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On the importance of viscous dietary fibre for acute and semi-acute glucose tolerance and appetite

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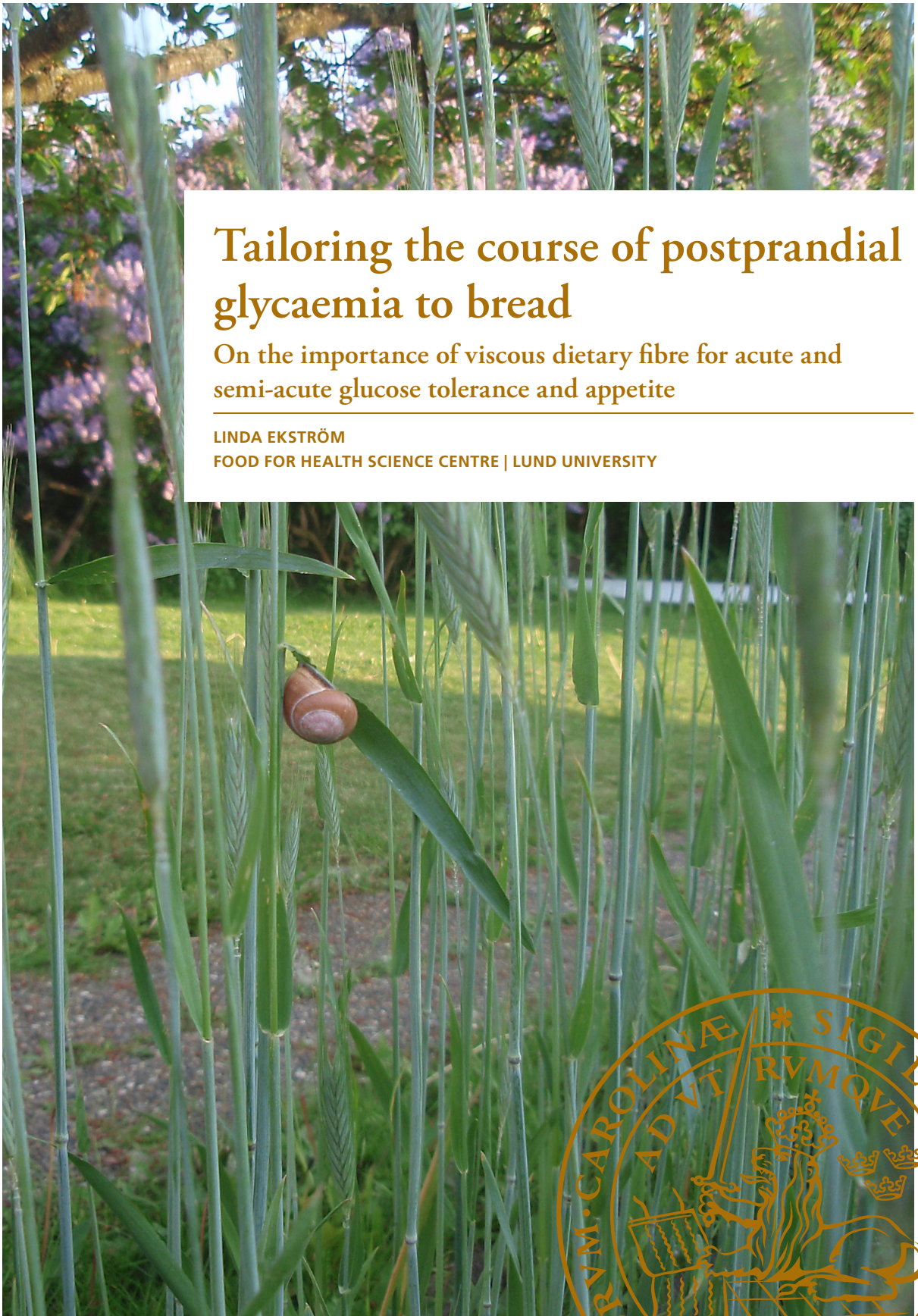

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On the importance of viscous dietary fibre for acute and semi-acute glucose tolerance and appetite

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FOOD FOR HEALTH SCIENCE CENTRE | LUND UNIVERSITY

Tailoring the course of postprandial glycaemia to bread

On the importance of viscous dietary fibre for acute and semi-acute glucose tolerance and appetite

Linda Ekström

LUNDS
UNIVERSITET

DOCTORAL DISSERTATION
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Faculty opponent

Professor Inga Thorsdottir, School of Health Sciences, University of Iceland, Reykjavik, Iceland

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Title and subtitle Tailoring the course of postprandial glycaemia to bread - On the importance of viscous dietary fibre for acute and semi-acute glucose tolerance and appetite

The prevalence of metabolic diseases such as type 2 diabetes mellitus (T2DM) is rapidly increasing all over the world. Frequent episodes of elevated postprandial blood glucose have been associated with oxidative stress and subclinical inflammation, and the importance of a tight glycaemic control has been identified as an important factor to maintain health and prevent T2DM, obesity and cardiovascular disease (CVD).

The aim was to investigate possibilities to tailor the course of postprandial glycaemia to bread in healthy adults in favour of reduced glycaemic index (GI) and increased glycaemic profile (GP) by inclusion of guar gum or β-glucans. GP is defined as the duration of the glucose curve above the fasting concentration divided by the incremental glucose peak. Effects on second meal glucose tolerance and appetite were also investigated. Furthermore, the potential use of in vitro measurements of starch hydrolysis rate (HI) and fluidity (FI) to predict course of postprandial glycaemia (GI and/or GP) was evaluated.

In paper I, white wheat-based bread was supplemented with whole grain maize flour and different types and amounts of guar gum. Supplementation with medium weight guar gum (mwGG) resulted in lower postprandial glycaemia and insulinaemia and improved acute appetite compared to the white wheat reference bread (WWB).

In paper II, three commercially available β-glucans from barley and oats were baked into yeast leavened bread products. Even a low level of high molecular weight (MW) β-glucan elicited a lowering effect on postprandial glycaemia, indicating that the β-glucan quality is of importance.

In paper III, mwGG and whole grain rye flour or high amylose maize starch (HAM) were combined in an effort to design bread products in favour of low but sustained glycaemia. The combination of mwGG and rye was superior, with improvements in subjective appetite. Additionally both mwGG in combination with whole grain rye flour and HAM led to improvements in biomarkers of appetite compared to the WWB.

In paper IV, pasta or WWB were provided for breakfast and a standardised lunch meal was given 4 h later. The pasta breakfast resulted in reduced glycaemic excursions, both acute and after a second meal, which demonstrates the importance of considering not only the ingredients but also the food processing conditions.

An indexed glycaemic profile (GPI) was introduced, allowing comparisons between studies. GPI was defined as GP for WWB divided by the GP for the test product taken by the same subject, multiplied by 100 and then presented as a mean of all individual values. GPI was better correlated to subjective appetite ratings compared to both GP and GI for the present studies.

Both the measures of HI and FI were related to GI, GPI, glucose iPeak, II and insulin iPeak (Spearman's partial correlation, papers I-III). HI seems to better predict the glycaemic response, defined as GI or GPI, compared to FI.

For the future, the importance of the course of glycaemia for long-term metabolic outcome should be evaluated, also including effects on weight regulation.

Key words: Postprandial glycaemia, appetite, prevention, antidiabetic food, inflammation, GI, glycaemic profile

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Contents

Abstract

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The aim was to investigate possibilities to tailor the course of postprandial glycaemia to bread in healthy adults in favour of reduced glycaemic index (GI) and increased glycaemic profile (GP) by inclusion of guar gum or β-glucans. GP is defined as the duration of the glucose curve above the fasting concentration divided by the incremental glucose peak. Effects on second meal glucose tolerance and appetite were also investigated. Furthermore, the potential use of in vitro measurements of starch hydrolysis rate (HI) and fluidity (FI) to predict course of postprandial glycaemia (GI and/or GP) was evaluated.

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For the future, the importance of the course of glycaemia for long-term metabolic outcome should be evaluated, also including effects on weight regulation.

Populärvetenskaplig sammanfattning

I detta arbete har olika aspekter kring brödprodukter som resulterar i ett långsamt blodsockersvar undersökts. Brödprodukterna har studerats i måltidsstudier, där friska frivilliga försökspersoner har fått äta en frukost bestående av det speciella brödet och vatten. Försökspersonerna har fått lämna blodprover vid ett antal tillfällen före och efter intaget av måltiden och bland annat blodsocker, insulin och upplevd aptit har mätts.

Antalet som drabbas av välfärdssjukdomar som typ 2 diabetes och hjärt-kärlsjukdomar ökar kraftigt. Metabola syndromet är ett förstadium till dessa sjukdomar och består av flera olika metabola störningar, t ex bukfetma, blodfettsrubbningar, högt blodtryck och förhöjt blodsocker. Metabola syndromet kan förebyggas genom lämplig kost och motion, t ex har mat som ger upphov till små svängningar i blodsockernivån visats vara skyddande. Glykemiskt index, eller GI, är ett begrepp som används för att rangordna kolhydratrika livsmedel efter hur snabbt kolhydratkomponenten tas upp från tarmen och bidrar till blodsockerhöjning efter måltid.

De testade brödprodukterna innehöll två olika typer av viskösa kostfibrer, guargummi och beta-glukaner, och en speciell typ av stärkelse från majs. Dessa ingredienser gjorde att bröden orsakade låga och välreglerade glukos- och insulinsvar hos försökspersonerna och blodsockerstigningen var lägre jämfört med efter ett referensbröd bakat av vitt vetemjöl. Dessutom höll sig blodsockervärdet över ingångsvärdet betydligt längre jämfört med referensbrödet. Det låga GI-värdet hängde samman med lägre upplevd hunger. Bröd bakat med guargummi och fullkornsråg minskade de upplevda hungerkänslorna och hade positiva effekter på PYY och ghrelin, vilka är två av de hormoner som styr aptitregleringen.

I en av delstudierna fick försökspersonerna äta antingen pasta eller vitt bröd till frukost. Pasta och bröd görs av samma råvaror men då pastan kavlas ut till en deg pressas stärkelse och proteinmolekyler ihop så att interaktioner bildas mellan dessa. Dessa krafter gör så att vår kropp inte kan bryta ner stärkelsen i pastan lika effektivt som i brödet. Fyra timmar efter respektive frukost fick försökspersonerna äta en och samma lunch, känd för att ge ett högt blodsockersvar. Efter pastafrukosten var blodsockersvarets ökning efter lunch hela 47 % lägre jämfört med den gång då de ätit det vita brödet till frukost.

Effekten av viskösa kostfibrer på blodsockersvaret är ett resultat av den viskositet som utvecklas under nedbrytningen i mag/tarmkanalen. Den högre viskositeten resulterar i att magsäcken töms långsammare och att näringsupptaget i tunntarmen blir fördröjt, jämfört med då inga viskösa fibrer ätits. I arbetet undersöktes också hur väl resultatet från två olika labb-metoder stämde överens med blodsocker-resultaten i måltidsstudierna. De två metoderna mäter flytbarheten respektive stärkelsens nedbrytningshastighet i brödprover som behandlats på ett sätt som efterliknar mag/tarmkanalen. Att mäta nedbrytningshastigheten verkar vara mer användbart än att mäta flytbarheten, då den förra gäller för en större mängd brödprodukter. Att enkelt kunna förutse om ett bröd kan ha effekt på blodsockernivån efter intag skulle underlätta processen att ta fram nya produkter.

Normalt äter vi flera gånger om dagen, varje dag, hela livet. Om vi kan underlätta kroppens arbete lite grann vid varje måltid minskar vi på sikt risken att drabbas av vår tids folksjukdomar. En förutsättning för att kunna göra detta är att den typen av produkter finns tillgängliga där vi handlar vår mat. Denna avhandling leder till att vi bättre förstår hur lösliga kostfibrer och val av stärkelse påverkar olika parametrar i kroppen och det är kunskap som kan användas till att öka antalet välsmakande livsmedel som ger konsumenten balanserat blodsocker.

List of papers

The thesis is based on the following papers, which will be referred to in the text using the Roman numerals given below.

Paper I — On the possibility to affect the course of glycaemia, insulinaemia, and perceived hunger/satiety to bread meals in healthy volunteers

L.M.N.K. Ekström, I.M.E. Björck, E.M. Östman

Food & Function 2013 4:4

Paper II — Oat β-glucan containing bread increases the glycaemic profile

Linda M.N.K. Ekström, Emma A.E. Henningsson Bok, Malin E. Sjöö, Elin M. Östman

Manuscript accepted for publication in Journal of Functional Foods

Paper III — An improved course of glycaemia after a bread-based breakfast is associated with beneficial effects on acute and semi-acute markers of appetite

L.M.N.K. Ekström, I.M.E. Björck, E.M. Östman

Food & Function 2016 7:2

Paper IV — Sustained glycaemia at breakfast improve glucose tolerance at a high-carbohydrate lunch

L.M.N.K. Ekström, I.M.E. Björck, E.M. Östman

Submitted Short Communication (European Journal of Nutrition, 31-08-2016)

The author's contributions

Paper I

The author, L. Ekström, was involved in the study design, developed and characterised the test products, coordinated the study, was responsible for sampling and analysis of blood glucose, serum insulin and subjective appetite ratings, evaluated the results and was responsible for writing the manuscript.

Paper II

The author, L. Ekström, was involved in the study design, development and characterisation of the test products and coordinated the study together with Emma Henningsson Bok. Ekström was responsible for the blood sampling, evaluated the results and was responsible for writing the manuscript.

Paper III

The author, L. Ekström, was responsible for the study design, developed and characterised the test products, coordinated the study, was responsible for the blood sampling, analysis of blood parameters and subjective appetite ratings, evaluated the results and was responsible for writing the manuscript.

Paper IV

The author, L. Ekström, was responsible for the study design, characterised the test products, coordinated the study, was responsible for and performed the blood sampling, preparation of breakfast and lunch meals, analysis of blood glucose, serum insulin, NEFA and TG as well as subjective appetite ratings, evaluated the results and was responsible for writing the manuscript.

Abbreviations

BMI – body mass index

CCK – cholecystokinin

CNS – central nervous system

CVD – cardiovascular disease

DF – dietary fibre

dwb – dry weight basis

EFSA – European Food Safety Authority

FI – fluidity index

GER – gastric emptying rate

GG – guar gum

GI – glycaemic index

GIP – glucose-dependent insulinotropic polypeptide

GLP-1 – glucagon-like-peptide-1

GLP-2 – glucagon-like-peptide-2

GP – glycaemic profile

H_2 – hydrogen gas

HAM – high amylose maize starch

HbA_{1c} – glycated haemoglobin

HDL – high-density lipoprotein

hGG – hydrolysed guar gum

HI – hydrolysis index

IFG – impaired fasting glucose

IGF – impaired glucose tolerance

IL-6 – interleukin 6

lwGG – low weight guar gum

MetS – metabolic syndrome

MW – molecular weight

mwGG – medium weight guar gum

NEFA – non-esterified fatty acids

OXM – oxyntomodulin

PHGG – partially hydrolysed guar gum

PP – pancreatic polypeptide

PYY – peptide YY

RDS – rapidly digestibly starch

RS – resistant starch

SCFA – short chain fatty acids

SDS – slowly digestibly starch

TG – triglycerides

TNF-α – tumour necrosis factor alpha

T2DM – type 2 diabetes mellitus

VAS – visual analogue scale

wgHiM – whole grain source of high amylose maize starch

ww – wet weight

WWB – white wheat bread

Background

Metabolic diseases – genesis, prevalence and prevention

T2DM is a progressive, metabolic disease characterised by multiple pathophysiological disturbances leading to chronic hyperglycaemia (Ferrannini & DeFronzo 2015). Diabetes-related complications affect different parts of the body such as eyes, brain, heart, kidney, nerves and limbs (Thondre 2013). Macrovascular complications, such as myocardial infarction and stroke, account for 80% of all deaths in T2DM patients (Ferrannini & DeFronzo 2015). Importantly, diabetic complications increase the healthcare costs by 250% compared to those of patients without complications (Liebl *et al* 2015).

The mean global prevalence of type 2 diabetes mellitus (T2DM) in 2010 was estimated to 6.4% and, in developing countries, a 69% increase is expected for the next 20 years. In the US, the estimated prevalence of T2DM among adults is 14%, and the prevalence of prediabetes is 38% (Menke *et al* 2015). In 2007, 20% of healthcare cost in the US was spent on care for diabetes patients (Leena & Jill 2010), and the societal burden for treatment of disorders related to the metabolic syndrome (MetS) is increasing continuously.

Obesity is the most important risk factor for developing T2DM and cardiovascular disease (CVD) and its prevalence has increased dramatically in the last decades (Blaak *et al* 2012). Socioeconomic factors are also associated with the risk of developing T2DM. In Rosengård, a low-income neighbourhood in Malmö, Sweden, as many as 45% of all subjects tested (n = 151) had impaired fasting glucose (IFG), impaired glucose tolerance (IGT) or T2DM (Bennet *et al* 2011). Furthermore, the official prevalence of T2DM in Sweden is 4%, but in the Rosengård cohort the prevalence was 21%, whereof 14% were already known and the rest detected at an oral glucose tolerance test.

Frequent postprandial episodes of elevated blood glucose have been associated with oxidative stress and subclinical inflammation, factors that increase the risk of developing T2DM as well as CVD (Blaak *et al* 2012). The extent of chronic hyperglycaemia, often measured as glycated haemoglobin (HbA_{1c}), is directly related to the risk of developing microvascular T2DM complications (Ferrannini & DeFronzo 2015). For macrovascular complications, postprandial glucose response is a better predictor of increased risk than fasting blood glucose (Decode

Study Group 2001), and it seems that the risk increase starts already within the normal blood glucose range (Ferrannini & DeFronzo 2015).

The progression from a healthy state to T2DM is a dynamic process that goes via prediabetes, see Table 1. Prediabetes is defined as IFG, IGT or elevated HbA_{1c} and is a result of impaired insulin sensitivity leading to hyperinsulinaemia (Kanat *et al* 2015). As β-cells fail to compensate for the elevated need of insulin, IFG and/or IGT and eventually T2DM develop. Interventions postponing or hindering β-cell failure is thus valuable in preventing individuals with prediabetes from entering the diabetic state. For individuals affected by T2DM, lifelong treatment is necessary. Treatment may include alterations in diet, increased physical activity, medication and blood glucose monitoring. Furthermore, the patient has to cope with the risk of severe complications (Karlsen *et al* 2012). Thus, it is important to identify individuals at high risk of T2DM in order to target them with preventive actions (Leena & Jill 2010).

Table 1
Diagnosis criteria for diabetes and prediabetes (Goldenberg & Punthakee 2013).

Category		**Fasting p-glucose**		**2 h p-glucose (capillary[b] blood)**		**HbA_{1c}[c]**
		(mmol/l)		(mmol/l)		(mmol/mol)
Healthy		< 6.1		< 7.8 (8.9)	or	< 42
Prediabetes	Impaired fasting glucose (IFG)	6.1 - 6.9	and	< 7.8 (8.9)	or	42 - 47
	Impaired glucose tolerance (IGT)	< 7.0	and	7.8 - 11.0 (8.9 – 12.1)	or	42 - 47
T2DM[a]		≥ 7.0	or	≥ 11.1 (12.1)	or	≥ 48

[a]The diagnosis T2DM is set after two readings in the T2DM range (on different days). HbA_{1c} should not be used for diagnosis in children, adolescents or during pregnancy.

[b]Capillary and venous sampling gives the same result in fasting state. However, in the postprandial state, capillary sampling result in somewhat higher values compared to venous sampling (Forster *et al* 1972).

[c]According to the method by International Federation of Clinical Chemistry (IFCC).

The individual risk of development of T2DM and CVD can be estimated from the occurrence of a set of risk factors, clustered in the MetS. The risk is present when 3 of the following 5 criteria are fulfilled (Lam & LeRoith 2015):

- enlarged waist circumference (population and country-specific ranges)
- elevated triglycerides (TG)

- decreased high-density lipoprotein (HDL)-cholesterol
- elevated blood pressure
- elevated fasting glucose

Drug treatment for any of the four latter parameters is an alternative indicator. In the definition of MetS, waist circumference is used as an indicator of obesity as it has shown good correlation to visceral adiposity, insulin resistance and development of T2DM and CVD (Lam & LeRoith 2015). Sub-clinical inflammation is increasingly recognised to have a role in the pathogenesis of MetS and its subsequent disorders such as T2DM and CVD (Ceriello 2000, Esser *et al*).

The potential of early prevention

It has been shown that lifestyle modifications are the most effective way to prevent or delay the onset of T2DM (Leena & Jill 2010). If overweight is prevalent, even a modest weight loss can reduce the risk. Also, moderate physical activity for at least 150 min per week reduces the risk, even if not leading to weight loss. Avoidance of prolonged sedentary behaviour such as sitting or lying down while *e.g.* working, driving, reading, playing games or watching TV, not smoking and moderate alcohol consumption are other favourable factors (Ardisson Korat *et al* 2014). The preventive value is better the earlier it starts and, ideally, prevention should start while healthy, to avoid the development even of the prediabetic state (Neumann *et al* 2014). A recent Swedish study showed that people already in the prediabetic state reported a lower health-related quality of life compared to healthy individuals (Neumann *et al* 2014).

The use of antidiabetic drugs, *e.g.* metformin and acarbose, has a preventive potential (Leena & Jill 2010). Metformin affects the hepatic glucose output and thus reduces the overall blood sugar level. Acarbose is an α-glucosidase inhibitor that reduces the activity of the brush border enzymes in the small intestine. This will delay the intestinal glucose absorption and lower the postprandial insulin response (Rudovich *et al* 2011). The use of acarbose in patients with IGT has been demonstrated to reduce the risk of progression to T2DM by 25% over 3.3 years, and the preventive effect was associated with a decreased postprandial rise in glucose after carbohydrate-rich meals, leading to less toxic effects of glucose (Chiasson *et al* 2002).

Besides the effect of entire diets on body weight (Ardisson Korat *et al* 2014), different food components such as dietary fibre (DF), whole grains, monounsaturated and n-6 polyunsaturated fatty acids have been associated with lowered risk of T2DM, (Thomas & Pfeiffer 2012). A recent study demonstrated that the intake of a multifunctional diet for 8 weeks improved several cardiometabolic risk factors in overweight or obese subjects (Tovar *et al* 2015).

The multifunctional diet, including functional components such as soybean, viscous fibres, long chain n-3 fatty acids, plant stanols, cinnamon, blueberries, vinegar and whey protein, was compared with a control diet low in the functional components. Both diets were designed in agreement with the Nordic Nutrition Recommendations. However, in the multifunctional diet prototype products known to lower postprandial glycaemia were included along with anti-inflammatory food factors. Hence, the multifunctional diet showed a remarkable reduction of several acknowledged risk factors in the MetS including inflammation, LDL-cholesterol and TG. A general CVD risk predictor, the Reynolds risk score, was reduced by 36% compared to the control diet, independent of changes in body weight. The results thus strengthen the importance of diet in preventive strategies and of quality characteristics of foods. Accordingly, an expert panel agreed that dietary approaches to lower postprandial glycaemia are of importance in reducing the risk of major chronic diseases and their related risk factors (Augustin *et al* 2015). Furthermore, Ardisson Korat *et al* (2014) stated that proper lifestyle modifications have the potential to prevent more than 90% of T2DM cases.

Glucose and appetite regulation

An optimal blood glucose level is crucial for normal functions in many cell types (Wasserman 2009). The blood glucose level is, therefore, tightly regulated by sophisticated mechanisms that remove or release glucose to the bloodstream when needed. To sustain metabolism in different tissues, *e.g.* the brain, glucose is constantly extracted from the blood. This is compensated for through hepatic glucose output (glycogenolysis and gluconeogenesis). After intake of a carbohydrate-rich meal the blood glucose concentration rises and insulin is released from the β-cells of the pancreas. The released insulin stimulates uptake of glucose from the blood to muscle, liver and adipose tissue and suppresses the hepatic output of glucose, resulting in overall lowered glycaemia. Hypoglycaemia, however, stimulates excretion of glucagon, the counter regulatory hormone that leads to increased blood sugar levels through breakdown of glycogen in liver and muscles as well as breakdown of muscles and adipose tissue if no glycogen is available.

The glucose-raising potential of carbohydrate containing foods varies substantially. This was described in 1981 when the glycaemic index (GI) was introduced as a way to describe the effect on blood glucose after intake of different carbohydrate-rich foods (Jenkins *et al* 1981). The GI is defined as the incremental area under the 2 h blood glucose curve after a test product, expressed as the percentage of the corresponding area after a reference product. The test product

and reference product should contain the same amount of available carbohydrates and be taken by at least 10 healthy subjects in a cross-over design under standardised conditions (Brouns *et al* 2005). Several studies have demonstrated an association between a high GI diet and increased risk of developing T2DM (Augustin *et al* 2015). A hypothetical model of the relation between high GI diets and the risk for T2DM is demonstrated in Fig. 1.

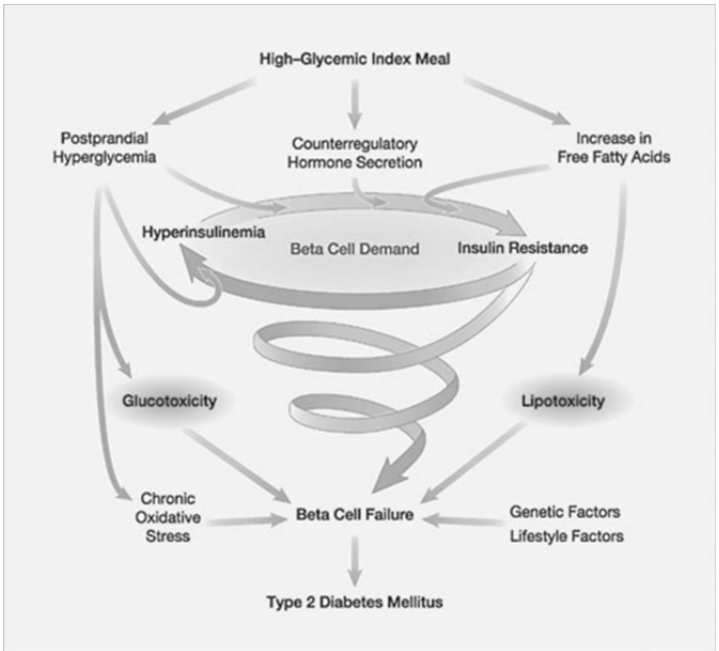

Figure 1
Hypothetical model of relation between high GI diet and the risk for T2DM (Ludwig 2002). Reprinted by permission from JAMA.

The postprandial state

After ingestion of carbohydrate-containing foods, plasma glucose rises quickly and insulin sensitive tissues will thus take up glucose and non-esterified fatty acids (NEFA), stimulate glycogenesis and lipogenesis, and suppress gluconeogenesis and lipolysis. If hyperglycaemia occurs during the first 2h, insulin can be elevated still after 2–4 h. This may result in a continuous fall in blood glucose and NEFA, resulting in hypoglycaemia. The latter stimulates excretion of counter regulatory stress hormones (glucagon, cortisol, catecholamines and growth hormones), which restore glycaemia through glycogenolytic and gluconeogenic pathways (Ludwig *et al* 1999). As insulin levels drop, circulating levels of NEFA will also return to fasting level or above (Jelic *et al* 2009). Nutrient absorption continues as long as

nutrients are present in the intestines, normally 2–4 h after meal. If slowly digestible starch (SDS) is ingested, the duration of the absorption will be prolonged compared to rapidly digested starch (RDS).

Epidemiologic studies have demonstrated that a higher intake of DF and whole grains is associated with lower weight gain compared to a lower DF intake, indicating that these components could affect appetite and/or food intake. Furthermore, several acute meal studies have reported decreased hunger, increased satiety and decreased voluntary food intake at a subsequent meal after intake of food items resulting in low postprandial blood glucose excursions (Augustin *et al* 2015). Hypoglycaemia, *per se*, is a signal of hunger and a rapid fall in blood glucose triggers a rapid return of hunger (Pawlak *et al* 2002).

It has been shown that a progressive increase in NEFA causes a dose-dependent inhibition of insulin-stimulated glucose disposal and insulin signalling in skeletal muscles (Belfort *et al* 2005). Thus, the lipid metabolism and plasma levels of NEFA have an important role in muscle glucose homeostasis. Increased levels of the counter-regulatory hormones released during hypoglycaemia as well as elevated NEFA-levels have been associated with increased risk of insulin resistance (Blaak *et al* 2012).

Elevated postprandial hyperglycaemia has been shown to promote sub-clinical (low grade) inflammation, leading to increased production of cytokines (*e.g.* interleukin 6 (IL-6) and tumour necrosis factor alpha (TNF-α)), which creates oxidative stress and endothelial dysfunction. Consequently, increased systemic inflammation has turned out to be an import risk factor in the development of T2DM and CVD (Galland 2010). Even within the normal range, elevated glucose levels have been shown to induce oxidative stress and inflammation as measured by IL-6, TNF-α and IL-8 in glucose-tolerant subjects (Blaak *et al* 2012). Furthermore, it has been demonstrated that a high GI bread meal activates the inflammatory marker nuclear factor-κB three-fold compared with a low GI pasta meal in healthy young subjects (Dickinson *et al* 2008). In healthy subjects, these inflammatory processes are normalised within 2–3 h but, in obese subjects with IGT and in T2DM, glucose-induced inflammatory response is stronger and lasts longer (Esposito *et al* 2003). Giacco *et al* (2015) recently demonstrated that 4–6 h of hyperglycaemia in T2DM patients caused persistent mitochondrial overproduction of reactive oxygen species for days after the glucose levels were normalised. Furthermore, there are indications that oxidative stress is increased as the glucose variability from peak to nadir is increased (Blaak *et al* 2012). In line with this, reduced glycaemic excursions offered relief for the β-cells and are thus proposed as an effective strategy to preserve or recover their function (Malin *et al* 2014).

