



LUND UNIVERSITY

Effects of Indigestible Carbohydrates and GI of Cereal Products on Glucose Metabolism, Satiety and Cognitive Function in Healthy Subjects; Emphasising mechanisms for glycaemic regulation at the acute, second and third meal

Nilsson, Anne

2007

[Link to publication](#)

Citation for published version (APA):

Nilsson, A. (2007). *Effects of Indigestible Carbohydrates and GI of Cereal Products on Glucose Metabolism, Satiety and Cognitive Function in Healthy Subjects; Emphasising mechanisms for glycaemic regulation at the acute, second and third meal*. [Doctoral Thesis (compilation), Division of Food and Pharma]. Division of Applied Nutrition and Food Chemistry, Lund University.

Total number of authors:

1

General rights

Unless other specific re-use rights are stated the following general rights apply:

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: <https://creativecommons.org/licenses/>

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

Effects of Indigestible Carbohydrates and GI of Cereal Products on Glucose Metabolism, Satiety and Cognitive Function in Healthy Subjects;

Emphasising mechanisms for glycaemic regulation at the acute, second and third meal

Anne Nilsson

September 2007

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



LUND UNIVERSITY

Akademisk avhandling för avläggande av teknologie doktorsexamen vid tekniska fakulteten, Lunds Universitet, kommer offentligen att försvaras fredagen den 7 september 2007 kl 09.15 i hörsal B, Kemicentrum, Getingevägen 60, Lund.

Fakultetsopponent: Prof Furio Brighenti, Department of Public Health, University of Parma, Italy.

Academic thesis which by due permission of the Faculty of Engineering at Lund University will be publicly defended on Friday 7th of September, 2007, at 09.15 in lecture hall B, Centre for Chemistry and Chemical Engineering, Getingevägen 60, Lund, for the degree of Doctor of Philosophy in Engineering. Faculty opponent: Prof Furio Brighenti, Department of Public Health, University of Parma, Italy.

© 2007 Anne Nilsson

Doctoral Thesis

ISBN 978-91-628-7219-9

Printed by Media-Tryck, Lund University
Lund, Sweden

ABSTRACT

The metabolic syndrome includes a cluster of dysfunctions that identifies subjects at high risk of developing type 2 diabetes and cardiovascular disease. The prevalence of the metabolic syndrome has increased markedly over the last two decades. Central to this syndrome is insulin resistance and hyperglycaemia, but also other manifestations, e.g. central obesity, dyslipidaemia, elevated blood pressure, imbalance in lipoproteins and pro-thrombotic factors, or sub-clinical inflammation, are involved.

Diets with a low glycaemic index (GI) have proven beneficial in prevention and treatment of diabetes, cardiovascular disease, and the metabolic syndrome. Furthermore, a whole grain diet has shown similar benefits adjunct to these diseases. Less is known regarding the effect of GI or other food characteristics on cognitive function. However, due to the significant differences in postprandial blood glucose profiles after a high- compared with a low-GI meal, cognitive functions during the postprandial phase could be expected to differ depending on the choice of carbohydrate food and time point in the postprandial phase.

The purpose of the present thesis was to evaluate the importance of GI features and/or contents of indigestible carbohydrates of cereal products on glycaemic regulation, metabolic risk markers, and cognitive function in healthy subjects. The test products were either evaluated in the acute postprandial phase, or following one or two subsequent standardised meals.

The results of the present thesis show that certain cereal products, with low GI and/or with a specific mixture of resistant starch (RS) and dietary fibre (DF) have the capacity to reduce glucose response not only in the acute phase, but also the incremental blood glucose responses following two consecutive meals during the course of a whole day, and in the perspective from a late evening meal to a subsequent breakfast. Boiled barley- and rye kernels, or a white wheat bread (WWB) added with RS and barley DF in an amount similar to that in barley kernels, possessed such properties. It was concluded that the GI features of the cereal based breakfast was the major determinant of glycaemia at the subsequent standardised lunch. Instead, improved glycaemic regulation within a 10.5 – 12.5 h perspective post the cereal test meal was associated with increased levels of colonically derived metabolites (e.g. plasma short chain fatty acids (SCFAs)), suggesting the involvement of colonic fermentation of indigestible carbohydrates present in the test meal.

A lowering of circulating FFAs was identified as a major factor for the improvements seen in glucose tolerance in the perspective from an evening test meal to breakfast. In addition, serum IL-6 was reduced and plasma adiponectin increased at breakfast following a barley kernel based evening meal, compared with an evening with white wheat bread (WWB). The barley kernel evening meal thus improved glucose tolerance and improved markers of inflammation and insulin sensitivity.

Following the evening test meals, markers of colonic fermentation (breath H₂) at breakfast were negatively correlated to the gastric emptying rate (GER) and positively correlated to satiety. A negative correlation was seen between GER and satiety post the breakfast. Further, the concentrations of SCFAs and GLP-1 were negatively correlated to the blood glucose response at breakfast. It is suggested that cereal products which promote colonic fermentation and GLP-1 release improve glucose tolerance and satiety in an overnight perspective.

The findings in the thesis provide evidence for a link between the gut microbial metabolism and key factors associated with improved insulin sensitivity. Further, the data obtained indicates that impaired cognitive function may be an early manifestation of the metabolic syndrome, since a significant decline in cognitive performance was displayed in middle-aged subjects with lower glucose tolerance, albeit still within a normal range. In addition, it was shown that a breakfast with low-GI features i.e. avoidance of a rapid decline in glycaemia (at 90 min), and maintenance of a net blood glucose increment in the late postprandial phase (at 170 min), was associated with improvements in measures of cognitive function (working memory and selective attention, respectively) at these time points.

The results in the thesis provide information to be used for tailoring of low-GI whole grain products, which facilitate glycaemic regulation and related parameters over the course of several meals, with beneficial implications on metabolic risk factors, weight control, and cognitive function.

LIST OF PAPERS

This thesis is based on the following papers:

- Paper I** *“Effects of GI and content of indigestible carbohydrates of cereal-based evening meals on glucose tolerance at a subsequent standardised breakfast”*.
A. Nilsson, Y. Granfeldt, E. Östman, T. Preston, and I. Björck
European Journal of Clinical Nutrition, 2006, 60 (9): 1092-1099
- Paper II** *“Effects of GI vs content of cereal fibre of the evening meal on glucose tolerance at a subsequent standardised breakfast”*.
A. Nilsson, E. Östman, T. Preston, and I. Björck
European Journal of Clinical Nutrition advanced online publication,
23 May 2007; doi: 10.1038/sj.ejcn.1602784
- Paper III** *“Importance of the composition of indigestible carbohydrates in the evening meal on glucose tolerance, satiety and inflammatory markers at a subsequent standardised breakfast”*.
A. Nilsson, E. Östman, J.J. Holst, and I. Björck
American Journal of Clinical Nutrition (submitted)
- Paper IV** *“Effect of cereal test breakfasts varying in GI and content of indigestible carbohydrates on day-long glucose tolerance in healthy subjects”*
A. Nilsson, E. Östman, Y. Granfeldt, and I. Björck
American Journal of Clinical Nutrition (accepted)
- Paper V** *“Effects of differences in postprandial glycaemia on cognitive functions in healthy middle-aged subjects”*.
A. Nilsson, K. Radeborg, and I. Björck
European Nutrition of Clinical Nutrition (accepted)

Reprints have been made with the permission of the publisher

MY CONTRIBUTION TO THE PAPERS

- Paper I** A. Nilsson was involved in the design of the study, coordinated the study, was involved in the analysis of the test products, prepared the test meals, collected the blood samples, took part in the analysis of the test parameters, performed the statistical calculations, evaluated the results, and wrote the manuscript.
- Paper II** A. Nilsson designed and coordinated the study, performed the analysis of the test products, prepared the test meals, collected the blood samples, took part in the analysis of the test parameters, performed the statistical calculations, evaluated the results, and wrote the manuscript.
- Paper III** A. Nilsson designed and coordinated the study, performed the majority of the analysis of the test products, prepared the test meals, collected the blood samples, took part in the analysis of the test parameters, performed the statistical calculations, evaluated the results, and wrote the manuscript.
- Paper IV** A. Nilsson designed the study and was responsible for the coordination and analysis of the test products, was responsible for the experimental work, performed the statistical calculations, evaluated the results, and wrote the manuscript.
- Paper V** A. Nilsson designed and coordinated the study, prepared the test meals, collected and analysed the blood samples, was involved in the statistical calculations and evaluations of the cognitive tests, and wrote the manuscript.

ABBREVIATIONS

(I)AUC	(Incremental) area under the curve
BDF	Barley dietary fibre corresponding to the total amounts in barley kernels
BMI	Body mass index
CHD	Coronary heart disease
CutOB	Ordinary barley kernels cut 1-2 times,
CVD	Cardiovascular disease
DF	Dietary fibre
FFAs	Free fatty acids
GER	Gastric emptying rate
GI	Glycaemic index
GIP	Gastric inhibitory peptide
GLP-1	Glucagon-like peptide 1
GT	Glucose tolerance
HAB	High amylose barley kernels
HBB	High beta-glucan barley kernels
HDL	High density lipoprotein
IDDM	Insulin dependent diabetes mellitus
IGT	Impaired glucose tolerance
IL-6	Interleukin-6
IRS	Insulin resistance syndrome
NIDDM	Non insulin dependent diabetes mellitus
OB	Ordinary barley kernels
1/2OB	Half a portion OB
ODF	Oat dietary fibre
OGTT	Oral glucose tolerance test
pGI	In vitro predicted GI
RS	Resistant starch
SA	Selective attention
SCFAs	Short chain fatty acids
WHO	World Health Organization
WM	Working memory
WMB	Whole meal barley
WWB	White wheat bread

CONTENTS

1. INTRODUCTION	1
2. BACKGROUND	3
2.1. THE METABOLIC SYNDROME; DEFINITIONS AND MECHANISMS	3
2.2. OXIDATIVE STRESS AND SUB CLINICAL INFLAMMATION	5
2.3. BLOOD GLUCOSE REGULATION	7
2.4. GLYCAEMIC INDEX (GI)	8
2.5. “SECOND-MEAL” EFFECTS ON GLUCOSE TOLERANCE	9
2.6. METABOLIC BENEFITS OF WHOLE GRAIN	10
2.7. COLONIC FERMENTATION OF INDIGESTIBLE CARBOHYDRATES	11
2.8. COGNITIVE FUNCTION IN RELATION TO GLYCAEMIA	11
3. OBJECTIVES	13
4. MATERIALS AND METHODS	14
4.1. TEST PRODUCTS AND MEALS	14
4.1.1. CEREAL BASED TEST PRODUCTS AND MEALS (PAPERS I – IV)	14
4.1.2. SIMULATED LOW-GI OR HIGH-GI MEALS (<i>PAPER V</i>)	18
4.1.3. STANDARDISED MEALS (<i>PAPERS I – IV</i>)	18
4.2. CHEMICAL ANALYSES OF TEST PRODUCTS	18
4.3. DETERMINATION OF GI	19
4.4. EXPERIMENTAL DESIGN	19
4.5. PHYSIOLOGICAL PARAMETERS	22
4.6. COGNITIVE PARAMETERS	25
4.6.1. WORKING MEMORY (WM) TEST	25
4.6.2. SELECTIVE ATTENTION (SA) TEST	25
4.7. CALCULATIONS AND STATISTICAL METHODS	26
5. RESULTS AND DISCUSSION	28

5.1. EFFECTS OF CEREAL BASED EVENING TEST MEALS ON GLUCOSE RESPONSE AND RELATED PARAMETERS AT THE FOLLOWING BREAKFAST	28
5.1.1. BLOOD GLUCOSE	28
5.1.1.1. <i>Importance of Glycaemic index</i>	28
5.1.1.2. <i>Importance of food structure</i>	34
5.1.1.3. <i>Importance of indigestible carbohydrates (DF and RS)</i>	34
5.1.1.4. <i>The role of colonic fermentation of indigestible carbohydrates</i>	36
5.1.2. SERUM INSULIN AND PLASMA GLUCAGON	40
5.1.3. PLASMA SHORT CHAIN FATTY ACIDS	40
5.1.4. PLASMA/SERUM FREE FATTY ACIDS AND SERUM TRIACYLGLYCEROLS	43
5.1.5. PLASMA INCRETINS (GLP-1 AND GIP)	46
5.1.6. PLASMA IL-6 AND IL-8	47
5.1.7. PLASMA ADIPONECTIN	48
5.1.8. GASTRIC EMPTYING RATE AND SATIETY	48
5.2. EFFECTS OF CEREAL BASED TEST MEALS ON GLUCOSE RESPONSE AND H₂ EXCRETION IN A DAYLONG VS OVERNIGHT PERSPECTIVE	50
5.2.1. EFFECTS OF TEST BREAKFASTS ON DAYLONG BLOOD GLUCOSE AND BREATH H ₂ CONCENTRATIONS	50
5.2.2. BLOOD GLUCOSE AND BREATH H ₂ CONCENTRATIONS 9.5 H AFTER A TEST BREAKFAST (DAYLONG) OR TEST DINNER (OVERNIGHT)	59
5.3. COGNITIVE PERFORMANCE IN RELATION TO POSTPRANDIAL GLYCAEMIA AND GLUCOSE TOLERANCE	62
6. CONCLUSIONS	67
7. FUTURE WORK	69
8. ACKNOWLEDGEMENTS	70
9. REFERENCES	71

1. INTRODUCTION

A western lifestyle, with a low physical activity and an almost constant access to food, offers a challenge to the food industry in supplying the market with a sufficient range of healthy and convenient food alternatives. Over the last century there has been a striking increase in diabetes, mainly diabetes type 2, which is strongly related to a sedentary lifestyle and obesity (Zimmet 1999). In Sweden, the prevalence of overweight in the population (16 - 84 years) has increased from 31 % of the men and 22 % of the women in 1980, to 41 % and 28 % for men and women, respectively, in 2001. Within this time frame, the prevalence of obesity increased from 5 % to 10 % (Persson, Sjöberg et al. 2004). The prevalence of overweight and obesity increases with age, and in Sweden, 60 – 65 % of men in the age of 45 – 74 years, and more than 50 % of the women, were overweight or obese in 2001 (Persson, Sjöberg et al. 2004). Even more alarming is the increase of overweight and obesity in the younger population. Consequently, among young people, 16 - 24 year of age, there has been a doubling of overweight and obesity from 1980 – 2001, and in 2001, 14 % of young women and 22 % of young men were overweight or obese.

The metabolic syndrome (also referred to as the insulin resistance syndrome, IRS) is a condition which includes a cluster of dysfunctions that identifies subjects at high risk of developing type 2 diabetes and cardiovascular disease (Chew, Gan et al. 2006). During the last two decades there has been a substantial increase in people with the metabolic syndrome (Eckel, Grundy et al. 2005). The increase in prevalence of the metabolic syndrome parallels the increase in obesity and type 2 diabetes (Eckel, Grundy et al. 2005). Currently, approximately 190 million people worldwide have type 2 diabetes, and the number is expected to rise to 300 millions by 2025 (Jazet, Pijl et al. 2003; Zimmet 2005). In addition, at least 300 million people have impaired glucose tolerance (IGT), a pre-diabetic state (Zimmet 2005). The dietary pattern is intimately linked to well being and health. It has thus been shown that certain foods or food components may have beneficial effects on metabolic risk factors. Consequently in a prospective study of 3157 subjects it was observed that among overweight adults, increased dairy consumption was inversely correlated with the metabolic syndrome (Pereira, Jacobs et al. 2002). The mechanism may be related to the fat quality, and a negative relation was demonstrated between milk-fat and hyperinsulinaemia among females with impaired fasting glucose (n = 4999, blood glucose \geq 5.6 but without diabetes

mellitus diagnosis) (Wirfalt, Hedblad et al. 2001). Besides milk-fat, intake of polyunsaturated omega-3 fatty acids (Ebbesson, Risica et al. 2005; Carpentier, Portois et al. 2006; Levy, Spahis et al. 2006) as well as monounsaturated fatty acids are negatively related to the prevalence of the metabolic syndrome (Rivellese, De Natale et al. 2002), whereas saturated fats are associated with increased insulin resistance. In addition intake of vegetables and fruits are negatively related to the metabolic syndrome (Esmailzadeh, Kimiagar et al. 2006).

In the choice of carbohydrate foods, foods that elicit a low blood glucose response, i.e. food with a low glycaemic index (GI), have proven beneficial in prevention and treatment of diabetes, cardiovascular disease and the metabolic syndrome (Fontvieille, Rizkalla et al. 1992; Järvi, Karlström et al. 1999; Jenkins, Kendall et al. 2002; Brand-Miller 2003; McKeown, Meigs et al. 2004). A whole grain diet has shown benefits similar to low-GI diets regarding prevention of type 2 diabetes and cardiovascular disease (Anderson, Hanna et al. 2000; Jenkins, Axelsen et al. 2000; McKeown, Meigs et al. 2002; Anderson 2003; Schulze, Liu et al. 2004). Furthermore, a low-GI diet combined with high intake of cereal fiber has shown particular advantageous in preventing diabetes type 2 in both women (Salmeron, Manson et al. 1997) and men (Salmeron, Ascherio et al. 1997). However, despite this knowledge, there is at present a shortage of low-GI cereal foods that in addition are rich in whole grain constituents. In the formulation of such foods, further studies are needed to clarify which food factors and physiological mechanisms that are involved in modulating metabolic parameters. More knowledge in this area will make possible tailoring of food products with magnified metabolic benefits. The present thesis addresses this specific topic.

