>p</i> = 0.017	-0.36, <i>p</i> = 0.002
H ₂ (AUC 120-270 min)				-0.24, <i>p</i> = 0.053	-0.34, <i>p</i> = 0.005
H ₂ (AUC 270-390 min)					-0.33, <i>p</i> = 0.007
Ghrelin (270 min)				-0.04, <i>p</i> = 0.77	0.33, <i>p</i> = 0.006

Ingestible carbohydrate content consists of total fiber + resistant starch in breakfast products. Spearman's partial correlation coefficients controlling for subjects and corresponding baseline values (two-tailed test). Significant correlations are shown in bold text.

biomarker of satiety, since it is confounded or moderated by several metabolic processes such as blood glucose and incretins. However, the current finding that a low postprandial insulin response correlates with improved satiety ratings and a lowered energy intake at a following meal is supported by several studies [18,21-25,40,41], with equi-carbohydrate portions and similar macronutrient compositions in the test foods; that is, at conditions comparable to those in the present study. Possibly, it is the absorption characteristics of the carbohydrates rather than insulin concentrations *per se* that affect satiety. A slow uptake of carbohydrates would lead to a prolonged

exposure of the small intestine to nutrients, thus extending the release of satiety peptides, e.g. GLP-1 [42-44]. Intake of food products containing slowly absorbable carbohydrates will require smaller amounts of insulin for the glucose uptake, thus limiting the risk of reactive hypoglycemia in the later postprandial phase. We have recently demonstrated that a product characterized by a low but prolonged blood glucose curve, described by a high Glycemic Profile (GP) was associated with lower insulin response, less postprandial hypoglycemia, and a smaller increase in late postprandial ghrelin [18]. In the present work, a high GP after a meal appears to positively affect appetite regulation, partly by reduced secretion of ghrelin. A high GP was associated with both a low postprandial concentration of ghrelin at 270 min, and a lower *desire to eat* both in the early and late postprandial phase (AUC 0-60 and 210-270 min). Furthermore, a lower concentration of ghrelin and a lower *Desire to eat* was related to a lower voluntary energy intake.

Rye products were characterized by higher GP than wheat products. Looking at specific rye products, RK induced significantly higher GP than WWB and WK. This is in line with our previous findings [18]. In the present work, the GP of the products were inversely related to the insulin response (II and incremental peak) but not to the GI. A prolonged blood glucose curve seen with high GP rye products could, besides a lowered insulin response, be a result of improved glucose tolerance induced by colonic fermentation [45]. Colonic

Table 4 Correlations between physiological parameters and certain product properties

	<i>r</i>	<i>p</i>
GP vs. II	-0.39	0.001
GP vs. Incremental insulin peak	-0.43	>0.001
GP vs. GI	-0.22	0.072
GP vs. Ghrelin (270 min)	-0.29	0.018
Indigestible carbohydrate (g). vs. H ₂ (AUC 120-270) min	0.46	>0.001
Indigestible carbohydrate (g). vs. H ₂ (AUC 270-390 min)	0.70	>0.001
GP vs. H ₂ (AUC 90-270) min	0.29	0.018
GP vs. HI (rye breads)	-0.11	0.50
GI vs. HI (rye breads)	0.121	0.48
II vs. HI (rye breads)	-0.10	0.56

Ingestible carbohydrate content consists of total fiber + resistant starch in breakfast products. Spearman's partial correlation coefficients controlling for subjects and corresponding baseline values (two-tailed test). Significant correlations are shown in bold text.

fermentation has recently been demonstrated to increase peripheral insulin sensitivity, 10 h after the test meal [46]. In the present study, a high breath hydrogen excretion was detected already in the 90-270 min phase after the test breakfast, and was found to correlate to a high GP. Additionally, the lowered blood glucose incremental peak seen with the high GP rye products could be explained by bioactive components, and by a lowered starch hydrolysis. Although no direct correlations between the GP, GI or II of the rye breads and the rate of in vitro starch hydrolysis (HI) could be found, the HI was lowered for the rye products compared to WWB. Obstructed amylolysis could therefore partly contribute to the low glucose incremental peaks and insulin responses of most rye products in the study. That the GP of RK was higher than that of WK suggests a facilitated glucose regulation following the RK breakfast. Interestingly, RK and WK induced similar GI values which indicate the importance of GP as a complement to GI, where the latter does not provide information about the course of glycemia.

Conclusions

Rye products, especially in the form of whole kernels, decrease both early and late appetite ratings after a breakfast meal, and lowers energy intake at a subsequent voluntary lunch. Our results suggest that a high content of indigestible carbohydrates and soluble fibers in the rye products may beneficially affect acute satiety through a bulking effect, and second meal satiety through a mechanism related to colonic fermentation by production of fermentation metabolites. Colonic fermentation might also contribute to an improved late glucose regulation. Indications of such a hypothesis stems from the correlation between a high breath hydrogen excretion and a high GP. A high GP was also related to lowered insulin response, late postprandial ghrelin secretion and desire to eat, thereby affecting a subsequent *ad libitum* meal. These findings indicate that the GP represent a nutritionally interesting entity in that it predicts metabolic responses as well as the satiating properties better than does the GI. This work provides information of a potential role in weight management for rye products. A high intake of rye products could contribute to a lowered energy intake, and thus protect against obesity. To evaluate this hypothesis, longer-term studies of rye products on metabolism and appetite regulation are needed.

List of abbreviations

AUC: total area under the curve; BMI, body mass index; CVD: cardiovascular diseases; EI: voluntary energy intake at lunch; ERB-lac: endosperm rye bread made with lactic acid; ERB: endosperm rye bread; FFA: free fatty acids; GI: glycemic index; GP: Glycemic profile; H₂: breath hydrogen; HI: hydrolysis

index; II: insulinemic index; RK: Rye kernels; T2D: type 2 diabetes; WGRB: whole grain rye bread; WGRB-lac: whole grain rye bread made with lactic acid; WK: wheat kernels; WWB: white (endosperm) wheat bread.

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Authors' contributions

LAHR coordinated the study and was responsible for the study design, the collection and analysis of the data, statistical analysis and for writing the paper. EMÖ was involved in the study design, interpretation of data and in writing the paper. IMEB was the guarantor and was involved in the study design, interpretation of data and writing of the paper. All authors have read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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