Another important aspect in relation to prevention of metabolic disorders is appetite regulation. The appetite sensations hunger, satiation and satiety regulate

food intake and they are created from complex interactions between the central nervous system (CNS) and peripheral sensations. The main origins of the latter are the gastrointestinal tract, liver and adipose tissues (Janssen *et al* 2011). The measurement of appetite sensations after a test meal could be useful to understand their satiating potential as well as to predict future energy intake (Drapeau *et al* 2007). As a means of measuring perceived satiety, the use of visual analogue scales (VAS) has shown to be reproducible (Flint *et al* 2000, Stubbs *et al* 2000). Furthermore, biomarkers of satiation and satiety have also been proposed as tools to evaluate the effect on appetite elicited by different food items (de Graaf *et al* 2004).

Two of these proposed biomarkers are the incretin hormones glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1), which are secreted from the gastrointestinal tract in response to nutrient ingestion. GIP is primarily released from K-cells in the duodenum and GLP-1 is primarily released from L-cells in the ileum. Both of them enhance the glucose-dependent insulin release from the pancreatic β-cells (Phillips & Prins 2011) and GLP-1 has been associated with reduced food intake and increased satiation, whereas GIP has been associated with increased satiety (de Graaf *et al* 2004). Lipids and carbohydrates are the most potent macronutrients stimulating incretin release, and the secretion is elevated just minutes after ingestion. Incretins also modulate the postprandial glucose handling. Whereas GLP-1 inhibits glucagon secretion and delays the gastric emptying rate (GER), both GIP and GLP-1 are reported to increase peripheral insulin sensitivity (Baggio & Drucker 2007). Furthermore, the ingestion of nutrients affects the release of other appetite-regulating gut peptides such as ghrelin, cholecystokinin (CCK) and peptide tyrosine tyrosine (PYY). Ghrelin is primarily secreted in the stomach and duodenum and seems to affect meal initiation and increase food intake (de Graaf *et al* 2004). CCK is released in the duodenum in response to presence of fat and protein. It delays GER, inhibits or reduce food intake and suppresses appetite and has thus been suggested as a biomarker of satiation (de Graaf *et al* 2004). PYY is released primarily in the colon and inhibits the release of neuropeptide Y, which is the most potent CNS stimulant of appetite.

Second-meal glucose tolerance

The glucose tolerance to a meal is not only affected by the meal itself, but also by previous food intake (Chowdhury *et al* 2015, Jenkins *et al* 1982, Staub 1921, Traugott 1922, Wolever *et al* 1988). It was observed that not only the amount but also the bioavailability of the carbohydrates at breakfast can influence the glucose tolerance at a lunch meal served 4 h later. If the first meal elicits a low GI, the response to the second meal has been suggested to be lower than if the first meal was of high GI character (Jenkins *et al* 1982). The improvements in glycaemia in

the perspective from breakfast to lunch have been associated with a number of interdependent mechanisms such as delayed gastric emptying and enhanced insulin secretion (Gonzalez 2014), higher insulin sensitivity (Wolever *et al* 1995), suppression of hepatic glucose production (Gonzalez 2014) and enhanced muscle glucose uptake (Jovanovic *et al* 2009).

However, not all meals with a low GI have proven to improve glucose tolerance in the perspective from breakfast to lunch. Pasta (Liljeberg & Björck 2000) or barley bread with lactic acid (Östman *et al* 2002a) given as breakfast meals improved the second-meal glucose tolerance at lunch compared to barley bread without lactic acid or white wheat bread (WWB), respectively. However, no effect on glucose tolerance was found after the intake of a low GI breakfast consisting of WWB and vinegar (Liljeberg *et al* 1999b), and the authors suggested that not only GI *per se*, but also the course of glycaemia is of importance for second-meal glucose tolerance.

Second-meal effects have also been reported following low GI meals rich in indigestible carbohydrates in the perspective from a late evening meal to breakfast (Nilsson *et al* 2006, Wolever *et al* 1988), or in the perspective from breakfast to dinner (Nilsson *et al* 2008b). This effect is related to the increased production of short-chain fatty acids (SCFA) produced during colonic fermentation of the indigestible carbohydrates provided in the first meal (Nilsson *et al* 2008a, Nilsson *et al* 2010, Wolever *et al* 1988).

The use of science-based diets in the prevention of T2DM and CVD offers a great potential. The dampening effect on postprandial glucose metabolism, both acutely and at a subsequent meal, can be part of the explanation for the positive effects found.

Digestion and absorption

A number of food factors affect the rate of intestinal digestion and, consequently, the uptake of glucose to the blood (Russell *et al* 2016). More specifically, the digestion process is affected by the processes of mastication, gastric motility, gastric emptying and small intestinal breakdown and absorption (Bornhorst & Paul Singh 2014). The interaction between the physiological processes and food factors are pictured schematically in Fig. 2.

Indigestible carbohydrates will pass the upper gastrointestinal tract and reach the colon, where they promote fermentation and produce SCFA and gases (Canfora *et al* 2015).

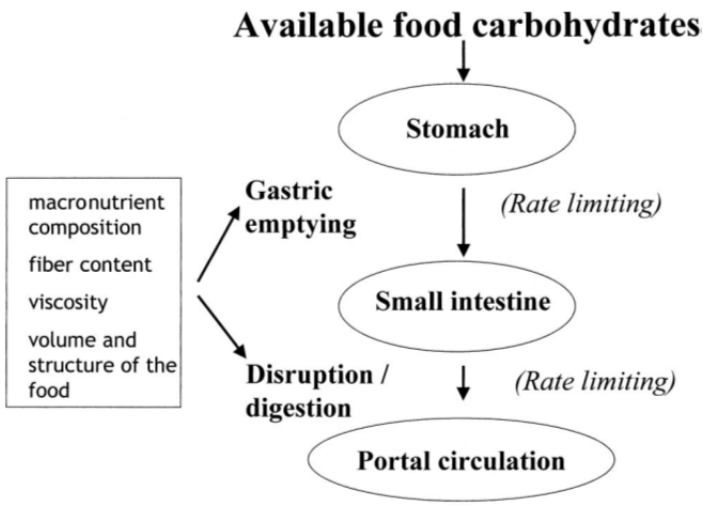

Figure 2
Factors affecting the rate of digestion and carbohydrate uptake in the gastrointestinal tract (Riccardi *et al* 2008). Reprinted by permission from American Society for Nutrition.

Gastric motility and gastric emptying rate

Gastric motility acts to crush and grind food particles so that they can pass the pyloric sphincter and enter the small intestine. The frequency, duration and intensity of the movements are to some extent affected by the food eaten and influence the rate of digestion (Bornhorst & Paul Singh 2014).

The GER is determined by a number of factors, related both to the subject, the ingested food and the gastric motility. Decreased GER extends the time period for intestinal glucose uptake and will thus result in a flattened glucose response.

Immediately after ingestion, an early-phase gastric emptying occurs that is determined by the meal volume. In the later postprandial period, GER correlates to the caloric content of the ingested meal, being reduced for a smaller calorie load at a given volume (Kwiatek *et al* 2009).

A high fat content (Clegg & Shafat 2009), a high content of DF (Hlebowicz *et al* 2007), addition of acetic acid or vinegar (Liljeberg & Björck 1998) or inclusion of sodium propionate in bread (Liljeberg & Björck 1996) reduces the GER. The increased viscosity resulting from addition of soluble DF has been shown to lower GER for liquids (Torsdottir *et al* 1989) as well as semi-solid foods (Zhu *et al* 2013). Furthermore, increased viscosity of the digesta will lead to an intestinal diffusion barrier, which delays absorption of glucose from the small intestine (Jenkins *et al* 1978).

Acute hyperglycaemia itself delays the rate of gastric emptying (Schvarcz *et al* 1997, Vollmer *et al* 2009) and hypoglycaemia accelerates it in order to facilitate glucose delivery to the intestines (Plummer *et al* 2014).

Food factors affecting amylase action

As the digesta enters the small intestine, pancreatic α-amylase starts to digest carbohydrates into dextrins, maltose, maltotriose and glucose (Bornhorst & Paul Singh 2014). All food factors that can reduce the α-amylase activity thus have the potential to slow down carbohydrate absorption and counteract a steep blood glucose increase. These factors can be related either to the raw material or to the processing conditions, and include a high degree of starch crystallinity, low degree of gelatinisation, highly organised food form or presence of certain organic acids (Björck *et al* 2000).

The presence of phytic acid (Schlemmer *et al* 2009) or polyphenols (Quek & Henry 2015) has been reported to reduce the α-amylase availability, possibly by binding to starch molecules or through an inhibitory effect on α-amylase.

If not acting directly on α-amylase activity, amylolysis can also be reduced by physical inaccessibility of the carbohydrates. Native starch packed in granules will thus be digested more slowly than gelatinised starch as the former is present in a crystalline and more ordered structure that is less for enzymatic digestion (Björck *et al* 2000). Upon retrogradation of gelatinised starch, crystalline starch ranging from completely enzyme-resistant starch (RS) to SDS is formed. RS is mainly formed from retrograded amylose and, thus, a higher amylose to amylopectin ratio in the starch source will give products with glucose-reducing potential (Björck *et al* 2000). Furthermore, it also promotes formation of SDS, with tightly packed linear amylose being more resistant to digestion compared to the branched amylopectin (Åkerberg *et al* 1998a). Retrogradation of starch can be promoted by temperature cycling (Leeman *et al* 2005) or baking for long time (20 h) at low temperature (120ºC) *i.e.* pumpernickel baking (Hallström *et al* 2011).

Other aspects of food structure, either of botanical origin, *e.g.* whole kernels (Liljeberg *et al* 1992) or physically induced, as in pasta where starch is entrapped within a gluten network (Colonna *et al* 1990, Granfeldt & Björck 1990), reduce the glycaemic response. Protein-starch interactions can also be induced by the presence of lactic acid during baking, as in sour dough fermentation (Östman *et al* 2002b).

The *in vitro* method for starch hydrolysis rate, measured as hydrolysis index (HI), has been suggested to predict possible effects on postprandial glycaemia elicited by obstructed amylolysis (Granfeldt *et al* 1992). The method measures starch

availability and diffusion hindrance and has demonstrated a potential in predicting glycaemia for a larger number of starch-rich products (Granfeldt *et al* 1992).

Diffusion barriers

A high content of viscous DF has been demonstrated to lower the glycaemic response to bread in several studies (Scazzina *et al* 2013). The effect on glycaemia by viscous DF results from increased digesta viscosity, which delays the GER (Benini *et al* 1994, Marciani *et al* 2000) and thickens the unstirred water layer presenting a greater barrier to absorption (Wood *et al* 1990).

Furthermore, viscous DF influences appetite regulation via both mechanical and nutrient-dependent factors. Mechanical factors include the lowering of energy density, increasing the need for mastication which results in more satiety mediating signals to the brain (Blundell & Halford 1994) and increasing stomach distension and thereby possibly triggering afferent vagal signals of fullness (de Graaf *et al* 2004). Nutrient-dependent factors result from increased interaction between nutrients and peptide-releasing cells in the mucosa, and increased small intestinal transit time due to increased viscosity of the digesta (Kristensen & Jensen 2011).

The measurement of *in vitro* fluidity of bread digests has been suggested as a method to predict possible effects on postprandial glycaemia elicited by increased digesta viscosity resulting from the addition of viscous DF (Östman *et al* 2006).

Guar gum

Guar gum (GG) is a soluble DF known to reduce GI and insulin responses (Butt *et al* 2007, Torsdottir *et al* 1989). GG is extracted from the endosperm of the guar bean (*Cyamopsis tetragonoloba*), an annual crop grown mainly in India and Pakistan (Butt *et al* 2007). GG is a water-soluble polysaccharide consisting of mannose and galactose units.

GG is used in a wide range of food as an emulsifier or stabiliser, usually in amounts of <1%. Considerably higher doses (1.8 to 15 g GG) have been shown to improve postprandial glycaemic response to an oral glucose load, with preparation method and timing of the delivery both affecting the results (Wolf *et al* 2002). The reducing effect of GG on glycaemia has been related to lowered GER (Leclere *et al* 1994) and the increase in viscosity created in the digesta after consumption (Ellis *et al* 1991).

The average molecular weight (MW) of the GG varies with production method and has been reported to be in the range of 0.25 to 5.1 million Da (Mudgil *et al* 2014). Partially hydrolysed GG (PHGG) is produced by enzymatic hydrolysis of GG using endo-β-D-mannosepyranose (Stewart & Slavin 2006) and thus has lower

MW compared to GG. As for GG, PHGG has also been shown to reduce the GI of white bread (Trinidad *et al* 2004), which indicates that other factors than viscosity could influence postprandial glycaemia. It has been shown that GG inhibits α-amylase in a direct, non-competitive way at the first stage of enzymatic starch degradation (Slaughter *et al* 2002).

β-glucans

β-glucans are another example of soluble DF. They are found in the cell walls of oats and barley and, to a lesser extent, in rye and wheat (Brummer *et al* 2014). Different barley varieties contain 2–20% β-glucans (dry weight basis) and the amount in oats varies between 3–8% (El Khoury *et al* 2012).

The molecular structures of β-glucans from barley and oats are very similar, with about 90% of the glucose arranged in units of three or four monomers linked by β-(1→4) (cellotriosyl or cellotetraosyl units) connected by β-(1→3) linkage. The rest are longer runs of consecutive β-(1→4) monomers (Wang & Ellis 2014).

The relative proportion of cellotriosyl or cellotetraosyl units varies depending on the β-glucan source. Oat β-glucans are generally more water-soluble (82%) and have longer molecular chains (MW 2 000-3 000 kDa) (Wang & Ellis 2014) compared to β-glucans from barley (20–50% water solubility (Izydorczyk *et al* 2000) and MW 200-2 660 kDa) (Cho & Samuel 2009)). Numerous studies have demonstrated that the MW of β-glucans is reduced during food processing such as baking (Izydorczyk *et al* 2000, Tosh *et al* 2008, Wood 2004, Åman *et al* 2004). The degradation is likely to be caused by β-glucanases originating from the β-glucan ingredient *per se*, or from other added ingredients.

β-glucans from oats and barley have repeatedly shown positive effects on GI, both alone and incorporated into different food items (Braaten *et al* 1991, El Khoury *et al* 2012, Kwong *et al* 2013). Less is known about their effects in the later postprandial phase.

Colonic fermentation

The intake of soluble DF (Weickert & Pfeiffer 2008), as well as RS (Topping & Clifton 2001) leads to increased colonic fermentation. The main end products of the colonic fermentation are SCFAs, mainly acetic, propionic and butyric acid, together with gases such as methane, hydrogen (H_2) and carbon dioxide (Pomare *et al* 1985). Increased formation of SCFAs has been associated with improved insulin sensitivity and modulation of gut hormone responses, both of which could have an impact on postprandial glycaemia (Nilsson *et al* 2010, Weickert & Pfeiffer 2008). Furthermore, SCFAs formed during fermentation of viscous DF have been reported to enhance the production of the appetite biomarkers GLP-1 and PYY

(Tolhurst *et al* 2012). This effect occurs when the ingested DF has reached the colon, *i.e.* at a subsequent meal (Isaksson *et al* 2011, Nilsson *et al* 2008b).

The course of glycaemia

There is an urgent need for food concepts with the potential to reduce risk factors linked to the MetS. One such measure includes modulation of postprandial glycaemia, appetite and inflammation. Much is known about the potential of reducing the postprandial response as measured using the GI. However, the later postprandial period also seems to be of interest for the potentially positive effects on health. In recent years the need for a measure describing the course of glycaemia beyond GI has been shown to be warranted. The reason for this was highlighted by Rosén *et al* who found that rye products often induced low but sustained net increment in blood glucose, which resulted in high AUC values and consequently increased GI values. When looking at the mean blood glucose curves for these products, it was evident that they had a reduced peak and a low but sustained net increment, beyond 120 min. Thus, Rosén *et al* introduced the glycaemic profile (GP) as a tool to discriminate between high peak/short duration and low peak/long duration. GP was defined as the duration of the glucose curve above the fasting concentration divided by the incremental glucose peak (Rosén *et al* 2009). Thus, the GP includes both peak response and duration of response above baseline, with a high GP representing a low iPeak and a steady duration above the fasting level.

Both hydrolysis index (HI) and fluidity index (FI) have previously been related to glycaemia measured as GI. So far, there is not enough data to determine to what extent these measurements also depict the overall course of glycaemia, including the late postprandial phase as well.

Objectives

The objectives of the present thesis were to:

Investigate possibilities to tailor the course of postprandial glycaemia to bread in healthy subjects in favour of reduced incremental peak (iPeak) and low but sustained net increment (low GI and high GP) by inclusion of guar gum or β-glucans.

Investigate whether bread products characterised by low but sustained postprandial glycaemia improve acute and semi-acute appetite in healthy subjects.

Investigate the role of low but sustained postprandial glycaemia on second-meal glucose tolerance and subjective appetite in healthy subjects using model meals based on similar ingredients.

Evaluate the potential use of *in vitro* measurements of starch hydrolysis rate and fluidity to predict the course of postprandial glycaemia (GI and/or GP) in healthy subjects.

Materials and methods

Test products

Breakfast products

White wheat bread (WWB) baked from 360 g water, 540 g white wheat flour with 10% protein (Vetemjöl, Kungsörnen AB, Järna, Sweden), 4.8 g dry yeast (Jästbolaget AB, Sollentuna, Sweden) and 4.8 g salt (Falksalt, AB Hanson&Möhring, Halmstad, Sweden) was used as the reference product in studies I, III and IV. The WWB was baked according to a standardised method using a Tefal home bread-baking machine and a program for white bread (Ekström *et al* 2013).

The test-bread products in paper I were prepared from wheat flour with 12% protein (Vetemjöl special, Kungsörnen AB, Järna, Sweden), dry yeast and salt, in combination with three different kinds of GG (mwGG, (MEYPRODOR®50, MW 50 kD)), lwGG (MEYPRODOR®5, MW 5 kD) (Danisco A/S, Denmark) and hGG, mean MW 20 kD (Sunfiber) (Azelis-Bröste AB, Mölndal, Sweden)) as well as Hi-maize® whole grain maize flour (Ingredion Incorporated, Bridgewater, NJ, USA). The breads containing hGG and lwGG were made using the same procedure as WWB in papers I, III and IV. For all wgHiM flour-containing breads, the dough was mixed in a bowl for 5 min, proved in a Tefal home bread-baking machine for 30 min, kneaded by hand for 15 s and again placed in the baking machine for another 30 min proving and 60 min baking.

The test-bread products in paper II were prepared from white wheat bakery flour with 11% protein (Pågens Extra Bagerivetemjöl, Pågen AB, Malmö, Sweden), dry yeast and salt, in combination with three different sources of β-glucans; whole grain barley flour from a variety high in β-glucan (approx. 15%) (National Starch, Manchester, England), coarse barley fibre (Lyckeby Culinar, Fjälkinge, Sweden) or refined oat fibre (Tate & Lyle Oat Ingredients, Kimstad, Sweden). Doughs were mixed (KitchenAid) for 7 min, proved in room temperature for 40 min and baked in a Tefal home bread-baking machine for 60 min. The WWB was baked from 270 g water, 450 g white wheat bakery flour, 4.5 g salt and 9.0 g dry yeast.

The test-bread products in paper III were made from wheat flour with 12% protein (Vetemjöl special, Kungsörnen AB, Järna, Sweden), mwGG (MEYPRODOR®50, Danisco A/S, Denmark) (the same as in paper I), dry yeast and salt, in combination with either HAM (Hi-Maize 260, Ingredion Incorporated, Bridgewater, NJ, USA) or whole grain rye flour milled from rye kernels (variety Visello, KWS LOCHOW GMBH, Bergen, Germany) using a laboratory mill (Perten laboratory mill 120, sieve 0.8 mm). The dough for the two test-breads were mixed in a bowl for 5 min, proved in a Tefal home bread-baking machine for 30 min, kneaded for 15 s by hand and placed in the bread machine for another 30 min proving followed by 60 min baking.

In all four studies, the bread products were allowed to cool directly after baking. The rye-containing bread in paper III was left for 16–18 h wrapped in a towel and put in a plastic bag. All other bread products were left for 1.5–2 h wrapped only in a towel. After cooling, the crust was removed and the crumb sliced and divided into portions wrapped in aluminium foil, put into plastic bags and stored in a freezer (-18ºC) until use. The day before use, the bread portions were taken from the freezer and thawed at ambient temperature, still wrapped in aluminium foil and in the plastic bag.

In paper IV, pasta (dried spaghetti made from durum wheat and white wheat flour, Kungsörnen, Järna, Sweden) was boiled for 8 min in 1 l of water containing 5.0 g NaCl, immediately before serving.

All breakfast meals included 250 ml of tap water.

Lunch meals

In paper III, a standardised lunch meal was served in *ad libitum* amounts 240 min after the start of the breakfast. The lunch meal consisted of regular spaghetti made from durum and normal wheat (Barilla Sweden AB, Filipstad, Sweden), ready-made frozen meatballs (ICA Handlarnas AB, Solna, Sweden), ketchup (Heinz) and fresh cucumber. The cucumber was served in slices of 2-3 mm, with the ends removed in order to standardize the ratio of peel to fruit flesh. The pasta was boiled for 8 min (1 l water, and 7 g NaCl per 100 g pasta), the water was then discarded and 8 g rape seed oil (Di Luca & Di Luca AB, Stockholm, Sweden) added per 100 g dry pasta. The meatballs were heated in a microwave oven at 850 W in 2 min cycles until they were evenly warm. Water (250 ml) was served with the lunch meal.

In paper IV, a standardised lunch meal was served 240 min after the start of the breakfast. The lunch meal consisted of 100.0 g meatballs (FELIX Små Delikatess Köttbullar, Orkla Foods Sverige AB, Eslöv, Sweden), heated in a microwave oven (1100W) for 1 min and 30 s, 55.0 g instant potato powder (FELIX Potatismos,

Orkla Foods Sverige, Eslöv, Sweden) reconstituted in 250 ml boiling water and 60.0 g frozen sweetcorn (Findus, Bjuv, Sweden), thawed at ambient temperature for 4 h. Water (250 ml) was served with the lunch meal.

Chemical analysis of the test products

Total, available and resistant starch

Potentially available starch was determined enzymatically according to the method developed by Holm *et al* (1986). Dried and milled samples of the test products were suspended with phosphate buffer and incubated with thermostable α-amylase (Termamyl 300 L, Novo Nordisk A/S, Denmark) in boiling water for 20 min in order to gelatinise the starch. Thereafter, subsamples were incubated with amyloglucosidase (Roche Diagnostics GmbH, Germany) at 60ºC for 30 min. The glucose content was then determined using a glucose oxidase/peroxidase reagent (GLOX) and the starch content was calculated from the amount of glucose by multiplying by 0.9. Samples were analysed in duplicate.

Total starch was determined after an initial solubilisation of retrograded starch in freshly prepared KOH according to the method by Björck and Siljeström (1992). Dried and milled samples were suspended with KOH and left at ambient temperature for 30 min. The content of starch was thereafter analysed using the same procedure as described above. The pasta was boiled as for use in the test breakfast meal and homogenised in the phosphate buffer before being mixed with KOH. Samples were analysed in duplicate.

Chemical analysis included in each paper is summarised in Table 2.

Table 2
Chemical analysis performed on the test products included in the different papers.

Paper	Total starch	Resistant starch	Potentially available starch	Dietary fibre	β-glucan	HI	FI
I	X	X				X	X
II			X	X	X	X	X
III	X	X				X	X
IV	X	X					

Resistant starch was determined using an *in vitro* method developed by Åkerberg *et al* (1998b). Six volunteers chewed the bread or pasta samples 15 times during 15 s. After the samples had been spitted out, the volunteers rinsed their mouths

with 5 ml water, which was also spitted into the sample beakers. Samples were then incubated at 37ºC with pepsin (Merck, Darmstadt, Germany) at pH 1.5 for 30 min, followed by incubation with pancreatin (Sigma, St. Louis, USA) and amyloglucosidase (Roche Diagnostics GmbH, Germany) at pH 5.0 and 40ºC for 16 h. After the incubation, the samples were precipitated with 60ºC ethanol and filtrated, using Celite 545 (Sigma-Aldrich St. Louis, USA) as filter aid. The indigestible residue of the filter cake was analysed for total starch according to the procedure described above (Björck & Siljeström 1992) giving the content of RS. Samples were analysed using six replicates, meaning that six individual chewers participated for every test product analysed. RS measured using this method includes all major forms of RS (resistant B-type starch, retrograded starch and physically inaccessible starch).

In papers I, III and IV, the amount of available starch was determined by subtracting the amount of RS from the amount of total starch and, in paper II, the results from analysis of potentially available starch were used.

Dietary fibre

Insoluble and soluble DF were determined using a gravimetric, enzymatic method according to Asp *et al* (1983). Dried and milled samples were hydrated in phosphate buffer and incubated with thermostable α-amylase (Termamyl 300 L, Novo Nordisk A/S, Denmark) in boiling water for 20 min for gelatinisation. Samples were then incubated with pepsin (Merck, Darmstadt, Germany) at pH 1.5 at 40ºC for 60 min and with pancreatin (Sigma, St. Louis, USA) at pH 6.8 at 40ºC for another 60 min. After the enzyme incubation, the samples were filtrated and insoluble DF collected in the filter cake, using Celite 545 (Sigma-Aldrich St. Louis, USA) as filter aid. The supernatant was thereafter treated with 60ºC ethanol for 60 min in order for the soluble fibre to precipitate. After the precipitation, samples were filtrated again, and soluble fibres collected in the filter cake. Samples were analysed in duplicates and DF-concentrations reported were corrected for ash (determined after burning samples) and protein (determined using a FlashEA 112m Thermo Fisher Scientific Inc., Waltham, MA, USA).

Concentration and molecular weight of β-glucan

The concentration of β-glucans was measured with an enzymatic kit (Megazyme, Ireland) according to AOAC method 995.16. Dried and milled samples were hydrated in buffer, incubated with lichenase enzyme and filtered. Subsamples were completely hydrolysed using purified β-glucosidase and the amount of liberated glucose was determined using a glucose oxidase/peroxidase reagent.

The MW of the β-glucans was analysed using high-performance size-exclusion chromatography (HPSEC) with calcoflour detection (Kim & Inglett 2006). Dried bread samples and β-glucan ingredients respectively, were wetted with ethanol (50% v/v) and dissolved in water with gentle stirring for 20 h. Samples were then filtered using a 45µm syringe glass filter before being injected into the HPSEC system (Agilent Technologies, Santa Clara, California, USA). A standard curve was prepared ranging from 40–359 kDa and the MW of the samples was calculated from the respective retention time.

Hydrolysis index

The rate of starch hydrolysis was determined using an *in vitro* procedure developed by Granfeldt *et al* (1992). Six volunteers started by chewing one sample each 15 times during 15 s. After spitting out the sample, they rinsed their mouth with 5 ml phosphate buffer, and the samples were then incubated with pepsin (Merck, Darmstadt, Germany) at 37ºC, pH 1.5 for 30 min. The samples were then neutralised and transferred to dialysis tubing (25 cm strips, width 45 mm, cut-off 12–14 kD, Spectrum Laboratories, Inc.), α-amylase (A-6255, Sigma Aldrich, Germany) was added and the volume adjusted. Each dialysis tube was incubated at 37ºC for 3 h in a 1 l beaker containing 800 ml phosphate buffer under gentle stirring. Every 30 min, aliquots were taken for analysis of reducing sugar (maltose equivalents) by the 3,5-dinitro salicylic (DNS) acid method. A standard curve was prepared using maltose. The degree of hydrolysis was calculated as the proportion of potentially available starch degraded to maltose using the conversion factor 0.95. Hydrolysis index was calculated by dividing tAUC 0-180 for the test product by that of WWB. Each sample was analysed in six replicates and within a paper, and the same six individual chewers participated for all test products analysed. Furthermore, in paper I, WWB was analysed twice, and a mean of the two analyses was used for calculations of HI.

In papers I–III, HI was used to predict GI as described by Leeman *et al* (2005).

Fluidity index

The fluidity was determined on bread samples subjected to *in vitro* digestion according to Östman *et al* (2006). The samples were initially crushed five times during 5 s using a mortar and pestle. Phosphate buffer was then poured onto the sample and it was crushed 15 times during 15 s. Thereafter, the samples were incubated with pepsin (Merck, Darmstadt, Germany) at 37ºC, pH 1.5 for 30 min, neutralised and incubated with α-amylase (A-6255, Sigma Aldrich, Germany) at 37ºC for 1 h. The fluidity was then measured on 45 ml aliquots using a Bostwick consistometer (24925-000, Christian Particle Technologies Ltd, UK), consisting of

a trough divided into two sections by a gate. The smaller section serves as a reservoir for the sample before starting the measurement, and the larger section is graded at every 0.5 cm. At time zero of the measurement the gate is released using a simple spring mechanism and the sample flows down the larger section. Measurements of the flowing distance were made in duplicates and readings were taken every 10 s for 1 min.

The fluidity index (FI) was calculated as:

$$FI = \frac{consistency_{reference\ bread}}{consistency_{test\ bread}} \times 100$$

$$Where\ consistency = \frac{1}{BU}$$

$$and\ BU = \frac{cm\ after\ 60\ s}{sample\ size\ in\ ml}$$

Meal studies

Test subjects

Healthy, young volunteers with normal body mass indices and without drug therapy were recruited to all four meal studies. Smoking or snuff use were exclusion criteria. In order to standardise the behaviour of the test subjects, they were asked to avoid alcohol, excessive physical activity and food rich in DF on the day before a test. For the duration of the study, the subjects were not allowed to use antibiotics or probiotics. In the late evening (21:00–22:00) prior to a test the subjects were instructed to eat a standardised meal consisting of a commercial white wheat bread with topping and drink of their own choice. However, the subjects were obliged to have an identical evening meal before each test. The subjects were otherwise instructed to maintain their regular lifestyle throughout the entire study.

Detailed information about the number of test products, the duration of experimental days and the participants is found in Table 3.

Table 3
Overview, number of test products, duration and characteristics of test subjects in the four studies.