2. BACKGROUND

2.1. The metabolic syndrome; definitions and mechanisms

There is currently no international consensus regarding the definition of the metabolic syndrome. An overview of factors involved is shown in **Figure 1**. According to WHO, (1999), the metabolic syndrome includes; hyperglycaemia/insulin resistance plus two or more of the four following additional criteria; central obesity, dyslipidaemia, elevated blood pressure, and micro-albuminuria. In 2005, the International Diabetes Federation (IDF) proposed a similar definition; central obesity plus two or more of the four criteria; hyperglycaemia, elevated triglycerides, reduced HDL-cholesterol, and elevated blood pressure (Chew, Gan et al. 2006). In addition, other definitions have been proposed e.g. by the National Cholesterol Education Program (NCEP), including three or more of the five criteria; central obesity, hyperglycaemia, elevated triglycerides, reduced HDL-cholesterol, and elevated blood pressure (Chew, Gan et al. 2006).

The prevalence of the metabolic syndrome has increased markedly over the last two decades, and is presently between approximately 35 and 39 % among adults in the US (Ford 2005). Insulin resistance seems to be the single most common cause of the syndrome (Eckel, Grundy et al. 2005; Zimmet 2005) and includes dysfunctions in insulin receptors and signalling pathways, and in glucose transport or glucose metabolism (Arner 2002). Insulin resistance is in addition also associated with several other abnormalities which are currently not included as a clinical criteria for the metabolic syndrome, e.g. imbalance in pro-thrombotic factors, increased inflammatory markers, obstructive sleep apnoea, and non-alcoholic fatty liver disease (Eckel, Grundy et al. 2005). Additionally, impairment of cognitive functions is increasingly being associated with type 2 diabetes and IGT (Messier, Tsiakas et al. 2003; Gallacher, Pickering et al. 2005).

The mechanisms underlying insulin resistance (IR) are not fully known, but a genetically caused disturbance of insulin-mediated glucose uptake and utilisation may be involved (Arner 2002), as well as dietary factors such as high intake of high-GI carbohydrate foods (McKeown, Meigs et al. 2004) and saturated fat (Rivellese, De Natale et al. 2002) and low intake of whole grain (McKeown, Meigs et al. 2004). One established physiological factor connected to IR is an increased concentration of circulating free fatty acids (FFAs), which are particularly increased

in obese subjects (Arner 2001). Insulin resistance at the level of the adipose tissue is characterised by adipocytes being resistant to the inhibitory effect of insulin of lipolysis, resulting in increased concentrations of FFAs in the circulation. Even though the visceral fat depots only contribute to approximately 10 % of the over all adipose tissue, this fat depot is particularly involved in the development of IR, especially at the level of the liver (Arner 2001). The rate of FFA turn-over is higher in the visceral fat depot, and in addition, in comparison to the subcutaneous depot this fat depot results in higher concentrations of FFAs in the portal vein, which in turn results in a decreased hepatic insulin extraction, and increased gluconeogenesis and triacylglyceride production. Elevated concentrations of FFAs cause IR also in the pancreas and the muscles, resulting in a decreased insulin secretion and glucose uptake (Arner 2002).

The metabolic syndrome

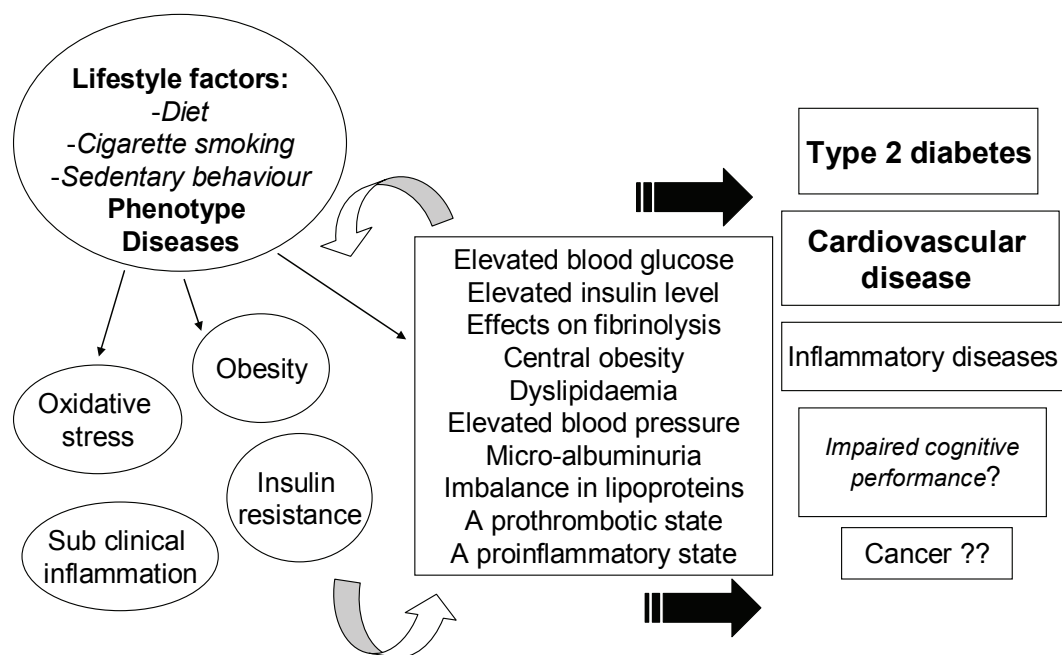


Figure 1: Factors involved in the etiology of the metabolic syndrome.

2.2. Oxidative stress and sub clinical inflammation

The choice of diet considerably influences the risk of developing cardiovascular disease. With respect to glucose metabolism, hyperglycaemia, and probably also elevated levels of circulating FFAs, induce increased concentrations of reactive oxygen- and nitrogen species (King and Loeken 2004), resulting in cell damage, endothelial dysfunction and vascular complications (Rask-Madsen and King 2007), as well as β -cell dysfunction (Evans, Goldfine et al. 2003; Robertson, Harmon et al. 2003; Miyazaki, Kawano et al. 2007). Consequently, oxidative stress is a possible mechanism in the patho-physiology of diabetes and cardiovascular disease (Ross 1999; King and Loeken 2004; Rask-Madsen and King 2007). Accordingly, adults with the metabolic syndrome displayed lower concentrations of several antioxidants in serum (Ford, Mokdad et al. 2003), suggesting a deficiency in dietary supply, or that antioxidants have been used to scavenge reactive species emanating from e.g. hyperglycaemia. Hyperglycaemia increases inflammation, possibly by factors involving increased production of reactive oxygen species (Haidara, Yassin et al. 2006). Oxidative stress is probably a key mediator of increased cytokine concentrations and low grade systemic inflammation (Esposito, Nappo et al. 2002), suggesting a role in the genesis of vascular damage (Burdge and Calder 2005). Acute hyperglycaemia induce increased concentration of circulating cytokines (TNF-alpha, IL-6, IL-18) also in healthy subjects (Esposito, Nappo et al. 2002). Low-grade inflammation has been connected to adverse effects on insulin sensitivity, glucose- and lipid metabolism, and blood pressure (Heliövaara, Teppo et al. 2005). A suggested relation between increased blood glucose concentrations, and circulating FFAs, respectively, and risk factors for the metabolic syndrome is schematically shown in **Figure 2**. In particular, frequent postprandial hyperglycaemic episodes appears to initiate cytokine production (Esposito, Nappo et al. 2002), indicating that tight blood glucose regulation may protect against cardiovascular disease (CVD) (Ceriello 2006). The importance of food choice in preventing ill-health related to sub-clinical inflammation (e.g. CVD) has been demonstrated in studies including a large number of subjects. Thus, data from The Nurses' Health Study cohort (1976) showed that a western diet (higher intake of red and processed meat, sweets, desserts, French fries, and refined grains), was positively correlated with inflammatory parameters e.g. plasma CRP, E-selectin, and interleukin 6 (IL-6), whereas a prudent dietary pattern (higher intake of fruit, vegetables, legumes, fish, poultry, and whole grain), was inversely related to the same parameters in women (Lopez-Garcia, Schulze et al. 2004). Further, the

plasma concentrations of IL-6 were positively related to the BMI. Similarly, in 1559 subjects, a positive correlation was observed between circulating concentrations of CRP and fibrinogen, respectively; and BMI. It was concluded that chronic sub-clinical inflammation may be one patho-physiological mechanism related to the increased risk of atherosclerotic disease associated with obesity (Festa, D'Agostino et al. 2001).

Prospective studies including large number of subjects have shown that an intervention with acarbose, (α -glucosidase inhibitor), importantly delays the onset of diabetes (type 2) (Delorme and Chiasson 2005), and reduce the risk of cardiovascular disease and hypertension in IGT subjects (Chiasson, Josse et al. 2003) The beneficial effect of acarbose is proposed to derive from reduced oxidative stress and subclinical inflammation, respectively, secondary to lowered postprandial blood glucose excursions (Delorme and Chiasson 2005; Båvenholm and Efendic 2006). The effect of acarbose is thus similar to that of a low GI diet. It could therefore be hypothesised that a diet with low GI, by virtue of maintaining a more tight blood glucose regulation, decreases oxidative stress and low grade inflammation.

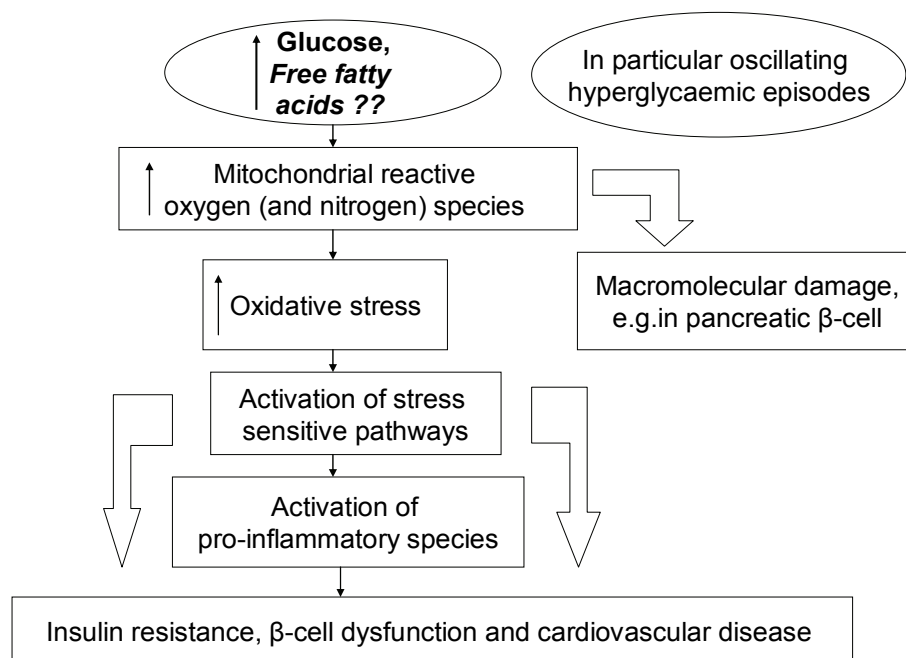


Figure 2. Proposed relation between postprandial blood glucose response, circulating FFAs and risk factors for the metabolic syndrome.

2.3. Blood glucose regulation

In healthy subjects, fasting blood glucose concentrations are maintained below 5.6 mmol/L (6.1 mmol/L in plasma) (WHO 1999) by glycogenolysis and gluconeogenesis in the liver, controlled mainly by glucagon released from pancreatic α -cells (Kruger, Martin et al. 2006). In the fed state, the blood glucose homeostasis is achieved by release of insulin from β -cells, amylin (co-released to insulin), and the incretine hormones; glucagon-like peptide-1 (GLP-1) and glucose-dependent insulintropic peptide (GIP), secreted by L- and K-cells of the intestinal wall, respectively, in response to the presence of food in the intestine (Kruger, Martin et al. 2006). Amylin suppresses postprandial glucagon secretion, contributes to regulation of gastric emptying and food intake, presumably by centrally mediated pathways (Kruger, Martin et al. 2006). GLP-1 enhances glucose-dependent insulin secretion, suppresses postprandial glucagon secretion and is involved in regulation of gastric emptying and food intake. In addition, GLP-1 has been shown to stimulate the formation of new beta cells in rodents (Holst and Orskov 2001). GIP enhances glucose-dependent insulin secretion and is involved in lipid metabolism (Yip and Wolfe 2000). Adiponectin, is an adipocyte derived peptide hormone that improves insulin sensitivity, has anti-inflammatory and anti-atherogenic effects, and is associated with a lower risk of ischemic heart disease (IHD) and type 2 diabetes (Pischon, Girman et al. 2005). Hormones involved in raising blood glucose concentrations are; epinephrine, cortisol, and growth hormones (Vander, Sherman et al. 2001). As discussed above, of importance in the regulation of blood glucose is also the concentration of circulating FFAs.

Despite normal fasting blood glucose, a subject may still display a pathological postprandial blood glucose response to a glucose tolerance test (75 g glucose). A blood glucose concentration remaining ≥ 7.8 mmol/L two hours after the glucose load is used as a definition of IGT, and a fasting value ≥ 6.1 mmol/L and/or postprandial blood glucose concentration ≥ 11.1 mmol/L classifies the subject as having diabetes mellitus (WHO 1999). In a cohort study on 625 subjects, fasting plasma glucose was inadequate in diagnosing diabetes and intermediate categories of glucose intolerance, whereas an oral glucose tolerance test (OGTT) proved to be a more sensitive tool in diagnosing IGT. An even better tool was, however, the use of both fasting glucose and OGTT (Chen, Mukherjee et al. 2002). A meta-analysis using five cohort studies including 6766 non-diabetic subjects, have further demonstrated that the two-hour blood glucose value after a glucose load (75 g) is a

better risk predictor for cardiovascular disease events, compared with fasting glucose concentrations (Qiao, Pyorala et al. 2002). Taken together these studies indicate that postprandial glucose clearance is predictive of metabolic disease risk. Measures to control the glycaemic features of foods and meals could therefore be expected to beneficially influence events involved in the etiology of the metabolic syndrome.

2.4. Glycaemic index (GI)

The glycaemic index (GI) is a concept used to classify carbohydrate-rich foods according to their effects on the postprandial blood glucose response. The GI of a product is determined using the 0 – 120 min positive incremental blood glucose area under the curve (IAUC), and calculated as the mean of individual ratios (Brouns, Björck et al. 2005) using WWB or glucose as reference products. For starchy foods, the differences in GI are related to a number of gastro-intestinal events e.g. differences in the rate of gastric emptying, and/or the rate of starch digestion and absorption in the small intestine. Food properties such as the degree of botanical integrity (Liljeberg, Granfeldt et al. 1992), dense food texture (e.g. spaghetti) (Granfeldt and Björck 1990), interaction between starch and other macronutrients (e.g. proteins) (Colonna, Barry et al. 1990), content and type of dietary fibre (Jenkins, Wolever et al. 1978; Liljeberg, Granfeldt et al. 1996), and organic acids, either added to the product or developed during sourdough baking (Liljeberg, Lönner et al. 1995), are all factors that influence the above gastro-intestinal events and hence the GI characteristics.

Due to an accumulating body of data showing beneficial effects of low-GI foods on glucose and lipid metabolism (Jenkins, Kendall et al. 2002; Brand-Miller 2003), a diet rich in such foods is increasingly being considered advantageous. Epidemiological data indicate that a low-GI diet has a protective role against development of type 2 diabetes (Salmeron, Ascherio et al. 1997; Salmeron, Manson et al. 1997), coronary heart disease (Liu, Willett et al. 2000; Jenkins, Kendall et al. 2002), and the metabolic syndrome (McKeown, Meigs et al. 2004). Some data also points to a positive association between dietary GI and the risk of colonic and breast cancers which is probably mediated by the lower insulin demand (Jenkins, Kendall et al. 2002). Insulin resistance is a characteristic feature of the metabolic syndrome (Arner 2001; Arner 2002), and impaired sensitivity to insulin at the level of adipose tissue plays a central role for the genesis of the metabolic abnormalities associated with this syndrome. An improved adipocyte insulin sensitivity has been demonstrated following a low-GI diet in patients with coronary heart disease

(Frost, Keogh et al. 1996; Frost, Leeds et al. 1998). Consequently, a low-GI diet appears to have a therapeutic potential in subjects with the metabolic syndrome, and a preventive potential by reducing insulin resistance and other risk factors associated with the genesis of the metabolic syndrome. The mechanisms whereby low-GI foods exert long-term metabolic benefits are not fully understood. However, the more distal absorption of carbohydrates, which prevents a high blood glucose response and the accompanying high insulin surge, as well as a rapid decline in blood glucose, has been implicated (Jenkins, Wolever et al. 1982). The avoidance of a rapid decline in blood glucose may prolong a suppression of release of FFAs to the circulation, thus avoiding deterioration of insulin sensitivity.