Paper	No. of visits	Lunch[a]	Duration	No. of subjects	Age[b]	BMI[b]
			(min)		year	(kg/m^2)
I	5	-	180	n = 12 (7 ♂[c], 5 ♀)	24.0 ± 1.5	23.3 ± 0.4
II	6	-	180	n = 13 (9 ♂, 4 ♀)	26.3 ± 0.7	22.6 ± 0.8
III	3	*ad libitum*	360	n = 19 (9 ♂, 10 ♀)	27.3 ± 1.4	21.7 ± 0.4
IV	2	standardised	360	n = 20 (8 ♂, 12 ♀)	23.7 ± 0.8	21.8 ± 0.4

[a]Lunch was served 240 min after start of the breakfast.

[b]Mean ± SEM

[c]One male was excluded due to several statistical outliers.

Study design

All studies were performed using a single-blind randomised crossover design. The test products were provided as breakfast meals after an overnight fast, approximately one week apart. All studies were approved by the regional ethical review board in Lund. The test subjects gave their informed written consent before the start of a study, and they were aware of the ability to withdraw from the study at any time, without giving a reason.

Breakfast meals

Test and reference products within a study should contribute the same amount of digestible carbohydrates, usually 50 g. However, in paper I, the test products contained a large proportion of indigestible material, and the portions were thus reduced to 37 g available starch (total starch - RS) in order to provide manageable portions. In papers III and IV, the meals were based on 50 g available starch (total starch - RS). In paper II, the meals were based on 53 g available starch (potentially available starch according to Holm *et al* (1986)). Chemical characteristics and portion sizes for all breakfast meals are presented in Table 4.

The breakfast portions, including 250 ml water, were served directly after the fasting samples had been taken. In paper III, 150 ml coffee, tea or water (without sweetener or milk) was served after the blood sampling at 120 min after the breakfast. The subjects chose which drink to consume at their first visit, and then stuck to it throughout the study. Thus the mid-morning drinks were individually standardised.

Table 4
Chemical characteristics and portion size of the test products.

Paper	Products	Total starch	Resistant starch	Potentially available starch	β-glucan	Dry matter	Portion size
		(% ww)	(% ww)	(% ww)	(% ww)	%	g
I	WWB	39.8	1.0	na[a]	na	52.0	95.6
	wgHiM	37.3	5.2	na	na	51.9	114.6
	wgHiMG1	35.0	5.4	na	na	51.4	123.8
	wgHiMG2	29.3	5.2	na	na	45.1	150.0
	wgHiMG3	26.7	5.2	na	na	43.9	174.2
II	WWB	na	na	44.8	na	54.3	117.7
	BB	na	0.4	32.6	3.1	51.7	-
	BF	na	1.4	32.7	2.1	51.8	-
	OF1	na	na	40.0	2.6	50.0	132.7
	OF2	na	na	37.5	3.7	49.0	141.3
	OF3	na	1.9	36.0	4.9	51.5	151.7
III	WWB	39.8	1.0	na	na	52.0	128.9
	HG	35.1	7.1	na	na	47.2	178.7
	VG	27.7	1.2	na	na	46.4	188.3
IV	WWB	41.9	0.9	na	na	52.2	122.0
	Pasta (boiled)	27.5	1.2	na	na	38.9	190.0[b]

WWB (white wheat bread), wgHiM (WWB with Hi-Maize® whole grain maize flour), wgHiMG1, wgHiMG2 and wgHiMG3 (wgHiM with 6%, 13% and 19% mwGG dry weight basis (dwb), respectively), BB (wheat-based bread with whole grain barley flour containing elevated amount of β-glucan), BF (wheat-based bread with barley fibre) OF1, OF2 and OF3 (wheat-based bread with refined oat β-glucan preparation in three different amounts), HG (bread containing HAM and mwGG), VG (bread containing whole grain rye (Visello) and mwGG).

[a]Not analysed.

[b]Corresponding to 77.4 g dry pasta.

Lunch meals

The *ad libitum* lunch meal in paper III and the standardised lunch meal in paper IV were both served 240 min after the breakfast. At the *ad libitum* lunch in paper III, the subjects were encouraged to eat at a comfortable pace until they were pleasantly full, with the target to reach the same level of satiation on every test occasion. Therefore, they were allowed to help themselves to the food, and the amount of each food item taken, as well as any leftovers, was recorded by the study leader. The subjects were seated separate from each other in order to avoid influences from the other participants during the meal. Food was available in surplus, and the amount of each item eaten by the subjects was recorded by the

study leader. At the standardised lunch in paper IV, the subjects were provided with the meal and were told to finish their portions at a comfortable pace, within 30 min.

Sampling and analysis of physiological parameters

At all visits, blood samples were taken in the fasting state and then continuously at predetermined time points during the study duration, see Table 5.

As the subjects arrived in the morning, they were asked to sit down and rest for 10–15 min, before the fasting sample was taken. In all papers, capillary finger-prick samples were taken for determination of blood glucose. Capillary samples were also used for determination of insulin (papers I, II and IV), and NEFA and TG (paper IV). In paper IV, insulin, NEFA and TG analyses were made within 3 h in order to avoid repeated thaw and freezing cycles. In paper III, a peripheral venous catheter was used for blood sampling for all parameters except glucose. The catheter was inserted as the subjects arrived and the fasting samples were taken 10–15 min after insertion.

Table 5
Time schedule for sampling of test variables analysed in the different studies.

	0[a]	**15**	**30**	**45**	**60**	**90**	**120**	**150**	**180**	**210**	**240[1]**	**255**	**270**	**285**	**300**	**330**	**360**
Glucose	I-IV	I-IV	I-IV	I-IV	I-IV	I-IV	I-IV	III	I-IV		III, IV		IV	IV	IV		IV
Insulin	I-IV	I-IV	I-IV	I-IV	I-IV	I-IV	I-IV		I, III, IV	III	III, IV		IV	IV	III, IV		III, IV
GLP-1[b]	III	III	III	III	III	III	III		III	III	III				III		III
PYY	III	III	III	III	III	III	III		III	III	III				III		III
GIP	III	III	III	III	III	III	III		III	III	III				III		III
Ghrelin	III	III	III	III	III	III	III		III	III	III				III		III
SCFA									III		III				III		III
TG	IV	IV	IV	IV	IV	IV	IV				IV		IV	IV	IV		IV
NEFA	IV		IV		IV		IV		III, IV		III, IV		IV	IV	IV		IV
Breath H_2	III		III		III	III	III	III	III	III	III		III		III	III	III
VAS	I-IV	I-IV	I-IV	I-IV	I-IV	I-IV	I-IV	III	I-IV	III	III, IV	III	III, IV	III, IV	III, IV	III	III, IV

[a]Variables at time 0 and 240 determined just before the serving of breakfast or lunch, respectively.

[b]Results from the analysis of GLP-1 was excluded due to problems with the sensitivity.

Glucose

Plasma glucose was determined in capillary whole blood immediately after sampling using a HemoCue Glucose 201^+ (HemoCue AB, Ängelholm, Sweden).

Insulin

Insulin was analysed in serum using an enzyme immunoassay kit (Mercodia AB, Uppsala, Sweden). In papers I and II, the measurements were performed on an integrated immunoassay analyser (CODA Open Microplate System, Bio-Rad Laboratories, Hercules, CA, USA). In paper IV the sample preparations were made manually using a microplate wash (ASYS Atlantis, Biochrom LTD, UK), a THERMO Star incubator (BMG LABTECH, Germany) and a SPECTROStar^{Nano} plate reader (BMG LABTECH, Germany). In paper III, plasma insulin was analysed using Milliplex MAP (Human Metabolic Hormone Magnetic Bead Panel, Millipore Corporation, Billerica, MA, USA). The details of the Milliplex MAP system are presented below.

Other hormones

In paper III, insulin, GLP-1 (GLP-1_{7-36}), GIP (GIP_{1-42} and GIP_{3-42}), PYY (PYY_{1-36} and PYY_{3-36}) and acyl (active) ghrelin were analysed in plasma using Milliplex MAP (Human Metabolic Hormone Magnetic Bead Panel, Millipore Corporation, Billerica, MA, USA). The Milliplex MAP technique is based on immunoassays on the surface of magnetic fluorescent-coded beads. The resulting chemoluminiscens was read on the Luminex 200 instrument (Luminex Corporation, USA) and evaluated with Milliplex analyst v3.4 (VigeneTech Inc., Charlisle, USA). The plasma was collected into tubes containing DPPIV-inhibitor (10 µg/ml blood, Millipore, St Chareles, USA) and Pefabloc (1 mg/ml blood, Roche Diagnostics, Mannheim, Germany). DPPIV-inhibitor was added for the determination of active GLP-1 and Pefabloc for the determination of active ghrelin.

NEFA and TG

NEFA and TG were determined in serum using enzymatic colorimetric methods NEFA C, ACS-ACOD method (Wako Chemicals GmbH, Germany) and LabAssay Triglyceride, GPO·DAOS method (WAKO Chemicals GmbH), respectively.

Markers of fermentation

SCFAs (acetate, propionate, isobutyrate and butyrate) were analysed in serum at 180, 240, 300 and 360 min using gas chromatography with a flame ionisation detector (Brighenti 1998).

Breath H_2 in exhaled air was measured as a marker of gut fermentation using Gastrolyser (Bedfont EC60 Gastrolyser, Rochester, UK).

Subjective appetite rating

In all papers, a 100 mm bipolar visual analogue scale (VAS) graded from "none" to "extreme" was used for subjective rating of *satiety*, *hunger* and *desire to eat* (Blundell *et al* 2010). In papers I-III, "pen and paper" were used and, in paper IV, the tests were performed electronically in personal laptops using the Adaptive Visual Analogue Scales (AVAS) software.

Calculations and statistical methods

Data are expressed as means ± standard errors of the mean (SEM) (paper I) or least square means (LSMs) ± SEM (papers II-IV).

The incremental and total areas under the curves (iAUC and tAUC, respectively) were calculated for each subject and test meal using the trapezoid model. GI and II were calculated from the iAUC 0-120 min for glucose and insulin respectively, using WWB as the reference (GI and II = 100).

Incremental peaks (iPeak) were calculated as the maximum postprandial increase from baseline (relevant for glucose, insulin and GIP). The GP was defined as the duration of the glucose curve above fasting concentration in the timespan from breakfast to lunch (0–180 or 0–240 min) divided by the iPeak (Rosén *et al* 2009). In the cases where the blood glucose did not go below the fasting value, the duration was set to the final sampling time. GP^2 was calculated in the same way as GP, with the exception that the duration was divided by the squared glucose iPeak. GraphPad Prism (version 6, GraphPad Software, San Diego, CA, USA) was used for graph plotting and area calculation.

In paper I, GI, II, GP and iPeak were analysed using a mixed model analysis of covariance (ANCOVA) with subjects as a random variable and corresponding baselines (fasting values) as covariates (MINITAB, release 16, Minitab Inc., State College, PA, USA). Differences between groups were identified by using Tukey´s multiple comparisons tests. Normality of the residuals was controlled using Anderson-Darling test and BoxCox transformation was performed if the residuals were not normally distributed.

Time × treatment interactions were analysed in paper I using a mixed model (PROC MIXED in SAS release 9.2, SAS Institute Inc, Cary, USA) with repeated measures and an autoregressive covariance structure.

The effect of reference and test meals on physiological responses in papers II–IV was evaluated using a linear mixed model ANCOVA (PROC MIXED procedure). Baseline, visit, treatment, time and treatment × time interaction were included as fixed effects. Subject was treated as random effect, and time and visit were

included as repeated effects. All models were tested for the normality of residuals using standard diagnostics to ensure that all variables met the assumptions for normal distribution and ln transformation was applied if necessary. To adjust for multiple comparisons of significant effects, Tukey-Kramer post hoc significance test was performed, and the Kenward-Roger correction was applied for reducing small sample bias. Calculations were performed using SAS (version 9.4, SAS Institute Inc., Cary, USA).

Correlation analysis was conducted in papers I–III to evaluate the relation among dependent measures with the use of Spearman's partial coefficients controlling for subjects and corresponding baselines (two tailed test) (SPSS software, version 19; SPSS Inc., Chicago, IL, USA).

For HI and FI, a mixed model analysis of variance (ANOVA) was used, with test subject and sampling occasion, respectively, as a random variable (MINITAB, release 16, Minitab Inc., State College, PA, USA).

Statistical significance was considered at a p-value < 0.05 (two-tailed).

Results and Discussion

Paper I

On the possibility to affect the course of glycaemia, insulinaemia, and perceived hunger/satiety to bread meals in healthy volunteers

L.M.N.K. Ekström, I.M.E. Björck, E.M. Östman

Food & Function 2013 4:4

The purpose of paper I was to investigate the possibilities of tailoring the course of postprandial glycaemia to give a low but sustained net increment (low GI and high GP) to bread products by using guar gum (GG). In addition to GG, a wholegrain-high amylose (Hi-Maize) flour (wgHiM) was added in order to study the effect of a slowly digestible and partially resistant starch fraction on the course of glycaemia. The potential use of *in vitro* measures for starch hydrolysis rate (HI) and fluidity (FI) in predicting the course of glycaemia (GI and/or GP) was also studied.

Low (lw), medium (mw) and high (h) molecular weight (MW) GG was incorporated in white wheat-based breads alone or together with wgHiM. The bread products were screened using the *in vitro* methods, and the most promising were included, together with WWB, in a meal study with healthy subjects.

The physicochemical characterisation of the test products revealed that FI was not affected by the use of wgHiM flour, hGG or lwGG. However, adding 6, 13 or 19% mwGG (dry weight basis, dwb) led to a stepwise decrease in FI. The use of wgHiM in combination with mwGG resulted in similar FI as when only mwGG was added. HI, on the other hand, was non-significantly reduced as wheat flour was replaced with wgHiM flour. However, the additions of mwGG (13 or 19% dry weight basis (dwb)) led to a significant reduction in HI, whereas the addition of lwGG or hGG had no impact. The addition of wgHiM markedly increased the level of RS but the addition of mwGG alone did not increase RS compared to WWB. It was observed, however, that when mwGG was combined with wgHiM, the content of RS (expressed as percentage of total starch) tended to increase stepwise as the amount of mwGG increased.

To conclude, FI decreased with an increasing amount of mwGG, but was not affected by the addition of lwGG or hGG. The addition of wgHiM *per se* did not affect FI. In addition, HI decreased with increasing amounts of mwGG. HI was not affected by the addition of either lwGG or hGG.

Considering the results from the physicochemical characterisation, the bread products containing wgHiM and wgHiM in combination with mwGG were selected for the meal study.

The bread products containing mwGG altered the course of glycaemia compared to the WWB, expressed as lower glucose iPeak, increased GP, lower II and insulin iPeak (see Table 6). However, only the medium and high doses of mwGG led to a GI different from that of WWB. The effect on glycaemia exerted by GG is assumed to result from the previously reported increased intestinal viscosity, leading to lower GER, reduced rate of starch breakdown and reduced rate of intestinal nutrient uptake (Ellis *et al* 1991, Wood *et al* 1990).

The highest level of mwGG used here was chosen on the basis of findings by Nilsson *et al* (2012). In that study, the inclusion of 15% GG (dwb) to white wheat-based bread resulted in improved cognitive performance, especially in the later postprandial period (75–235 min) compared to WWB. In the present study it was found that lower amounts of mwGG also give rise to the smooth postprandial blood glucose profile that was associated with the effect on cognitive performance.

The white wheat-based bread with addition of only wgHiM did not affect postprandial glycaemia or insulinaemia compared to WWB, despite its higher content of RS. This is in contrast to products with elevated amylose content and increased RS-levels that have previously been associated with reduced postprandial glycaemia and/or insulinaemia (Björck *et al* 2000, Granfeldt *et al* 1995, Hallström *et al* 2011). However, not all studies with high amylose ingredients have shown effect on both glucose and insulin (Bodinham *et al* 2010).

It should be noted that the reduction in GI/II for products based on high-amylose ingredients is probably not a result of the RS *per se*, but rather a result of a slowly digestible starch (SDS) fraction formed simultaneously with RS (Raigond *et al* 2014). Anderson *et al* (2010) studied the effect on glycaemia and appetite using 4 different types of maize starch in a preload meal, where one of the treatments was the same as in paper I. They characterised the different starch fractions using the method published by Englyst *et al* (1992), including estimations of rapidly digested starch (RDS), SDS and RS. Interestingly, the wgHiM had the lowest level of SDS (10% of total starch) compared to HiM260 and regular maize starch (13 and 33%, respectively) as well as the highest content of RS (66 compared to 48 and 40%, respectively). It is possible, however, that the presence of whole grain DF in the wgHiM flour disturbed the co-formation of SDS normally seen when using HAM. However, the mechanism for a potential obstructed formation of SDS in the case of the wgHiM remains to be elucidated.

The three bread products with different levels of mwGG induced a greater *feeling of fullness* compared to the WWB (tAUC 0-180). Furthermore, the two breads with the largest amount of mwGG induced a lower *feeling of hunger* and the product with the highest level of mwGG induced a decreased *desire to eat*. Overall, the subjective *feeling of fullness* was positively correlated to GP and negatively correlated to both GI and II. This is very interesting and demonstrates that the mwGG-mediated alteration in course of glycaemia was associated with improvements in appetite. It can be anticipated that the benefits on appetite regulatory properties with the mwGG and wgHiM-containing bread products resulted from increased need for mastication, increased stomach distension, decreased GER as well as increased production of gut hormones.

A linear reduction was found for glucose and insulin responses as well as for appetite ratings with an increased amount of mwGG, *i.e.* a dose-response behaviour. This has previously been demonstrated for insulin, but not for glucose, after incorporation of GG in biscuits (Ellis *et al* 1988).

FI and HI were strongly correlated to the physiological responses measured in the meal study. Previous research has shown that HI is a good predictor of GI values for cereal, legume and potato products (Leeman *et al* 2005) and in this study we can see that this is valid also for mwGG-containing products. In addition, the results indicate that also FI could be used for prediction of GI and GP in the case of mwGG-mediated viscosity effects on glycaemia.

To conclude, although WWB with addition of wgHiM did not affect postprandial glycaemia compared to WWB, the inclusion of both mwGG and wgHiM in bread products resulted in a low but sustained net increment in postprandial glycaemia. Furthermore, appetite ratings were improved after the mwGG-containing bread products. Both HI and FI were correlated to the different measures of glycaemia, indicating a possibility to predict glycaemic responses after intake of bread products containing whole grain high amylose flour and mwGG.

Paper II

Oat β-glucan containing bread increases the glycaemic profile

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The purpose of paper II was to investigate possibilities to tailor the course of glycaemia to bread using commercially available cereal ingredients with high β-

glucan content claiming to either lower postprandial glycaemia or to have β-glucans of high MW. Three different β-glucan containing ingredients were chosen and incorporated into yeast leavened bread. The breads were analysed for β-glucan concentration and MW. Products meeting the criteria of having β-glucans with MW above 250 kDa were included in a meal study with healthy subjects, using WWB as reference.

The potential use of *in vitro* measures for starch hydrolysis rate (HI) and fluidity (FI) in predicting the course of glycaemia (GI and/or GP) was also evaluated.

The β-glucan ingredients were: whole grain barley flour with elevated content of β-glucans, a barley fibre and a refined oat β-glucan (OF). As expected, the MW of β-glucans in the bread products were lower than in the starting material, with a more pronounced degradation in the case of both barley β-glucan ingredients. Only the breads made with β-glucans from oats meet the meal study inclusion criteria of MW > 250 kDa. To evaluate the potential role of increased doses on glucose and insulin responses, the meal study included wheat-based bread products with 5.2, 7.5 and 9.6% (dwb) β-glucans, as well as plain WWB.

The OF ingredient proved to be very effective in lowering postprandial glycaemia as all OF-products displayed lower glucose and insulin iAUCs (0–120 min) and iPeaks compared to WWB. Both the amount and MW of the solubilised β-glucans in the gastrointestinal tract influence the effect on glycaemia (Wood *et al* 2000). Therefore, it was interesting to see that even the lowest level of OF was able to reduce GER and, thus, glycaemia and insulinaemia substantially, with no further reduction apparent with increased levels. However, the highest β-glucan level prolonged the time period for net glucose increment above fasting, and thus generated a higher GP compared to the WWB.

The more pronounced MW degradation in the barley bread products is probably a result of greater β-glucanase activity in the barley β-glucan ingredients (Andersson *et al* 2004). In oats, a heat treatment *i.e.* kilning process, is undertaken to inactivate endogenous enzymes, *e.g.* lipases (Ames, Storsley, & Tosh, 2015), and thus the high MW of the β-glucans is better preserved. It is noteworthy that the coarse barley fibre ingredient had such a low MW in the starting material that no effect on glycaemia could be expected, even without further degradation during yeast bread preparation. Interestingly, the producer claims that they use a production process that does not reduce the β-glucan MW from its state in the grain.

The results obtained in paper II illustrate the importance of knowing the β-glucan MW if it should be used in yeast leavened bread products. Having a high raw material MW allows for some degradation during the bread preparation process. Furthermore, as the reduction in β-glucan MW occurs during mixing and fermentation, these processes should preferably be kept controlled in order to reduce the degradation.

The β-glucan-containing products displayed lower FI-values compared to the WWB, regardless of the β-glucan MW. For the OF-products, FI decreased with increasing dose of β-glucan. Only the medium and high level OF-products displayed lower HI compared to WWB.

Current European legislation allows health claims related to blood glucose regulation based solely on the ratio between β-glucans and available carbohydrates in a meal. Furthermore, the claim is valid for β-glucans originating both from oats and barley. Four g β-glucans per 30 g available carbohydrates has been considered as the lowest dose to reduce postprandial glycaemia (Carlo Agostoni *et al* 2011). It should thus be noted that the lowest level of OF β-glucan in paper II also resulted in significant lowering of both glycaemia and insulinaemia, despite the fact that a portion only contained 3.3 g β-glucans (corresponding to 1.9 g β-glucans per 30 g available starch). Furthermore, a recent review demonstrated that the glucose-reducing potential of β-glucans is more strongly related to the content of β-glucans alone than to the ratio of β-glucans to available carbohydrates (Tosh, 2013). The present results demonstrate that lower levels of high MW oat β-glucans, measured both as dosage in a meal and amount in relation to available starch, also reduce postprandial blood glucose and, thus, highlight the need for revision of the current legislation.

Paper III

An improved course of glycaemia after a bread-based breakfast is associated with beneficial effects on acute and semi-acute markers of appetite

L.M.N.K. Ekström, I.M.E. Björck, E.M. Östman

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The purpose of paper III was to investigate whether bread products characterised by low but sustained glycaemia improve acute and semi-acute appetite in healthy subjects. Based on previous findings, guar gum of medium molecular weight (mwGG) was used in order to obtain the desired effect on glycaemia. In addition, high-amylose maize (HAM) or whole grain rye was added to the bread products in order to further strengthen the glucose lowering effect and/or add various substrates for gut microbial fermentation.

The potential use of *in vitro* measures for starch hydrolysis rate (HI) and fluidity (FI) in predicting the course of glycaemia (GI and/or GP) was also evaluated.

In paper I it was shown that the inclusion of 13% mwGG (dwb) in bread products increased GP and reduced GI compared to WWB. When comparing different rye

varieties, Visello resulted in promising results on both glycaemia and insulinaemia (Rosén *et al* 2011b). Furthermore, rye appeared to promote gut fermentative activity at an earlier time point after ingestion compared to other cereals (Grasten *et al* 2000, Nilsson *et al* 2008b). On the other hand, RS-rich HAM has been shown to increase the colonic fermentation at a somewhat later stage compared to rye (Li *et al* 2010, Topping & Clifton 2001). Thus, two test products containing mwGG in combination with either Visello rye flour (VG) or HAM (HG) were prepared and included in a meal study together with WWB. An *ad libitum* lunch meal was served 240 min after the breakfast where the subjects were allowed to eat until they felt pleasantly full. The amount of food eaten as well as any left-overs were recorded.

The low but sustained net increment in glycaemia (low GI/high GP) and insulinaemia by inclusion of a medium dose mwGG seen in paper I was confirmed. The effect on GI and II was of the same magnitude as for the medium dose mwGG bread in paper I (wgHiMG2). The expected lowering of GER was further validated by the reduced GIP-levels compared to after WWB-intake.

In line with paper I, there were effects on subjective appetite by the mwGG-containing bread products. Consequently, the intake of VG resulted in a significant decrease in *feeling of hunger* from breakfast to lunch compared to WWB. There were tendencies also for an increased *feeling of fullness* ($p = 0.10$) and decreased *desire to eat* ($p = 0.14$) after the VG breakfast. In addition, the HG breakfast tended to result in improved appetite ratings compared to the WWB. Furthermore, there was a borderline significance ($p_{meal} = 0.058$) in lowered voluntary energy intake after the VG breakfast compared to the WWB.

In addition to subjective appetite ratings, some biomarkers of appetite were included as objective measures of appetite regulation. PYY is an acute signal of satiety that rises after food intake and has been acknowledged as important in appetite regulation (Batterham & Bloom 2003, Cooper 2014). The present results showed that the *feeling of fullness* was positively correlated to plasma PYY-levels just before starting lunch, and negatively correlated to *feeling of hunger* and *desire to eat*. After the *ad libitum* lunch, PYY tAUC (240–360 min) was significantly higher after the VG breakfast compared to the WWB. Furthermore, PYY tAUC (240–360 min) was negatively correlated to subjective *feeling of hunger* after lunch. The overall mean for PYY (0–360 min) was higher after both VG and HG, compared to the WWB. The increased PYY-levels are likely a result of prolonged GER. The present study indicates that the inclusion of mwGG, rye and/or HAM could be useful for stimulation of endogenous production of PYY.

Another piece of evidence for improved appetite regulation by VG and HG is the fact that both reduced the relative increase, from nadir to 240 min, of the hunger hormone, ghrelin. Furthermore, the ghrelin level at 240 min was positively correlated to the energy intake at lunch. This is in line with research

acknowledging ghrelin as an acute hunger signal in the pre-prandial period (Müller *et al* 2015, Wren *et al* 2001). Furthermore, the difference in ghrelin from 240 min to nadir was correlated to both glycaemia and insulinaemia (iAUC 0–120 min), indicating that well-regulated glycaemia and insulinaemia could counteract oscillations in ghrelin and thus reduce hunger.

As markers of gut fermentation, breath H_2 excretion and plasma SCFA were studied. Breath H_2 increased after lunch following the VG breakfast and the increase was related to increased satiety and reduced hunger ratings after lunch (240–360 min). This is in line with a study where breath H_2 was stimulated by a prebiotic DF and linked to lowered hunger ratings (Cani *et al* 2009). The increase in breath H_2 was, however, not accompanied by an increase in plasma SCFA. The latter could possibly be due to formation of other fermentation products, *e.g.* lactate or succinate, which were not measured. It is also possible that the combination of mwGG and rye led to entrapment of the readily fermented rye fraction and, thus, delayed the SCFA production beyond the studied timespan (*i.e.* 360 min). In contrast, we found increased butyrate levels in serum as early as 4 h after the intake of the HG product and, to our knowledge, such an early increase has not been reported before. It is hypothesised that SCFAs act as a regulator of appetite through the gut-brain axis (Canfora *et al* 2015).

In the present study we found increased levels of both ghrelin and PYY directly after intake of the test products. It is possible that the test products can also result in effects in a later perspective as the intake of RS could have longer term systemic effects even though no acute effects are shown. As an example, 30 g of RS per day for a period of 4 weeks has been demonstrated to increase insulin sensitivity (Robertson *et al* 2005). The presence of RS-promoting ingredients, *e.g.* HAM, have been demonstrated to improve glucose metabolism beyond the effect on GI given by the co-formation of SDS (Bindels *et al* 2015), which is discussed in paper I above. A possible mechanism for that effect is increased formation of SCFAs by gut fermentation, which, in turn, could increase the formation of intestinal PYY and GLP-1. The increased insulin sensitivity after 4 weeks of RS supplementation is proposed to be a result of elevations in systemic concentrations of ghrelin and SCFAs (Robertson *et al* 2005).

At the time of lunch, NEFA level was significantly lower after the VG breakfast compared to the WWB. In case of the HG breakfast there was only a tendency of reduced NEFA levels. Interestingly, at the time of lunch NEFA level was positively correlated to GI and negatively correlated to GP. This is in line with previous studies demonstrating that a prolonged digestive phase suppresses the level of NEFAs in the later postprandial phase (Wolever *et al* 1995). The benefit of NEFA suppression is that it improves insulin sensitivity at the time of a subsequent meal (Wolever *et al* 1995).

The study design in this paper (III) does not allow separation of effects from mwGG and rye or HAM. However, considering that both HG and VG showed very similar results for glucose, insulin, ghrelin, GIP and PYY, it is reasonable to assume that the mwGG was mainly responsible for the effects. If the increase in SCFAs is caused by the presence of RS only, or by the combination of RS and mwGG, cannot be determined here. However, the lack of SCFA-increase after VG-intake indicates that it is not promoted by the mwGG *per se*.

Both HI and FI were strongly correlated to glucose and insulin responses (iAUC 0–120) and GP, which is in line with the results from paper I. HI was more strongly correlated to GP than to glucose iAUC, whereas the opposite was the case for FI.

To conclude, bread products containing mwGG and rye or HAM resulted in a low but sustained net increment in glycaemia and had an appetite-regulating potential. Judging from appetite ratings and PYY-levels, the combination of mwGG and rye seemed superior in reducing post-meal hunger. However, both products reduced the oscillations in ghrelin levels from breakfast to lunch compared to the WWB. The tendency of reduced energy intake at the subsequent *ad libitum* lunch is promising and deserves further investigation.

Paper IV

Sustained glycaemia at breakfast improve glucose tolerance at a high-carbohydrate lunch

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In paper IV, the purpose was to investigate whether two breakfast meals differing in course of glycaemia had different effects on second-meal glucose tolerance and subjective appetite ratings in healthy subjects.

Two breakfast meals produced from the same raw materials were included, WWB with high GI and low GP, and pasta (Pasta) with low GI and supposedly high GP. A standardised lunch meal was served 4 h after the start of the breakfast.

The Pasta breakfast generated the expected increase in GP and lowered GI, II as well as reduced glucose and insulin iPeaks, compared to WWB. Pasta also resulted in significantly lower overall insulin responses (-19%, 0–360 min) compared to the WWB. At the time of lunch (240 min) both glucose and insulin were higher after the Pasta breakfast than after the WWB, as a result of their late net

increments. This is probably a result of the reduced amylolytic availability of the starch in the Pasta, which is caused by a compact food structure (Granfeldt & Björck 1990). The incremental glucose response after the standardised lunch (*i.e.* normalised using the value at 240) was reduced by 47% after Pasta compared to the WWB (iAUC 240-360), and there was a tendency of reduced insulin response during the same time interval (-15%, $p = 0.07$). The reduced glucose and insulin responses after Pasta were associated with lower subjective ratings of *desire to eat* ($p = 0.004$) compared to the WWB breakfast. Furthermore, the sustained increment in late glycaemia after Pasta coincided with smaller oscillations in the NEFA levels, which suggest improved insulin sensitivity at the time of the second meal.

To conclude, the Pasta meal, resulting in lower GI/II, lower glucose and insulin iPeak as well as higher GP compared to the WWB, reduced incremental glycaemia at a standardised subsequent meal. The low and sustained net increment in glycaemia after the Pasta breakfast resulted in improved appetite, which was demonstrated by lowered overall ratings of *desire to eat* compared to the WWB.

General discussion

Tailoring postprandial course of glycaemia in healthy subjects

The high values obtained for GP in the present thesis indicate that the bread products increased the duration over fasting value and/or decreased the glucose iPeak on an individual level compared to the WWB. Thus, the addition of certain soluble DFs offers tools to tailor the course of glycaemia after bread intake. Data on glucose and insulin for the tested products are compiled in Table 6. Glucose iPeak was significantly reduced by 26 to 56% compared to WWB by the incorporation of 6–19% mwGG in bread (dwb) whilst the glucose iPeak reduction was 35 to 37% by the addition of 5–10% oat β-glucans. Although there was a tendency, the duration of the glucose response was not significantly increased by the addition of mwGG or oat β-glucans. This led to an increase in GP values from 73 to 227% by mwGG and from 43 to 66% by the addition of oat β-glucans, as compared to WWB. Furthermore, whereas no significant lowering of GI was found for 6% mwGG inclusion in bread, the GI was significantly reduced by 33 and 41% by the addition of 13 and 19% mwGG, respectively. In the case of oat β-glucans, the GI-values were reduced by 32–37% when added at levels between 5–10%.

Table 6
Responses of glucose and insulin after all products in papers I–IV.

Paper	Products	GI[a]	Glucose iPeak	GP[b]	II[a]	Insulin iPeak
		%	Δ mM	min/mM	%	Δ nM
I	WWB	100 a	2.7 ± 0.2 a	45 ± 6 a	100 a	0.17 ± 0.03 a
	wgHiM	107 ± 15 a	2.7 ± 0.4 a	53 ± 11 ab	94 ± 25 ab	0.15 ± 0.03 ab
	wgHiMG1	87 ± 11 ab	2.0 ± 0.3 b	109 ± 25 bc	87 ± 20 bc	0.10 ± 0.02 bc
	wgHiMG2	59 ± 10 c	1.2 ± 0.2 c	142 ± 26 c	38 ± 8 c	0.05 ± 0.01 c
	wgHiMG3	67 ± 10 bc	1.3 ± 0.2 bc	147 ± 15 c	36 ± 15 c	0.04 ± 0.01 c
II	WWB	100 a	4.3 ± 0.3 a	35 ± 5 a	100 a	0.35 ± 0.04 a
	OF1	64 ± 5 b	2.8 ± 0.3 b	52 ± 5 ab	71 ± 14 b	0.20 ± 0.04 b
	OF2	68 ± 5 b	2.9 ± 0.3 b	50 ± 5 ab	68 ± 14 b	0.23 ± 0.04 b
	OF3	63 ± 5 b	2.7 ± 0.3 b	58 ± 5 bc	61 ± 14 b	0.21 ± 0.04 b
III	WWB	100 a	3.2 ± 0.2 a	51 ± 10 a	100 a	0.35 ± 0.03 a
	HG	66 ± 6 b	1.9 ± 0.2 b	95 ± 10 b	44 ± 4 b	0.22 ± 0.03 b
	VG	61 ± 6 b	1.8 ± 0.2 b	88 ± 11 b	59 ± 4 c	0.25 ± 0.04 b
IV	WWB	100 a	2.8 ± 0.2 a	51 ± 12 a	100 a	0.21 ± 0.02 a
	Pasta	74 ± 6 b	1.9 ± 0.2 b	93 ± 12 b	53 ± 5 b	0.11 ± 0.02 b

WWB (white wheat bread), wgHiM (WWB with Hi-Maize® whole grain maize flour), wgHiMG1, wgHiMG2 and wgHiMG3 (wgHiM with 6%, 13% and 19% mwGG dry weight basis (dwb), respectively), BB (wheat-based bread with whole grain barley flour containing elevated amount of β-glucan), BF (wheat-based bread with barley fibre) OF1, OF2 and OF3 (wheat-based bread with refined oat β-glucan preparation in three different amounts), HG (bread containing HAM and mwGG), VG (bread containing whole grain rye (Visello) and mwGG).

Values are mean ± SEM (paper I) or LSMs ± SEM (paper II-IV). Products within each paper not sharing the same letters were significantly different, $p<0.05$ (ANCOVA followed by Tukey's test.

[a]0–120 min

[b]0–180 min

Both mwGG and oat β-glucans can be used in order to lower the iPeak, increase the duration and thereby increase the GP of bread products. The mwGG seems to cause a greater reduction in glucose iPeak compared to the OF β-glucan. The addition of mwGG to breads resulted in a very sticky crumb, giving a very slippery feel in the mouth when eaten. The OF β-glucan, on the other hand, did not affect the crumb texture negatively, and some subjects actually rated the OF β-glucan breads higher than the WWB due to its denser but still white, soft crumb.

It should be noted that the nutritional value of a bread supplemented with GG is different from breads produced from *e.g.* whole grains. Many studies have demonstrated that the consumption of whole grains improve health, probably due to its high content of nutrients and phytochemicals (Slavin 2004). Modulation of

viscosity by the use of GG can certainly also be done in whole grain based products.

Glycaemic profile (GP) as estimate of course of glycaemia

The glycaemic response to a meal may vary between subjects due to several factors, *e.g.* age, sex, ethnicity, BMI, insulin sensitivity and β-cell function (Wolever *et al* 2015). However, as the GI is a comparison between the glycaemic response of the test and reference food in individuals, the differences mentioned are considered to be evened out (Wolever *et al* 2015). The same factors are also likely to influence the iPeak and duration and thus the measure of GP.

So far, GP-values using WWB as the reference have been published in 8 studies, resulting in values for more than 50 separate meals, see Table 7. The GP-values for WWB range from 35 to 51 (*i.e.* 30% variability) and it is thus questionable whether GP-values can be compared between studies. Recently, Greffeuille *et al* (2015) published GP data for three different types of pasta, whereof two contained faba-bean. They used glucose solution as reference, which resulted in GP 35. This is in line with the GP obtained for WWB in paper II. The different pasta products had significantly higher GP values ranging from 56 to 66, while the pasta in paper IV resulted in GP 93.

Rye products have been found to reduce II but not GI values (Juntunen *et al* 2003) (Leinonen *et al* 1999). In the work by Rosén *et* al (Rosén *et al* 2009, Rosén *et al* 2011a, Rosén *et al* 2011b, Rosén *et al* 2011c) it was investigated whether differences between the glycaemic response to rye and wheat products were better described using GP instead of GI. Several of the rye products tested induced low but sustained incremental glycaemia, leading to unfairly high GI values. In these cases, the use of GP could be used to discriminate the different outcomes from each other. In the present work, the purpose instead was to increase the GP of a product as much as possible. In the work by Rosén *et al*, GP was measured for the time period 0–180 min (Rosén *et al* 2009, Rosén *et al* 2011b, Rosén *et al* 2011c) or 0–270 min (Rosén *et al* 2011a). In papers I and II, GP was measured for the time period 0–180 min but, in papers III and IV, it was possible also to calculate GP for 0–240 min. In cases where the glucose value remained above fasting value for the whole time period, the duration was set to 180 or 240 min, respectively. Using the time period 0–240 min instead of 0–180 min in papers III and IV resulted in the same significant differences between test and reference meals, even though marginally higher values were obtained (5–20% increase). Based on these results, it seems to be of minor importance whether the time period 0–180 min or 0–240 min is used for the calculation of GP for bread and pasta.

Indexed glycaemic profile (GPI)

In order to facilitate comparisons between studies, an indexed glycaemic profile (GPI) was created and calculated as the GP for WWB divided by the GP for the

test product taken by the same subject. GPI was thus calculated for each subject, multiplied by 100 and then presented as a mean of all individual values. GPI inverses the concept of GP, and indexes it by taking GP^{-1}_{test}/ GP^{-1}_{ref} to make the result more easily comparable with GI. This is equivalent to taking GP_{ref} /GP_{test}. With this definition, it could be expected that individual variations in glycaemic excursions will be evened out, as for GI.

GPI will be 100 for the reference product, and a lower value is the result of a reduced iPeak and/or extended duration over the fasting value for the meal compared to the reference product in an individual. A value of GPI significantly lower than 100 will thus be regarded as more beneficial than a value close to 100.

In Table 7 the GPI has been calculated for all products in papers I–IV as well as those presented by Rosén el al. In addition, mean GPI-values have been correlated to II, appetite ratings and *in vitro* measures (HI and II). For the papers in this thesis, the GPI ranges from 35 to 103 and, for the work by Rosén, from 54 to 126.

For the work in this thesis, GPI was better correlated to II than either GI or GP. Furthermore, GPI was similarly or better correlated to appetite scores than GP.

In the work by Greffeuille *et al* (2015), mentioned above, a glycaemic profile index was also calculated. They had, however, inverted the numbers in the equation, *i.e.* they divided the GP_{test} by GP_{ref}. As a result they considered a higher value to indicate a better course of glycaemia. Their inverted result yields GPI.

The use of GPI has advantages compared to GP, when evaluating postprandial course of glycaemia. The possibility to compare values between different studies is an obvious advantage. Furthermore, the indexation also strengthens the comparative value within a study. The use of GPI deserves further attention as a complement to GI. It would be interesting to determine GPI for a broader range of products, and to evaluate its possible relations to acute and semi-acute physiological variables as well as longer-term effects in humans.

To conclude, both mwGG and oat β-glucans can be used to lower the iPeak, increase the duration and thereby increase the GP, or reduce the GPI, of bread products. The mwGG seems to cause a more prominent reduction in glucose iPeak compared to the OF β-glucan. As judged from general appearance, the addition of mwGG to breads resulted in a sticky crumb, giving a very slippery feel in the mouth when eaten. The OF β-glucan, on the other hand had not this negative effect on the crumb texture, some subjects actually appreciated the OF β-glucan breads more than the WWB due to its denser but still white and soft, crumb.

It should be noted that the nutritional value of a bread supplemented with GG is different from breads produced from *e.g.* whole grains. The consumption of whole grains has been demonstrated to be protective against cancer, CVD, T2DM and obesity in many studies, probably due to its high content of nutrients and phytochemicals (Slavin 2004). Modulation of viscosity by the use of GG should therefore preferably also be done in whole grain based products.

Table 7
Compilation of published data for GI, GP, iAUC (0-120 min)§, glucose iPeak and indexed GP (GPI).

Paper	Product	GI	GP	GPI	iAUC	Glucose iPeak
(Rosén *et al* 2009)	WWB^{+MG}	100 a	37 ± 6 b	100 ab	168 ± 18 a	3.3 ± 0.3 a
	WWP (white wheat porridge)	77 ± 10 ab	35 ± 5 b	124 ± 26 a	119 ± 13 ab	3.1 ± 0.2 ab
	ERB (endosperm rye bread)	64 ± 8 b	69 ± 10 a	59 ± 8 b	104 ± 16 b	2.1 ± 0.2c
	ERP (endosperm rye porridge)	70 ± 6 b	50 ± 6 ab	81 ± 11 ab	103 ± 8 b	2.5 ± 0.1 bc
	WGRB (wg rye bread)	71 ± 10 ab	51 ± 7 ab	75 ± 6 ab	119 ± 22 ab	2.5 ± 0.3 bc
	WGRB-lac (WGRB with lactic acid)	74 ± 10 b	74 ± 10 a	54 ± 8 b	114 ± 11 b	2.2 ± 0.2 c
	WGRP (wg rye porridge)	72 ± 10 b	40 ± 7 b	108 ± 14 ab	110 ± 14 b	2.7 ± 0.2 abc
	RBB (rye bran bread)	87 ± 7 ab	36 ± 3 b	112 ± 19 a	147 ± 23 ab	3.3 ± 0.3 a
(Rosén *et al* 2011a)	WWB	100 a	49 ± 7 b	100 a	212 ± 25 a	3.9 ± 0.4 a
	ERB (endosperm rye bread)	77 ± 8 ab	59 ± 10 ab	100 ± 4 a	160 ± 19 ab	3.2 ± 0.3 ab
	ERB-lac (ERB with lactic acid)	64 ± 9 b	78 ± 9 ab	98 ± 3 a	136 ± 24 b	2.5 ± 0.3 b
	WGRB (wg rye bread)	79 ± 14 ab	75 ± 13 ab	101 ± 4 a	148 ±19 ab	2.7 ± 0.2 b
	WGRB-lac (WGRB with lactic acid)	64 ± 7 b	65 ± 9 ab	97 ± 4 a	132 ± 18 b	2.6 ± 0.2 b
	RK boiled rye kernels	73 ± 8 ab	94 ± 13 a	100 ± 2 a	151 ± 23 b	2.5 ± 0.3 b
	WK boiled wheat kernels	68 ± 9 ab	51 ± 7 b	100 ± 3 a	145 ± 24 b	3.0 ± 0.4 b
(Rosén *et al* 2011c)	WWB^{+MG}	100 a	47 ± 3 a	100 a	220 ± 25 a	3.7 ± 0.3 a
	wg rye bread, var. D. Zlote	96 ± 9 a	40 ± 3 a	123 ± 12 a	202 ± 23 a	3.7 ± 0.3 a
	wg rye bread, var. H. Loire	96 ± 10 a	46 ± 3 a	112 ± 13 a	193 ± 18 a	3.5 ± 0.2 a
	wg rye bread, var. Nikita	91 ± 11 a	42 ± 5 a	122 ± 11 a	185 ± 25 a	3.5 ± 0.3 a
	wg rye bread, var. Rekrut	84 ± 7 a	41 ± 3 a	126 ± 15 a	171 ± 17 a	3.5 ± 0.2 a
	wg rye bread, var. Amilo	79 ± 5 a	50 ± 6 a	118 ± 21 a	170 ± 22 a	3.1 ± 0.3 a

Paper	Product	GI	GP	GPI	iAUC	Glucose iPeak
(Rosén *et al* 2011b)	WWB	100 a	42 ± 3 a	100 a	208 ± 15 a	3.8 ± 0.2 a
	wg rye bread, var. Amilo	90 ± 8 ab	54 ± 7 a	87 ± 7 a	176 ±13 ab	3.1 ± 0.2 b
	wg rye bread, var. Evolo	92 ± 8 ab	53 ± 5 a	85 ± 6 a	177 ± 12 ab	3.2 ± 0.2 b
	wg rye bread, var. Kaskelott	88 ± 9 ab	48 ± 4 a	94 ± 8 a	174 ± 14 ab	3.2 ± 0.2 ab
	wg rye bread, var. Picasso	80 ± 8 b	52 ± 5 a	85 ± 6 a	159 ± 16 a	2.9 ± 0.2 b
	wg rye bread, var. Visello	79 ± 8 b	60 ± 10 a	84 ± 10 a	152 ± 13 a	2.9 ± 0.2 b
	wg rye bread, commercial blend	95 ± 8 ab	48 ± 7 a	105 ± 11 a	188 ± 14 ab	3.4 ± 0.2 ab
I	WWB	100 a	45 ± 6 a	100 a	126.4 ± 16 a	2.7 ± 0.2 a
	wgHiM	107 ± 15 a	53 ± 11 ab	102 ± 14 a	130.7 ± 26 a	2.7 ± 0.4 a
	wgHiMG1	87 ± 11 ab	109 ± 25 bc	60 ± 14 b	113.4 ± 19 ab	2.0 ± 0.3 b
	wgHiMG2	59 ± 10 c	142 ± 26 c	39 ± 6 b	71.5 ± 12 c	1.2 ± 0.2 c
	wgHiMG3	67 ± 10 bc	147 ± 15 c	35 ± 7 b	87.3 ± 16 bc	1.3 ± 0.2 bc
II	WWB	100 a	35 ± 5 a	100 ab	248 ± 21 a	4.3 ± 0.3 a
	OF1	64 ± 5 b	52 ± 5 ab	75 ± 9 bc	155 ± 21 b	2.8 ± 0.3 b
	OF2	68 ± 5 b	50 ± 5 ab	78 ± 12 abc	159 ± 21 b	2.9 ± 0.3 b
	OF3	63 ± 5 b	58 ± 5 bc	64 ± 7 c	159 ± 21 b	2.7 ± 0.3 b
III	WWB	100 a	51 ± 10 a	100 a	175.7 ± 20 a	3.2 ± 0.2 a
	HG	66 ± 6 b	95 ± 10 b	61 ± 10 b	114.1 ± 16 b	1.9 ± 0.2 b
	VG	61 ± 6 b	88 ± 11 b	64 ± 9 b	106.5 ± 17 b	1.8 ± 0.2 b
IV	WWB	100 a	51 ± 12 a	100 a	144.5 ± 16 a	2.8 ± 0.2 a
	Pasta	74 ± 6 b	93 ± 12 b	73 ± 10 b	104.6 ± 15 b	1.9 ± 0.2 b

WWB^{MG+}, the same as WWB except for the addition of 2% monoglycerides (flour basis), wg (whole grain). Detailed description of the products studied in the work by Rosén *et al* can be found in the respective publication.

Appetite in relation to course of glycaemia

The findings of the present thesis suggest that improved postprandial course of glycaemia is associated with improved appetite regulation. Consequently, four out of five of the bread products containing mwGG (papers I and III) resulted in improved subjective appetite ratings in the period from breakfast to lunch, compared to WWB. This is in line with previous research where the addition of 2.5 g of GG (medium viscosity) to a meal taken three times a day has been shown to prevent increase in appetite, hunger and desire to eat (Kovacs *et al* 2001) and to increase satiety (Kovacs *et al* 2002). It has also been suggested that the satiating potential of GG causes improvement in weight management (Butt *et al* 2007).

In paper III, some relevant appetite biomarkers were also measured. The level of ghrelin at the time of lunch (240 min) was positively correlated to the voluntary energy intake at lunch. This is in line with a previous report (Erdmann *et al* 2004) and thus constitutes another piece of evidence that ghrelin may assist in understanding changes in energy intake. Interestingly, increased *desire to eat* after lunch (tAUC 240–360 min) coincided with increased levels of ghrelin after lunch (iAUC 240–360). The correlation between the ghrelin recovery (nadir to 240 min) and glycaemia and insulinaemia (iAUC 0–120 min), respectively, is also noteworthy, indicating that well-regulated glycaemia and insulinaemia could counteract oscillations in ghrelin and thus reduce hunger. A similar relation was previously reported for rye products by Rosén *et al* (2011a).

The improvement in appetite regulation by the mwGG-containing bread products was also supported by the changes in PYY-levels, which were negatively correlated to *feeling of hunger* after the *ad libitum* lunch meal (tAUC 240–360 min). It has previously been reported that intravenous infusion of PYY reduces appetite and energy intake (Batterham & Bloom 2003). The effect of meal-stimulated PYY is less studied although a correlation between subjective *feeling of hunger* and plasma PYY has previously been found (Cooper 2014).

The results on circulating levels of ghrelin and PYY support the results obtained from the subjective appetite measures. In another study on β-glucan-enriched bread, consistency was found between subjective appetite ratings and alterations in levels of ghrelin and PYY (Vitaglione *et al* 2009), but this has not been found in all studies. It has been found, however, that results from subjective appetite ratings are very often confirmed by the measured energy intake (de Graaf *et al* 2004). The VG breakfast in paper III led to a borderline significant decrease in voluntary intake at a subsequent *ad libitum* lunch (-7% compared to the WWB, $p = 0.058$). Rye kernels have previously been found to improve satiety ratings and decrease voluntary food intake at a subsequent lunch (Rosén *et al* 2011a). The VG bread and the rye kernels had similar characteristics for GI, II and GP, although the VG

bread had a lower GPI compared to the rye kernels, (61 and 100, respectively). None of the studies however, had voluntary food intake as its main outcome and was therefore not sufficiently powered for this variable. This fact can be the reason for the different outcomes in the two studies. It has been suggested that at least 26 subjects are needed to detect a difference in energy intake of 500 kJ with a power of 0.8 if a paired design is used (Gregersen *et al* 2008). Papers I and II had postprandial glycaemia and insulinaemia as primary study outcome and the number of test subjects was determined to give enough statistical power regarding these measures ($n \geq 10$) (Brouns *et al* 2005). Postprandial appetite scores need a larger number of participants ($n \geq 18$) in order to have enough power to reveal a 5 mm difference in mean values (Flint *et al* 2000).

Mechanisms of relevance for improved appetite

Foods giving lower glycaemic excursions have been shown to induce higher satiety compared to those giving higher glycaemic excursions (Bornet *et al* 2007). This effect is probably caused by differences in digestion and thus absorption, where prolonged contact with nutrients in the small intestine leads to increased release of satiety signals, *e.g.* GLP-1. Previously, it has been shown that pasta (Liljeberg & Björck 2000), as well as lactic acid containing bread (Östman *et al* 2002a) offers a second-meal effect on glucose tolerance, but the addition of vinegar to WWB (Liljeberg *et al* 1999a) does not. All three examples give rise to a low GI, but the effect caused by acetic acid comes from a delayed gastric emptying and not reduced amylase activity, which is the case for the two other products. The latter creates a low iPeak but a sustained increment in the late glycaemia (*i.e.* a high GP). Interestingly, the addition of acetic acid influences appetite positively. Often, food items giving rise to reduced glycaemic excursions are higher in fibre and it has been debated whether the effect on satiety results from the lower postprandial glycaemia *per se* or from the presence of fibres. In paper IV, the pasta breakfast led to improved appetite ratings (*i.e.* lower *desire to eat*). The effect on appetite was not a result of dietary fibres as both the reference bread and the pasta were manufactured from the same low fibre ingredient (*i.e.* wheat flour). The effect on ghrelin and PYY given by bread products resulting in prolonged digestion (paper III) is discussed above. Furthermore, it is possible that RS from wgHiM (paper I) or HAM (paper III) can influence appetite by increasing the levels of gut hormones. In rats, it has been demonstrated that the continuous addition of RS to the diet increases the endogenous levels of PYY and GLP-1 (Keenan *et al* 2006).

When combining appetite data from papers I, III and IV, significant but weak correlations were obtained between all glycaemic and insulinaemic parameters studied, with the exception of *feeling of hunger* and GP, see Table 8.

In paper III, correlations were also observed between increased breath H_2 and increased *feeling of fullness* and decreased *feeling of hunger* after lunch. However,

there were no correlations between breath H_2 and voluntary energy intake. Colonic fermentation has been proposed to affect appetite as increased concentrations of SCFAs could stimulate the colonic L-cells to produce PYY and GLP-1 (Cani *et al* 2009). It is possible that the systemic effects of appetite resulting from fermentation in paper III are delayed beyond the studied time frame.

Table 8
Correlations for subjective appetite versus measures of glycaemia and insulinaemia.

Paper		***Feeling of fullness*[a]**		***Feeling of hunger*[a]**		***Desire to eat*[a]**	
		r	P	r	p	r	p
I	GI	**-0.43**	**0.001**	0.24	0.090	**0.36**	**0.009**
	GP	**0.44**	**0.001**	-0.18	0.205	-0.267	0.053
	GPI	**-0.42**	**0.002**	0.19	0.164	**0.28**	**0.039**
	Glucose iPeak	**-0.50**	**< 0.001**	0.26	0.063	**0.33**	**0.018**
	II	**-0.44**	**0.001**	**0.48**	**< 0.001**	**0.50**	**< 0.001**
	Insulin iPeak	**-0.48**	**< 0.001**	**0.36**	**0.008**	**0.33**	**0.017**
III	GI	-0.24	0.072	0.24	0.074	**0.27**	**0.046**
	GP	0.24	0.074	-0.14	0.322	-0.24	0.072
	GPI	-0.25	0.070	0.18	0.198	**0.29**	**0.035**
	Glucose iPeak	-0.24	0.072	**0.32**	**0.016**	**0.38**	**0.004**
	II	-0.19	0.165	0.08	0.560	0.16	0.239
	Insulin iPeak	-0.11	0.429	0.216	0.114	0.22	0.114
IV	GI	-0.10	0.545	0.30	0.064	**0.40**	**0.012**
	GP	0.26	0.115	0.15	0.350	**-0.56**	**< 0.001**
	GPI	-0.26	0.115	-0.15	0.350	**0.56**	**< 0.001**
	Glucose iPeak	-0.26	0.115	-0.15	0.350	**0.56**	**< 0.001**
	II	-0.10	0.545	0.10	0.55	**0.60**	**< 0.001**
	Insulin iPeak	-0.10	0.545	0.10	0.55	**0.60**	**< 0.001**
I, III and IV	GI	**-0.28**	**0.001**	**0.29**	**< 0.001**	**0.38**	**< 0.001**
	GP	**0.42**	**< 0.001**	-0.08	0.365	**-0.20**	**0.014**
	GPI	**-0.28**	**< 0.001**	**0.22**	**0.007**	**0.35**	**< 0.001**
	Glucose iPeak	**-0.34**	**< 0.001**	**0.29**	**< 0.001**	**0.39**	**< 0.001**
	II	**-0.27**	**0.001**	**0.39**	**< 0.001**	**0.46**	**< 0.001**
	Insulin iPeak	**-0.28**	**0.001**	**0.35**	**< 0.001**	**0.36**	**< 0.001**

Spearman's partial correlation coefficients controlling for subjects and corresponding baseline values, p values < 0.05 were regarded as significant. Significant correlations are shown in bold text.

[a]Considers tAUC 0–180 min (paper I and II) or tAUC 0-240 min (paper III and IV).

Subjective satiety was also measured in paper II, but no differences were found between any of the meals and the results are not reported. The use of β-glucan in bread has previously demonstrated effects on appetite and reduced energy intake at a subsequent meal (Cloetens *et al* 2012). Paper II was powered for measurement of postprandial glycaemia and insulinaemia, which could explain the lack of effect on subjective appetite.

Food intake is a complex process that is driven by metabolic processes as well as personal liking and external stimuli (Blundell *et al* 2010). A limitation of the studies within this thesis is that the subjects were not asked to rate the palatability of the test products, and the statistical calculations cannot therefore be corrected for differences in palatability that may have affected the subsequent satiety (De Graaf *et al* 1999).

The present meal studies demonstrated acute effects on appetite. The potential effect elicited by the tested bread products on subjective appetite and voluntary food intake must be further evaluated in longer term studies. It is possible that lower caloric intake at one meal can be compensated for at one or more subsequent meals, which would counteract any possible effect on *e.g.* weight maintenance (Almiron-Roig *et al* 2013). Reduced weight is, however, not necessary for substantial improvements in metabolic parameters after intake of a multifunctional diet (Tovar *et al* 2015).

Prediction of course of glycaemia using *in vitro* methods

The availability of reliable prediction methods could be expected to facilitate development of food products with health merits. In papers I–III, the *in vitro* measurements of starch hydrolysis rate (HI) and fluidity (FI), respectively, were correlated with different measures describing course of glycaemia (*i.e.* GI, glucose iPeak, GP and GPI). The correlations are presented in Table 9.

Table 9
Correlations for HI and FI versus measures of glycaemia and insulinaemia (Papers I–III).

Paper		**HI**		**FI**	
		r	p	r	p
I-III	GI	0.62	< 0.001	0.62	< 0.001
	GP	-0.16	0.045	-0.06	0.426
	GPI	0.67	< 0.001	0.65	< 0.001
	Glucose iPeak	0.70	< 0.001	0.63	< 0.001
	II	0.74	< 0.001	0.64	< 0.001
	Insulin iPeak	0.52	< 0.001	0.45	< 0.001

Spearman's partial correlation coefficients controlling for subjects and corresponding baseline values, p-values < 0.05 were regarded as significant.

The HI and FI methods are both mimicking important steps in the *in vivo* digestion of foods. Whereas the HI method gives an estimation of starch bioavailability and diffusivity (Granfeldt *et al* 1992), the FI-method estimates the fluidity after removing starch-induced viscosity (Östman *et al* 2006). None of the methods

captures effects on GER. Furthermore, the FI method is relevant only in the case of viscosity-mediated effects on glycaemia.

Both HI and FI were significantly correlated to all measures of glycaemia and insulinaemia using Spearman's partial correlation. Based on the r-values obtained in the pooled correlations, it seems as though both HI and FI could be useful in the prediction of glycaemia, with the exception of GP, for the studied products. HI has previously been studied for a broader range of products (Granfeldt *et al* 1992) and can therefore at this point be considered more robust and allows better predictions compared to FI.

The relations between mean values of different measures of glycaemia (GI, GP, and GPI) and HI or FI for the bread products studied in papers I–III were studied in order to be able to make equations for prediction, see Fig. 3. For the present data, HI and FI were better at predicting GI compared to GP and GPI. Higher R^2-values were found when using FI rather than HI. The correlations using the pooled data contain much more information as every individual value is included in the calculations. Tests were made to see whether a regression equation including both FI and HI for the bread products studied in papers I–III would make the predictions stronger and more accurate. The regression coefficient statistics indicated, however, that the inclusion of both measures did not improve the predictive value compared to when only one of the parameters was included.