2.5. “Second-meal” effects on glucose tolerance

As judged from the literature, low-GI foods appear to have the capacity to improve glucose tolerance at a “second-meal” ingested 4h after the low-GI meal, hence, reducing the glycaemic and insulinaemic responses over two consecutive meals (Jenkins, Wolever et al. 1982; Trinick, Laker et al. 1986; Liljeberg, Åkerberg et al. 1999; Liljeberg and Björck 2000). An improvement in second-meal glucose tolerance following low-GI starchy foods has also been observed in the perspective from a late dinner to breakfast the following morning (Wolever, Jenkins et al. 1988; Thorburn, Muir et al. 1993; Granfeldt, Wu et al. 2006). The mechanism for a “second-meal effect” with low-GI foods in the perspective from a test breakfast to lunch (4 h) is mediated by the more slow and distal intestinal carbohydrate uptake in the case of low-GI foods. These lente features contribute to a postponed in-between-meal fasting and a suppression of free fatty acids (FFAs); hence improving glucose tolerance at the time of the subsequent lunch-meal (Liljeberg and Björck 2000). Also the mechanism for benefits on glucose tolerance from dinner to breakfast has been related to suppression of FFAs (Thorburn, Muir et al. 1993; Wolever, Bentum-Williams et al. 1995). However, the cause for reduced FFAs is likely to vary depending on the time frame in-between meals. Food properties like the presence of fermentable dietary fibre may be involved in the second-meal effect from an evening meal to breakfast (Thorburn, Muir et al. 1993). It has also been suggested that the GI characteristics of the dinner meal *per se* can predict the glucose response to a standardised test meal the following morning (Wolever, Jenkins et al. 1988). However, in a recent study, low-GI characteristics of the evening meal *per se* did not seem to influence glucose tolerance the following morning, and only a low-GI meal, which in addition was rich in indigestible

carbohydrates, significantly lowered blood glucose and insulin responses at a subsequent breakfast (Granfeldt, Wu et al. 2006).

2.6. Metabolic benefits of whole grain

Although not a prerequisite, many low-GI foods e.g. legumes, pumpernickel bread, barley, and bulgur are rich in indigestible carbohydrates, i.e. resistant starch (RS) and dietary fibre (DF). It has been shown that high carbohydrate diets rich in DF increase peripheral insulin sensitivity and lower plasma glucose, insulin, and cholesterol concentrations (Fukagawa, Anderson et al. 1990). Substantial evidences from cohort studies have demonstrated that diet rich in whole grain decrease the risk of developing coronary heart disease (CHD) (Anderson, Hanna et al. 2000; McKeown, Meigs et al. 2002). Furthermore, a whole grain diet has been implicated as protective against development of type 2 diabetes and cardiovascular disease (Anderson, Hanna et al. 2000; Jenkins, Axelsen et al. 2000; McKeown, Meigs et al. 2002; Anderson 2003; Schulze, Liu et al. 2004). Diets rich in whole grain and diets with a low GI thus exert similar benefits adjunct to the metabolic syndrome, and it could be hypothesised that low-GI foods, which in addition also are rich in “whole grain” constituents could be particularly advantageous. Thus, it has previously been shown that low-GI bread products rich in cereal DF significantly improved insulin economy in young women at risk of developing type 2 diabetes i.e. with a history of gestational diabetes (Östman, Frid et al. 2006). The role of specific cereal DF fractions is of particular interest in this respect, and different sources and types of DF give rise to different physiological effects. Consequently, viscous soluble DF have traditionally been associated with benefits on metabolism, whereas insoluble non viscous fibre, e.g. wheat bran, instead have been assigned advantages emanating from their effects locally in the colon and e.g. reduce the risk of colorectal cancer (Harris and Ferguson 1993). It is well known that viscous soluble fibre lower the rate of glucose delivery to the blood, and accordingly, reduce the postprandial blood glucose response and hormonal surge (Jenkins, Wolever et al. 1978; Tappy, Gugolz et al. 1996; Würsch and Pi-Sunyer 1997; Jenkins, Kendall et al. 1998; Jenkins, Jenkins et al. 2002). In addition, soluble fibres have also shown beneficial effects on blood lipids; risk factors for development of cardiovascular disease (Jenkins, Kendall et al. 2002). Moreover, whole grain foods are, in addition to being rich in DF, also rich in associated potentially bioactive components, such as minerals, plant stanols and sterols, vitamins and antioxidants (Anderson 2003; Slavin 2003). Consequently, the mechanisms for the beneficial effects of a whole grain diets is probably multi-factorial, and remain to be elucidated.

2.7. Colonic fermentation of indigestible carbohydrates

Indigestible carbohydrates (RS and DF), reach the colon and constitute a substrate for colonic bacteria (Rumessen 1992). Colonic fermentation of indigestible carbohydrates results in formation of microbial metabolites, particularly short-chain fatty acids (SCFAs, mainly acetic, propionic, and butyric acids), and gases (e.g. carbon dioxide and hydrogen (H₂)) (Cummings, Pomare et al. 1987; Cummings, Macfarlane et al. 2001). Moreover, combining different types of indigestible carbohydrates, e.g. including various DF and RS in a diet, have been shown to induce synergistic effects on colonic fermentation, which may affect the total level of SCFAs formed as well as the pattern and the site of SCFAs release in the rat hindgut (Henningsson, Bjorck et al. 2002). H₂ produced during colonic fermentation is absorbed into the circulation and exhaled in breath and can be used as an indicator of colonic fermentation (Rumessen 1992). It has been suggested that SCFAs produced during colonic fermentation of indigestible carbohydrates may have beneficial implications on glucose metabolism (Anderson and Bridges 1984; Thorburn, Muir et al. 1993; Berggren, Nyman et al. 1996).

2.8. Cognitive function in relation to glycaemia

Glucose is the main fuel for the brain. Although constituting only 2 % of the total body weight, the brain uses approximately 20% of the body's energy at rest (Benton and Parker 1998). A period of intensive cognitive demand has been shown to result in a measurable decrease in peripheral blood glucose levels, caused by an increased neural energy expenditure (Scholey, Harper et al. 2001; Scholey, Laing et al. 2005). The brain's energy stores are small, and without glucose supply, the brain reservoir would be depleted from glucose within approximately 10 min. Although a number of substrates e.g. vitamins, minerals, and anti-oxidants, are important for optimal function of CNS (Bourre 2006), optimal cognitive function do require maintenance of an adequate blood glucose concentration. A number of studies have demonstrated advantageous effects on cognitive functions when providing glucose in the form of a glucose drink (Messier 2004). Not surprisingly, improved cognitive functions has also been observed after ingesting a breakfast, compared with remaining in a fasting state (Benton and Parker 1998; Pollitt, Cueto et al. 1998; Martin and Benton 1999). These findings points to a relation between cognitive performance and systemic blood glucose concentrations. Due to the significant differences in postprandial blood glucose profiles after a high- compared with a low-GI meal, cognitive functions during the postprandial phase could be

expected to differ depending on the choice of carbohydrate food. However, in addition to the choice of food, also the glucose tolerance of the subject has to be taken into consideration. Consequently, it is evident from the literature that type 2 diabetes is associated with an increased risk of cognitive dysfunction (Strachan, Deary et al. 1997; Ryan and Geckle 2000; Gallacher, Pickering et al. 2005). An accumulating body of data also shows a relation between decreased cognitive functions and IGT (non diabetic), especially in elderly persons (Messier, Gagnon et al. 1997; Convit, Wolf et al. 2003; Messier, Tsiakas et al. 2003). It can therefore be hypothesised that also cognitive decline may be included as an additional dysfunction connecting to the metabolic syndrome.

3. OBJECTIVES

The aim of the present thesis was to study the impact of various whole grain cereal products on glycaemic regulation, satiety, metabolic risk factors, and cognitive functions in healthy subjects. The cereal products varied in GI features and in content of indigestible carbohydrates and were evaluated in acute and/or semi acute meal studies. In particular, the importance of product characteristics for glucose tolerance and related parameters was evaluated either in the perspective from breakfast to subsequent standardised lunch- and dinner meals, or from evening meal to a subsequent standardised breakfast.

4. MATERIALS AND METHODS

4.1. Test products and meals

4.1.1. Cereal based test products and meals (*Papers I – IV*)

Included in *Papers I - IV* were cereal based test products. An overview of the test meals (*Papers I – V*) is shown in **Table 1**. The test products in *Paper I* were; boiled wheat kernels (Durum wheat, Hven-durum HB, Hven, Sweden), boiled barley kernels (Pot barley, Goudas Food Products, Concord, Ontario, Canada), spaghetti (Kungsörnen, Järna, Sweden), and spaghetti with added wheat bran (9.4 g DF, Fiberform[®], Tricum). The test products were chosen and processed to elicit similar and low GIs (GI 52 – 54) but with different type and/or amounts of indigestible carbohydrates (DF + RS). The GI characteristics and contents of RS and insoluble-, soluble-, and total DF in the test meals in *Papers I – IV* are shown in **Table 2**.

Five test meals were included in *Paper II*. The test meals differed in GI (GI 58 – 100) and contents of DF (2.6 g – 22.6 g DF). The RS content was low in all test meals and varied between 0.5 - 2.8 g. The test meals were: spaghetti or white wheat bread (WWB) added with amounts of DF extracted from barley (BDF) (Lyckeby Stärkelsen, Kristianstad, Sweden) corresponding to the total DF content in one portion of intact barley kernels included in *Paper I* (9.8 g barley DF/meal); spaghetti added with a matched content of DF from oats (ODF) (9.8 g added DF/meal, OatWell[®], Swedish Oat Fiber AB, Väröbacka, Sweden); spaghetti added with twice the amount of DF from barley (2*BDF) (19.6 g added DF/meal). In addition, barley kernels were milled to flour, and included in a whole meal porridge (WMB porridge). The same barley kernel variety was used for the porridge and the barley kernel used in *Paper I*. The oat DF was included in the study as a comparison of barley DF with another DF, also rich in beta glucans.

In *Paper III* the test products consisted of breads with the main carbohydrate ingredients from; barley kernels (OB bread) (non-specified, ordinary Swedish barley provided from Lantmännen Food, Stockholm, Sweden), a half portion of the same bread with ordinary barley kernels (1/2OB bread), barley kernels which were cut 1 - 2 times (CutOB bread, made from same barley kernels), a barley kernel variety with elevated amounts of amylose (HAB bread) (Karmosé, Svalöf Weibull AB, Svalöv, Sweden), a barley kernel variety with elevated amounts of beta-glucans (HBB bread) (mutant 13, Svalöf Weibull AB, Svalöv, Sweden), WWB added with

resistant starch (RS 2) from a high RS corn starch (WWB+RS) (Hi-maize® 1043, Biomin AB, Göteborg, Sweden), or WWB added with the same RS and in addition also added with dietary fibre extracted from barley (WWB+RS+DF) (Lyckeby Stärkelsen, Kristianstad, Sweden). The RS and DF added was supposed to correspond to the total amounts of RS and DF that naturally occur in a portion of ordinary intact barley kernels.

The test meals included in *Paper IV* were; wheat kernels, rye kernels, oat kernels, barley kernels, whole grain barley flour porridge (made from flour of the same barley kernels), and WWB with addition of barley DF (Lyckeby Stärkelsen, Kristianstad, Sweden). The cereal kernels used were commercially available non specified Swedish varieties. The barley and oat kernels were kindly provided by Finax (Finax AB, Helsingborg, Sweden), and the wheat- and rye kernels from Nord Mills (Nord Mills AB, Malmö, Sweden). The amount of barley DF added to the WWB meal was intended to correspond to the total DF content naturally occurring in an equi-starch portion of boiled barley kernels.

In *Papers I – II* and *IV*, the cereal kernels were boiled and consumed intact, and the dietary fibres included were sprinkled on the spaghetti (*Paper I*) or mixed in water and consumed with the meals (*Paper II and IV*). In *Paper III*, the test cereals, DF, and RS were included in breads. Cereal kernels were included in breads in proportions (weight %) of 90/10 kernels/white wheat flour. In *Papers I – IV*, a white wheat bread was included as a reference product. The size of all the test meals, except for the half serving in *Paper III*, corresponded to 50 g available carbohydrates.

Water was consumed with the test meals in *Papers I - IV*; 150 ml in *Paper I*, 250 ml in *Papers II* and *IV*, and unlimited amounts with the evening meal in *Paper III*. An amount of 250 ml water was consumed with the standardised meals. Tea/coffee or additional water (150 ml, no milk or sugar) was consumed immediately after the test evening meals in *Paper I* and also immediately after breakfast in *Papers I – II*. In *Paper IV*, the participants were served 150 ml water and 150 ml tea/coffee (no milk or sugar) or water two hours after the standardised breakfast and lunch, respectively. In the case of tea/coffee/water, each subject took the same drink throughout the study.

Table 1. Test meals included in Papers I – V

<i>Paper I</i>	<i>Paper II</i>	<i>Paper III</i>	<i>Paper IV</i>	<i>Paper V</i>
WWB	WWB	WWB	WWB	Bolus glucose drink
Boiled barley kernels	WWB+BDF	OB bread	WMB porridge	Sipping glucose drink
Boiled wheat kernels	Spaghetti+BDF	CutOB bread	Boiled wheat kernels	
Spaghetti	Spaghetti+2*BDF	1/2OB bread	Boiled oat kernels	
Spaghetti + Wheat bran	Spaghetti+ODF	HBB bread	Boiled rye kernels	
	WMB porridge	HAB bread	Boiled barley kernels	
		WWB+RS	WWB + barley DF	
		WWB+RS+DF		

WWB: white wheat bread, BDF: barley dietary fibre, ODF: oat dietary fibre, WMB: whole meal barley, OB: Ordinary barley kernels, CurOB: ordinary barley kernels cut 1 - 2 times, 1/2OB: half a portion OB, HBB: high beta-glucan barley kernels, HAB: high amylose barley kernels

Table 2. GI characteristics and contents of resistant starch (RS) and insoluble-, soluble-, and total dietary fibre (DF) in the test meal in *Papers I - IV*.

Test meals	GI	RS	Dietary fibre			RS + DF
			Insoluble	soluble	total	
	%	<i>g/meal</i>	<i>g/meal</i>	<i>g/meal</i>	<i>g/meal</i>	<i>g/meal</i>
<i>Paper I</i> ¹						
WWB	100	0.5	1.6	1.4	3.0	3.6
Wheat kernels	54	10.4	8.5	1.6	10.1	20.5
Barley kernels	54	7.3	5.5	4.3	9.8	17.0
Spaghetti	52	2.6	2.2	0.9	3.1	5.7
Spaghetti + wheat bran	52	2.6	11.4	1.1	12.5	15.1
<i>Paper II</i> ²						
WWB	100	0.5	1.4	1.2	2.6	3.1
WWB+BDF	(100)	0.6	6.1	6.2	12.3	12.9
Spaghetti+BDF	(58)	2.8	6.3	6.6	12.9	15.7
Spaghetti+2*BDF	(58)	2.8	11.0	11.6	22.6	25.4
Spaghetti+ODF	(58)	2.8	6.5	6.2	12.7	15.5
WMB porridge	97	0.7	4.7	3.8	8.5	9.2
<i>Paper III</i> ³						
WWB	100	1.3	2.0	0.6	2.6	3.9
OB bread	52	9.5	7.8	2.9	10.7	20.1
CutOB bread	55	8.8	8.1	2.5	10.5	19.4
1/2OB bread	52	4.7	4.	1.2	5.3	10.0
HAB bread	52	22.0	10.9	5.2	16.1	38.1
HBB bread	50	30.9	26.7	23.4	50.1	81.0
WWB+RS	76	8.0	2.5	1.0	3.4	11.5
WWB+RS+BDF	88	8.8	5.4	4.9	10.3	19.1
<i>Paper IV</i> ⁴						
WWB	100	0.8	2.3	2.0	4.3	5.1
Wheat kernels	79	5.0	5.9	2.1	8.0	13.0
Rye kernels	73	3.7	10.6	3.6	14.2	17.9
Oat kernels	85	4.0	5.4	2.5	7.9	11.9
Barley kernels	49	8.0	6.2	2.9	9.1	17.1
WWB+BDF	93	0.8	6.6	6.3	12.9	13.7
WMB porridge	112	1.7	6.6	2.6	9.2	10.9

1: GIs of the wheat and barley kernels were predicted using an in vitro method according to Granfeldt et al (1992) and GI calculated as $GI = 8.198 + 0.862 HI$.