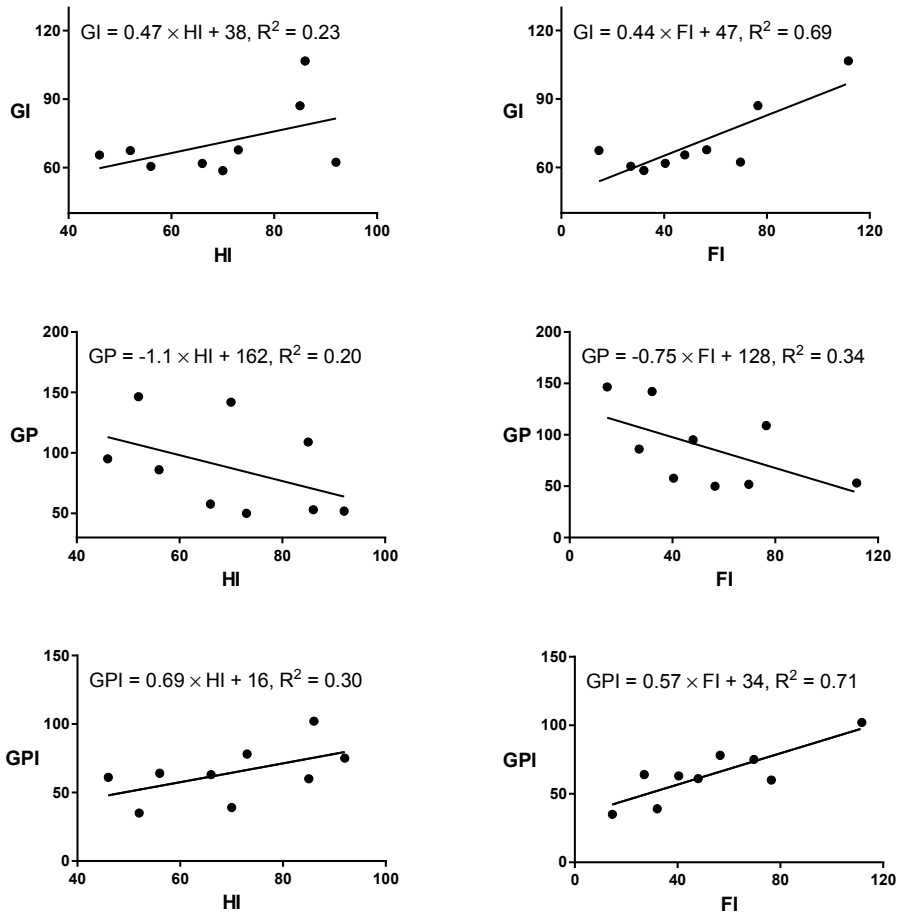

Figure 3
Relationship between different measures of glycaemia (GI, glucose iPeak, GP, and GPI) and HI or FI for the bread products studied in papers I–III. Relations involving GP will have different direction compared to those involving GI and GPI.

Data for HI, FI, predicted GI and GPI are found in Table 10, together with actual GI and GPI obtained in papers I–III.

Table 10
HI, FI, and predicted GI together with the actual GI values obtained in papers I–III. Predicted values were calculated as described by Leeman *et al (2005)* and using relations with FI as displayed in Fig. 3.

Paper	Products	HI	FI	Pred GI Leeman *et al*	GI predicted from HI	GI predicted from FI	GI
I	WWB	100 a	100 a	-	-	-	100 a
	wgHiM	86 ± 2 b	112 b	85	78	96	107 ± 15 a
	wgHiMG1	85 ± 9 bc	76 c	84	78	80	87 ± 11 ab
	wgHiMG2	70 ± 3 c	32 d	70	71	61	59 ± 10 c
	wgHiMG3	52 ± 2 d	15 e	54	62	54	67 ± 10 bc
II	WWB	100 a	100 a	-	-	-	100 a
	OF1	92 ± 3 a	70 b	90	81	78	64 ± 5 c
	OF2	73 ± 4 b	57 cd	73	72	72	68 ± 5 bc
	OF3	66 ± 3 b	40 e	66	69	65	63 ± 5 bc
III	WWB	100 a	100 a	-	-	-	100 a
	HG	46 ± 2 b	48 b	48	60	68	66 ± 6 b
	VG	56 ± 3 b	27 c	57	64	59	61 ± 6 b

WWB (white wheat bread), wgHiM (WWB with Hi-Maize® whole grain maize flour), wgHiMG1, wgHiMG2 and wgHiMG3 (wgHiM with 6%, 13% and 19% mwGG dry weight basis (dwb), respectively), BB (wheat-based bread with whole grain barley flour containing elevated amount of β-glucan), BF (wheat-based bread with barley fibre) OF1, OF2 and OF3 (wheat-based bread with refined oat β-glucan preparation in three different amounts), HG (bread containing HAM and mwGG), VG (bread containing whole grain rye (Visello) and mwGG).

Values are means ± SEM, products not sharing the same letter were significantly different within each paper, $p < 0.05$ (ANOVA (HI and FI) or ANCOVA (GI) followed by Tukey's test). Differences for FI consider the flowing distance after 60 s ($n = 2$)

Taken together, the thesis results indicate that the two *in vitro* methods HI and FI can be used to facilitate the decision regarding which products deserve further investigation during development of low glycaemic bread products. Both HI and FI were found to be correlated to the glycaemic response after intake. HI has been studied for a greater range of products and therefore HI seems more robust and useful. It should, however, be pointed out that promising results from either the HI or FI methods are no guarantee for effects on glycaemia. In the present work, the wgHiM bread (paper I) had significantly lower HI compared to WWB but gave no effect on glycaemia, and the OF1 bread (paper II) had an effect on glycaemia despite there being no significant reduction in HI.

Conclusions

Both guar gum and oat β-glucans can be used to tailor the course of glycaemia after bread intake. The inclusion of medium weight guar gum (mwGG) in bread products resulted in a dose-dependent lowering of both postprandial glycaemia and insulinaemia. In contrast, a low level of high MW β-glucan elicited a lowering effect on postprandial glycaemia and insulinaemia, and the addition of more did not lead to further benefits. The use of mwGG increased the glycaemic profile (GP) by 140–230% compared to WWB, while the use of the oat β-glucan ingredient led to more moderate increases (43–66%). This indicates that not only the amount, but also the quality of the DF is of importance. The increase in GP was higher than that which has earlier been obtained for rye bread. Furthermore, an indexed glycaemic profile (GPI) has been introduced, with advantages compared to GP. GPI makes it possible to compare values obtained in different studies.

Bread products with a low but sustained net increment in glycaemia improved acute and semi-acute appetite compared to WWB. However, the appetite-regulating effects differed slightly depending on whether mwGG was combined with whole grain rye or high amylose starch. A combination of mwGG with whole grain rye had the strongest appetite-regulating potential as judged from decreased overall *feeling of hunger* from breakfast to lunch, as well as increased levels of the satiety peptide PYY after the subsequent *ad libitum* lunch. Furthermore, the mwGG/rye bread reduced the oscillations in ghrelin levels from breakfast to lunch, which is also considered to be a desirable characteristic to avoid hunger at the time of a subsequent meal. The latter was also true for the bread with mwGG and high amylose starch. The HG product led to increased levels of serum SCFA, more specifically serum butyrate, already 4 h after breakfast. This is interesting since colonic fermentation has been shown to modulate appetite (Cani *et al* 2009). Although the combination of rye and mwGG had the more pronounced effect on appetite, with effects on both subjective appetite ratings and biomarkers of appetite, high amylose starch and mwGG also have interesting features.

White wheat-based bread is acknowledged for its comparatively high postprandial glycaemic excursions. However, when instead processing white flour into pasta, the resulting product displays reduced glycaemic excursions, both acute and after a second meal. This was demonstrated when comparing WWB and pasta, and demonstrates the importance of considering the preparation process.

For the bread products studied in papers I–III, both the measure of starch hydrolysis rate (HI) and fluidity (FI) were related to the course of glycaemia measured as GI or GPI. The use of HI seems to be more useful in *e.g.* product development, as its results are valid for a broader group of starch-containing products such as breads (based on wheat, barley, rye or maize), pasta, rice and legumes.

Future perspectives

In order to further evaluate the impact of postprandial course of glycaemia in healthy subjects on metabolic outcome and appetite regulation, both semi-acute and longer-term studies are needed. These should preferably include more extensive evaluations of metabolic risk markers *e.g.* inflammatory markers (*e.g.* IL-6, IL-18, adiponectin), hormones involved in appetite regulation (*e.g.* OXM, ghrelin, PYY, GLP-1), GLP-2 and NEFA. There is convincing evidence that low GI and/or low glycaemic load (GL) diets reduce the risk of T2DM and coronary heart disease. For the new characterisation measures GP and GPI, their effect in relation to major health outcomes is not known and needs to be studied. The appetite-regulating potential of food items providing a low but sustained net increment in glycaemia seen in the present thesis is interesting, with possible effects on food intake and weight maintenance, and possibly weight loss. This possibility is an interesting field for further studies.

When it comes to GPI as a predictor of overall course of glycaemia, further work needs to be done in order to evaluate its potential. For example, a broader range of carbohydrate rich products should be studied. This extended work should preferably include use of *in vitro* prediction models such as HI and FI, as the predictive value of these methods could then be explored simultaneously.

For future studies aiming to evaluate the impact of course of glycaemia on metabolic outcomes and appetite, it is important to have access to a large variety of consumer-friendly products. In order to ease product development, tools for fast and reliable product optimisation are needed. Future research should focus on different quality aspects of DF, *e.g.* the β-glucan MW and the viscosity-raising potential of different guar gum preparations. This should ideally be done in parallel with the development of products, so that parameters of importance for the effect postprandial glycaemia are maintained throughout the processing.

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PAPER

Linda M. N. K. Ekström *et al.*

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On the possibility to affect the course of glycaemia, insulinaemia, and perceived hunger/satiety to bread meals in healthy volunteers

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Linda M. N. K. Ekström,* Inger M. E. Björck and Elin M. Östman

Frequent hyperglycaemia is associated with oxidative stress and subclinical inflammation, and thus increased risk of cardiovascular disease. Possibilities of modulating glycaemia, insulinaemia and perceived satiety for bread products were investigated, with emphasis on the course of glycaemia expressed as a glycaemic profile (defined as the duration of the glucose curve above the fasting concentration divided by the incremental glucose peak). For this purpose white wheat bread was supplemented with whole grain corn flour with an elevated amylose content and different types and levels of guar gum. The bread products were characterised *in vitro* for release of starch degradation products and content of resistant starch. Fibre related fluidity following enzyme hydrolysis was also studied. By combining medium weight guar gum and whole grain corn flour with an elevated amylose content, the course of glycaemia, insulinaemia and subjective appetite ratings were improved compared to the reference white wheat bread. In addition, the combination beneficially influenced the content of resistant starch. Fluidity measurements showed potential to predict the glycaemic profile.

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Introduction

Frequent postprandial episodes of elevated blood glucose are associated with oxidative stress and subclinical inflammation, factors that both increase the risk of developing type 2 diabetes (T2D) and cardiovascular diseases (CVD).[1] The type and composition of food products affect the metabolic responses. Low glycaemic index (GI) foods are characterised by a slow digestion and/or absorption of the carbohydrate moiety which lowers the postprandial blood glucose and insulin responses.[2] Observational studies have indicated that a diet rich in low GI food is associated with lowered inflammation[1,2] and reduced risk for developing CVD,[3] thus with potential benefit adjunct to T2D. Interestingly, low GI foods have been shown to be less prone to trigger acute inflammation in healthy young subjects, using nuclear factor-κB as a marker.[4] This indicates that postprandial metabolism and avoidance of elevated postprandial glycaemia may be advantageous. Examples of food features that may be exploited are enclosure of intact cereal grains, whole grain rye flour and various dietary fibres (DF) to the products, since they are known to lower acute postprandial glycaemic and/or insulinaemic responses.[5]

The GI is an established concept of ranking carbohydrates according to their blood glucose raising potential in the 2 h postprandial period. As a means to describe the glycaemia also in the later postprandial phase (beyond 120 min) the glycaemic profile (GP) was recently introduced and defined as the duration of the glucose curve above the fasting concentration divided by the incremental glucose peak (iPeak).[6] A high GP has been associated with less postprandial hypoglycaemia and lower insulin response, as well as with improved appetite regulation.[7] A low but sustained net increment in blood glucose has also shown to reduce glycaemia after a subsequent standardised meal.[8] Furthermore, combining low GI features with a high content of resistant starch (RS) and DF in a meal have shown to improve the glucose tolerance in a 10 h perspective.[9]

One mechanism that can be used to prolong the digestion and absorption phase and increase the GP of bread products is the viscosity introduced by certain DF.[10] Guar gum (GG), isolated from the Indian cluster bean (*Cyamopsis tetragonoloba*), is an example of such a water-soluble DF with documented glucose- and insulin lowering properties.[11] Doses in the range of 1.8 to 15 g of GG have been reported to reduce the postprandial glycaemic response to a carbohydrate meal, but there are also studies where no effect has been found.[12] The molecular weight and size distribution of the galactomannans in the GG-preparation affect their rheological properties[13] and enzymatic treatment leading to partial hydrolysation reduces viscosity. However, also hydrolysed GG has been shown to reduce the GI of white bread,[14] indicating that effects other than the viscosity may add to the acute benefits seen on glucose metabolism. One strategy to predict the glycaemic responses to products supplemented with viscous DF is to measure the physiologically

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relevant viscosity; that is omitting the viscosity caused by digestible nutrients such as starch. Hence, measurement of fluidity index (FI) of breads containing barley β-glucans correlated very well with measured GI-values; the lower the FI the lower the GI.[15]

By definition, RS is not digested in the upper gastrointestinal tract, instead it is used as substrate by the colonic microbiota.[16] However, in parallel with the formation of RS an intermediate fraction of slowly digestible starch appears to be formed.[17] This starch fraction will be completely hydrolysed in the intestines but during a longer time span than readily digestible starch.

The purpose of the present work was to investigate possibilities to tailor the course of glycaemia, and increase the RS content of bread products. For this purpose whole grain corn flour with elevated amylose content was combined in wheat flour bread with various forms and levels of GG; medium/low molecular weight (mwGG/lwGG) or hydrolysed (hGG). The products were evaluated *in vitro* for the RS content, fluidity and rate of starch hydrolysis, and the most promising ones were included in a following meal study with healthy subjects. Glycaemia, insulinaemia and appetite ratings were evaluated in the postprandial phase. In particular, the GP was measured to depict potential effects on the course of glycaemia. A white wheat bread (WWB) was used as the reference product.

Experimental

Raw materials and recipes

Hi-maize® whole grain corn flour was obtained from Ingredion Incorporated (Bridgewater, NJ, USA), medium (MEYPRODOR®50) and low (MEYPRODOR®5) molecular weight GG were kindly provided by Danisco A/S (Denmark). Hydrolysed GG (Sunfiber) was kindly provided by Azelis-Bröste AB (Mölndal, Sweden) and dry yeast was obtained from Jästbolaget AB (Sollentuna, Sweden).

WWB was made from wheat flour with 10% protein (Vetemjöl, Kungsörnen AB, Järna Sweden) while the other products were made from wheat flour with 12% protein (Vetemjöl special, Kungsörnen AB, Järna, Sweden). The latter was used to improve loaf size of the test bread products. Wheat breads with 9% (wet weight, ww) of hGG, lwGG and mwGG, respectively, were prepared (hG3, lwG3, mwG3). Furthermore, wheat breads containing 3 and 6% mwGG (ww), respectively, were prepared (mwG1, mwG2). WgHiM flour was included in wheat bread in the highest amount possible, still resulting in an acceptable product regarding crumb structure and taste (wgHiM). To the wgHiM bread mwGG was included in 3 levels, 3, 6 and 9% ww (wgHiMG1, wgHiMG2, wgHiMG3). Detailed recipes for the different breads are presented in Table 1.

The WWB and wheat breads with hGG and lwGG were made in a home baking machine (Tefal, home bread) using a program for white bread (including kneading for 5 min, resting for 5 min, kneading for 20 min, rising for 70 min with 10 s of kneading after 15 and 31 min, baking for 52 min, in total a process of 2 h and 32 min). All wgHiM containing breads were made with a uniform procedure where the dough was mixed in a mixing bowl for 5 min, proofed in a home baking machine (Tefal, home bread) for 30 min, kneaded for 15 s by hand and again placed in the baking machine for 30 min proofing and 60 min baking.

After baking, each bread was wrapped in a towel and left to cool for 2 h. The crust was then removed and the crumb sliced and portions wrapped in aluminium foil, put into plastic bags and stored in a freezer (−18 °C) until use. The day before usage the bread portions were taken from the freezer and thawed at ambient temperature, still wrapped in aluminium foil and in the plastic bag.

Chemical analysis

Prior to the analysis of available and total starch, the bread samples were dried and milled to pass through a 0.5 mm screen (Cyclotec, Tecator, Höganäs, Sweden). Measurements of RS and hydrolysis index (HI) were performed on the product "*as is*".

The amount of total starch was determined according to Björck and Siljeström,[18] RS was analysed according to Åkerberg *et al.*[19] and the HI was determined according to an *in vitro* procedure based on chewing.[20] The available starch content of each serving was calculated by subtracting the amount of RS from that of total starch. The chemical characterisations of the breads are shown in Table 2. The energy content of each test meal was calculated using available carbohydrates (analysed) and estimated fat and protein contents, respectively (17 kJ per g protein and available carbohydrates; 37 kJ per g fat). The compositions of the test meals are presented in Table 3, with the amount of wgHiM and mwGG estimated from the recipes and weight of bread loafs before and after baking.

Fluidity measurements

Physiological digestion of the bread products was simulated using the initial steps of an *in vitro* procedure developed to predict the rate of starch hydrolysis.[20] However, the second amylolysis step used to simulate small intestinal digestion was reduced to 1 h. By this procedure, product viscosity which can be anticipated to be degraded during *in vivo* digestion in the upper gastrointestinal tract was removed.

The fluidity of the digesta was then measured using a Bostwick consistometer (45 ml aliquots) according to the method previously described by Östman *et al.*[15]

Study design

Twelve healthy non-smoking volunteers (7 men and 5 women) aged 24 ± 1.5 (mean ± SEM) years with normal body mass indices (23.3 ± 0.4 kg m^{-2}) and without drug therapy participated in the study. All subjects had normal fasting blood glucose concentrations (5.5 ± 0.05 mM). The subjects were recruited in October 2010 and the study was performed during November and December 2010. All test subjects gave their informed consent and were aware of the possibility of withdrawing from the study at any time. Approval of the study was obtained by the regional ethical review board in Lund, Sweden (registration number 556/2008). The subjects were instructed to maintain their regular life-style throughout the entire study. The day prior to a test the participants were told to avoid

Table 1 Ingredients in the different breads[a]

Ingredient (g per bread)	WWB	hG3	lwG3	mwG1	mwG2	mwG3	wgHiM	wgHiMG1	wgHiMG2	wgHiMG3
Water	360	360	360	416	442	480	360	360	442	460
Wheat flour 10% protein	540	—	—	—	—	—	—	—	—	—
Wheat flour 12% protein	—	540	540	500	440	400	340	300	240	200
WgHiM flour	—	—	—	—	—	—	200	200	200	200
Guar gum	—	71	71	25	50	71	—	25	50	71
Dry yeast	4.8	4.8	4.8	6.3	6.3	6.3	5.0	5.0	5.0	5.0
NaCl	4.8	4.8	4.8	5.0	5.0	5.0	5.0	5.0	5.0	5.0

[a] WWB (white wheat bread), hG3 (WWB with 9% added hydrolysed guar gum), lwG3 (WWB with 9% added low molecular weight guar gum), mwG1 (WWB with 3% added medium molecular weight guar gum (mwGG)), mwG2 (WWB with 6% added mwGG), mwG3 (WWB with 9% added mwGG), wgHiM (WWB bread containing Hi-maize® whole grain corn flour), wgHiMG1 (wgHiM with 3% added mwGG), wgHiMG2 (wgHiM with 6% added mwGG) and wgHiMG3 (wgHiM with 9% added mwGG).

Table 2 Chemical characterisation of bread products

Product	Total starch[a] (% of ww)	Resistant starch[b] (% of ww)	Resistant starch (% of total starch)	Hydrolysis index[c] (%)	Fluidity index	Pred GI
WWB	39.8	1.0 a	2.6	100 a	100	—
mwG1	35.8	0.9 ab	2.6	83 ± 9 abc	79	82
mwG2	31.1	0.4 b	1.3	67 ± 6 cd	37	67
mwG3	28.0	0.9 ab	3.0	69 ± 3 cd	21	69
hG3	38.7	—	—	96 ± 4 a	103	94
lwG3	37.5	—	—	95 ± 9 ab	100	93
wgHiM	37.3	5.2 c	14.0	86 ± 2 abc	112	85
wgHiMG1	35.0	5.4 c	15.5	85 ± 9 abc	76	84
wgHiMG2	29.3	5.2 c	17.9	70 ± 3 bcd	32	70
wgHiMG3	26.7	5.2 c	19.7	52 ± 2 d	15	54

[a] Result presented as mean ($n = 2$). [b] Result presented as mean ($n = 6$). [c] Result presented as mean ± SEM ($n = 6$, mwG2 $n = 5$, wgHiMG1 $n = 4$). Values within a column not sharing the same letter were significantly different, $p < 0.05$ (ANOVA followed by Tukey's test).

Table 3 Compositions of the breakfast meals[a]

Product	Fresh weight (g per portion)	Energy content (kJ per portion)	WgHiM flour (g per portion)	Guar gum (medium weight) (g per portion)	Total starch (g per portion)	Resistant starch (g per portion)
WWB	95.6	897	0	0	38.9	1.1
wgHiM	114.6	1009	28.5	0	42.7	6.0
wgHiMG1	123.8	999	30.5	3.8	43.3	6.7
wgHiMG2	150.0	1027	35.9	9.0	43.9	7.8
wgHiMG3	174.2	1084	41.8	14.8	46.5	9.1

[a] Energy content calculated using available carbohydrates (analysed) and estimated fat and protein contents. Amount of wgHiM flour and guar gum estimated from recipes, total starch and resistant starch calculated from analysed values. Values within a column not sharing the same letter were significantly different, $p < 0.05$ (ANOVA followed by Tukey's test).

alcohol, excessive physical activity and food rich in DF. In the late evening (21.00–22.00) prior to a test the subjects were told to eat a standardised meal consisting of a commercial white wheat bread with topping and drink of their own choice. The subjects were instructed to take the same evening meal at all times. The test products were provided as breakfast meals in random order approximately one week apart. The subjects arrived in the laboratory at 07.45 on the test day after an overnight fast. Capillary fasting blood samples were taken prior to the breakfast at time 0. Thereafter the test meals, contributing with 37 g of available starch, were served with 250 g of tap water. The test subjects were told to finish the meal within 14 min. Capillary blood samples were then taken at 15, 30, 45, 60, 90, 120 and 180 min after the beginning of the breakfast for analysis of blood glucose and serum insulin. The subjects were also asked to rate their subjective *feeling of hunger*, *satiety* and *desire to eat* on a bipolar visual analogue scale directly after each blood sampling. During the experiment the subjects were not allowed to eat or drink anything except for the breakfast provided and they were told to remain seated as much as possible.

Blood analysis

Blood glucose concentrations were determined in capillary whole blood using a blood-glucose analyser (HemoCue Glucose 201$^+$ Analyser, HemoCue AB, Ängelholm, Sweden). Samples for insulin analysis were collected in BD Microtainer SST Tubes and were kept at room temperature for approximately 30 min before being centrifuged for 5 min (5000 rpm, 20 °C, Eppendorf mini spin, F-45-12-11). Serum was then frozen at −18 °C until analysis. The serum insulin measurements were 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).

Calculations and statistical methods

The FI was calculated as: $(\text{consistency}_{\text{test bread}})/(\text{consistency}_{\text{reference bread}}) \times 100\%$, where consistency is the reciprocal of the fluidity (1/Bostwick units (BU)) and where BU indicates the flowing distance (cm) of the sample after 60 s divided by the sample size (ml).

One subject was excluded from the meal study due to several statistical outliers in the insulin responses (Grubb's test) and one subject could not complete because of an antibiotic treatment. Data were therefore analysed with $n = 11$ with the exception of wgHiM where $n = 10$. Data are expressed as means ± SEM.

The incremental area under the curve (iAUC) was calculated for each subject and test meal for glucose and insulin responses using the trapezoid model and excluding all values below the fasting level. For the rate of starch hydrolysis (0–180 min), *feeling of hunger*, *feeling of satiety* and *desire to eat*, the total area under the curve (tAUC) was calculated using the same model. GI and insulinaemic indices (II) were calculated from the iAUC 0–120 min for glucose and insulin respectively, using WWB as the reference (GI and II = 100). HI was calculated from tAUC 0–180 min using a mean of two WWB replicates as the reference.[20] The GP, defined as the duration of the glucose curve above the fasting concentration divided by the iPeak,[7] was also calculated. Therefore, iPeaks for glucose and insulin, respectively, were calculated as the maximum postprandial increase from baseline. The GI, II, GP and iPeak data were analysed using a mixed model analysis of covariance (ANCOVA) with subjects as a random variable and corresponding baselines (fasting values) as covariates. For HI and FI, a mixed model analysis of variance (ANOVA) was used with test subject or sampling occasion as a random variable (MINITAB, release 16, Minitab Inc., State College PA). Differences between groups were identified by using Tukey's multiple comparison tests. The distribution of the residuals was controlled with the Anderson–Darling test. For the insulin iPeak, the residuals were not normally distributed and therefore a BoxCox transformation was performed on the data before the ANCOVA. The result for the insulin iPeak is presented as original data. Time × treatment interactions were analysed using a mixed model (PROC MIXED in SAS release 9.2, SAS Institute Inc., Cary, USA) with repeated measures and an autoregressive covariance structure. Correlation analysis was conducted to evaluate the relationship between dependent measures with the use of Spearman's partial coefficients controlling for subjects and the corresponding baselines (two tailed test) (SPSS software, version 19; SPSS Inc., Chicago, IL, USA). $p < 0.05$ was considered to be statistically significant.

Results and discussion

In vitro characterisation of bread products

The addition of mwGG to WWB did not increase the amount of RS formed compared to WWB, however breads containing whole grain corn flour with an elevated amylose content had a higher RS content, 5.2–5.4% (ww) compared to WWB (1.0%, ww). There were no significant differences in RS (ww) between the wgHiMG1–3 breads, but the amount tended to increase stepwise for the three levels of mwGG when RS was expressed as a percentage of total starch (Table 2).

The use of 200 g of wgHiM flour as replacement for wheat flour in the wgHiM bread product led to a reduction of HI by 14% compared to WWB (Table 2). The HI for the hG3 and the lwG3 breads, respectively, was not different from WWB. Both 6 and 9% (ww) of added mwGG to WWB and wgHiM, respectively, significantly decreased the HI compared to WWB. The combination of wgHiM and mwGG tended to result in a lower (not significant) HI compared to only mwGG at the same level of incorporation. Regression analysis was performed to investigate and model the relationship of HI and level of mwGG for all breads tested, the levels 0, 3, 6 and 9% of added mwGG were used as a predictor; the regression resulted in the following relationship: HI = 93.2 − 3.7mwGG-level ($p > 0.001$).

The incorporation of hGG and lwGG to the WWB did not affect the FI compared to WWB but the addition of mwGG decreased FI, the more the mwGG the lower the FI, see Table 2. The wgHiM bread had a slightly higher FI than WWB, but the use of wgHiM flour did not affect the FI for breads when combined with mwGG. The hG3 and the lwG3 did not differ from WWB regarding the FI.

Choice of test bread products in the meal study. The analysis results revealed that there were no differences in HI or FI between mwGG bread with and without addition of whole grain corn flour with an elevated amylose content. However, the RS-content of the wgHiM products was substantially higher than the corresponding bread based on white wheat. Thus, the four bread products containing 24% wgHiM (ww) with three different levels of added mwGG (3, 6 and 9% (ww)) (wgHiM, wgHiMG1, wgHiMG2 and wgHiMG3) were selected for the meal study. The WWB was included as a reference product. For these bread products, the flow distance at 60 s was significantly different between all samples, resulting in FI-values ranging from 15 to 112 (ANOVA followed by Tukey's test). Furthermore, the predicted GI, calculated from HI as described by Leeman *et al.*,[21] decreased with increased addition of mwGG. For the chosen bread products, there was a positive correlation between HI and FI ($r = 0.906$, $p < 0.001$). In addition, the HI and the amount of RS per test portion were negatively correlated ($r = -0.983$, $p < 0.001$) and so were the HI and the amount of mwGG in the portions ($r = -0.959$, $p < 0.001$).

Meal study

Postprandial glucose responses. The mean incremental glucose responses and the corresponding data are shown in Fig. 1 and Table 4, respectively. Both wgHiMG2 and 3 displayed lower GI compared to WWB and wgHiM. WWB and wgHiM did not differ significantly from each other. All products containing mwGG resulted in lower glucose iPeaks compared to WWB and wgHiM, respectively. The wgHiMG1 and 2 elicited iPeaks that differed from each other, whereas no significant differences were found between WWB and wgHiM. The GP was higher for all breads containing mwGG compared to WWB, but no significant differences were seen between the different mwGG-levels or between WWB and wgHiM. Treating the dose of mwGG in the bread portions as a continuous variable (values 0, 1, 2 and 3), there was a linear reduction of postprandial glycaemia with increasing content of mwGG ($p < 0.001$, Table 4).

A time × treatment interaction was found for glucose (0–180 min, $p < 0.0001$). At 15 min, wgHiMG2 induced a lower level of glucose than WWB and wgHiM, respectively. At 30 and 45 min, both wgHiMG2 and 3 induced lower glucose levels than WWB and wgHiM, respectively. At 180 min, wgHiMG3 had a higher incremental glucose level than WWB and wgHiM, respectively.