1.GI of spaghetti was determined in healthy subjects. 2: GIs of the spaghetti and WWB were determined in healthy subjects (Östman, Granfeldt, Björck, unpublished observations). GI of barley porridge was determined in healthy subjects (Liljeberg et al (1996)). 3: GIs of the products were predicted using an in vitro method (Granfeldt, Björck et al. 1992) and GI calculated as $GI = 6.272 + 0.912 * HI = GI$ (Leeman, Bårström et al. 2005). 4: GIs of the products were determined in vivo using the recommended procedure by Brouns, Björck et al. (2005) .

4.1.2. Simulated low-GI or high-GI meals (*Paper V*)

In *Paper V*, two test meals, based on a pure glucose solution, were administered to simulate high- or low-GI meals, respectively. The glucose solution consisted of glucose (50 g) dissolved in water (450 ml). To mimic a high-GI meal, the glucose was administered as a bolus drink, and the subjects consumed the glucose solution within 10 - 12 min. To mimic a low-GI meal, the glucose solution was instead divided into six equal loads which were consumed through continuous sipping during 150 min.

4.1.3. Standardised meals (*Papers I – IV*)

Standardised meals containing WWB (Jätterasken, Pågen AB, Malmö, Sweden) were included in *Papers I – III* (breakfast) and *Paper IV* (breakfast and dinner). In *Paper III* paracetamol was added to the recipe (1 g/portion) as a tool to measure the gastric emptying rate. In *Paper IV* a standardised lunch meal was included consisting of mashed potatoes (Felix Basmos, Procordia Food AB, Eslöv, Sweden) and 100 g meat-balls (Ica frozen meat-balls, Ica AB, Solna, Sweden). The WWB and mashed potatoes portions were equivalent to 50 g potentially available starch.

4.2. Chemical analyses of test products

In *Papers I – II* the contents of potentially available starch in the test products was analysed with an enzymatic method according to Holm *et al.* (1986), including steps of boiling in 20 min with a thermo-stabile α -amylase (Termamyl[®], Novozymes, Denmark) and incubation with amyloglucosidase (Roche Diagnostics, Indianapolis, USA) for 30 min at 60°C. The released glucose was then determined with a glucose oxidase peroxidase reagent, and the concentration was spectrophotometrically measured. In *Papers III – IV*, the available starch in the products was calculated by subtracting RS from total starch. The method for the analyse of total starch differed from the described method in the paragraph above by an additional step including incubation with KOH for 30 min prior to incubation with Termamyl (Björck and Siljeström 1992). RS content (*Papers I – IV*) was determined according to Åkerberg *et al.* (1998). To mimic physiological conditions, chewing of the product was included as a pre step before incubation with enzymes (pepsin, pancreatine, amyloglucosidase). After 16 h incubation, the RS was precipitated with ethanol and collected in a filter. The RS concentration was analysed as the total starch in the filter. DF content (insoluble and soluble) (*Papers I – IV*) was analysed according to Asp *et al.* (1983).

4.3. Determination of GI

The GI of the products in *Paper IV* was determined in vivo using the 0 – 120 min positive incremental blood glucose areas under the curves (IAUC). The GI were calculated as the mean of individual ratios (Brouns, Björck et al. 2005) using WWB as a reference product; i.e. for each person the glucose IAUCs after the test products were divided with the IAUC after the WWB. The GI of the products in *Paper I* (with exception for the spaghetti) and *Paper III* were predicted from hydrolysis indices (HI) using an in vitro method with an initial chewing step to mimic eating conditions (Granfeldt, Björck et al. 1992), and GI calculated from the equation: $GI = 6.272 + 0.912 * HI$ (Leeman, Bårström et al. 2005).

4.4. Experimental design

An overview of the experimental design in the different papers is shown in **Figure 3 – 5**. In addition the blood glucose response to the breakfasts in *Paper V* is shown in **Figure 5**. All studies included healthy volunteers with normal BMIs. The mean age of the subjects in *Papers I – IV* was 25.3 ± 3.8 years (mean \pm s.d) and the body mass indices 22.1 ± 2.2 kg/m² (mean \pm s.d). In *Paper V* the mean age of the participants was 59.2 ± 6.9 years (mean \pm s.d) and the BMI 23.8 ± 2.7 kg/m² (mean \pm s.d). In *Papers I – III*, the subjects consumed the test meals at 9.30 pm and in *Paper IV, Series 2*, at 10.30 pm. The time for the test meals in *Paper IV* was set to achieve the same time interval in-between the test meals and standardised meals in *Series 1 and Series 2*. After the evening meals, the subjects were fasting until the standardised breakfast. The subjects arrived to the department at 7.45 am In *Papers I - III*, an intravenous cannula was inserted into an antecubital vein for blood sampling. In *Papers IV and V*, the blood analyses were instead performed on capillary blood. In all *Papers (I – V)*, the breakfast was served at 8.00 am. The experimental period in *Papers I – III and V* was three hours; and in *Papers IV (Series 2)* two hours. The experimental period in *Paper IV, Series 1* was started at 8.00 am and ended at 7.30 pm. In *Paper IV*, blood glucose was determined during two hours postprandial the breakfast, lunch, and dinner, respectively.

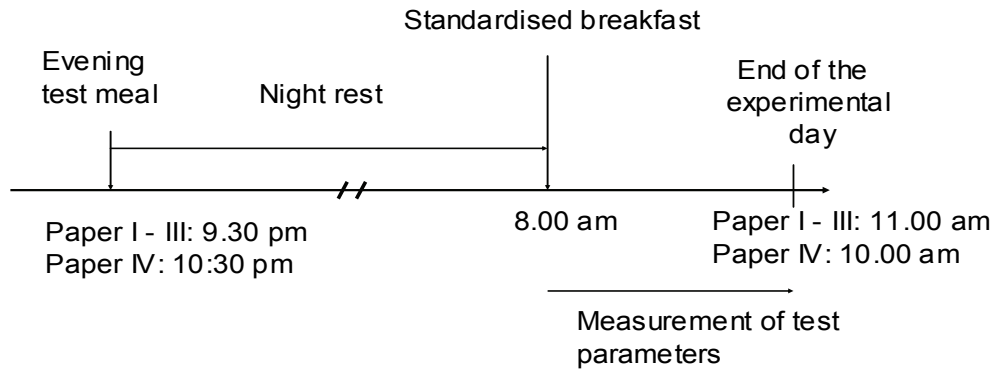


Figure 3. Experimental design in *Papers I – IV (Paper IV Series 2)*.

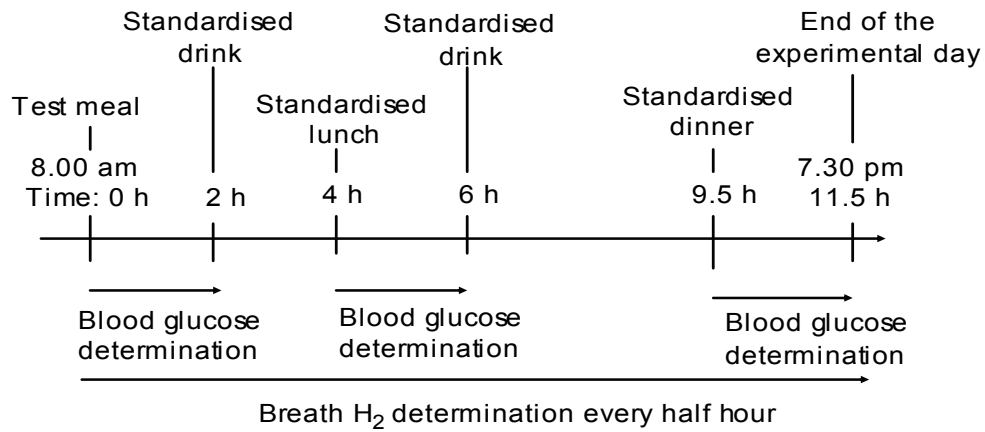


Figure 4. Experimental design in *Paper IV, Series 1*.

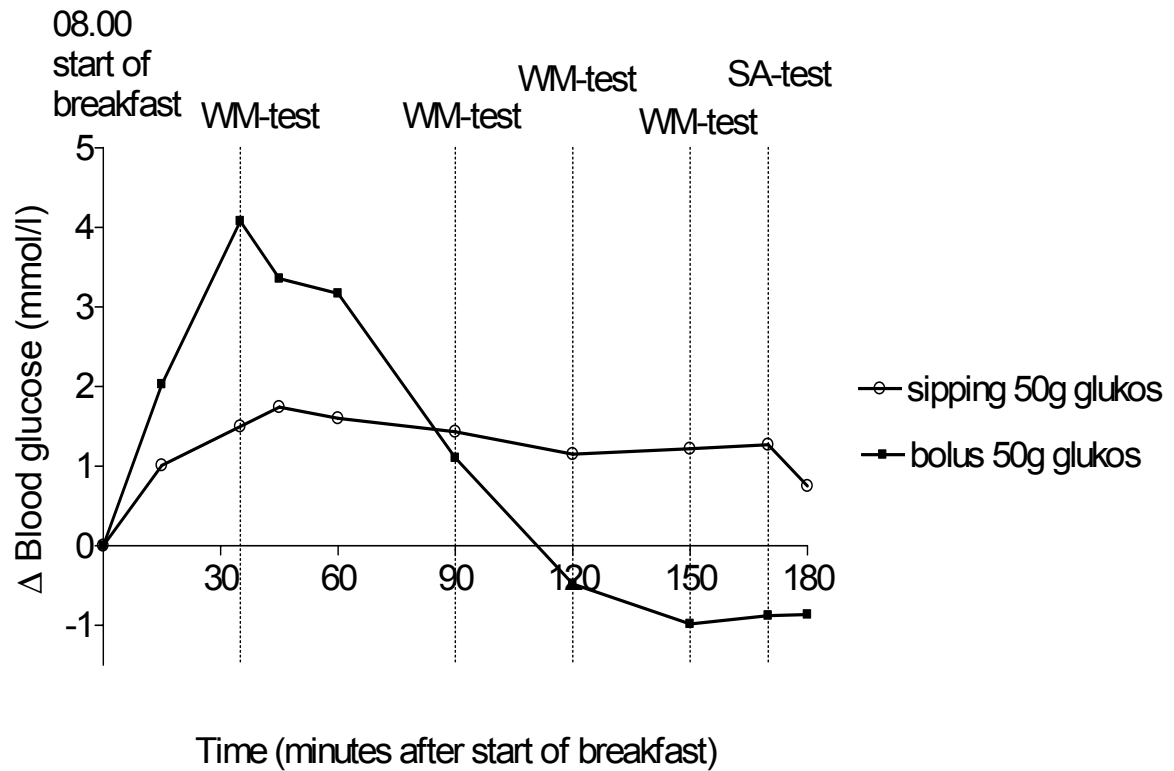


Figure 5. Mean blood glucose incremental changes (Δ) after the two simulated test breakfasts, and the time schedule for the cognitive tests. WM: Working memory, SA: Selective attention.

4.5. Physiological parameters

An overview of the test parameters included in the different papers is presented in **Table 3**. The venous blood samples were centrifuged and plasma/serum were separated and stored in a freezer (< -20 °C) until analysed.

Table 3. Test parameters in the different Papers.

<i>Paper I</i>	<i>Paper II</i>	<i>Paper III</i>	<i>Paper IV</i>	<i>Paper V</i>
B-glucose	B-glucose	B-glucose	B-glucose	B-glucose
S-insulin	S-insulin	S-insulin	Breath H ₂	Glucose tolerance
S-FFAs	S-FFAs	S-FFAs		WM-test
P-SCFAs	P-SCFAs	S-triacylglycerol		SA-test
Breath H ₂	Breath H ₂	P-glucagon		
		P-GIP, P-GLP-1		
		S-IL-6, S-IL-8		
		P-adiponectin		
		S-paracetamol		
		Satiety		
		Breath H ₂		

WM: Working memory, SA: selective attention, B: blood, S: serum, P:, plasma.

Blood glucose was analysed in venous whole blood (*Papers I and II*). The blood was collected in a sodium fluoride tube, and then immediately transferred to a tube containing a NaOH solution where the proteins were precipitated with addition of ZnSO₄. After centrifugation the supernatant was separated and glucose concentrations were determined immediately with a glucose oxidase-peroxidase reagent. The absorbance of the red-coloured GLOX solutions were monitored spectrophotometrically (450 nm). In *Papers III – V*, finger-prick capillary blood samples were instead used for determination of blood glucose using HemoCue® B-glucose equipment (HemoCue AB, Ängelholm, Sweden).

Serum insulin (Papers I – III) was determined by using an integrated immunoassay analyzer (CODA Open Microplate System; Bio-Rad Laboratories, Hercules, CA) with a solid phase two-site enzyme immunoassay kit (Insulin ELISA 10-1113-01, Mercodia AB, Uppsala, Sweden).

Serum/plasma free fatty acids (FFAs) were determined in serum (*Paper I*) or plasma (*Papers II - III*), with an enzymatic colorimetric method (NEFA C 994 - 75409, ACS-ACOD method, WAKO Chemicals GmbH, Germany).

Plasma GIP and GLP-1 (Paper III) were determined after extraction of plasma with 70 % ethanol (by vol, final concentration) (Krarup, Madsbad et al. 1983). The carboxyl-terminal directed antiserum R 65 were used for the GIP radioimmunoassay, which cross-reacts fully with human GIP but not with the so called GIP 8000, whose chemical nature and relation to GIP secretion is uncertain. Human GIP and ¹²⁵I human GIP (70 MBq/nmol) were used for standards and tracer. The plasma concentrations of GLP-1 were measured (Orskov, Rabenhøj et al. 1994) against standards of synthetic GLP-1 7-36 amide by using antiserum code no. 89390, which is specific for the amidated carboxyl terminus of GLP-1 and, therefore, does not react with GLP-1-containing peptides from the pancreas. The results of the assay accurately reflect the rate of secretion of GLP-1 because the assay measures the sum of intact GLP-1 and the primary metabolite, GLP-1 9-36 amide, into which GLP-1 is rapidly converted (Deacon, Pridal et al. 1996). The sensitivity for both GIP and GLP-1 assays was < 1 pmol/L, the intraassay CV was <6 % at 20 pmol/L, and the recovery of standards (which was added to plasma before extraction) was ≈100% when corrected for losses inherent in the plasma extraction procedure.

Serum Triacylglycerols (Paper III) were analysed with a Serum Triglyceride Determination kit (SIGMA, Saint Louis, MO, USA). Triacylglycerol concentrations were determined by subtracting circulating glycerol (not bound in triacylglycerols) from total glycerol.

Plasma adiponectin concentrations were measured at breakfast following the OB bread and the WWB, respectively (*Paper III*). Plasma adiponectin concentrations were determined with a solid phase two-site enzyme immunoassay kit (Mercodia Adiponectin ELISA, Mercodia AB, Uppsala, Sweden).

Plasma glucagon (Paper III) was analysed using a glucagon RIA kit (LINCO Research, MO, USA).

Paracetamol was included in the WWB breakfast bread (1 g/portion) in *Paper III* as a tool to measure the gastric emptying rate. Serum paracetamol was determined with an enzymatic assay kit (Paracetamol Enzyme Assay Kit, Cambridge Life Sciences Ltd., UK).

Serum IL-6 and *serum IL-8* were analysed using enzyme immunoassay kits (TiterZyme[®] – EIA, Assay Designs, MI, USA). The procedure for the determination of serum IL-6 concentration was modified such that no dilution of serum was performed prior to the analysis. IL-6 concentrations were measured at breakfast following the OB bread, HBB bread and the WWB, respectively, and IL-8 concentrations were measured at breakfast following the OB bread and the WWB, respectively (*Paper III*).

Plasma short chain fatty acids (SCFAs, acetate, propionate, butyrate) concentrations (*Papers I – II*) were analysed with GC/MS (Hewlett Packard 5890 II GC with an Optic II PTV and CTC A200S auto sampler and VG Trio-1000 quadrupole MS with EI ionisation) following purification by ultra filtration (Amicon Centriplus centrifugal filter device YM-30, 30 kDa cut-off, Millipore (UK) Ltd., Watford, UK) and solid phase extraction (Bakerbond SPE column, 200 mg SDB-2, 3 ml capacity, Mallinckrodt Baker UK, Milton Keynes, UK) (Morrison, Cooper et al. 2004).

Breath hydrogen was measured as an indicator of colonic fermentation. In *Papers I – II* breath H₂ excretion was sampled by blowing gently through a straw into a glass vial, and analysed with an automated continuous-flow isotope ratio mass spectrometer (HYDRA, PDZ Europe, Crewe) (Slater and Preston 2004). In *Papers III – IV* breath H₂ was analysed using an EC 60 gastrolyzer (Bedfont EC60 Gastrolyzer, Rochester, England).

Satiety was estimated in a postprandial phase (*Paper III*) using a subjective bipolar rating scale.

Classification of glucose tolerance in “lower” versus “higher” glucose tolerance was made based on the blood glucose response following an oral bolus glucose load (50 g) (*Paper V*). The difference in mmol/L between the highest and the lowest blood glucose values up to 3 h after intake were taken as a measure of the efficiency in blood glucose regulation. The median of the differences was calculated, and the subjects with a difference between their highest and lowest glucose value above the median were classified as with “lower glucose tolerance” and the subjects beneath with “higher glucose tolerance”.

4.6. Cognitive parameters

4.6.1. Working memory (WM) test

The tests of WM employed in the present thesis (*Paper V*) represent an extension of the methodology originally developed by Radeborg *et al.* (1999), and measures the capacity for simultaneous storing and processing of information. The WM-tests were oral, and each test composed of 12 sets of four short declarative sentences that could be either semantically meaningful of the type ‘the boy brushed his teeth’, or not meaningful, such as ‘the rabbit struck the idea’. The sentences were read one by one, and immediately after each sentence, the subject had to indicate if the sentence was semantically meaningful or not. After each set of four sentences, the subjects had to repeat, in any order, the first noun in each of the four sentences. Four WM-tests were included at each experimental day. One WM-test took approximately 6 minutes to perform and was scored for the number of correctly remembered nouns.

4.6.2. Selective attention (SA) test

Selective attention (SA) was measured using a computerized test consisting of 72 pictures, each shown for two seconds on the screen. The pictures consisted of one square, divided into four smaller squares. An example of what could be seen on the screen during the test is shown in **Figure 6**. One of the smaller squares was red and one green, resulting in a total of 12 unique picture combinations. The subjects had to remember the positions of the colored squares and to compare each new picture with the preceding one. Each time a new picture emerged on the screen, either the green, the red or none of the colored squares could be in the same position as in the previous picture. Within two seconds, the subjects were supposed to indicate which of the three possible alternatives that occurred for each new picture by pressing one of three different keys on the keyboard. The SA-test took approximately 10 minutes to perform and was scored for the number of correct responses, and also, the time to deliver the correct answer (reaction time).

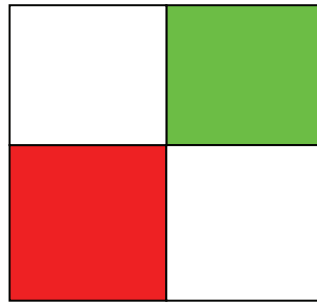


Figure 6. Illustration of the computerized test of SA. The subject was supposed to remember the positions of the coloured squares in the previous picture and mark with one of three keys on the keyboard if the green, the red or none of the coloured squares in the current picture was in the same position compared with the previous picture. Each picture was shown for two seconds on the screen.

4.7. Calculations and statistical methods

The results are expressed as means \pm SEM. GraphPad Prism (version 4.03; GraphPad Software, San Diego, CA, USA) were used for graph plotting and calculation of each individual areas. *Papers I – IV*: significant differences in test parameters depending on test meals were assessed with analysis of variance (ANOVA general linear model) followed by Tukey’s pairwise multiple comparison method for means, or if noted in the text by Dunnett’s test with the WWB as a control, in MINITAB Statistical Software (release 13 for windows; Minitab inc., State College, PA, USA). Differences between the test meals at different time points were analysed by using a mixed model (PROC MIXED in SAS release 8.01; SAS Institute Inc, Cary, NC) with repeated measures and an autoregressive covariance structure. Spearman’s rank correlation was used to study relations between test parameters, and if noted in the text Person’s test, based on group means, was used. A correlation for each subject was calculated and from these values the mean value of Spearman’s correlation coefficient was obtained (*Papers II – IV*). To determine the P – value, a permutation test was performed using MATLAB with the null hypothesis that no correlations existed (the alternative hypothesis was that the data was correlated).

Paper V: Repeated measures analysis of variance (ANOVA) was used to determine the influence of test meals on blood glucose response, with test meal, order of the test meals, and time as independent variables. The effect of higher versus lower glucose tolerance (as stated in this thesis) was investigated by ANOVA with order of test meals and higher versus lower glucose tolerance as independent variables, and performance on tests of working memory and selective attention as dependent variables. Calculations of correlations were performed with Pearson's correlation, followed by Fisher's test to determine P – values. The effect of the test meals on the cognitive tests was investigated by repeated measures ANOVAs at the test points, with order of test meal and test meal as independent variables, the difference between the highest and lowest glucose values (measurement of glucose tolerance) as covariate, and performance on WM-test and SA-test as dependent variables. The influence of the test meal on WM and SA during the first experimental day was determined by ANOVA with meal as independent variable, the measure of glucose tolerance as covariate, and performance on WM-test and SA-test as dependent variables. Statistical calculations were performed in Stat View 5.0 (Abacus Concepts, Inc., Berkeley, CA, 1992) and SuperAnova 1.11 (Abacus Concepts, Inc., Berkeley, CA, 1991). Although statistical analyses are adjusted for glucose tolerance, the mean values in the results are presented without being adjusted.

In *Papers I – III* and *V*, values of $P < 0.05$ were considered statistically significant. In *Paper IV*; $P \leq 0.05$ were considered statistically significant.

5. RESULTS AND DISCUSSION

5.1. Effects of cereal based evening test meals on glucose response and related parameters at the following breakfast

5.1.1. Blood glucose

5.1.1.1. Importance of Glycaemic index

The postprandial glucose responses after the standardised breakfast following the different evening test meals in *Papers I – III* are displayed in **Figures 7 - 9**, and the incremental areas (IAUCs) (0 – 120 min) are shown in **Tables 4 - 6**. In *Paper I*, all test products were processed to have similar and low GI (52 – 54), but differed in amounts and composition of indigestible carbohydrates. Two wheat varieties (one ordinary Swedish variety and one durum wheat variety) and different boiling conditions were tested to achieve a GI within the above range, as judged from predictions using an in-vitro enzymatic assay. In addition, the GI of commercial pre-boiled and cooked wheat kernels (Matvete, Frebaco, Lidköping, Sweden) was also estimated in vitro. A decrease in cooking time from 40-, 35-, 30-, to 20 min lowered the predicted GI (pGI) of the ordinary wheat kernels from 86, 82, 74, to 45, respectively. The pre-boiled wheat kernels boiled for 5 min elicited a pGI of 82. By choosing a durum wheat variety, and 20 min boiling time, the pGI of boiled wheat kernels (pGI = 54) became similar to that determined for barley kernels boiled for 40 min (GI = 54) and the spaghetti (GI = 52). The considerable variations in pGI obtained by changing the boiling time indicate that food processing has a profound effect on the GI of the prepared food. Despite the low GI of boiled wheat kernels or the spaghetti evening meal, whether added with wheat bran to simulate the total DF level in the barley kernels or not, only the boiled barley kernel evening meal improved the glucose tolerance at breakfast compared with WWB (-46 % IAUC 0 – 120 min, $P < 0.05$) (*Paper I*). Similarly, addition of dietary fibres extracted from oats or barley to a low-GI spaghetti evening meal and/or a high-GI WWB evening meal, in amounts similar to the content of DF in the barley kernels used in *Paper I*, was without effect on glucose tolerance at a proceeding breakfast meal compared with WWB (*Paper II*). Interestingly, alike an evening meal with barley kernel bread (OB bread), a WWB added with *both* barley DF and RS (WWB+RS+DF), significantly lowered the blood glucose response at breakfast with approximately 26 % (IAUC 0 – 120 min)

compared with the corresponding area after the WWB evening meal reference (*Paper III*). The amounts of DF and RS added to the WWB corresponded to the total content of DF and RS intrinsic to the OB bread. In contrast to the low-GI characteristics of the OB bread, the pGI of WWB+RS+DF product was high (pGI=85). Consequently, taken together, the results from *Papers I - III* indicate that *a*; the GI of the evening meal *per se* was a poor predictor of the overnight effect on glucose tolerance within the range of GI features studied, *b*; barley DF, oat DF or wheat bran added to a low-GI meal (spaghetti) in amounts similar to the content in boiled barley kernels had no effect on the overnight glucose tolerance, *c*; enrichment of WWB with barley DF and RS in certain amounts improved overnight glucose tolerance, to the same extent as did the boiled barley kernels containing a similar amount of intrinsic DF and RS, implicating a mechanism related to the presence of specific indigestible carbohydrates. These results of this thesis contradict previous conclusions that the GI features of the late dinner meal *per se* affected glucose response to a standardised test meal the following morning (Wolever, Jenkins et al. 1988). However, the low-GI evening meals included by these authors were either lentils or composite meals with lentils, pearled barley and green peas, thus containing appreciable amounts of indigestible carbohydrates.

Figure 7, *Paper I*

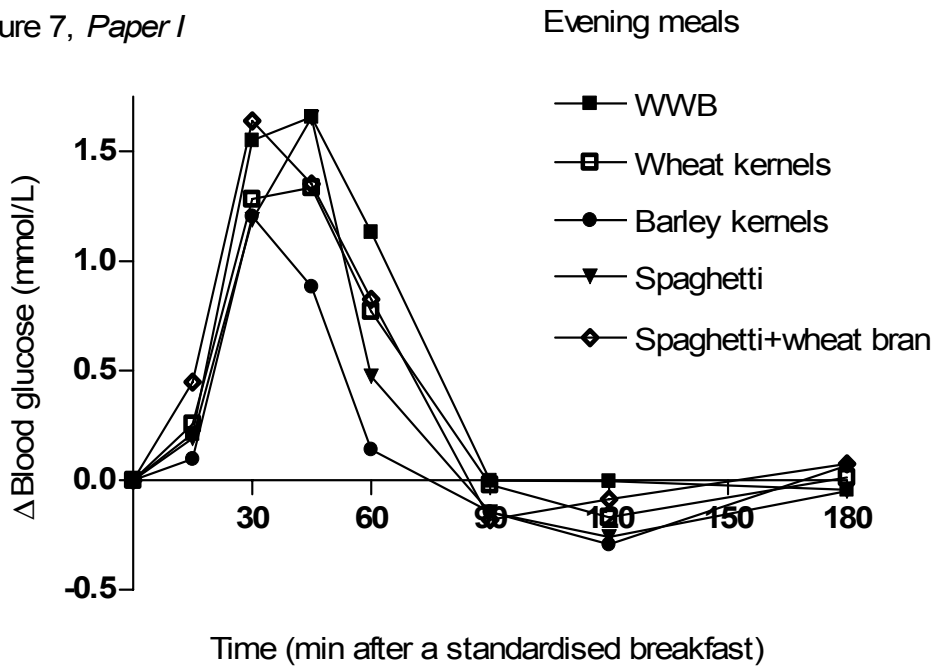


Figure 8, *Paper II*

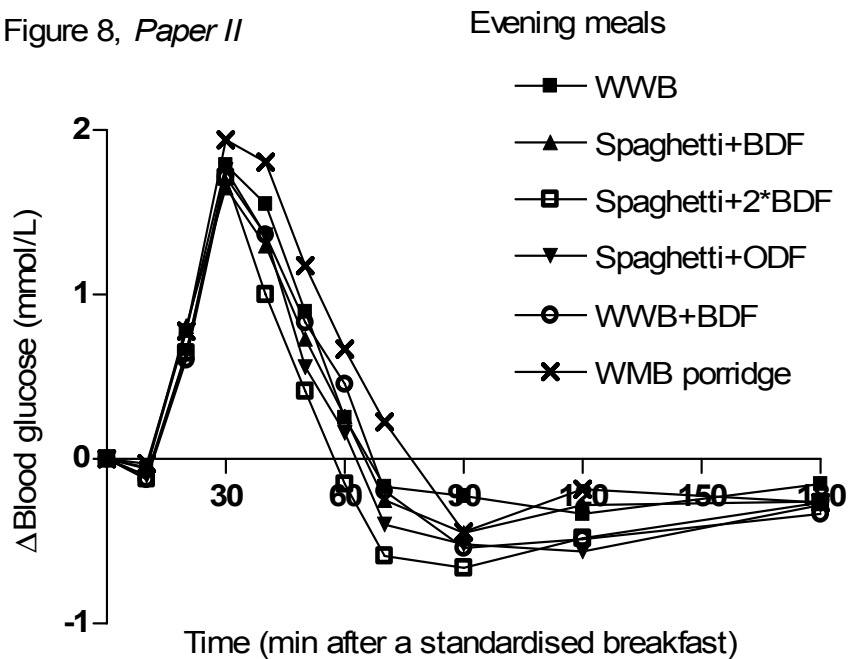


Figure 7 – 8 *Papers I – II*. Mean incremental change (Δ) in blood glucose concentrations after a standardised breakfast, following different evening test meals. WWB: white wheat bread, BDF: barley dietary fibre, ODF: oat dietary fibre, WMB: whole meal barley.

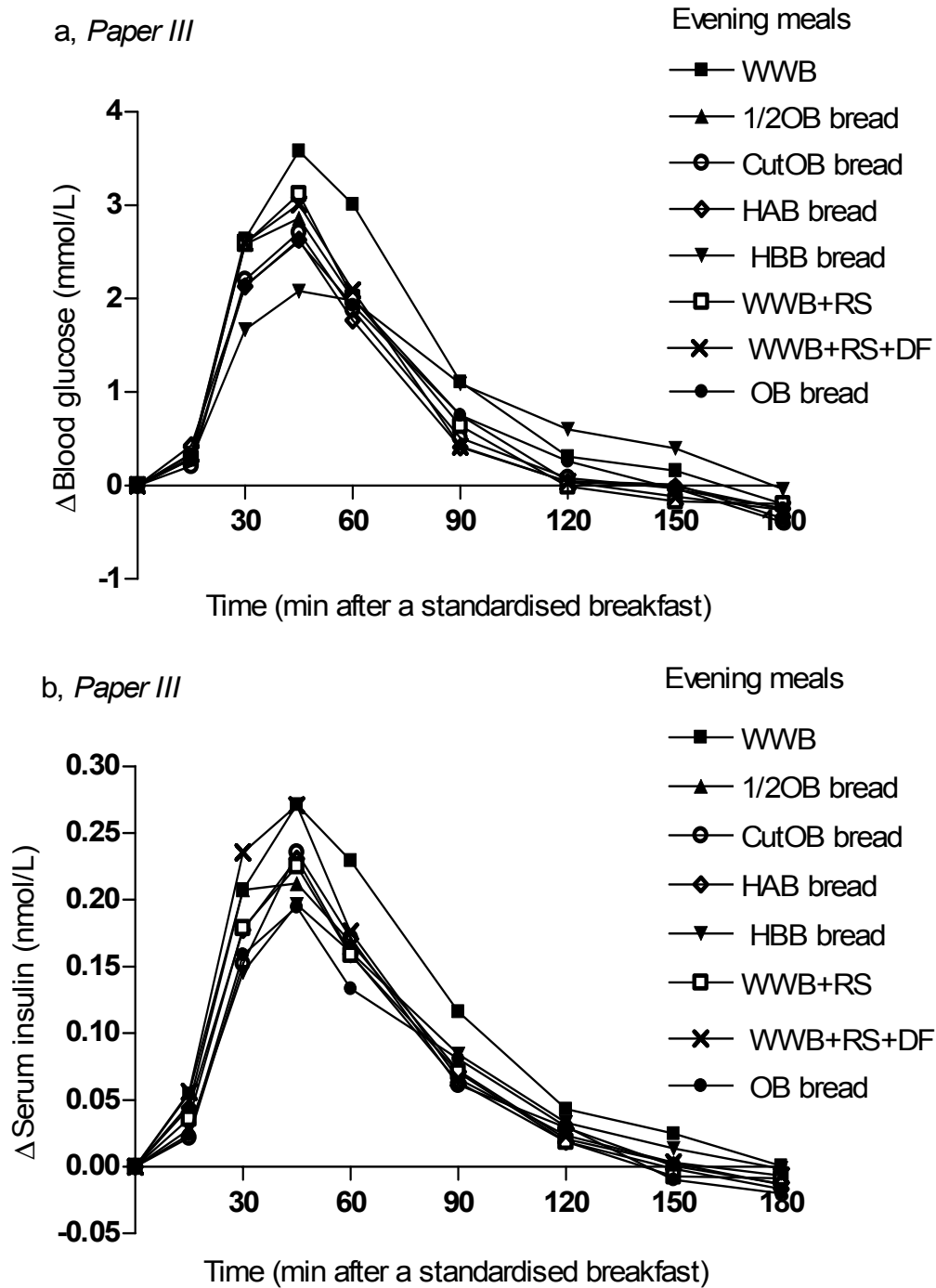


Figure 9, Paper III. Mean incremental change (Δ) in (a) blood glucose and (b) serum insulin after a standardised breakfast, following different evening test meals.

WWB: white wheat bread, OB: Ordinary barley kernels, CutOB: ordinary barley kernels cut 1 - 2 times, 1/2OB: half a portion OB, HBB: high beta-glucan barley kernels, HAB: high amylose barley kernels.

Table 4, Paper I. Blood glucose and serum insulin response to the standardised breakfast, following different evening test meals.