The GI was negatively correlated with the amount of GG in the portions and with the GP ($r = -0.509$ and -0.576, respectively, $p < 0.001$). The GI was positively correlated with FI and HI ($r = 0.509$ and 0.518, respectively, $p < 0.001$). The GP was negatively correlated with both FI and HI ($r = -0.719$ and -0.738, respectively, $p < 0.001$).

Postprandial insulin responses. The mean incremental insulin responses and the corresponding data are shown in Fig. 1 and Table 4, respectively. Both wgHiMG2 and 3 displayed lower II than WWB and wgHiM, whereas WWB and wgHiM did not differ. The iPeaks for insulin were lower for wgHiMG2 and 3 compared with WWB and wgHiM, respectively. Treating the dose of mwGG in the bread portions as a continuous variable (values 0, 1, 2 and 3), there was a linear reduction of postprandial insulinaemia with increasing content of mwGG ($p < 0.001$), see Table 4.

A time × treatment interaction was found for insulin (0–180 min, $p < 0.0001$). WgHiMG2 induced a lower mean incremental insulin level than WWB at 15, 30, 45 and 60 min. In addition, wgHiMG2 and wgHiMG3 elicited lower insulin responses than WWB and wgHiM at 30 and 45 min. At 60 min, wgHiMG3 induced a lower insulin response than WWB.

The insulin iPeak was positively correlated with both II and FI ($r = 0.801$, respectively 0.695, $p < 0.001$). The GP was negatively correlated with both II and insulin iPeak ($r = -0.675$, respectively -0.711, $p < 0.001$).

Subjective appetite ratings. All test products containing mwGG induced a higher *feeling of fullness* than the WWB (tAUC 0–180 min, Fig. 2). In addition, the two breads with the highest amounts of mwGG induced a lower *feeling of hunger* compared to WWB. The *desire to eat* was lower after ingestion of the wgHiMG3 bread than after WWB (tAUC 0–180 min). Just after the completion of the breakfast (tAUC 0–15 min) there were no significant differences in the *feeling of fullness* or *desire to eat*, but the subjects reported lower *feeling of hunger* for the two breads with the highest amounts of mwGG compared to WWB (data not shown). Treating the dose of mwGG in the portions as a continuous variable (values 0, 1, 2 and 3), there was a linear increase in *feeling of fullness* with increasing content of mwGG ($p < 0.001$), and a linear decrease in *feeling of hunger* and *desire to eat* ($p < 0.002$ and 0.001, respectively).

A main effect was found for both *feeling of fullness* and *feeling of hunger*, $p = 0.02$ and $p = 0.01$, respectively. However, no significant time × treatment interactions were found for *feeling of fullness, feeling of hunger* or *desire to eat* (0–180 min, $p = 0.82$, 0.52, and 0.95, respectively).

The *feeling of fullness* (0–180 min) was negatively correlated with both GI and II ($r = -0.426$ and -0.436, respectively, $p = 0.001$) and positively correlated with GP ($r = 0.437$, $p = 0.001$). The *feeling of hunger* (0–180 min) was positively correlated with II ($r = 0.477$, $p < 0.001$). The *desire to eat* (0–180 min) was positively correlated with both GI and II ($r = 0.358$, $p = 0.009$ and 0.501, $p < 0.001$, respectively).

In this study we found that addition of mwGG to breads containing wgHiM significantly improved the course of glycaemia compared to WWB, as judged by the lowered GI and glucose iPeak, as well as increased GP. Furthermore, the II and insulin iPeak, respectively, were reduced for all mwGG-containing bread products compared to WWB and this is another indicator of improved blood glucose regulation. The GP for the bread with the highest level of mwGG was more than twice as high as those previously reported for rye breads and boiled rye kernels.[7] However, the addition of wgHiM *per se* did not affect the glycaemic or insulinaemic responses.

The highest level of mwGG used in the present study was chosen based on findings by Nilsson *et al.*,[22] where this level

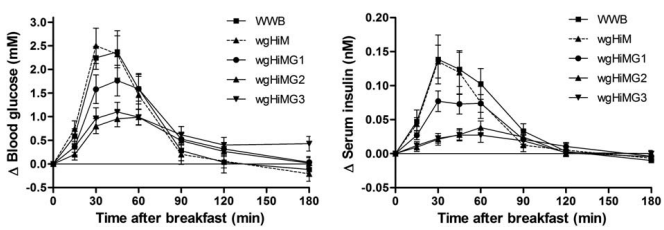

Fig. 1 Mean incremental change (Δ) in blood glucose and serum insulin. Values are mean ± SEM, $n = 11$ (wgHiM $n = 10$).

Table 4 Glucose and insulin responses after the intake of the test products[a]

Product	Glucose iAUC (0–120 min) (min mM)	GI (%)	Glucose iPeak (⊿ mM)	GP (min mM^{-1})	Insulin iAUC (0–120 min) (min mM)	II (%)	Insulin iPeak (⊿ nM)
WWB	126.4 ± 16	100 a	2.7 ± 0.2 a	45 ± 6 a	8.13 ± 1.4	100 a	0.17 ± 0.03 a
wgHiM	130.7 ± 26	107 ± 15 a	2.7 ± 0.4 a	53 ± 11 ab	6.85 ± 1.6	94 ± 25 ab	0.15 ± 0.03 ab
wgHiMG1	113.4 ± 19	87 ± 11 ab	2.0 ± 0.3 b	109 ± 25 bc	5.15 ± 0.75	87 ± 20 bc	0.10 ± 0.02 bc
wgHiMG2	71.5 ± 12	59 ± 10 c	1.2 ± 0.2 c	142 ± 26 c	2.67 ± 0.71	38 ± 8 c	0.05 ± 0.01 c
wgHiMG3	87.3 ± 16	67 ± 10 bc	1.3 ± 0.2 bc	147 ± 15 c	2.26 ± 0.61	36 ± 15 c	0.04 ± 0.01 c

[a] Values are means ± SEM, $n = 11$ ($n = 10$ for wgHiM). Products in the same column not sharing the same letter are significantly different, $p < 0.05$ (ANCOVA followed by Tukey's test).

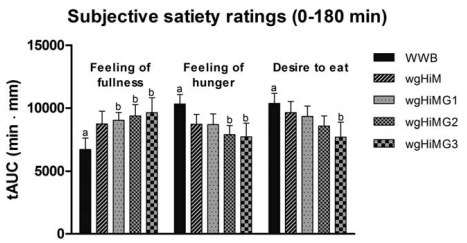

Fig. 2 Subjective appetite ratings, columns within a group not sharing the same letter were significantly different. Products not displaying any letter were not different from any other test products ($p < 0.05$, ANCOVA followed by Tukey's test). Values are means ± SEM, $n = 11$ ($n = 10$ for wgHiM).

elicited a lower iPeak for glucose and a maintained net increment in the late postprandial phase compared to WWB. Interestingly, in that study the improved course of glycaemia was accompanied by an improved cognitive function in the later postprandial phase.[22] The two lower mwGG-levels were chosen in order to evaluate a possible dose–response effect on glycaemia. The GI of wgHiMG3 was not as low as predicted from HI-measurements and, in addition, the GI of wgHiMG2 was lower than expected from HI-data. However, when calculated on the basis of each individual there was a linear reduction of iAUC for both glucose and insulin with increasing levels of mwGG. It could be hypothesised that the amount of mwGG included in wgHiMG2 was enough to lower the gastric emptying rate to such an extent that the even higher level of mwGG added to wgHiMG3 was not able to cause any further reduction. This is supported, especially in the first 120 min, by the course of glycaemia, where the responses are very similar to both products. During the last hour the larger dose of mwGG continued to release glucose whereas the mean glucose level declined after the intake of wgHiMG2 during the same period. Furthermore, the GP of both products was similar and this also speaks in favour of wgHiMG2 giving a high enough mwGG-level to obtain beneficial effects on glycaemia. If we were to repeat the study, an inclusion level of mwGG between wgHiMG1 and wgHiMG2 would have been interesting to see if a product with the GP between 109 (wgHiMG1) and 142 (wgHiMG2) could be created. Studying the correlations it seems like the FI would be a better predictor of GP than of the GI. This is probably due to the fact that the GP takes into account both the peak value and the time span during which the glucose value stays over the fasting value. This indicates that GP could be a better predictor of a beneficial glycaemic regulation than GI, as it considers also the later postprandial phase. The linear relationship also existed for the subjective satiety ratings, as the mwGG in the products increased, the *feeling of hunger* and *desire to eat* were lowered and the *feeling of fullness* increased. There are several suggested explanations to why fibre-mediated viscosity reduces the postprandial glycaemic and insulinaemic responses, such as reduced gastric emptying rate, altering of intestinal motility, slower diffusion rate of starch digestion products and reduced α-amylase accessibility.[23] In addition, it has been shown *in vitro* that GG inhibits α-amylase in a direct, non-competitive way in the first stage of the enzymatic degradation of starch.[13] If ingested as a liquid preload, the GG appears to develop higher viscosity, and the timing of the meal appears to be important for the glycaemic effect.[12] Incorporation of GG into bread products overcomes potential variability in results due to timing but may result in a product with an unpleasant mouth feel.[12] However, the combination of mwGG and wgHiM used in the present study resulted in a product that we considered as acceptable in terms of mouth feel and taste.

Besides its positive effect on the course of glycaemia, mwGG also influenced the subjective satiety ratings in a dose–response manner. In fact we found a positive correlation between *feeling of fullness* and GP. Viscous DF has previously been reported to influence appetite regulation both *via* mechanical and nutrient dependent factors.[24,25] Although not further investigated, we anticipate that the inclusion of mwGG and wgHiM in the test products increased the need for mastication, increased stomach distension as well as increased the production of gut released hormones like GLP-1, due to a prolonged transit time. A limitation of the study was that the subjects were not asked to rate the palatability of the test products, the palatability of an ingested food could affect subsequent satiety.[26]

Breads containing wgHiM flour had an increased amount of RS compared with the WWB reference. Even though the mwGG-containing breads had similar contents of wgHiM the content of RS increased with an increased level of mwGG, ranging from 14.0 to 19.7% of total starch. This is noteworthy and may

suggest enhanced formation and/or limited degradation of RS from the wgHiM in the presence of mwGG. Furthermore, the increased RS formation was expected to be accompanied by an increased amount of slowly digestible starch[17] which could add to the viscosity related effect of mwGG on the course of glycaemia. We noted that the addition of mwGG to the recipe resulted in a very stiff dough, which might reflect a decreased availability of water and a reduced gelatinisation.[27] The HI decreased with the increased addition of mwGG in the bread, but it was not affected by the addition of either lwGG or hGG. The result for mwGG was assumed to be a consequence of a higher viscosity of the digesta, leading to diffusion barriers and obstructed amylolysis. Consequently, the breads containing mwGG had an increased viscosity, measured as fluidity; the more mwGG the lower the fluidity and the FI. The reduced HI could also, at least partially, be due to an inhibitory effect of α-amylase of GG as previously shown *in vitro*.[13] However, this was not the case for hGG and lwGG, of which none was significantly different from the WWB. HI has previously been shown to be a good predictor of GI values for cereals, legumes and potato products.[21] Also in the present study there was a correlation between the measured GI and that predicted from the HI-analysis for the mwGG bread. Furthermore, our results indicate that FI could be an interesting alternative to HI for the prediction of GI and GP in this type of bread with GG-mediated viscosity.

Although not investigated in the present study, both RS and DF originating from wgHiM or the GG could be expected to reach the colon and promote formation of short chain fatty acids (SCFA) by the gut microflora.[28] An increased formation of SCFA has been shown to correlate with enhanced glycaemic regulation in a second meal perspective.[29] The time frame of the present study did not allow for conclusions to be drawn about the possible impact on colonic fermentation. However, we hypothesise that the combination of wgHiM and mwGG in addition to the acute effects presented here also might have exerted a positive effect on health, by improving colonic health and glycaemic regulation also at subsequent meals, as seen previously with barley products rich in RS and DF.[9]

Conclusion

To conclude, the novelty of the present study is that a combination of medium weight guar gum and whole grain corn flour with an elevated amylose content increased the RS-content and improved the course of glycaemia to bread. The wgHiM *per se* did not positively influence the GP but in combination with mwGG both glycaemic and insulinaemic responses were lowered and GP was increased compared to WWB. There was a reciprocal correlation between GP and FI, indicating a predictive value of FI in this type of bread products. Interestingly, the prototype bread products also had a positive effect on the appetite ratings, but whether the improved appetite scores could lead to a reduction in voluntary food intake at a subsequent snack or meal remains to be elucidated.

Competing interests

The authors declare no competing financial interest.

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Oat β-glucan containing bread increases the glycaemic profile

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ABSTRACT

A net postprandial glucose increment beyond 2 h has been shown to improve glucose and appetite regulation at a subsequent meal. Such an improved glycaemic profile (GP) has been reported for bread containing guar gum. In the present study three commercially available β-glucans from barley and oat were baked into yeast leavened bread products. Only oat beta-glucan containing bread met the criteria of β-glucan molecular weight and was included in a meal study. The three levels of oat β-glucans reduced the GI and glucose iPeak by 32–37% compared to a white wheat reference bread. Furthermore, the highest oat β-glucan level increased GP by 66% compared to the reference bread. It is concluded that the oat β-glucans were suitable for use in baking, since the MW remained relatively high. Thus, the oat ingredient showed an interesting potential to be used when tailoring the glycaemic profile of bread products.

1. Introduction

Elevated postprandial blood glucose is associated with oxidative stress and subclinical inflammation, factors known to increase the risk of developing type 2 diabetes mellitus and cardiovascular disease (Galland, 2010). Thus, reduced postprandial glycaemic excursions are of importance for the prevention of those diseases (Blaak et al., 2012). The importance of late postprandial glycaemia has gained more interest in recent years, and as a measure of the same, the concept of glycaemic profile (GP) was introduced in 2009 (Rosén et al., 2009). GP is defined as the duration of the net glucose increment above fasting, divided by the incremental peak value for glucose. In previous work it has been suggested that GP for different bread products showed a better correlation to insulin index (II) than glycaemic index (GI) did (Ekström, Björck, & Östman, 2013; Rosén, Östman, & Björck, 2011). In addition, high GP breakfast meals have been associated with improved appetite regulation, described as lowered energy intake or increased plasma levels of PYY, at a subsequent voluntary lunch meal (Ekström, Björck, & Östman, 2016; Rosén et al., 2011).

Bread is a starch rich staple food and many studies have been carried out to improve its glycaemic characteristics. So far, one successful strategy has been to add different forms of dietary fibre (DF) (Ekström et al., 2013; Scazzina, Siebenhandl-Ehn, & Pellegrini, 2013).

Oat and barley β-glucans, in *e.g.* bread, pasta, hot and cold breakfast cereals or beverages, have repeatedly been shown to positively influence blood glucose response after a meal due to viscosity development in the gut (Tosh, 2013). The effect on postprandial glycaemia is depending on β-glucan molecular weight (MW), solubility and the amount ingested (Regand, Tosh, Wolever, & Wood, 2009). Numerous studies have demonstrated that the MW of β-glucans is reduced during food processing, like *e.g.* baking (Izydorczyk, Storsley, Labossiere, MacGregor, & Rossnagel, 2000; Tosh, Brummer, Wolever, & Wood, 2008; Wood, 2004; Åman, Rimsten, & Andersson, 2004). The degradation is likely to be caused by β-glucanases originating from the β-glucan ingredient *per se*, or from other added ingredients.

The content of β-glucans in different barley varieties is 2–20% (dry weight basis) and in oat varieties 3–8% (El Khoury, Cuda, Luhovyy, & Anderson, 2012). The molecular structure of β-glucans from barley and oat is very similar, being a linear polymer of D-glucose with β-(1→3) and β-(1→4) glucosidic linkages (Wang & Ellis, 2014). Oat β-glucans are, however, generally more water soluble (82%) and have higher molecular weights (MW) (2000–3000 kDa) (Wang & Ellis, 2014) compared to β-glucans from barley (20–50% water solubility (Izydorczyk et al., 2000) and MW 200–2660 kDa) (Cho & Samuel, 2009)). The higher MW of oat β-glucans in processed oats could be a result of the kilning process *i.e.* heat treatment, undertaken in oats to inactivate endogenous enzymes, *e.g.* lipases (Ames, Storsley, & Tosh, 2015).

Abbreviations: GP, glycaemic profile; II, insulinaemic index; GI, glycaemic index; DF, dietary fibre; MW, molecular weight; GER, gastric emptying rate; WWB, white wheat bread; RS, resistant starch; FI, fluidity index; HI, hydrolysis index

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The purpose of the present work was to investigate possibilities to tailor the course of glycaemia of bread products with particular focus on the later postprandial phase (beyond 120 min) using some commercially available β-glucans. Three different β-glucan ingredients, where the producer claimed a high MW and/or beneficial effect on postprandial glycaemia, were chosen and incorporated into yeast leavened bread. Based on the review by Tosh (2013), bread products meeting the criteria of having β-glucans with MW above 250 kDa were included in a meal study, using white wheat bread as reference product.

Furthermore, in vitro fluidity and rate of starch hydrolysis were analysed, as measures of prediction for intestinal viscosity and glucose regulating potential.

2. Materials and methods

2.1. Bread products

White wheat bakery flour (Pågens Extra Bagerivetemjöl) was kindly provided by Pågen AB (Malmö, Sweden), whole grain barley flour from a variety with elevated β-glucans (approx. 15%) (BB) was kindly provided by National Starch (Manchester, England), coarse barley fibre (BF) was kindly provided by Lyckeby Culinar (Fjälkinge, Sweden) and refined oat fibre (OF) was kindly provided by Tate & Lyle Oat Ingredients (Kimstad, Sweden). Dry yeast (Jästbolaget AB, Sollentuna, Sweden) and salt (Falksalt, AB Hanson&Möhring, Halmstad, Sweden) were bought in the local supermarket.

The three different cereal ingredients with high β-glucan content were included, one by one, in bread, targeting a level of 3.5% β-glucans (per wet weight (ww)). The test products were based on white wheat flour with addition of BB, BF and OF, respectively. Manufacturers' data was used to calculate the incorporation level of the different β-glucan preparations. The recipes are presented in Table 1. White wheat bread (WWB) was used as the reference product.

Each dough was mixed in a KitchenAid™ Artisan (5KSM150, St. Joseph, Michigan, USA) for seven minutes, proofed in room temperature for 40 min and baked in a home baking machine (Tefal, home bread) for 60 min. After baking, the breads were left to cool for 1.5–2 h, wrapped in a towel. The crust was then removed, the crumb sliced and divided into portions wrapped in aluminium foil, put into plastic bags and stored in a freezer (−18 °C). The day before usage, either for analysis or meal study, the bread portions were taken from the freezer and thawed at ambient temperature, still in the plastic bags.

Table 1
Ingredients in the different breads.

Ingredient (g/bread)	WWB	BB	BF	OF
Water	270	345	345	350
Wheat flour	450	271	290	386
Whole grain barley flour	–	179	–	–
Course barley fibre	–	–	160	–
Refined oat fibre	–	–	–	64
Dry yeast	9	9	9	9
NaCl	4.5	4.5	4.5	4.5

.All breads are based on white wheat flour with either only white wheat flour (WWB), with whole grain barley flour (BB), with coarse barley fibre (BF) or with refined oat fibre (OF).

2.2. Chemical analyses and in vitro characterisation

Bread samples were dried and milled to pass through a 0.5 mm screen (Cyclotec, Tecator, Höganäs, Sweden) prior to the analysis of available starch (Holm, 1986), insoluble and soluble fibres (Asp, Johansson, Hallmer, & Siljestrom, 1983) and β-glucans (Megazyme, Ireland (AOAC method 995.16)). Measurements of resistant starch (RS) (Åkerberg, Liljeberg, Granfeldt, Drews, & Björck, 1998), rate of starch hydrolysis (Granfeldt, Björck, Drews, & Tovar, 1992) and fluidity (Ekström et al., 2013) were performed on frozen and thawed bread products. Fluidity is a measure of fibre-mediated viscosity whilst the HI method measure starch bioavailability.

MW of the β-glucans was analysed using high performance size exclusion chromatography (HPSEC) with calcoflour detection (Kim & Inglett, 2006; Rimsten, Stenberg, Andersson, Andersson, & Åman, 2003; Suortti, 1993). Approximately 0.15 g dried bread samples and β-glucan fractions, respectively, were wet with ethanol (50% v/v) and dissolved in 25 ml water with gentle stirring at ambient temperature for 20 h. Samples (approximately 0.3 g β-glucan per litre) were then filtered using a 45 μm syringe glass filter before injected into the HPSEC system (Agilent Technologies, Santa Clara, California, USA).

2.3. Study design

Thirteen healthy volunteers (9 men and 4 women) aged 23–30 years (26.3 ± 0.7; mean ± SEM) with body mass indices 18–28 kg/m^2 (22.6 ± 0.8) participated in the study. All subjects had normal fasting plasma glucose levels (5.2 ± 0.05 mM). The study was performed from March to June 2012. All test subjects gave their informed consent and were aware of the possibility of withdrawing from the study at any time. Approval of the study was obtained by the Regional ethical review board in Lund, Sweden (registration number 556/2008). Tobacco users were not included in the study. The participants were not allowed to use antibiotics or probiotics during two weeks before and throughout the study, otherwise they were told to maintain their regular life-style.

The day before each experiment, the test subjects should avoid strenuous exercise, alcohol and food with high fibre content (*e.g.* whole grain breads, whole kernels, fibre enriched pasta, cabbage, etc.). Furthermore, the subjects were instructed to take the same evening meal at 18.00 at all days prior to an experiment, and to eat a standardized meal consisting of a commercial white wheat bread with topping and drink of their choice in the late evening (21.00–22.00).

The bread products were provided as breakfast meals in random order, separated by at least one week. The subjects arrived in the laboratory at 07.45 after an overnight fast. Capillary blood samples were taken prior to the breakfast at time 0. Thereafter, either of the test meals, contributing with 53 ± 0.5 g of available starch, was served with 250 g tap water. The subjects were instructed to finish the meal within 15 min. Capillary blood sampling then followed at 15, 30, 45, 60, 90, 120 and 180 min after the beginning of the meal. During the experiment, the subjects were not allowed to eat or drink anything except for the breakfast provided, and they were told to remain seated as much as possible.

2.4. Blood analysis

Plasma glucose concentrations were determined in whole blood using a HemoCue Glucose 201$^+$ Analyser (HemoCue AB, Ängelholm, Sweden). Samples for insulin analysis were collected in

BD Microtainer SST Tubes and were left in room temperature for approximately 30 min before being centrifuged for 5 min (5000 rpm, 20 °C, eppendorf mini spin, F-45-12-11). The serum was then frozen at −18 °C until analysis. Insulin measurements were performed at 0, 15, 30, 45, 60, 90 and 120 min 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).

2.5. Statistical analysis and calculations

Fluidity index (FI) was calculated from Bostwick units (BU) using the following equation: $(\text{consistency}_{\text{reference bread}})/(\text{consistency}_{\text{test bread}}) \times 100$, where consistency is 1/BU and BU is flowing distance after 60 s divided by the sample size in ml. WWB was used as the reference ($FI_{WWB} = 100$) and all measurements of flowing distance were made in duplicates (Ekström et al., 2013; Östman, Rossi, Larsson, Brighenti, & Björck, 2006). A lower FI indicates a higher viscosity and a higher FI indicates a lower viscosity compared to reference. Rate of starch hydrolysis (0–180 min), described as hydrolysis index (HI), was calculated as the percentage of the total area under the curve (tAUC 0–180 min) for each test bread, using the trapezoid model and WWB as the reference (Granfeldt et al., 1992), predicted GI was calculated from HI (Leeman, Bårström, & Björck, 2005). A mixed model analysis of variance (ANOVA) was used for HI and FI, with test subject and sampling occasion, respectively, as random variables (MINITAB, release 16, Minitab Inc., State College, PA, USA).

Data are expressed as least square means (LSMs) and standard errors of the mean (SEM), n = 13. For all subjects and test meals the incremental area under the curve (iAUC) was calculated for glucose and insulin responses using the trapezoid model and excluding all values below the fasting level. Glycaemic (GI) and insulinaemic indices (II) were calculated from the iAUC 0–120 min for glucose and insulin, respectively, using WWB as the reference (GI and II = 100). Incremental peaks (iPeaks) for glucose and insulin, respectively, were calculated as the maximum postprandial increase from baseline for each subject. Furthermore, the glycaemic profile (GP), defined as the duration of the glucose curve above fasting concentration from 0 to 180 min, divided by the iPeak, was calculated (Rosén et al., 2011). GraphPad Prism (version 6, GraphPad Software, San Diego, CA, USA) was used for graph plotting and area calculation.

The effect of reference and test meals on physiological responses was evaluated using a linear mixed model ANCOVA (PROC MIXED procedure). Baseline, visit, meal, time and meal × time interaction were included as fixed effects. Subject was treated as random effect and time and visit were included as repeated effects. All models were tested for the normality of residuals using standard diagnostics to ensure that all variables met the assumptions for normal distribution, ln transformation was necessary for insulin. To adjust for multiple comparisons of significant effects, Tukey-Kramer *post hoc* significance test was performed and the Kenward-Roger correction was applied for reducing small sample bias. Calculations were performed using SAS (version 9.4, SAS Institute Inc., Cary, USA). Correlation analysis was conducted to evaluate the relation among dependent measures with the use of Spearman's partial coefficients controlling for subjects and corresponding baselines (two tailed test) (SPSS software, version 19; SPSS Inc., Chicago, IL, USA). Statistical significance was considered at a p-value < 0.05 (two-tailed).

3. Results

3.1. In vitro characterisation of bread products

The MWs of the β-glucan preparations and the bread products are presented in Table 2. The β-glucan degradation was more pronounced in the two bread products based on barley β-glucan ingredients compared to the bread with oat β-glucan. In the BB bread the MW was 20% of that in the raw material and in the BF bread the MW was 45% of that in the raw material. The OF bread gave two separated peaks with 75 and 50% of the raw material MW, respectively. Only the oat β-glucan ingredient resulted in bread with MW above 250 kDa and where eligible for further evaluation in the meal study. Since only oat β-glucan bread met the criteria for the meal study, two more doses were added to be able to see a possible dose-response effect. Thus, bread products targeting 2.5 and 4.5% (ww) oat β-glucans were also prepared. OF1 (target 2.5% β-glucan) was prepared from 64 g OF, 386 g wheat flour, 380 g water, 9 g dry yeast and 4.5 g salt and OF3 (target 4.5% β-glucan) from 116 g OF, 334 g wheat flour, 405 g water, 9 g dry yeast and 4.5 g salt. MW was analysed also for these bread products and gave two separate peaks with 282 and 174 kDa, respectively, for OF1, and 291 and 205 kDa for OF3. The mean MW for peak 1 of OF1, OF2 and OF3 was 286 ± 3 kDa and for peak 2 184 ± 11 kDa.

The characteristics of the breads are presented in Table 3. The final concentration of β-glucans in the bread products differed somewhat from the estimations based on manufacturers' data.

All test bread products had lower FI than WWB and FI decreased with increasing amounts of OF. A regression analysis including all OF products resulted in the following relationship: FI = 104 − 13 × β-glucan-concentration (% ww) (p = 0.033). Both OF2 and OF3 displayed significantly lower HI-values compared to WWB.

3.2. Glucose and insulin responses

The mean incremental glucose and insulin responses and corresponding data are shown in Fig. 1 and Table 4. The mean fasting concentrations for glucose and insulin did not differ between meals. For glucose, there was a significant meal effect (p = 0.001). All three OF-breads displayed lower glucose iAUCs (0–120 min) and glucose iPeaks compared to WWB. Furthermore, OF3 displayed higher GP compared to the WWB. Significant differences in blood glucose were observed at specific time points (time × meal interaction, p < 0.0001). At 30 min OF3 induced a lower glucose response than WWB and at 45 and 60 min, all three OF-breads induced a lower glucose response

Table 2
Molecular weight of β-glucans in raw materials and breads.

Product	MW[1]
Raw material	*kDa*
Whole grain barley flour (used in BB)	680
Coarse barley fibre (used in BF)	102
Refined oat fibre (used in OF)	376
Bread products	
BB	130
BF	44
OF peak 1	282
OF peak 2	174

[1] Molecular weight obtained from the HPSEC chromatograms. All breads are based on white wheat flour with either only white wheat flour (WWB), with whole grain barley flour (BB), with coarse barley fibre (BF) or with refined oat fibre (OF).

Table 3
Characteristics of the breads.

Bread product	Available starch[1]	RS[2]	RS	Insoluble fibre	Soluble fibre	β-glucan[3]	Dry matter	HI[4]	FI	Predicted GI[5]	β-Glucan	Weight
	%	%	% of total starch	%	%	%	%				g per portion	g per portion
WWB	44.8	–	–	2.3	1.2	–	54.3	100 a	100 a	–	0	118
BB	32.6	0.4 a	1.2	5.3	3.7	3.1	51.7	99 ± 5 a	50 d	97	–	–
BF	32.7	1.4 b	4.3	4.7	3.1	2.1	51.8	94 ± 4 a	64 bc	92	–	–
OF1	40.0	–	–	2.6	3.6	2.6	50.0	92 ± 3 a	70 b	90	3.4	133
OF2	37.5	–	–	2.5	4.9	3.7	49.0	73 ± 4 b	57 cd	73	5.2	141
OF3	36.0	1.9 c	4.9	2.8	6.1	4.9	51.5	66 ± 3 b	40 e	66	7.5	152

Result presented as mean [1](n = 2), [2](n = 6), [3](n = 2), [4]result presented as mean ± SEM (n = 6), [5]prediction of GI using HI. Values within a column not sharing the same letter were significantly different, $p < 0.05$ (ANOVA followed by Tukey's test). RS (resistant starch), HI (hydrolysis index), FI (fluidity index). All breads are based on white wheat flour with either only white wheat flour (WWB), with whole grain barley flour (BB), with coarse barley fibre (BF) or with refined oat fibre (OF).