Evening test meals	IAUC (0 – 120 min)			
	Blood glucose		Serum insulin	
	<i>mmol·min/L</i>	<i>Change (%)</i> ¹	<i>nmol·min/L</i>	<i>Change (%)</i> ¹
WWB	90.4 ± 15.6 ^a	0	13.6 ± 1.7	0
Wheat kernels	79.1 ± 16.2 ^{ab}	-13	13.5 ± 1.4	-1
Barley kernels	48.9 ± 9.1 ^b	-46	11.9 ± 1.6	-13
Spaghetti	67.3 ± 10.1 ^{ab}	-26	13.7 ± 1.6	1
Spaghetti + wheat bran	86.0 ± 15.1 ^a	-5	13.7 ± 1.5	1

WWB: white wheat bread. Values in a column with different superscript letters are significantly different ($P < 0.05$).

¹ Change (%) compared to the WWB.

Table 5, Paper II. Blood glucose and serum insulin response to the standardised breakfast, following different evening test meals.

Evening test meals	IAUC (0 – 120 min)			
	Blood glucose		Serum insulin	
	<i>mmol·min/L</i>	<i>Change (%)</i> ¹	<i>nmol·min/L</i>	<i>Change (%)</i> ¹
WWB	62.9 ± 9.4 ^{ab}	0	14.5 ± 1.3	0
WWB+BDF	65.2 ± 11.0 ^{ab}	4	14.7 ± 1.5	1
Spaghetti+BDF	58.2 ± 9.7 ^{ab}	-7	15.8 ± 1.4	9
Spaghetti+2*BDF	45.9 ± 8.1 ^a	-27	14.8 ± 1.6	2
Spaghetti+ODF	51.5 ± 7.5 ^{ab}	-18	13.7 ± 1.4	-6
WMB porridge	78.7 ± 11.5 ^b	25	16.4 ± 1.7	13

WWB: white wheat bread, BDF: barley dietary fibre, ODF oat dietary fibre, WMB whole meal barley. Values in a column with different superscript letters are significantly different ($P < 0.05$).

¹ Change (%) compared to the WWB.

Table 6, Paper III: Blood glucose-, serum insulin-, and plasma glucagon response (IAUC 0 – 120 min) to the standardised breakfast, following different evening test meals.

Evening meal	IAUC (0 – 120 min)			Mean value (0 – 120) min
	Blood glucose <i>mmol·min/L</i>	Serum insulin <i>pmol·min/L</i>	Plasma glucagon <i>pg/ml</i>	
WWB	211.6 ± 23.8 ^a	17.3 ± 2.9 ^a	0	56.4 ± 4.8
WWB+RS	167.2 ± 21.3 ^{ab}	12.5 ± 1.3 ^{ab}	-28	59.0 ± 4.5
1/2OB bread	160.6 ± 16.4 ^{ab}	13.3 ± 1.5 ^{ab}	-23	62.3 ± 4.3
WWB+RS+DF	156.8 ± 17.0 ^{b†}	14.8 ± 1.7 ^{ab}	-14	60.2 ± 4.6
OB bread	152.1 ± 17.0 ^{b†}	11.6 ± 1.3 ^b	-33	63.8 ± 4.1 [*]
HBB bread	149.9 ± 19.3 ^{b†}	12.2 ± 2.3 ^{ab}	-29	65.2 ± 4.0 [*]
CutOB bread	142.2 ± 14.1 ^{b††}	12.2 ± 1.3 ²	-29	63.5 ± 4.9 [*]
HAB bread	135.5 ± 11.6 ^{b†††}	12.6 ± 1.2 ^{ab}	-27	62.5 ± 4.8

WWB: white wheat bread, OB: Ordinary barley kernels, CutOB: ordinary barley kernels cut 1 - 2 times, 1/2OB: half a portion OB, HBB: high beta-glucan barley kernels, HAB: high amylose barley kernels. Values in a column with different superscript letters are significantly different.

[†]: $P < 0.05$, ^{††}: $P < 0.01$, ^{†††}: $P = 0.001$.

¹ Change (%) compared to the WWB.

² $P = 0.05$ between the WWB and CutOB.

* $P < 0.05$, significantly different compared with the WWB (ANOVA followed by Dunnetts test with the WWB as a control)

5.1.1.2. Importance of food structure

A late evening meal with boiled barley kernels was particularly prone to lower glucose response at a following standardised breakfast, compared with a WWB evening meal ($P < 0.05$, -46 % in *Paper I*, and -33 % in *Paper IV*). In addition, the enclosure of barley kernels into bread (*Paper III*) did not deteriorate the beneficial effect on glucose tolerance seen with boiled barley kernels. Interestingly, the barley kernels which were cut (1 – 2 times) prior to the baking of the bread displayed similar benefits on overnight glucose tolerance as did the whole barley kernels (*Paper III*). This is an important finding when aiming at exploiting the benefits of barley kernels in the form of new and palatable low-GI cereal products with optimal benefits on blood glucose regulation. On the other hand, destroying the structure of the barley kernels by milling and then cooking the whole grain flour to a porridge, resulted in a product devoid of the benefits displayed by the whole barley kernels (*Paper II*). These results indicate the importance of maintaining the botanical structure and/or the dietary fibre matrix.

5.1.1.3. Importance of indigestible carbohydrates (DF and RS)

Both barley and oats are rich in viscous β -glucans. It has previously been shown that β -glucans lowers the acute glycaemia after a meal in normal (Behall, Scholfield et al. 2006; Granfeldt, Nyberg et al. 2007), as well as in type 2 diabetic subjects (Tappy, Gugolz et al. 1996). Also RS (e.g. as present in high amylose corn starch) has been shown to lower the acute postprandial glycaemia (Behall, Scholfield et al. 2006). In *Paper I* it was observed that an evening meal with boiled barley kernels reduced the glycaemic response to a standardised breakfast compared with WWB ($P < 0.05$), whereas boiled wheat kernels were devoid of effect. The total amounts of indigestible carbohydrates were similar, but the composition differed. Consequently, the barley kernels contained a higher content of soluble DF (mostly β -glucans), whereas the wheat kernels were richer in insoluble DF (**Table 2**). This indicates the potential importance of specific prebiotic mixtures. The role of indigestible carbohydrates on colonic fermentation and as related to glucose tolerance is discussed below (see section: 5.1.1.4.) In *Paper II*, it was shown that addition of isolated DF from barley or oat to a high-GI (WWB) or a low-GI (spaghetti) evening meal, in quantities (9.8 g) corresponding to the amount of DF in barley kernels included in *Paper I*, did not improve glucose tolerance at breakfast compared with a WWB evening meal. Taken together with the lack of effect of a whole grain barley flour porridge on overnight glucose tolerance (*Paper II*), the

overnight benefits with the barley kernel evening meal probably cannot be assigned to the content of barley DF *per se*. Possibly, the considerable amounts of RS intrinsic to the barley kernel product may have contributed to the substantial improvement in overnight glucose tolerance seen with this product. Due to the milling procedure when preparing the whole grain barley flour porridge, the content of RS in a portion of barley porridge decreased from 7.3 g/portion to 0.7 g/portion, when using 50 g available starch as basis for comparison. RS has previously been associated with beneficial effects on glucose tolerance and/or insulin sensitivity in healthy subjects. Thus, a RS intake (type 2 RS) corresponding to 30 g/d for four weeks, or 60 g RS the day prior to the experimental day, improved glucose tolerance and/or insulin sensitivity measured with clamp procedure or estimated (minimal model) by use of ratio of postprandial serum insulin to blood glucose, respectively (Robertson, Currie et al. 2003; Robertson, Bickerton et al. 2005).

Although not significant, a spaghetti evening meal enriched with elevated amounts, i.e. twice the content of barley DF present in the barley kernel product, tended to lower (-27 %, n.s.) the postprandial glucose responses (IAUC, 0 - 120 min) in the morning compared with WWB, and significantly lowered the blood glucose IAUC compared with an evening meal with whole grain barley flour porridge ($P < 0.05$) (*Paper II*). Consequently, both the type and amount of prebiotic carbohydrates ingested in the evening may be of importance for the glucose tolerance the proceeding morning.

To further evaluate the importance of the level and/or composition of the indigestible carbohydrate fractions present in barley kernels; two additional barley genotypes with different prebiotic mixtures were included. One variety contained elevated amounts of β -glucans (HBB), and the other was a high amylose barley genotype (HAB) and yielded increased levels of RS in the finished product. Breads were made containing barley kernels from ordinary barley kernels (OB) as well as from the genotypes; HBB and HAB, respectively (*Paper III*). Of the evening test meals in *Paper III*, the HAB bread resulted in the lowest postprandial glucose response (IAUC 0 - 120 min) to the subsequent standardised breakfast. The HAB bread displayed a more pronounced capacity to lower the breakfast glucose response (IAUC 0 - 120 min) compared with WWB (-36 %, $P = 0.001$) than did the evening meal with OB bread (-28 %, $P < 0.05$). The result indicates that the elevated level of RS in the HAB bread meal (22.0 g vs 9.5 g in the OB bread meal) resulted in additional benefits with respect to overnight glucose tolerance.

The HBB bread evening meal resulted in a different postprandial glucose profile at breakfast, with a lower and more prolonged net increment in blood glucose concentration, compared with the other test meals in *Paper III* (**Figure 9**). This was probably due to the high content of indigestible carbohydrates in the HBB bread evening meal. In addition to elevated amounts of β -glucans, a serving of HBB bread was also a rich source of insoluble DF and RS (**Table 2**). The WWB with addition of RS only, or half a portion size of the OB bread, had no effect on over-night glucose tolerance. Taken together with the finding that the evening meal with WWB+RS+DF lowered the glucose response to the standardised breakfast, compared with WWB, it is possible to conclude that the prebiotic mixture present in the evening meal is of crucial importance for the over-night effect on glycaemia. Moreover a certain level of prebiotics has to be reached to elicit the effect. The results opens for interesting possibilities to utilise supplementation with prebiotic carbohydrates in the tailoring of foods capable of promoting lower glycaemic excursions, also at a subsequent meal ingested after 10h. As judged from the lack of over-night benefits with half a portion size, it can be concluded that a low carbohydrate intake per se not is preferable in this respect.

5.1.1.4. The role of colonic fermentation of indigestible carbohydrates

The mean breath H_2 excretion at the standardised breakfasts in *Papers I – III* is displayed in **Table 7** and **Figure 10**. At the time of the breakfast, the H_2 excretion was significantly higher ($P<0.05$) after the barley kernel evening meal compared with all the other evening test products, with the exception of spaghetti + wheat bran ($P = 0.08$) (*Paper I*). Consequently, the evening test meal that resulted in the lowest blood glucose response at the standardised breakfast (IAUC 0 – 120 min) also elicited the most prominent colonic fermentation of indigestible carbohydrates, as indicated from measurement of breath H_2 . The pattern was similar in *Paper II and III*. Accordingly, the spaghetti meal with elevated levels of barley DF (2*BDF) resulted in the highest mean breath H_2 excretion (0 – 180 min) ($P<0.05$) and lowest postprandial glucose response at breakfast (IAUC 0 – 120 min) (*Paper II*). Also, the HBB bread and HAB bread evening meals induced a higher colonic activity at breakfast the following morning, expressed as mean breath H_2 excretion (0–180 min, $P<0.0001$ and $P<0.01$, respectively), and lower blood glucose response compared with the WWB evening test meal (*Paper III*). Furthermore, the mean breath H_2 excretion after the standardised breakfast (0 – 180 min) was lowest after the evening meals with WWB, 1/2OB bread, and WWB+RS, i.e. the test products that were without effect on overnight glucose tolerance (*Paper III*). These results

strongly imply that an evening meal promoting colonic fermentation of indigestible carbohydrates beneficially modulates the glucose tolerance after the subsequent breakfast. Results from the present thesis that further strengthen the relation between colonic fermentation and glucose response are those, showing that the glucose response at the standardised breakfast (IAUC 0 – 120 min) was negatively correlated to the mean H₂ excretion (0 – 180 min) at this meal ($r = -0.25$, $P < 0.05$, *Paper III*). In addition, the fasting breath H₂ excretion was positively correlated to the postprandial glucose areas (0 – 180 min) *below* the fasting value ($P < 0.05$, *Paper II*). A steep drop in postprandial blood glucose is generally not considered desirable. However, the standardised breakfast consumed in *Paper II* consisted of WWB and water, only. Probably, the hypoglycaemia could have been expected to be less pronounced in the case of a composite breakfast.

Taken together these results indicate a close relationship between events related to colonic fermentation of indigestible carbohydrates and glucose regulation in the perspective from an evening meal to breakfast.

Table 7. Breath H₂ excretion in *Papers I – III* at a standardised breakfast, following different evening test meals.

<i>Evening test meals</i>	Mean H ₂ excretion 0 – 20 min
<i>Paper I</i>	
	<i>Δppm</i>
WWB	3.0 ± 3.7 ^a
Wheat kernels	2.6 ± 5.0 ^a
Barley kernels	27.7 ± 9.8 ^b
Spaghetti	1.3 ± 5.2 ^a
Spaghetti +wheat bran	6.6 ± 7.8 ^{ab}
Mean H ₂ excretion 0 - 180 min	
<i>Paper II</i>	
	<i>Δppm</i>
WWB	-17.9 ± 4.6 ^a
WWB+BDF	-2.3 ± 4.5 ^{ab}
Spaghetti+BDF	12.3 ± 4.5 ^{bc}
Spaghetti+2*BDF	23.3 ± 5.8 ^c
Spaghetti+ODF	5.6 ± 6.1 ^{bc}
WMB porridge	-5.5 ± 6.3 ^{ab}
Mean H ₂ excretion 0 - 180 min	
<i>Paper III</i>	
	<i>ppm</i>
WWB	14.6 ± 3.2 ^a
OB bread	24.6 ± 4.9 ^{abc}
CutOB bread	22.4 ± 4.0 ^{ab}
1/2OB bread	12.5 ± 2.7 ^{ab}
HAB bread	31.0 ± 4.8 ^{bc}
HBB bread	44.2 ± 6.2 ^c
WWB+RS	17.8 ± 3.9 ^{ab}
WWB+RS+BDF	24.0 ± 4.0 ^{ab}

WWB: white wheat bread, BDF: barley dietary fibre, ODF: oat dietary fibre, WMB: whole meal barley, OB: Ordinary barley kernels, CutOB: ordinary barley kernels cut 1 - 2 times, 1/2OB: half a portion OB, HBB: high beta-glucan barley kernels, HAB: high amylose barley kernels. In *Papers I - II*, the breath H₂ excretion is presented as incremental concentrations with the basal value determined immediately prior to the evening test meals. In *Paper III* the results are presented as true concentrations obtained at 0 – 180 min postprandial the standardised breakfast. Values in a column with different superscript letters are significantly different, $P < 0.05$.

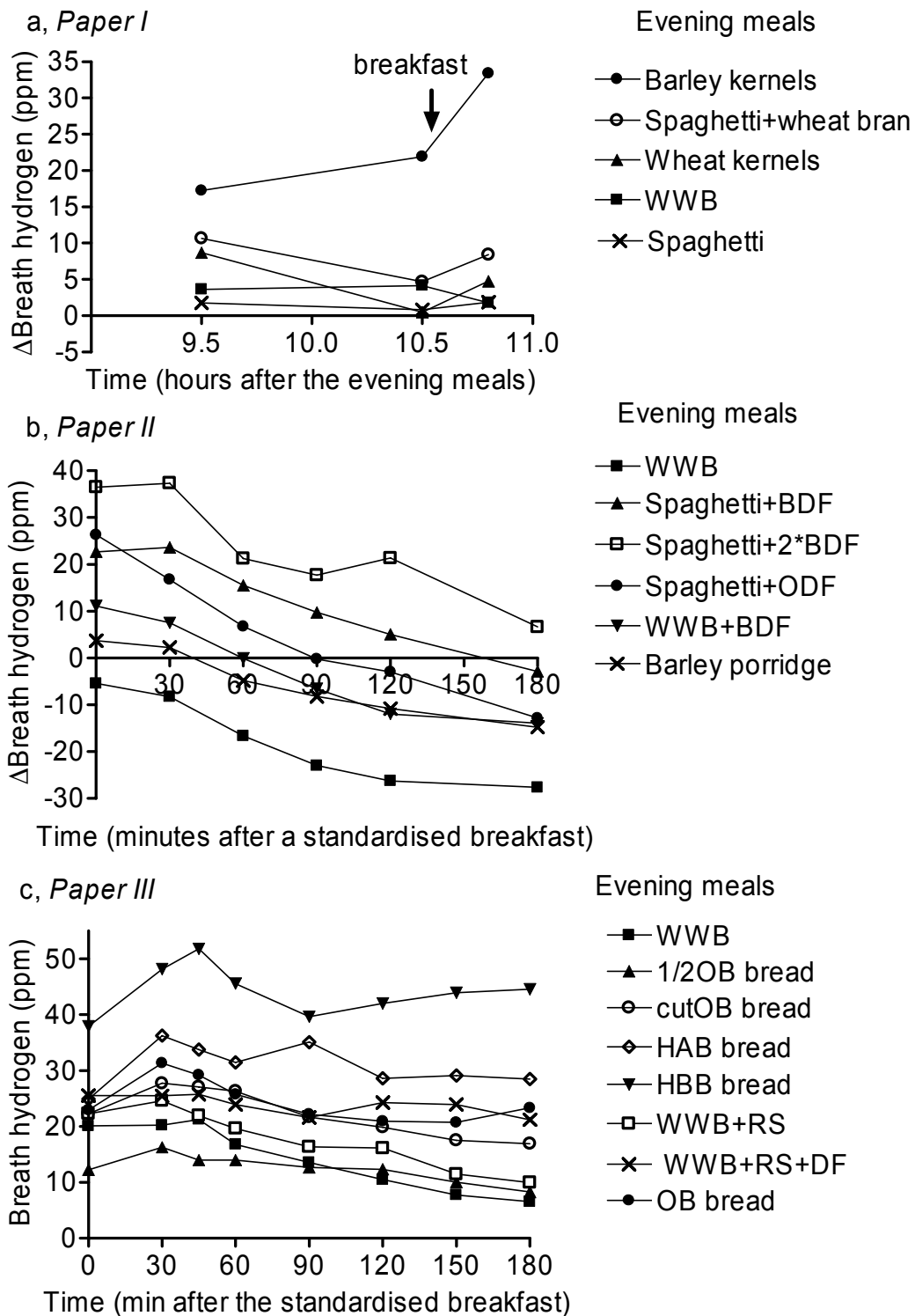


Figure 10. WWB: white wheat bread, BDF: barley dietary fibre, ODF: oat dietary fibre, WMB: whole meal barley, OB: Ordinary barley kernels, CutOB: ordinary barley kernels cut 1 - 2 times, 1/2OB: half a portion OB, HBB: high beta-glucan barley kernels, HAB: high amylose barley kernels. Breath H₂ exertion at breakfast following different cereal based evening test meals. In *Papers I - II* (figure a – b), the results are presented as delta values with the basal values determined immediately prior to the evening test meals. In *Paper III* (figure c) the results are presented as true values.

5.1.2. Serum insulin and plasma glucagon

Although certain cereal based evening meals significantly lowered the blood glucose response at a standardised breakfast (AUC 0 - 120 min) by 26 – 46 % compared with WWB, the effect on serum insulin was less pronounced. In *Papers I - II*, no significant differences were seen in serum insulin concentrations at breakfast, irrespectively of the preceding evening test meal. In *Paper III*, it was only the OB bread evening meal that resulted in significantly lower (-33 %, $P < 0.05$) serum insulin response to the standardised breakfast IAUC (0 – 120 min), compared with WWB. Compared with WWB, there was a strong tendency towards a lower insulin response also after the CutOB bread (-29 %, $P = 0.05$) (**Figure 9b**, **Table 6**) (*Paper III*). The less pronounced effect on serum insulin may be explained by increased insulin sensitivity, i.e. the insulin was more efficient in inducing clearance of blood glucose after certain evening test meals.

The postprandial plasma glucagon concentrations after the standardised breakfast are presented in **Table 6** (*Paper III*). After a slight increase in plasma glucagon concentrations in the early postprandial phase (up to 15 min), the glucagon concentrations were suppressed to a level beneath the fasting value from approx. 45 min and throughout the 120 min postprandial period. Expressing the result as mean values (0 – 120 min), the plasma glucagon concentration after the standardised breakfast was lower following the WWB evening meal compared with the evening meals with HBB bread ($P < 0.01$), OB bread ($P < 0.05$), and CutOB bread ($P < 0.05$), respectively (ANOVA followed by Dunnetts test with the WWB as a control). The mean plasma glucagon concentrations were negatively related to the blood glucose IAUCs (0 – 120 min) ($r = -0.23$, $P < 0.05$), thus reflecting the postprandial glucose response at the standardised breakfast.

5.1.3. Plasma short chain fatty acids

Previous results regarding effects of SCFAs on glucose metabolism are not conclusive. Some longer-term studies have shown beneficial effects of orally administered propionate on glucose metabolism in both man (Venter, Vorster et al. 1990) and animals (Boillot, Alamowitch et al. 1995; Berggren, Nyman et al. 1996). In contrast, others have failed to show benefits of SCFAs on glucose metabolism in healthy subjects following rectal (Wolever, Brighenti et al. 1989) or gastric (Laurent, Simoneau et al. 1995) infusion of propionate, acetate, or a combination of these. However, the results in the present thesis support a relation between plasma SCFAs produced during colonic fermentation, and glucose tolerance. The

plasma SCFAs (*Papers I – II*) are compiled in **Table 8 - 9**. Accordingly, the mean concentration (0 - 180 min) of plasma propionate was significantly higher in the morning following an evening meal with barley kernels compared with an evening meal with WWB ($P<0.05$, ANOVA followed by Dunnetts test, *Paper I*). Furthermore, a negative correlation between plasma propionate (0 - 180 min) and the blood glucose peak value after the standardised breakfast ($r = -0.25$, $P<0.05$), and a negative correlation between mean plasma acetate (0 - 180 min) and the glucose IAUC (0 - 120 min) ($r = -0.36$, $P<0.05$) was observed (*Paper I*, unpublished observation). Also, a significant negative correlation between blood glucose response after the standardised breakfast (total AUC 0 - 180 min) and plasma propionate (0 - 30 min) was found in *Paper II* ($r = -0.24$, $P<0.05$). In addition, a negative correlation was found between plasma butyrate (0 - 30 min) and glucose IAUC (0 - 120 min) ($r = -0.98$, $P<0.001$, $n = 6$, Person's test, based on group means). In *Paper II* it was found that the plasma acetate, and also the total plasma SCFAs concentration (sum of acetate, propionate and butyrate), was higher in the morning after an evening meal with spaghetti+ODF or WWB+BDF compared with a barley porridge evening meal ($P<0.05$). An evening meal with spaghetti+ODF also resulted in higher plasma acetate and total plasma SCFAs concentrations in the morning compared with WWB ($P<0.05$). Evening meals with spaghetti+ODF or spaghetti+2*BDF resulted in higher concentrations of plasma butyrate at the subsequent breakfast compared with an evening meal with barley porridge ($P<0.05$). Accordingly, the plasma butyrate concentrations were highest in the morning after the evening meals that induced the lowest blood glucose responses at breakfast. In contrast, plasma butyrate concentration was lowest after the evening meal that elicited the highest blood glucose response post the subsequent breakfast. The presence of colonic derived metabolites in plasma was thus associated with improved glucose tolerance.

Table 8 *Paper I.* Plasma SCFAs at breakfast following consumption of different evening test meals.

Evening meals	Plasma SCFAs			
	Total SCFAs	Acetate	Propionate	Butyrate
	$\mu\text{mol/L}$	$\mu\text{mol/L}$	$\mu\text{mol/L}$	$\mu\text{mol/L}$
WWB	144.8 ± 9.5	137.1 ± 9.5	6.4 ± 0.2	1.4 ± 0.3
Wheat kernels	168.5 ± 11.6	160.3 ± 11.4	6.9 ± 0.4	1.5 ± 0.4
Barley kernels	160.1 ± 6.9	151.6 ± 7.0	7.7 ± 0.5*	1.6 ± 0.3
Spaghetti	159.3 ± 8.1	151.5 ± 8.2	6.3 ± 0.3	1.4 ± 0.4
Spaghetti + wheat bran	155.5 ± 8.9	146.7 ± 8.7	7.2 ± 0.3	1.6 ± 0.4

* Significantly higher compared with WWB $P < 0.05$ (analyses of variance followed by Dunnetts comparisons with the reference WWB as a control).

Table 9 *Paper II.* Plasma SCFAs at breakfast following consumption of different evening test meals.

Evening meals	Plasma SCFAs			
	Total SCFAs	Acetate	Propionate	Butyrate
	$\mu\text{mol/L}$	$\mu\text{mol/L}$	mmol/L	$\mu\text{mol/L}$
WWB	157.3 ± 14.0 ^{ac}	148.3 ± 13.6 ^{ac}	7.6 ± 0.9 ^a	1.4 ± 0.1 ^{ab}
WWB+BDF	201.2 ± 15.4 ^{ab}	190.4 ± 14.3 ^{ab}	9.3 ± 1.6 ^a	1.5 ± 0.3 ^{ab}
Spaghetti+BDF	195.4 ± 14.6 ^{abc}	185.1 ± 14.1 ^{abc}	8.8 ± 1.5 ^a	1.5 ± 0.3 ^{ab}
Spaghetti+2*BDF	188.9 ± 13.3 ^{abc}	179.1 ± 13.0 ^{abc}	7.9 ± 0.9 ^a	1.9 ± 0.4 ^a
Spaghetti+ODF	216.7 ± 14.8 ^b	205.5 ± 13.8 ^b	9.4 ± 1.5 ^a	1.8 ± 0.6 ^a
WMB porridge	149.5 ± 12.6 ^c	141.8 ± 11.7 ^c	6.7 ± 1.2 ^a	1.0 ± 0.4 ^b

WWB: white wheat bread, BDF: barley dietary fibre, ODF: oat dietary fibre, WMB: whole meal barley. Values in a column with different superscript letters are significantly different, $P < 0.05$.

5.1.4. Plasma/serum free fatty acids and serum triacylglycerols

The plasma/serum concentrations of FFAs in *Papers I* and *III* are displayed in **Figure 11 a - b**. Consumption of barley kernels in the evening was accompanied by a significant reduction in fasting plasma FFA levels in the morning compared with WWB ($P < 0.05$, *Paper I*). Further, evening meals consisting of OB bread, HAB bread, CutOB bread, and WWB+RS also lowered fasting serum FFA concentrations compared with the WWB ($P < 0.0001$, $P < 0.0001$, $P < 0.001$, and $P < 0.01$, respectively, *Paper III*). The postprandial blood glucose response (IAUC 0 – 120 min) post the standardised breakfast was highly and positively correlated to the fasting FFA concentration; *Paper I*: $r = 0.52$, $P < 0.01$ (unpublished observations), *Paper II*: $r = 0.29$, $P < 0.05$, *Paper III*: $r = 0.37$, $P < 0.01$).

The relation between lowered FFAs and increased glucose tolerance has also been observed by others examining the effects of various test breakfasts at a standardised lunch (Wolever, Bentum-Williams et al. 1995) or from an evening test meal to a standardised breakfast (Thorburn, Muir et al. 1993). The concentration of circulating FFAs is known to affect insulin sensitivity, and increased FFA levels are associated with impairment of insulin sensitivity in a dose dependent manner also within a modest range (from 440 to 695 $\mu\text{mol/L}$, healthy lean subjects) (Belfort, Mandarino et al. 2005). In contrast, a decrease in FFA concentrations (using Acipimox, a lipolysis inhibitor) from 329 to 128 $\mu\text{mol/L}$, was found to improve insulin sensitivity in healthy lean subjects (Santomauro, Boden et al. 1999). The results of the present thesis strongly suggest that suppression of FFAs is a major factor involved in the overnight benefits seen after certain evening meals on breakfast glucose tolerance. The reason for the overnight lowering of serum FFAs with some cereal based evening test meals is not clear, and several factors may be involved. Firstly; the prolonged fed state after “lente” low-GI foods, thereby maintaining suppression of FFAs, has been suggested as a mechanism for improved glucose tolerance from breakfast to a standardised lunch (Wolever, Bentum-Williams et al. 1995). However, the in between meal period in the present thesis was 10.5 h and therefore probably too long to exert a significant suppression of FFA concentrations. Secondly; it is possible that a higher concentration of fasting plasma adiponectin, as seen in the morning after the OB bread evening meal in *Paper III*, may affect the FFA concentrations. Thus, it has been shown that adiponectin promote an increased rate of FFA clearance from the blood, and improves insulin sensitivity (Jazet, Pijl et al. 2003; Tschritter, Fritsche et al. 2003). Thirdly; it has been shown that an acute increase in plasma IL-6, as occurring in

response to hyperglycaemia (Haidara, Yassin et al. 2006), is associated with increased plasma FFAs, that may persist for several hours (van Hall, Steensberg et al. 2003). The positive correlation between serum IL-6 and serum FFA observed in *Paper III* supports such a relation. Finally, it is possible that the inverse correlations seen between SCFAs and postprandial glucose response may involve mechanisms influencing plasma concentrations of FFA. Accordingly, rectally infused SCFAs (acetate and propionate) have been shown to lower plasma FFA concentrations in man (Wolever, Brighenti et al. 1989; Wolever, Spadafora et al. 1991). The results regarding IL-6 and adiponectin are discussed below in the sections 5.1.6 and 5.1.7, respectively.

No significant differences in serum triacylglycerols were seen at breakfast depending on the test meals the previous evening (*Paper III*).

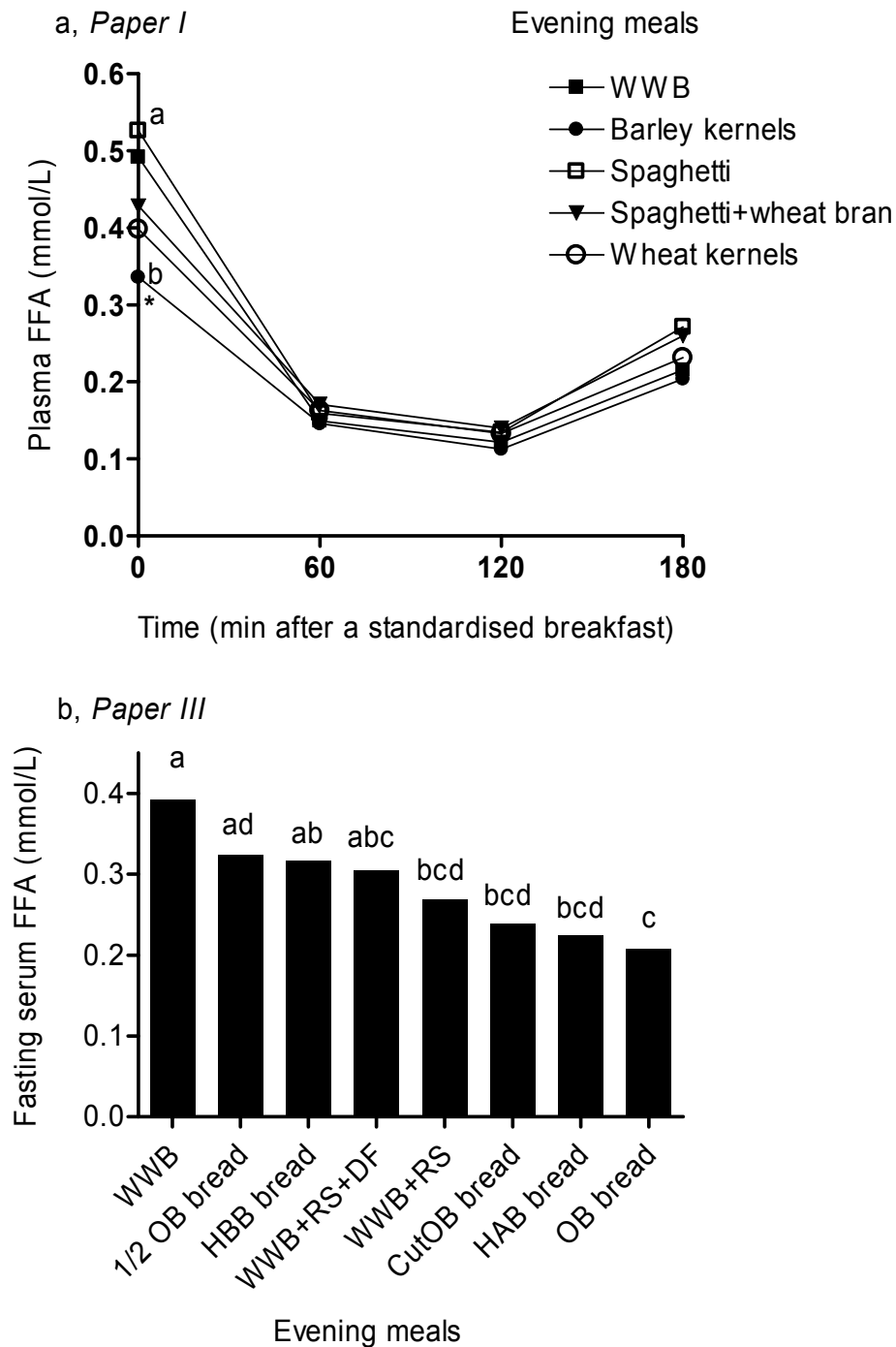


Figure 11. (a) Postprandial plasma FFA concentrations after a standardised breakfast in *Paper I* and (b) fasting serum FFA concentrations in *Paper III*. Values/bars with different letters are significantly different ($P < 0.05$).

* Significantly lower compared with WWB ($P < 0.05$, analyses of variance followed by Dunnett's comparisons).