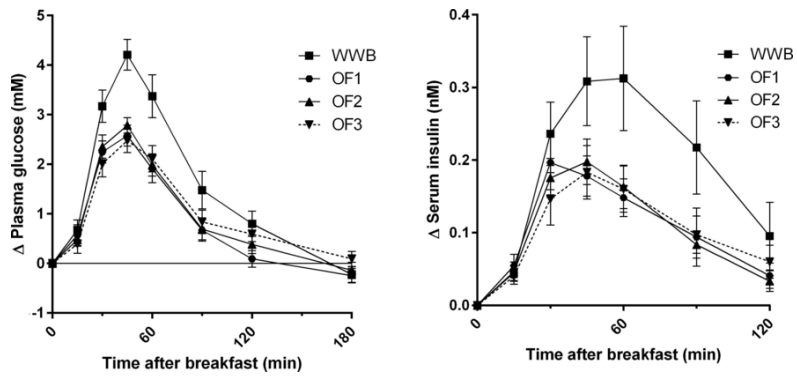

Fig. 1. Mean incremental change (Δ) in plasma glucose and serum insulin. Values are mean ± SEM, n = 13. White wheat bread (WWB), bread based on white wheat flour with addition of refined oat fibre in three different amounts (OF1 (2.6% ww), OF2 (3.7% ww) and OF3 (4.9% ww)).

Table 4
Glucose and insulin responses after intake of the breads.

Product	Glucose iAUC	GI	Glucose iPeak	Δ-Glucose iPeak	GP	Insulin iAUC	II	Insulin iPeak	Δ-Insulin iPeak
	(0–120 min)					(0–120 min)			
	min mM	%	Δ mM	mM	min/mM	min mM	%	Δ nM	nM
WWB	248 ± 21 a	100	4.3 ± 0.3 a		35 ± 5 a	23.4 ± 3.2 a	100	0.35 ± 0.04 a	
OF1	155 ± 21 b	64 ± 5	2.8 ± 0.3 b	−1.6	52 ± 5 ab	12.1 ± 3.2 b	71 ± 14	0.20 ± 0.04 b	−0.15
OF2	159 ± 21 b	68 ± 5	2.9 ± 0.3 b	−1.4	50 ± 5 ab	13.6 ± 3.2 b	68 ± 14	0.23 ± 0.04 b	−0.12
OF3	159 ± 21 b	63 ± 5	2.7 ± 0.3 b	−1.7	58 ± 5 bc	13.7 ± 3.2 b	61 ± 14	0.21 ± 0.04 b	−0.14

Values are LSMs ± SEM, n = 13. Products in the same column not sharing the same letter are significantly different, $p < 0.05$ (ANCOVA followed by Tukey's test). Glycaemic index (GI), glycaemic profile (GP), insulinaemic index (II), white wheat bread (WWB), bread based on white wheat flour with addition of refined oat fibre in three different amounts (OF1, OF2 and OF3).

than WWB. Overall, using the amount of oat β-glucan as a continuous variable, a linear reduction in postprandial glycaemia with increasing oat β-glucan content was found ($p = 0.001$).

For insulin there was a significant meal effect ($p < 0.0001$) with the three OF breads eliciting lower insulin iAUC (0–120) and lower insulin iPeak values compared to WWB. No significant time × meal interaction was found for insulin (0–120 min, $p = 0.076$).

Both GI and II was negatively correlated with the amount of β-glucan in the portions ($r = −0.53$ and $−0.40$, respectively $p < 0.01$), and positively correlated with FI and HI ($r = 0.53$ (GI) and 0.40 (II), respectively $p < 0.01$). GP was positively correlated with the amount of β-glucan in the portions ($r = 0.43$, $p < 0.01$), and negatively correlated with FI and HI ($r = −0.43$, $p < 0.01$).

4. Discussion

Both postprandial glycaemia and insulinaemia were significantly improved after ingestion of all OF breads as estimated from the lowered GI, glucose iPeak as well as II and insulin iPeak. An increased late increment in glycaemia in combination with decreased iPeak after the OF-breads was indicated by GP increases in the range of 43–66% compared to the WWB. Interestingly, the different levels of OF (2.6%, 3.7% and 4.9%) resulted in very similar values of GI and II, as well as iPeaks for glucose and insulin. However, the bread containing 4.5% β-glucan increased GP compared to the WWB, indicating that a higher dose resulted in an even better ability to maintain glucose above the fasting level. The effect on postprandial glycaemia elicited by β-glucans is a result of the increased intestinal viscosity which results in decreased GER, reduced rate of α-amylase induced starch hydrolysis and reduced intestinal nutrient uptake (Braaten et al., 1991; Tosh, 2013). The rheological behaviour of β-glucans is complex and depends on source and technological pre-treatments, as well as concentration (Dongowski et al., 2005). From studies of glucose containing beverages it has been concluded that increasing either concentration or MW of β-glucans in the solution lead to increased viscosity (Kwong, Wolever, Brummer, & Tosh, 2013b), which in turn lead to a greater reduction in glycaemic response (Regand et al., 2009; Wood, Beer, & Butler, 2000). In the present study, it is possible that already the lowest level of OF β-glucans was able to reduce GER to its extreme, with no possibility of further reduction by increased levels.

Fibre-mediated viscosity, as measured by FI, has previously demonstrated potential in predicting postprandial glycaemia for guar gum containing bread products (Ekström et al., 2013; Ekström et al., 2016). Despite the differences in β-glucan MW and concentration, all β-glucan containing products in the present study displayed lower FI compared to WWB. For the OF-products, FI decreased with increasing dose of β-glucan. It is an obvious disadvantage that the FI measure cannot differentiate products containing β-glucans of various MW. However, as there were correlations between FI and physiological responses in the case of oat β-glucans, its potential use for prediction of intestinal viscosity deserves further evaluation. The fluidity was evaluated by use of a Bostwick consistometer and thus, the results cannot be directly translated into other rheological measures (Perona, 2005). It is possible that both viscosity and gel formation affects fluidity and these measures are not separated using the consistometer. Viscous solutions are formed by entanglement of β-glucan polymer chains whereas weaker hydrogen bonds formed between sequences of repeated β-(1→3)-cellotriosyl units leads to gel formation. It has been demonstrated that gel formation is diffusion limited and thus occurs more effectively between lower MW β-glucans (Kwong, Wolever, Brummer, & Tosh, 2013a). Thus, the effect on FI elicited by the barley β-glucan products could be a result of increased viscosity as well as gel formation. According to a recent study, viscous glucose solutions influenced postprandial glycaemia, whereas glucose gels did not (Kwong, Wolever, Brummer, & Tosh, 2013a).

HI is an in vitro measure of starch availability and diffusion hindrance that has previously demonstrated potential in predicting glycaemia for a larger number of starch rich products compared to FI (Granfeldt et al., 1992). Although there was a positive correlation between HI and the physiological responses, HI was significantly reduced only for OF2 and OF3 compared with WWB. As all OF products had a significantly lowering effect on postprandial glycaemia, this indicates that HI underestimates the effect on glycaemia for this type of oat β-glucan containing products. For OF1 and OF2, the predicted GI did not match with the actual GI. This could probably be explained by the effect of viscosity, which is not fully covered in the method of HI, which is the basis of the predicted GI.

Commercially available β-glucans varies considerably in MW, and it turned out that one of the tested preparations had such a low MW that no effect on glycaemia could be expected. The more pronounced β-glucan MW degradation in the barley bread products is probably a result of greater β-glucanase activity in those preparations (Andersson et al., 2004). Also the wheat flour may have contained β-glucanases, however, as the same wheat flour was used in all bread products and the degradation in the oat β-glucan bread was less prominent, this was probably of less importance. The present results demonstrates that when adding barley β-glucans to bread products with the purpose of lowering postprandial glycaemia, attention should be paid to initial MW and measures taken to minimize β-glucan degradation.

Current European legislation allows health claims related to blood glucose regulation based solely on the ratio between β-glucans and available carbohydrates in a product. Furthermore, the claim is valid for β-glucans originating both from oats and barley. Four g β-glucans per 30 g available carbohydrates has been considered as the lowest dose to reduce postprandial glycaemia (Agostoni, Pagona Lagiou, Hildegard Przyrembel, & Verhagen, 2011). It should be noted that also the lowest level of OF β-glucan gave significant lowering of both glycaemia and insulinaemia in the present study, despite the fact that a portion only contained 3.3 g β-glucans (corresponding to 1.9 g β-glucans per 30 g available starch). In the light of the present results and a recent review showing that the glucose reducing potential of β-glucans is more strongly related to the content of β-glucans alone than to the ratio of β-glucans to available carbohydrates (Tosh, 2013), it appears as if the legislation needs to be updated.

It is concluded that already the lowest dose of the studied oat β-glucan ingredient demonstrated a strong potential in tailoring postprandial glycaemia after incorporation in yeast leavened breads. One important reason for this being the relatively low decrease in MW during baking. The highest concentration of oat β-glucans more than doubled GP compared to the reference bread. The results also highlight that quality aspects like MW of β-glucans must be considered to support health claims relating to their effects on postprandial blood glucose reduction.

Conflict of interest

The authors declare no competing interests.

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Paper III

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An improved course of glycaemia after a bread based breakfast is associated with beneficial effects on acute and semi-acute markers of appetite†

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The prevalence of type 2 diabetes mellitus is rapidly increasing all over the world and a diet promoting reduced glycaemic excursions in the postprandial phase may help to prevent the disease. In the present study guar gum (GG) and whole grain rye flour or high amylose maize starch (HAM) was combined to design bread products giving low and sustained glycaemia. A meal study was performed with young, healthy subjects and in addition to glucose and insulin, also subjective appetite ratings and biomarkers of appetite, voluntary energy intake at a second meal and markers of fermentation were studied. The combination of GG and rye was superior with improvements in subjective appetite whereas both test products lead to improvements in biomarkers of appetite compared to the white wheat bread reference. The inclusion of GG, rye and/or HAM in bread products show great potential in lowering risk factors associated with insulin resistance and improving acute and semi-acute appetite.

Background

The prevalence of type 2 diabetes mellitus (T2DM) is rapidly increasing all over the world.[1] A recent review acknowledged the importance of diet and lifestyle modifications in prevention of T2DM.[2] More specifically, the importance of a diet leading to reduced glycaemic excursions in the postprandial phase has been identified as a prerequisite in order to maintain metabolic health and prevent T2DM, overweight and cardiovascular disease (CVD).[3]

Lower glycaemic excursions can be achieved by a conscious choice of ingredients in carbohydrate rich foods or meals. Both physiological factors and inherent food properties, *e.g.* enzymatic availability, botanical, physical or chemical structure of the food,[4,5] presence of certain dietary proteins[6] and/or indigestible or slowly digestible carbohydrates[7,8] are of importance for the glycaemic response. However, the metabolic response to a meal is not only affected by its type and composition, but also by previous food intake.[8–10]

Glycaemic index (GI) is used to rank the glycaemic effect of carbohydrate rich foods during the first 2 h after a meal, and low GI's represent lower glycaemic excursions. In order to take into account also the course of glycaemia beyond 120 min, we recently introduced the concept of glycaemic profile (GP). Consequently, GP considers the duration of the glucose response and the incremental peak.[11] Based on previous findings for products with low GI and high GP[11–13] it is hypothesised that carbohydrate rich foods with a low but sustained net increment in glycaemic response, *i.e.* low GI and high GP, induce metabolic benefits both acutely and at a subsequent meal.

Rye products have repeatedly shown to lower insulin responses, regardless of their glycaemic responses.[14,15] When comparing five different rye varieties grown in Sweden,[16] Visello rye was one of the more promising candidates to lower both postprandial glycaemia and insulinaemia. Furthermore, rye appears to promote colonic fermentative activity at an earlier point in time than other cereals.[17,18]

Guar gum (GG) was recently shown to increase GP of bread at three different inclusion levels[13] and the suggested mechanism is by increasing viscosity in the upper small intestine.[19] The same study showed that a combination of GG and whole grain high amylose maize starch (HAM) in bread resulted in a pronounced formation of RS.[13] RS is assumed to increase colonic fermentation at a somewhat later stage during the digestion, than rye.[20] An increased amylose content also leads to formation of a slowly digestible starch fraction that affects the course of glycaemia.[21] However, at equivalent available starch basis, an increased RS-level did not influence the acute glycaemia *per se.*[13]

We hypothesized that food products modulated to give a low but sustained net increment in glycaemia (low GI/high GP) and promote early gut fermentation will lower risk factors associated with insulin resistance and improve acute and semi-acute appetite. Thus, in addition to glucose and subjective appetite ratings, we studied insulin, biomarkers of appetite,

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voluntary energy intake at a second meal and markers of fermentation after bread meals containing GG and either HAM or whole grain Visello rye.

Methods

Raw materials and recipes

HAM (Hi-Maize) was obtained from Ingredion Incorporated (Bridgewater, NJ, USA), medium molecular weight GG (MEYPRODOR®50) was kindly provided by Danisco A/S (Denmark) and dry yeast was obtained from Jästbolaget AB (Sollentuna, Sweden). Rye kernels (Visello) were obtained from KWS LOCHOW GMBH (Bergen, Germany). White wheat bread (WWB) was made from wheat flour with 10% protein (Vetemjöl, Kungsörnen AB, Järna, Sweden). The breads with HAM and GG (HG) and Visello rye whole grain flour and GG (VG), respectively, were made from wheat flour with 12% protein (Vetemjöl special, Kungsörnen AB, Järna, Sweden) to improve loaf volume. The Visello rye kernels were milled to whole grain flour using a laboratory mill (Perten laboratory mill 120, sieve 0.8 mm) before baking.

The WWB was made in a home baking machine (Tefal, home bread) using a program for white bread as previously described.[13] The HG and VG breads were made with a uniform procedure where the dough was mixed in a bowl for 5 min, proofed in a home baking machine (Tefal, home bread) for 30 min, kneaded for 15 s by hand and placed in the bread machine for another 30 min proofing followed by 60 min baking. The recipes are presented in Table 1.

After baking, WWB and HG breads were left to cool for 2 h wrapped in a towel, whereas the VG breads were left for 16–18 h wrapped in a towel in a plastic bag. Thereafter, the crust was removed, the crumb sliced and portions wrapped in aluminium foil, put into plastic bags and stored in a freezer (−18 °C) until use. The day before usage, either for analyses or in the meal study, bread portions were taken from the freezer and thawed at ambient temperature, still wrapped in aluminium foil and in the plastic bag.

Composition of the lunch

In order to measure the voluntary energy intake, an *ad libitum* meal was served at 240 min after the start of the breakfast. The ordinary Swedish lunch meal consisted of regular spaghetti made from durum wheat and normal wheat (Barilla Sweden AB, Filipstad, Sweden), ready-made frozen meatballs (ICA Handlarnas AB, Solna, Sweden), ketchup (Heinz) and fresh cucumber. The cucumber was served in slices, 2–3 mm thick, with the ends removed in order for all slices to have the same ratio of peel to fruit flesh. The pasta was boiled for 8 min (1 l water, and 7 g NaCl per 100 g pasta) the water was then discarded and 8 g rape seed oil (Di Luca & Di Luca AB, Stockholm, Sweden) added per 100 g dry pasta. The meatballs were heated in a microwave oven at 850 W in 2 min cycles until they were evenly warm.

Chemical analysis

Prior to the analysis of available and total starch[22] the bread samples were air dried and milled to pass through a 0.5 mm screen (Cyclotec, Tecator, Höganäs, Sweden). Measurements of RS,[23] rate of starch hydrolysis[24] and fluidity[13] were performed on the product "as is". Available starch content of the servings was calculated by subtracting RS from total starch. The chemical characteristics of the breads are shown in Table 2. The energy content of the three test meals was calculated based on available carbohydrates (analysed) and estimated fat and protein contents using 17 kJ per g protein and available carbohydrates, and 37 kJ per g fat. The composition of the test breads are presented in Table 3, with the amount of HAM and GG estimated from the recipes and weight of bread loafs before and after baking.

Study design

Nineteen healthy non-smoking volunteers (9 men and 10 women) aged 27.3 ± 1.4 years (mean ± SEM) with normal body mass indices (21.7 ± 0.4 kg m^{-2}) and without drug therapy, participated in the study. All subjects had normal fasting blood glucose concentrations (5.4 ± 0.06 mmol l^{-1}). The recruitment of test subjects and the study trials were performed from September to December 2011. All test subjects gave their informed consent and were aware of the possibility of withdrawing from the study at any time. Approval of the study was obtained by the regional ethical review board in Lund, Sweden (registration number 2011/507). The subjects

Table 1 Ingredients in the test and reference breads

Ingredient (g per bread)	WWB	HG	VG
Water	360	445	460
Wheat flour 10% protein	540	—	—
Wheat flour 12% protein	—	280	105
Hi-Maize (HAM)	—	160	—
Visello rye flour	—	—	360
Guar gum (GG)	—	50	55
Dry yeast	4.8	5.0	9.6
NaCl	4.8	5.0	5.0

WWB (white wheat bread), HG (bread containing HAM and GG), VG (bread containing whole grain Visello rye flour and GG).

Table 2 Chemical characteristics of test and reference breads

Chemical characteristics	WWB	HG	VG
Total starch[a] (% of ww)	39.8	35.1	27.7
Resistant starch[b] (% of ww)	1.0	7.1	1.2
Resistant starch (% of total starch)	2.6	20.2	4.3
Dry matter content (%)	52.0	47.2	46.4
Hydrolysis index (HI)[c] (%)	100 a	46 ± 2 b	56 ± 3 b
Fluidity index (FI)	100 a	48 ± 1 b	27 ± 1 c
Predicted GI from HI	—	48	57

[a] Result presented as mean (n = 2). [b] Result presented as mean (n = 6). [c] Result presented as mean ± SEM (n = 5). Values within the same lines not sharing the same letters were significantly different, $p < 0.05$ (ANOVA followed by Tukey's *post hoc* test).

Table 3 Composition of the breakfast meals

Composition of breakfast	WWB	HG	VG
Fresh weight (g per portion)	128.9	178.7	188.3
Energy content (kJ per portion)[a]	1208	1074	1246
Hi-Maize (g per portion)	—	33.6	—
Guar gum (g per portion)	—	10.5	11.6
Total starch (g per portion)	51.3	62.7	52.2
Resistant starch (g per portion)	1.3	12.7	2.2

[a] Energy content calculated using available carbohydrates (analysed) and estimated fat and protein content. Amount of Hi-maize and GG is calculated from the recipes, total starch and RS calculated from respective analysis.

were instructed to maintain their regular life-style throughout the entire study. The day prior to a test the participants were told to avoid alcohol, excessive physical activity and food rich in dietary fibre (DF). In the late evening (21.00–22.00) prior to a test the subjects were instructed to eat a standardized meal consisting of white wheat bread with topping and drink of their own choice. However, the subjects were obliged to have an identical evening meal before each test. The test and reference products were provided as breakfast meals in random order approximately one week apart. The subjects arrived in the laboratory at 07.45 on the test day after an overnight fast. A peripheral venous catheter (BD Venflon Dickinson, Helsingborg, Sweden) was inserted into an antecubital vein. Capillary plasma glucose and venous blood samples were taken in the fasting state, after which the test meals, contributing with 50 g of available starch, were served with 250 g of tap water (time 0). The subjects were told to finish the meal within 14 min. Blood samples were then taken at 15, 30, 45, 60, 90, 120, 150, 180, 210 and 240 min after the beginning of the breakfast. After the blood sampling at 120 min, 150 ml coffee, tea or water was served. The same drink was then used for each participant throughout the study. After the sampling at 240 min the lunch meal was served in a buffet style. Further (venous) blood samples were taken at 300 and 360 min after breakfast. The participants were told to eat until they were pleasantly full and try to reach the same level of satiation at every test occasion. Therefore they were allowed to take the food by themselves and the amount of food was recorded by the study leader. Water (250 ml) was served with the lunch meal. The subjects were asked to rate their subjective feeling of hunger, satiety and desire to eat on a bipolar visual analogue scale (VAS) directly after each blood sampling. During the experiment the subjects were not allowed to eat or drink anything except for the food provided and they were told to remain seated as much as possible.

Blood analysis

Plasma glucose concentrations were determined in capillary whole blood at all time points before lunch using a HemoCue Glucose 201+ Analyser (HemoCue AB, Ängelholm, Sweden). Serum samples were collected in 3.5 ml SST tubes and plasma samples in 2.0 ml EDTA tubes pre-treated with inhibition mix (2 mg Pefablock (Roches) and 20 μl DPPIV (Millipore) in each test tube). The inhibition mix was added to each tube by a syringe no more than 4 days before the usage and the tubes were then stored in 8 °C. Tubes for serum were centrifuged for 10 min (2000 G, 4 °C) after 30 min of clotting. Test tubes for plasma were kept on ice before and after sampling and these tubes were centrifuged for 10 min (1000 G, 4 °C) as soon as possible. Blood samples were then frozen in aliquots at −18 °C until analysis.

NEFA was measured in serum at 180 and 240 min by an enzymatic colorimetric method (NEFA C, ACS-ACOD method, WAKO Chemicals GmbH, Germany).

Insulin, ghrelin (active), GIP (total) and PYY (PYY$_{1-36}$ and PYY$_{3-36}$) were measured by MILLIPLEX MAP (Human Metabolic Hormone Magnetic Bead Panel, Millipore Corporation, Billerica, MA, USA) at all time points.

As an indicator of colonic fermentation, breath hydrogen (H_2) excretion was measured every 30 min during the entire test day using a Gastrolyser (Bedfont EC60 Gastrolyser, Rochester, UK). Short chain fatty acids (SCFA – acetate, propionate, isobutyrate and butyrate) in serum were analysed at 180, 240, 300 and 360 min using gas chromatography.[25]

Calculations and statistical methods

Data are expressed as least square means (LSMs) and standard errors of the mean (SEM). One subject was not able to finish the VG portion so the data was analysed with n_{WWB} and n_{HG} = 19 and n_{VG} = 18.

The incremental- and total areas under the curves (iAUC and tAUC, respectively) were calculated for each subject and test meal using the trapezoid model. GI and insulinaemic index (II) were calculated from the iAUC 0–120 min for glucose and insulin respectively, using WWB as the reference (GI and II = 100). HI was calculated from tAUC 0–180 min using WWB as the reference. The predicted GI was calculated from HI as described by Leeman *et al.*[26] The result for fluidity index (FI) was calculated as: (consistency$_{\text{reference bread}}$)/(consistency$_{\text{test bread}}$) × 100%, where consistency is the reciprocal of the fluidity (1/Bostwick Units (BU)) and BU indicates the flowing distance of the sample after 60 s in cm, divided by the sample size (ml).[13,27]

Incremental peaks (iPeak) for glucose, insulin and GIP were calculated as the maximum postprandial increase from baseline. The GP was defined as the duration of the glucose curve above fasting concentration in the timespan from breakfast to lunch (0–240 min) divided by the iPeak.[11] GraphPad Prism (version 6, GraphPad Software, San Diego, CA, USA) was used for graph plotting and area calculation.

The effect of reference and test meals on physiological responses was evaluated using ANCOVA (PROC MIXED procedure). Baseline, visit, treatment, time and treatment × time interaction were included as fixed effects. Subject was treated as random effect and time and visit were included as repeated effects. All models were tested for the normality of residuals using standard diagnostics to ensure that all variables met the assumptions for normal distribution and ln transformation was applied if necessary (the case for insulin, ghrelin and GIP). To adjust for multiple comparisons of significant effects,

Tukey-Kramer *post hoc* significance test was performed, the Kenward-Roger correction was applied for reducing small sample bias. Calculations were performed using SAS (version 9.4, SAS Institute Inc., Cary, USA).

For HI a mixed model analysis of variance (ANOVA) was used with test subject as a random variable. The same procedure was used for FI, but in this case the replicate was used as random variable (MINITAB, release 16, Minitab Inc., State College PA).

Correlation analysis was conducted to evaluate the relation among dependent measures with the use of Spearman's partial coefficients controlling for subjects and corresponding baselines (two tailed test) (SPSS software, version 22; SPSS Inc., Chicago, IL, USA). Statistical significance was considered at a p-value <0.05 (two-tailed).

Results

Glucose responses at breakfast

The fasting concentrations for plasma glucose did not differ between the treatments (Table 4). There was no significant treatment effect (p = 0.16) among the meals (Table 4), however, a time × treatment interaction was found (p < 0.0001) (Fig. 1). HG and VG both resulted in significant lower GI and glucose iPeak, as well as higher GP, compared to the WWB.

Insulin and NEFA responses

The fasting concentrations for plasma insulin did not differ between the treatments (Table 4). HG and VG resulted in significantly lower overall insulin responses (0–360 min, p = 0.003) compared to the WWB (Table 4). Furthermore, there was a significant time × treatment interaction (p < 0.0001) (Fig. 1). II and insulin iPeak was significant lower for HG and VG compared to the WWB, with II for HG also being significantly lower than that of VG.

Incremental insulin responses after intake of the *ad libitum* lunch meal (iAUC 240–360) were significantly lower after VG compared to HG breakfast (p = 0.017), whereas WWB did not differ from any of the two (WWB compared to HG p = 0.88 and WWB compared to VG p = 0.067, respectively).

VG induced a lower concentration of NEFA than WWB at 240 min (p = 0.014), whereas HG did not differ from any of the two products.

Ghrelin

There was no significant treatment effect for ghrelin between the meals (p = 0.70). However, a significant time × treatment interaction was found (p < 0.0001) (Fig. 2). The mean plasma ghrelin level decreased to a nadir at 54 ± 3 min, with a significantly smaller relative decrease for HG and VG compared to WWB. HG and VG had a significantly lower relative increase from the nadir to 240 min at lunch time, compared with WWB. Ghrelin at 240 min was positively correlated to the energy intake at lunch (r = 0.297, p = 0.028).

GIP

HG and VG resulted in significantly lower overall GIP responses (0–360 min, p < 0.0001) compared to WWB (Table 4). There was a significant time × treatment interaction for GIP (p < 0.0001) (Fig. 2). HG and VG resulted in signifi-

Table 4 Metabolic responses after intake of the test products[a]

Test variables	Subjects (n)	WWB	HG	%[b]	VG	%[b]
Breakfast (0–240 min)						
Glucose, fasting value (mmol l^{-1})	$19_{WWB,\ HG}$, 18_{VG}	5.3 ± 0.1	5.4 ± 0.1	0	5.5 ± 0.1	3
Glucose, overall mean 0–120 (mmol l^{-1})		6.3 ± 0.1	6.1 ± 0.1	−3	6.1 ± 0.1	−4
Glucose, iPeak 0–240 (Δ mmol l^{-1})		3.2 ± 0.2 a	1.9 ± 0.2 b	−41	1.8 ± 0.2 b	-42
GI (%)		100 a	66 ± 6 b	−35	61 ± 6 b	−39
Glucose, GP (min mmol l^{-1})		51 ± 10 a	95 ± 10 b	87	88 ± 11 b	75
Insulin, fasting value (nmol l^{-1})	$19_{WWB,\ HG}$, 18_{VG}	0.078 ± 0.008	0.083 ± 0.008	5	0.072 ± 0.008	−8
Insulin, overall mean 0–360 (nmol l^{-1})		$0.17 \pm 2 \times 10^{-4}$ a	$0.14 \pm 2 \times 10^{-4}$ b	−18	$0.15 \pm 2 \times 10^{-4}$ ab	−10
Insulin, iPeak 0–240 (Δ nmol l^{-1})		0.35 ± 0.03 a	0.22 ± 0.03 b	−39	0.25 ± 0.04 b	−29
II (%)		100 a	44 ± 4 b	−56	59 ± 4 c	−41
Ghrelin, Δ nadir (at time 54 ± 3 min)	$19_{WWB,\ HG}$, 18_{VG}	75.5 ± 5.3 a	51.7 ± 5.3 b	−31	54.2 ± 5.4 b	−28
Ghrelin, relative increase from nadir to 240 min (%)		54.3 ± 3.0 a	38.0 ± 3.0 b	−30	37.9 ± 3.1 b	−30
GIP, overall mean 0–360 (ng l^{-1})	$19_{WWB,\ HG}$, 18_{VG}	51.2 ± 1.1 a	38.9 ± 1.1 b	−24	40.2 ± 1.1 b	−21
GIP, iPeak 0–240 (ng l^{-1})		64.8 ± 5.1 a	36.0 ± 5.2 b	−44	34.1 ± 5.3 b	−47
GIP, iAUC 0–240 (min ng l^{-1})		7898 ± 840 a	4042 ± 840 b	−49	4219 ± 840 b	−47
PYY, overall mean 0–360 (ng l^{-1})	$18_{WWB,\ HG}$, 17_{VG}	72.4 ± 3 a	82.3 ± 3 b	14	88.6 ± 3 b	22
NEFA, 240 min (mmol l^{-1})	$19_{WWB,\ HG}$, 18_{VG}	0.28 ± 0.03 a	0.23 ± 0.03 ab	−20	0.17 ± 0.03 b	−41
Lunch (240–360 min)						
GIP, iPeak 240–360 (ng l^{-1})		168.5 ± 11.8	168.0 ± 11.4	0	163.6 ± 13.8	−3
s-Acetate, 240 min (μmol l^{-1})	$19_{WWB,\ HG}$, 18_{VG}	334 ± 14	317 ± 14	−5	312 ± 15	−7
s-Propionate, 240 min (μmol l^{-1})		10.5 ± 0.4	10.8 ± 0.4	3	10.4 ± 0.4	−3
s-Isobutyrate, 240 min (μmol l^{-1})		12.0 ± 0.6 ab	12.9 ± 0.6 a	7	11.3 ± 0.6 b	−6
s-Butyrate, 240 min (μmol l^{-1})		16.1 ± 0.9 a	19.2 ± 0.9 b	19	15.8 ± 1.0 a	−2

[a] Values are LSMs ± SEM. Products in the same line not sharing the same letter are significantly different, p < 0.05 (ANCOVA followed by Tukey's *post hoc* test). [b] The percent change is calculated as the difference from HG and VG to the WWB.

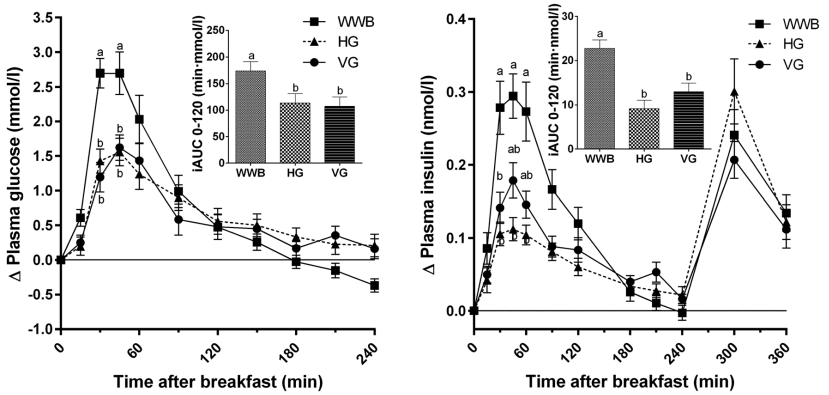

Fig. 1 Mean incremental changes (Δ) and iAUC 0–120 min in plasma glucose and insulin (mean and LSMs ± SEM, respectively), n = 19, (VG n = 18).

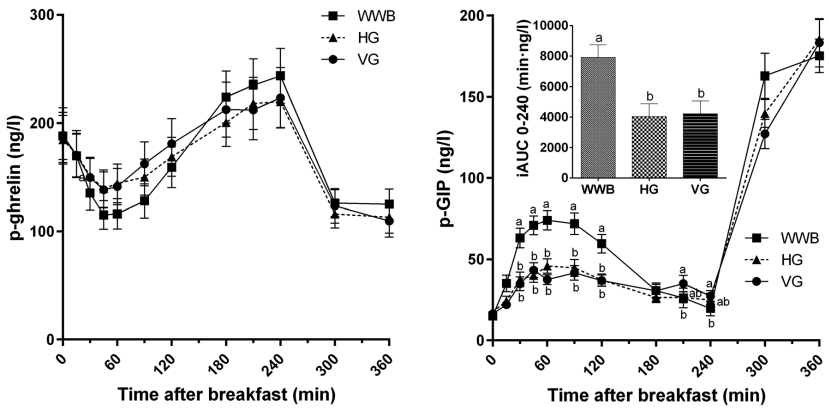

Fig. 2 Postprandial change in ghrelin and GIP (mean ± SEM) and iAUC 0–240 min for GIP (LSMs ± SEM), n = 19, (VG n = 18).

cantly lower iAUC and iPeak values for GIP compared to the WWB in the timespan from breakfast to lunch.

PYY

HG and VG resulted in significantly lower overall PYY response (0–360 min, p = 0.0002) compared to WWB (Table 4). There was no significant time × treatment interaction (p = 0.0938). The tAUC in the time period after the *ad lib* lunch (tAUC 240–360) was significantly higher after the VG breakfast compared to WWB (Fig. 3).

Breath H_2 and s-SCFA

There was no significant treatment effect for breath H_2 (p = 0.11), however, a significant time × treatment interaction was found (0–360 min, p = 0.008) (Fig. 3). In the period after lunch (240–360 min), the VG breakfast tended to give a higher iAUC for H_2 compared to the WWB and HG (p = 0.058).

The amount of acetate, propionate and isobutyrate in serum did not differ between any of the products throughout the test day. The HG breakfast gave rise to a higher concentration of s-butyrate at 240 min compared to WWB and VG, see Table 4.

Subjective appetite ratings and energy intake at the *ad libitum* lunch meal

VG resulted in significantly lower overall feeling of hunger compared to the WWB in the period from breakfast to lunch (p = 0.017) (Table 5 and ESI Fig. 1†), but no differences were

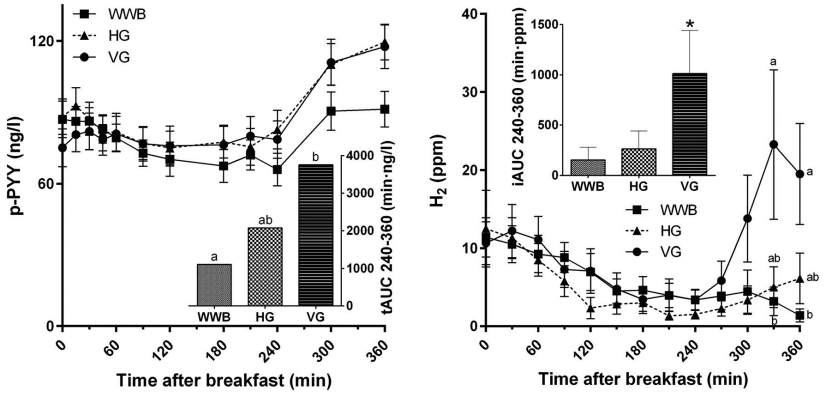

Fig. 3 Postprandial changes and i- or tAUC 240–360 min for PYY and breath H_2 (mean and LSMs ± SEM, respectively). PYY n = 18, (VG n = 17), breath H_2 WWB n = 16, HG n = 18, VG n = 14, * p = 0.058.

Table 5 Subjective appetite ratings from breakfast to lunch[a] and voluntary intake at the *ad lib* lunch

Test variables	WWB	HG	%[b]	VG	%[b]
Feeling of fullness, overall mean 0–240 min (mm)	35.1 ± 4	39.9 ± 4	14	44.1 ± 4	26
Feeling of hunger, overall mean 0–240 min (mm)	58.6 ± 4 a	53.8 ± 4 ab	−10	47.0 ± 4 b	−20
Desire to eat, overall mean 0–240 min (mm)	63.4 ± 4	57.2 ± 4	−11	54.3 ± 5	−16
Energy intake, voluntary lunch (kJ)	3586 ± 200	3603 ± 200	0	3326 ± 202	−7

[a] Products in the same line not sharing the same letter are significantly different, values are LSMs ± SEM, n = 19, (VAS and voluntary lunch intake, VG n = 18). [b] The percent change is calculated as the difference from HG and VG to the WWB.

found for feeling of fullness or desire to eat. No significant time × treatment interaction was found for feeling of fullness, feeling of hunger or desire to eat (0–240 min, p = 0.65, 0.93 and 0.41, respectively).

There were no difference in energy intake at the voluntary lunch (p = 0.087) (Table 5).

Correlations

Correlations between responses of glucose, insulin and appetite biomarkers as well as subjective appetite ratings and HI/FI are presented in ESI Table 1.† Both glucose and insulin (iAUC 0–120) were positively correlated to NEFA (240 min), GIP (iAUC 0–120), HI and FI, and negatively correlated to ghrelin (difference nadir to 240 min) and PYY (240 min). For GP most correlations were similar but with opposite signs. Insulin (iAUC 0–120) was correlated to feeling of satiety (tAUC 0–240) and both insulin and glucose (iAUC 0–120) were correlated to desire to eat (tAUC 0–240). Correlations between subjective appetite ratings and appetite biomarkers in the period from breakfast to lunch are presented in ESI Table 2† and those between subjective appetite ratings, appetite biomarkers and breath hydrogen excretion after lunch in ESI Tables 3 and 4.†

Discussion

In the present study we confirm that the inclusion of 10% GG (flour basis) in bread products reduces GI and increases GP compared with white wheat reference bread. Interestingly, by combining GG with other fermentable substrates, *i.e.* rye flour or HAM, differences in appetite variables and markers of fermentation were observed. We also found correlations between biomarkers of appetite (ghrelin and PYY) and measures of glucose and insulin (glucose iAUC 0–120, GP and insulin iAUC 0–120).

The glucose iPeak for both VG and HG was lowered by 1.3 and 1.4 mmol l^{-1} (−41 and −44%, respectively) compared to the WWB reference. Previously, a bread with similar concentrations of GG, combined with whole grain high amylose maize flour, lowered the iPeak with 1.5 mmol l^{-1} (−55%) when given in a smaller portion (37 g available carbohydrates).[13] It should be noted that both of these reductions meet the recently suggested guidelines for minimum differences in postprandial glycaemia to achieve metabolic improvements in T2DM pathogenesis.[3] Furthermore, the guidelines also emphasize the importance of a lowered insulin response and in the present study, the insulin iPeaks were significantly reduced by

29 and 37%, respectively for HG and VG, compared to WWB and the total insulin excursion was reduced by 18 and 12%, respectively. Thus, the ingredients and/or combinations could be further exploited in future development of bread products that could reduce postprandial glycaemic and insulinaemic excursions. As methods of prediction, both HI and FI were well correlated to glucose and insulin responses (iAUC 0–120). In a previous study we saw that FI and HI were better predictors of GP compared to GI.[13] This was, however, not the case in the present study where only HI correlated better to GP compared to glucose iAUC, whereas FI did not. This could possibly be a result from the inclusion of rye in the VG products, since previous observations in our lab on rye containing products indicates that the behaviour of rye in fluidity measurements is different from other cereals and GG.

In the present study we found reduced GIP-levels after the HG and VG breakfasts compared to WWB, and we interpret them as reflecting a lowered gastric emptying rate (GER) caused by GG. This is in line with a study reporting lower levels of GIP and decreased GER after intake of a high viscosity meal containing 3.3 g GG compared to a low viscosity meal without GG.[28] The present study design does, however, not allow us to isolate separate effects relating only to GG and, thus, we cannot exclude that also RS or rye could affect the GIP levels. Decreased GER can also contribute to increased satiety by prolonging the period of gastric distension after a meal.[28] The significantly higher levels of PYY after HG and VG breakfast meals were thus likely to be caused by prolonged gastric emptying and over-all transit time. Thus, the inclusion of GG, rye and/or HAM seems to be useful in the attempt to stimulate endogenous production of PYY.

The feeling of fullness was positively correlated to PYY-levels just before starting lunch, and at the same time the feeling of hunger and desire to eat were negatively correlated to PYY-levels. After lunch, the PYY was negatively correlated to subjective feeling of hunger, a correlation also reported by others.[29]

A significantly lower relative increase in ghrelin from nadir to 240 min was found after the HG and VG breakfasts compared to WWB. The ghrelin level at 240 min was positively correlated to the energy intake at lunch, which is in line with a recent review, indicating that ghrelin is an acute hunger signal in the pre-prandial period.[30]

After lunch, increased levels of breath H_2 was found following the rye containing VG breakfast indicating increased gut fermentative activity.[31] This is in line with previous studies of rye where increased H_2 excretion was found from 4 to 8 h after consumption.[12,32] However, in the present study, the increase in H_2 excretion was not accompanied by an increase in plasma SCFA. Possibly, this could be due to the formation of other fermentation products, *e.g.* lactate, not measured here. In the present study, increased breath H_2 at 240 min was related to increased satiety and reduced hunger after lunch (240–360 min), but not to the voluntary energy intake. This could possibly indicate that the systemic effects of an increase in breath H_2 are delayed.

The HG breakfast increased the butyrate levels already after 4 h and to our knowledge this is the first study reporting such early increases in peripheral levels of a gut fermentation mediated metabolite in response to an acute meal. It has been demonstrated, though, that a late evening meal consisting of high amylose barley bread, as well as 4 weeks of rye bread consumption, prior to a wheat bread breakfast results in higher levels of butyrate and or propionate.[33,34] No increase in SCFA was found after the consumption of VG breakfast, but preliminary data by Jakobsdottir *et al.*[35] indicated an increase in SCFA around lunch time after having rye bread for breakfast. One possibility is that the current combination of rye with GG may have retained the easily fermentable rye fraction, leading to a possible delay in SCFA production beyond our studied time span. It has been hypothesised that SCFA act as a regulator of appetite and food intake through the gut-brain axis.[36] In the present study we did, however, not find any correlations between SCFA and subjective appetite or food intake at the subsequent lunch.

HAM has previously been shown to have positive effects on insulin sensitivity and fatty acid (FA) metabolism,[37] and the effect of RS on glucose tolerance can be due to mechanisms involving muscle uptake of FA. However, the lower insulin secretion following HG breakfast in the present study was not accompanied by significant reduction of NEFA. Instead it was VG that significantly lowered NEFA at the time of lunch, an effect displayed by rye products also in a previous study.[12] A prolonged digestive phase has earlier been shown to suppress the levels of NEFA in the late postprandial phase[38] and we found correlations between improved course of glycaemia (low GI/high GP) and lower NEFA-values at 240 min. Interestingly, we also found a positive correlation between the levels of NEFA and ghrelin at lunch time. Ghrelin favours oxidation of FA as energy source[39] and this might have contributed to the increase in NEFA at lunch time after the WWB breakfast.

Conclusion

By combining GG with whole grain rye or HAM, bread products with low and sustained glycaemia were obtained. Furthermore, the combination of GG and rye stimulated PYY excretion after a subsequent *ad lib* meal. The combination of GG and rye was superior with improvements in subjective appetite. The tendency of reduced energy intake at the subsequent *ad lib* lunch warrants further investigation.

Competing interests

The authors declare no competing financial interests.

Acknowledgements

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Paper IV

Submitted manuscript

Sustained glycaemia at breakfast improve glucose tolerance at a high-carbohydrate lunch

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Abstract

In the present study it is hypothesised that a late increment in postprandial glycaemia leads to improved glucose tolerance at a second meal.

Key words: Second meal glucose tolerance, glycaemic profile, appetite

Introduction

Many studies over the last years have shown that the composition of carbohydrates in a meal may not only affect acute glycaemia and insulinaemia (Thondre 2013) but additionally also influence glucose tolerance at a subsequent meal (Gonzalez 2014, Liljeberg *et al* 1999). Reduced postprandial glycaemic excursions are of importance for the prevention of type 2 diabetes mellitus, overweight and cardiovascular disease, conditions that increase worldwide (Blaak *et al* 2012). Data also suggest that the insulin response to a meal may be important in short-term appetite regulation (Flint *et al* 2007). The glycaemic index (GI) was developed to describe postprandial glycaemia up to 120 min. To also consider the period beyond 2 hrs the concept of glycaemic profile (GP), *i.e.* the duration of a glucose net increment (up to at least 180 min) divided by its highest incremental peak (iPeak), was introduced (Rosén *et al* 2009).

The present work hypothesises that a low but sustained postprandial glycaemia, manifested by a high GP, could be a better indicator than a low GI with respect to the effect on second meal glucose tolerance. Thus, two breakfast meals were studied; white wheat bread (WWB) with high GI and low GP, and pasta (Pasta) with low GI and supposedly high GP. A standardized meal was given 4 h after the breakfast. Glycaemia, insulinaemia, non-esterified fatty acids (NEFA) and triglycerides (TG) as well as subjective appetite ratings were studied for 360 min.

Materials and methods

Raw materials, test meals and chemical characteristics

Standard white wheat flour, dry yeast, NaCl, dried spaghetti (durum wheat) and frozen sweetcorn were obtained from a local store. Commercially fried and deep frozen meatballs (FELIX Små Delikatess Köttbullar) and instant mashed potatoes (FELIX Potatismos) were kindly provided by Orkla Foods Sverige AB (Eslöv, Sweden).

The WWB was made in a home baking machine (Tefal, home bread) as previously described (Ekström *et al* 2013). Immediately before serving, pasta was boiled for 8 min in 1 l of water containing 5.0 g NaCl. Both

breakfast meals constituted of 50 g available carbohydrates (122 g WWB, 77.4 g dry pasta (approx. 190 g boiled)) and were served with 250 ml water. The standardized lunch consisted of 100 g meatballs, heated in a microwave oven (1100 W) for 1 min and 30 s, 55.0 g instant potato powder reconstituted in 250 ml boiling water and 60 g frozen sweetcorn (thawed at ambient temperature), served with 250 ml of water.

Total starch analysis (Björck & Siljeström 1992) was performed on WWB (dried and milled (IKA A11 basic)) and Pasta (boiled and homogenized in phosphate buffer (IKA Ultra turrax T25 basic, 21.5 rpm for 90 s)). WWB consisted of 41.9% and Pasta 27.5% total starch (wet weight, ww). Resistant starch (RS) analysis (Åkerberg *et al* 1998) was performed on the products 'as eaten' and WWB consisted of 0.9% and Pasta 1.2% RS (ww). The available starch contents were calculated by subtracting the amount of RS from that of total starch. Estimated energy content was 1144 and 1102 kJ for WWB and Pasta, respectively.

Study design

Twenty healthy non-smoking volunteers (8 men and 12 women) aged 23.7 ± 0.8 years (mean ± SEM) with normal body mass indices (21.8 ± 0.4 kg/m^2) and without drug therapy, participated in the study. All subjects had normal fasting blood glucose concentrations (5.4 ± 0.09 mM). Recruitment and study trials were performed from September to December 2014 in accordance with a previous study (Ekström *et al* 2013). All test subjects gave their informed consent and were aware of the possibility of withdrawing from the study at any time. Approval of the study was obtained by the Regional ethical review board in Lund, Sweden (2011/507). Capillary samples for determination of blood glucose (HemoCue Glucose 201+ Analyser, HemoCue AB, Ängelholm, Sweden), insulin (enzyme immunoassay kit, Mercodia AB, Uppsala, Sweden), NEFA (enzymatic colorimetric method, NEFA C, ACS-ACOD method, WAKO Chemicals GmbH, Germany) and TG (enzymatic colorimetric method, LabAssay Triglyceride, GPO·DAOS method, WAKO Chemicals GmbH) were taken at fasting and 15, 30, 45, 60, 90, 120, 180 and 240 min after starting the breakfast. After the sampling at 240 min the standardized lunch meal was served. The subjects were asked to finish the portion in a comfortable pace. Further capillary samples were taken at 270, 285, 300 and 360 min after breakfast. The subjects were asked to rate their subjective *feeling of hunger*, *satiety* and *desire to eat* on a bipolar visual analogue scale (VAS) directly

after each blood sampling. In order to avoid repeated thaw and freezing cycles, all blood parameters were analysed within 3 hours.

Calculations and statistical methods

The incremental- and total areas under the curves (iAUC and tAUC, respectively) were calculated (GraphPad Prism, ver 6, GraphPad Software, San Diego, CA, USA) for each subject and test meal using the trapezoid model. In the case when subjective appetite ratings were missing, they were extrapolated from the values right before and after. GI and insulinaemic index (II) were calculated from the corresponding iAUC 0-120 min, using WWB as the reference (GI and II = 100). Insulin iAUC 0-180 min include only 19 subjects due to analytical errors. Incremental peaks (iPeak) were calculated as maximum increase from baseline to 240 min. GP was defined as the duration of the glucose curve above fasting concentration (0-240 min) divided by the iPeak (Rosén *et al* 2009). The results were evaluated using a linear mixed model ANCOVA (PROC MIXED procedure) (SAS, version 9.4, SAS Institute Inc., Cary, USA). Fasting value, visit, time, meal and time × meal interaction were included as fixed effects. Subject was treated as random effect and visit and time were included as repeated effects. The models were tested for the normality of residuals and ln transformation was necessary for insulin. Data are expressed as least square means (LSMs) and standard errors of the mean (SEM). P-values ≤ 0.05 (two-tailed) were considered to be statistically significant.

Results and discussion

The mean incremental glucose and insulin responses and corresponding data for glucose, insulin, lipids and appetite ratings are shown in Fig. 1 and Tab. 1. There were no differences in mean fasting values prior to the meals for any of the studied parameters. The Pasta breakfast generated lower GI (-26%), II (-48%) and glucose- and insulin iPeaks (-31 and -47%, respectively) and an increase in GP (108%) compared to the WWB. Additionally, also the overall insulin responses (0-360 min, $p = 0.0189$) were lowered by Pasta. Interestingly, the difference in GI was less pronounced than the increase in GP which can be explained by the late increment caused by Pasta, resulting in a relatively high iAUC (0-120 min). This feature was also associated with a significantly higher mean blood

glucose and insulin levels just before lunch (240 min) after Pasta ($p < 0.001$ and $p < 0.01$, respectively) compared to WWB (time × meal interaction, $p < 0.0001$ for both glucose and insulin). The incremental glucose response after the standardized lunch (*i.e.* normalised using the value at 240) was reduced by 47% after Pasta compared to the WWB (iAUC 240-360), and there was a tendency of reduced insulin response after the standardized lunch (-15%, $p = 0.07$). Furthermore, the reduced overall glucose and insulin responses after Pasta were associated with lower subjective ratings for *desire to eat* ($p = 0.004$) compared to the WWB breakfast. Low GI foods have been shown to induce higher satiety compared to high GI foods (Bornet *et al* 2007). It has, however, been debated if this effect is due to the lower postprandial glycaemia *per se* or the presence of dietary fibres. In the present study it can be excluded that the effect on satiety is due to dietary fibres as the two test products were manufactured from the same ingredient, low in dietary fibre. The sustained increment in late glycaemia after Pasta coincided with smaller oscillations in the NEFA levels (time × meal interaction, $p < 0.0001$, Fig. 2). Suppressed NEFA concentrations has been associated with improved insulin sensitivity (Wolever *et al* 1995) and may result from a number of interdependent mechanisms such as delayed gastric emptying rate, enhanced insulin secretion, suppression of hepatic glucose production (Gonzalez 2014) and enhanced muscle glucose uptake (Jovanovic *et al* 2009). There were no differences in TG in the present study (meal effect, $p = 0.10$, time × meal interaction, $p = 0.92$), which is in contrast to a previous study comparing pasta and WWB, where TG were reduced at lunch and the following 90 min post pasta breakfast(Liljeberg & Björck 2000).

Previously, it has been shown that pasta (Liljeberg & Björck 2000), as well as lactic acid containing bread (Östman *et al* 2002) elicits a second meal improvement in glucose tolerance. However, although the addition of vinegar to WWB lowered the GI it did not result in any significant decrease in second meal glucose tolerance (Liljeberg *et al* 1999). When using data from the study with vinegar added to WWB, the estimated GP (0-180 min) was not significantly different to that of the control WWB without vinegar. This indicates that not only a low GI per se, but in combination with a high GP could be of importance for improved second meal glucose tolerance.

In the present study we demonstrate that a low GI food also characterised by a high GP improve glucose tolerance at a standardised subsequent meal compared to WWB. This feature enables a possibility to decrease postprandial glucose excursions over the day, still allowing for occasional high GI meals. In addition, the Pasta breakfast lead to lower overall ratings

of *desire to eat*. We suggest that the concept of GP could be used along with GI as a tool to optimise postprandial glycaemia.

Table 1
Metabolic responses and appetite ratings after intake of reference and test product.

Test variables[1]	WWB	Pasta	%[2]
Blood glucose			
GI (%)	100 a	74 ± 6 b	-26
iPeak (0-180 min)3 (Δ mM)	2.8 ± 0.2 a	1.9 ± 0.2 b	-31
GP (0-180 min) (min/mM)	51 ± 12 a	93 ± 12 b	80
GP (0-240 min) (min/mM)	55 ± 16 a	115 ± 16 b	108
iAUC (240-360 min) (min·mM)	150 ± 14 a	80 ± 14 b	-47
overall mean (0-360 min) (mmol/L)	6.47 ± 0.1	6.32 ± 0.1	-2
Serum insulin			
II (%)	100 a	53 ± 5 b	-48
iPeak 0-240 (Δ nM)	0.21 ± 0.02 a	0.11 ± 0.02 b	-47
iAUC 240-360 (min·nM)	18.0 ± 2	15.3 ± 2	-15
overall mean (nmol/L)	0.15 ± 0.01 a	0.12 ± 0.01 b	-19
Blood lipids			
NEFA, overall mean (mmol/L)	0.32 ± 0.01	0.32 ± 0.01	0
TG, overall mean (mmol/L)	0.92 ± 0.06	0.87 ± 0.06	-6
Appetite ratings			
Feeling of fullness, overall mean (mm)	48.5 ± 3.7	49.7 ± 3.7	3
Feeling of hunger, overall mean (mm)	41.3 ± 3.5	37.2 ± 3.5	-10
Desire to eat, overall mean (mm)	49.4 ± 3.8 a	42.5 ± 3.8 b	-14

Products in the same column not sharing the same letter are significantly different. [1]Values are LSMs ± SEM, n = 20. [2]The percent change for pasta compared to WWB. [3]Equal for timespan 0-240 min.

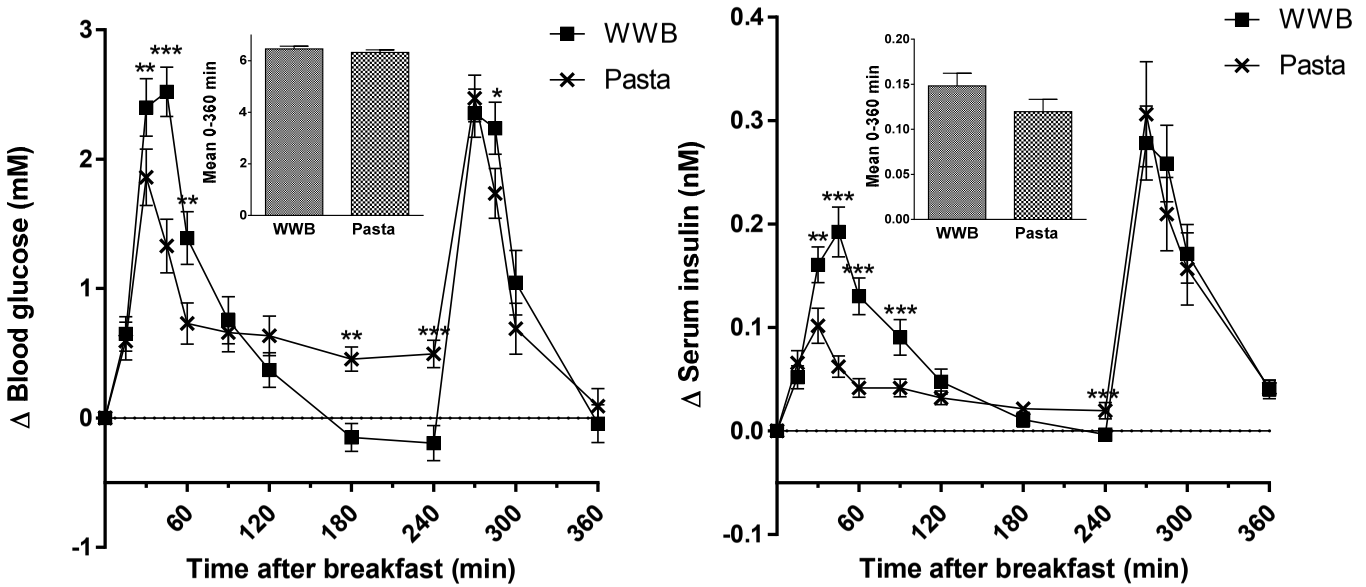

Figure 1
Mean incremental change (Δ) in blood glucose and serum insulin. Values are mean ± SEM, n = 20. Values marked with * are significantly different (* $p < 0.05$, ** $p < 0.01$ and ***$p < 0.001$).

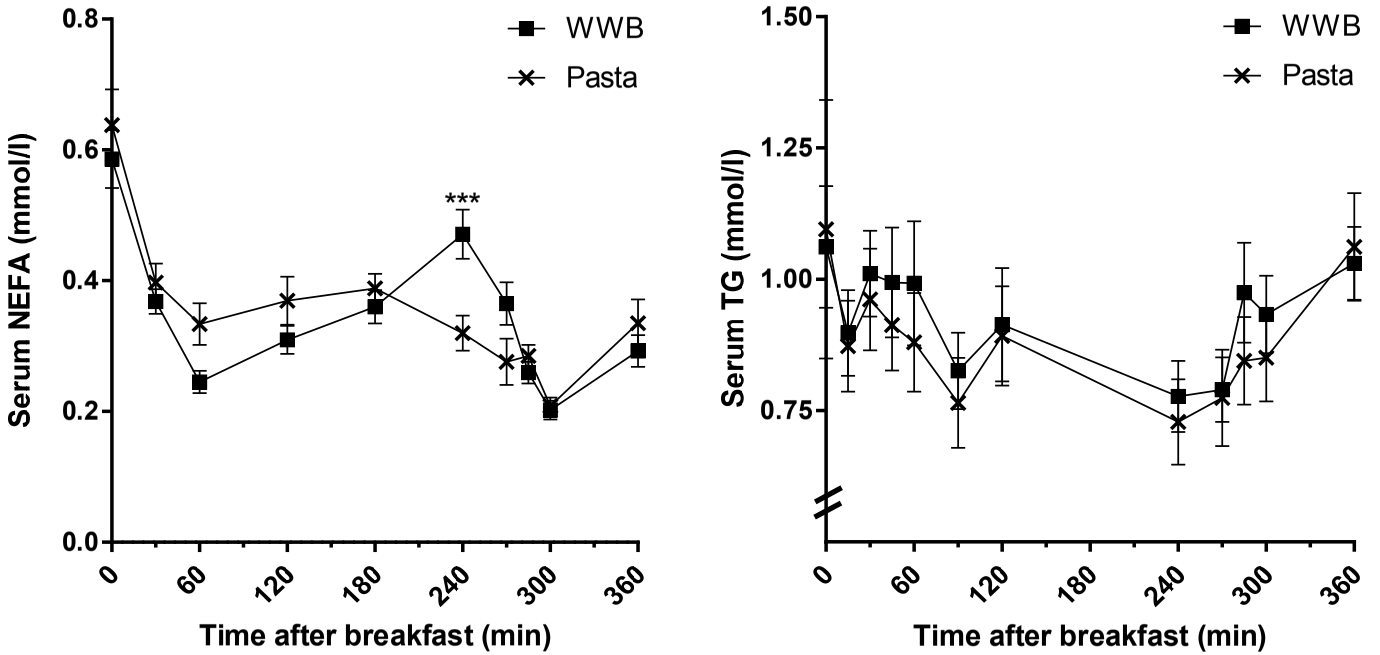

Figure 2
Absolute change in serum NEFA and TG. Values are mean ± SEM, n = 20. Values marked with * are significantly different (* $p < 0.05$, ** $p < 0.01$ and ***$p < 0.001$).

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