5.1.5. Plasma incretins (GLP-1 and GIP)

GIP and GLP-1 are insulinotropic hormones that are released from the intestinal mucosa in response to a meal. GLP-1 also regulates the postprandial glucose response by inhibiting the gastric emptying rate (GER) (Nauck, Niedereichholz et al. 1997). According to previous studies in animals, certain DF promote secretion of GLP-1; an effect suggested to be mediated by bacterial colonic fermentation and formation of SCFAs (Reimer and McBurney 1996; Cani, Daubioul et al. 2005). In the present thesis, the total GLP-1 response (AUC 0 – 120 min) after the standardised breakfast was significantly higher following the OB bread evening meal compared with the evening meal with WWB (3513 ± 333 and 2843 ± 265 pM*min, respectively, $P < 0.05$, *Paper III*). The blood glucose response (IAUC 0 – 120 min) after the standardised breakfast was negatively correlated to the plasma GLP-1 response (AUC 0 – 120 min) ($r = -0.26$, $P < 0.05$, *Paper III*). In contrast, no relation was observed between GLP-1 and insulin response. The lack of such a relation may indicate that GLP-1 facilitated blood glucose regulation through other mechanisms than through enhanced insulin release, e.g. through a reduced GER (healthy humans) (Nauck, Niedereichholz et al. 1997), increased glucose disposal (type 2 diabetic elderly subjects), or an improved hepatic insulin sensitivity (diabetic mice model) (Cani, Knauf et al. 2006). In the latter study, endogenous stimulation of GLP-1 secretion was achieved through oligo-fructose supplementation, and was suggested as a therapeutic tool in the treatment of type 2 diabetes. A possible mechanism for these benefits of oligofructose probably relates to its fermentation in the colon leading to formation of SCFAs (Cani, Daubioul et al. 2005).

The plasma GIP response in the later postprandial phase after the standardised breakfast AUC (60–120 min) was significantly higher following the evening meal with HBB bread (2610 ± 340 pM*min) compared with the evening meal with WWB (2030 ± 225 pM*min) ($P < 0.01$). At the end of the postprandial period (120 min after the standardised breakfast), the plasma GIP concentration was higher after the evening meals with HBB bread, ($P < 0.01$) and the OB bread ($P < 0.05$), compared with the WWB (*Paper III*).

The results from the present thesis thus support a theory relating indigestible carbohydrates and/or production of colonic fermentation metabolites to the release of incretine hormones. Interestingly, the OB bread evening meal increased GLP-1 AUC at breakfast with more than 20% compared with WWB, suggesting a substantial endogenous stimulation.

5.1.6. Plasma IL-6 and IL-8

When analysing plasma IL-6 over the postprandial phase after the standardised breakfast, it was shown that an evening meal consisting of OB bread resulted in a lower mean concentration (0 – 180 min), compared with a WWB evening meal (13.6 ± 1.0 and 16.3 ± 1.0 pg/ml, respectively ($P < 0.01$, *Paper III*). This is an interesting finding, and indicates anti-inflammatory properties of the OB bread product. An increase in pro-inflammatory markers is increasingly being associated with the metabolic syndrome. Thus, it has been shown that IL-6, CRP, and VCAM-1 were positively correlated with the degree of glucose intolerance (Deepa, Velmurugan et al. 2006). It is known that acute hyperglycaemia increase inflammation, possibly through a mechanism involving increased production of reactive oxygen species (Haidara, Yassin et al. 2006). In particular, frequent postprandial hyperglycaemic episodes appear to be especially prone to initiate cytokine production (TNF-alpha, IL-6, IL-18) (Esposito, Nappo et al. 2002), and it could therefore be hypothesised that a low-GI diet decreases oxidative stress and lowers inflammation, by maintaining a more tight blood glucose regulation. Low-GI foods capable of reducing blood glucose excursions also at a second-meal could be expected to be particularly beneficial in this respect. A majority of low-GI products appear to have such properties in a 4 h perspective, e.g. from breakfast to lunch (Jenkins, Wolever et al. 1982; Trinick, Laker et al. 1986; Liljeberg, Åkerberg et al. 1999; Liljeberg and Björck 2000). More knowledge concerning food mechanisms capable of improving glycaemic regulation over an extended time frame, for example over the course of a whole day or overnight, could be utilised to magnify these benefits. As judged from the observations made in the present thesis, low-GI cereals rich in specific fermentable carbohydrates might be especially advantageous in reducing risk factors for the metabolic syndrome. The reduced glycaemia with low-GI foods may *per se* act to suppressing pro-inflammatory cytokines. However, in the case of barley kernel products it can also be hypothesised that SCFAs produced during colonic fermentation of DF and RS may have contributed to the anti-inflammatory properties. Accordingly, dietary fibres have shown beneficial effects in preventing inflammatory conditions in the colon through a mechanism related to colonic production of SCFAs (Rodriguez-Cabezas, Galvez et al. 2003). Butyrate and propionate are utilized as energy substrates for the colonocytes, and interestingly, these SCFAs were shown to downregulate different mediators of inflammation such as pro-inflammatory cytokines. Propionate and butyrate seemed to act synergistically on the immune response (animal studies and

in vitro models) (Rodriguez-Cabezas, Galvez et al. 2003). Since anti-inflammatory properties of SCFAs have been shown locally in the colon, it cannot be excluded that colonically derived SCFAs also may have systemic anti-inflammatory properties. This effect may have contributed to the observed effect of the OB bread on IL-6 in the present thesis.

No significant differences in plasma IL-8 were seen at breakfast depending on the test meals the previous evening (*Paper III*).

5.1.7. Plasma adiponectin

Adiponectin is a hormone secreted by the adipocytes that regulates energy homeostasis and glucose and lipid metabolism (Yamauchi, Kamon et al. 2002). Obesity and type 2 diabetes are associated with decreased plasma adiponectin concentrations. Adiponectin has shown to suppress FFA concentrations in the blood, increase insulin sensitivity and suppress hepatic glucose production (Jazet, Pijl et al. 2003). Interestingly, adiponectin has been associated with anti-inflammatory properties (Jazet, Pijl et al. 2003). In a study of 3640 non-diabetic men (60 – 79 years) adiponectin was inversely associated with insulin resistance (HOMA-IR), the metabolic syndrome, triglycerides, CRP, and positively associated with HDL-cholesterol, (Wannamethee, Tchernova et al. 2007). In the present thesis, analysis of plasma adiponectin was performed after the standardised breakfast following evening meals consisting of OB bread or WWB, respectively (*Paper III*). It was observed that the fasting concentrations of plasma adiponectin were higher following the evening meal containing OB bread compared with the evening meal with WWB (8.5 ± 0.8 and 7.8 ± 0.7 $\mu\text{g/ml}$, respectively, $P < 0.05$). This finding is consistent with the improved overnight glucose tolerance and reduced concentrations of circulating FFAs and reduced expression of pro-inflammatory marker (IL-6) observed following the evening meal with OB bread.

5.1.8. Gastric emptying rate and satiety

The GER after the standardised breakfast (judged from the appearance of paracetamol in postprandial serum IAUCs, 0 – 90 min), was reduced after the evening meal with HBB bread (IAUC 2.23 ± 0.3 $\text{mmol} \cdot \text{min/L}$) compared with evening meals with WWB, WWB+RS, and 1/2OB bread, respectively (IAUC: 3.43 ± 0.4 , 3.42 ± 0.3 , and 3.34 ± 0.2 $\text{mmol} \cdot \text{min/L}$, respectively, $P < 0.05$). After the evening meal with WWB, the serum paracetamol concentrations at breakfast reached the peak value at 60 min, whereas the other evening test meals resulted in a continues increase of this marker up to the last test point at 90 min after the

breakfast, indicating a faster GER after the WWB. In addition to a reduced GER, the evening meal with HBB bread also resulted in a higher satiety score (IAUC 0 – 180 min) after the standardised breakfast compared with all the other evening meals (HAB bread $P<0.05$, WWB and CutOB bread $P<0.01$; and versus the other evening meals $P<0.001$) (*Paper III*).

In the present thesis, a negative correlation was observed between H_2 excretion (0–180 min) and serum paracetamol concentrations (IAUC 0 – 90 min) ($r = -0.24$, $P<0.05$), indicative of an involvement of colonic fermentation in modulating GER at breakfast (*Paper III*). Further, there was a positive correlation between breath H_2 excretion (0 – 180 min) and the satiety (AUC 0 – 180 min) post breakfast ($r = 0.27$, $P<0.01$), suggesting a role of colonic fermentation and associated metabolites in modulating also the satiety; possibly through a mechanism involving a lowered rate of gastric emptying. Consequently, the rated satiety at breakfast was inversely related to the GER, as measured from serum paracetamol concentrations (IAUC 0 – 90 min) post this particular meal ($r = -0.23$, $P<0.05$).

It has previously been demonstrated that the presence of SCFAs in the colon probably can act to reduce the GER (Cuche, Cuber et al. 2000; Cherbut 2003). The effect on motility seems to depend on the concentration of SCFAs in the colonic lumen, and most likely sufficient quantities of SCFAs can be reached during colonic fermentation of indigestible carbohydrates present in food (Cherbut 2003). This “colonic break” mechanism may provide an explanation to a reduced GER at breakfast following ingestion of an evening meal containing fermentable carbohydrates. In addition, as discussed above in the section 5.1.5. GLP-1 has been shown to lower the GER (Nauck, Niedereichholz et al. 1997), and it has been suggested that colonic SCFA may reduce GER through a stimulation of GLP-1 release (Reimer and McBurney 1996; Cani, Daubioul et al. 2005).

In a meta-analysis of nine studies of the acute effect of intravenously infused GLP-1, it was shown that GLP-1 infusion was associated with increased satiety (visual analogue scales) and reduced energy intake in a dose dependent manner in both lean and overweight subjects. The increased satiety was suggested to be secondary to a lowered GER (Verdich, Flint et al. 2001). These results indicate that the incretin hormones may participate in weight regulation, e.g. by reducing GER. The appearance of the 0 – 120 min blood glucose curve at breakfast after the HBB bread (*Paper III*), with a low and prolonged net increment, is probably partly related to a reduced GER following the test breakfast. The fact that a significant negative correlation was observed between GER and subjective rating of satiety in

the present thesis makes it plausible that the higher satiety obtained after the standardised breakfast, following the HBB bread evening meal was the result of a lower GER. Taken together, the results in this thesis suggest beneficial effect on blood glucose and weight regulation of low-GI whole grain products mediated by a reduced GER, in response to metabolites formed upon colonic fermentation.

5.2. Effects of cereal based test meals on glucose response and H₂ excretion in a daylong vs overnight perspective

5.2.1. Effects of test breakfasts on daylong blood glucose and breath H₂ concentrations

The test meals were consumed at breakfast, and blood glucose and breath H₂ were determined for 2 h after the test breakfast, a standardised lunch, and a standardised dinner meal, respectively. Included as test breakfasts were the four most common Swedish cereals, i.e. wheat kernels, oat kernels, rye kernels and barley kernels (*Paper IV, Series I*). Also included were breakfasts with WMB porridge (made from the same kernels that were consumed intact), WWB+BDF, and a WWB reference meal. An overview of the postprandial blood glucose increments during the course of the experimental day is shown in **Figure 12**, and the GIs and incremental areas (0 – 120 min) after breakfast, lunch and dinner, respectively, are presented in **Table 10**. Also shown in Table 10 are the changes (%) in areas compared with the WWB. The incremental blood glucose responses after the breakfast, lunch and dinner meal, respectively, is shown in **Figure 13 a–c**.

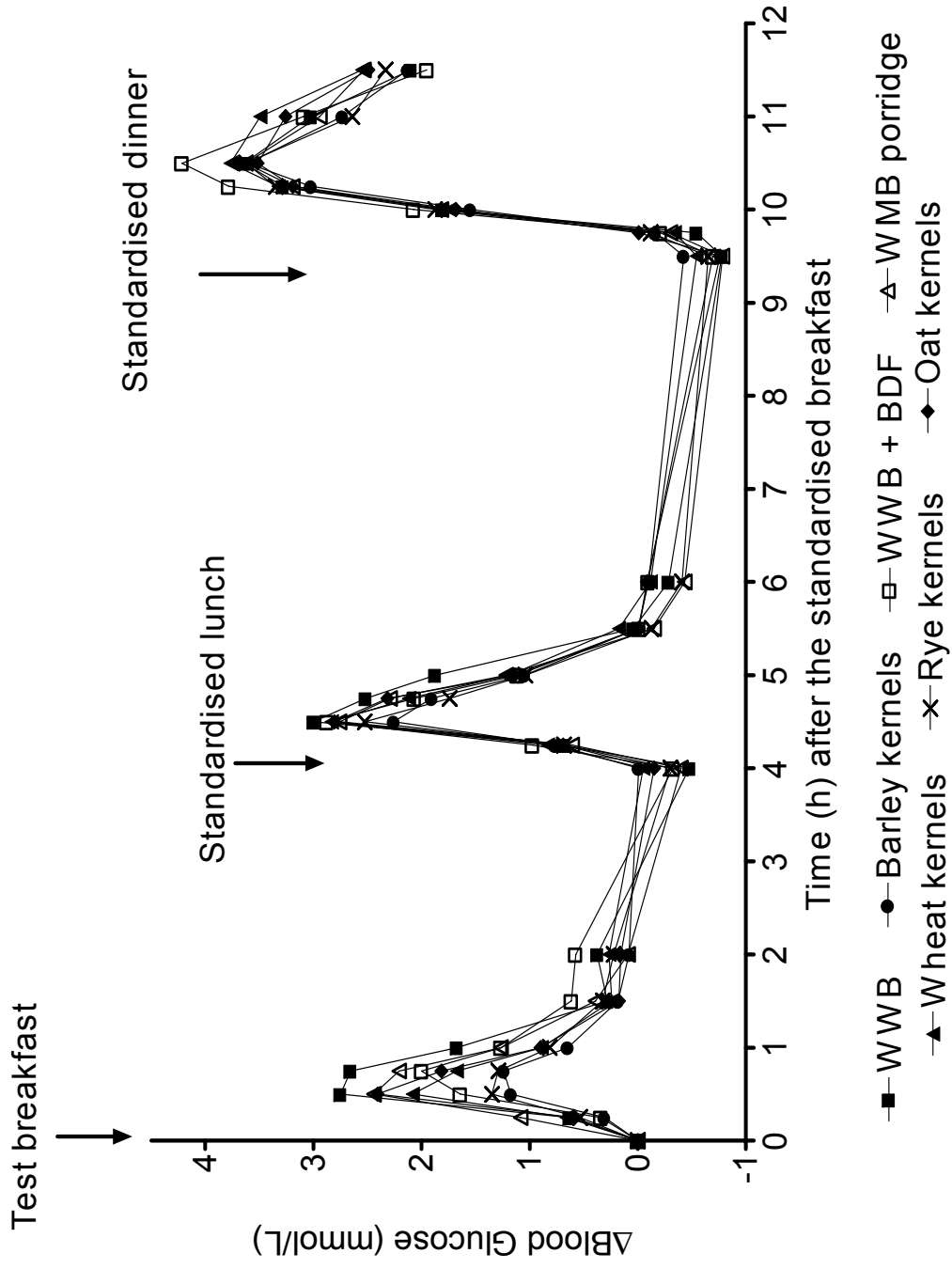


Figure 12. Overview of the blood glucose responses during the course of the experimental day (*Paper IV*)

Table 10. GI characteristics and postprandial blood glucose responses after the test breakfast, standardised lunch, and standardised dinner, respectively (*Paper IV*).

Test meals	GI %	IAUC (0 - 120 min)		
		Breakfast (mmol·min/L)	Lunch (mmol·min/L)	Dinner (mmol·min/L)
WWB	100 ^a	145.4 ± 22. ^a	192.5 ± 28.9 ^a	360.6 ± 36.3 ^a
WMB porridge	112 ± 25 ^{ab}	134.6 ± 20.6 ^{ab}	157.0 ± 17.8 ^{ab}	366.9 ± 23.9 ^a
Wheat kernels	79 ± 13 ^{abc}	100.4 ± 17.5 ^{abc}	127.4 ± 14.2 ^{bt}	361.0 ± 33.5 ^a
Oat kernels	85 ± 13 ^{abc}	105.0 ± 15.1 ^{abc}	137.1 ± 17.0 ^{ab}	372.6 ± 34.7 ^a
Rye kernels	73 ± 19 ^{bctt}	80.6 ± 11.3 ^{bctt}	130.7 ± 21.2 ^{bt}	345.8 ± 36.0 ^a
Barley kernels	49 ± 7 ^{cttt}	68.2 ± 11.8 ^{cttt}	107.7 ± 14.4 ^{bttt}	309.6 ± 25.6 ^a
WWB+BDF	93 ± 15 ^{abc}	116.0 ± 14.8 ^{abc}	156.2 ± 21.9 ^{ab}	379.7 ± 17.6 ^a

Areas in a column not sharing the same superscript letters are significantly different.

[†]; $P < 0.05$, ^{††}; $P < 0.01$, ^{†††}; $P < 0.001$ compared to WWB. In addition, at breakfast $P < 0.01$ between barley kernels and the WMB porridge.

['] Change (%) in postprandial IAUC compared with the reference WWB

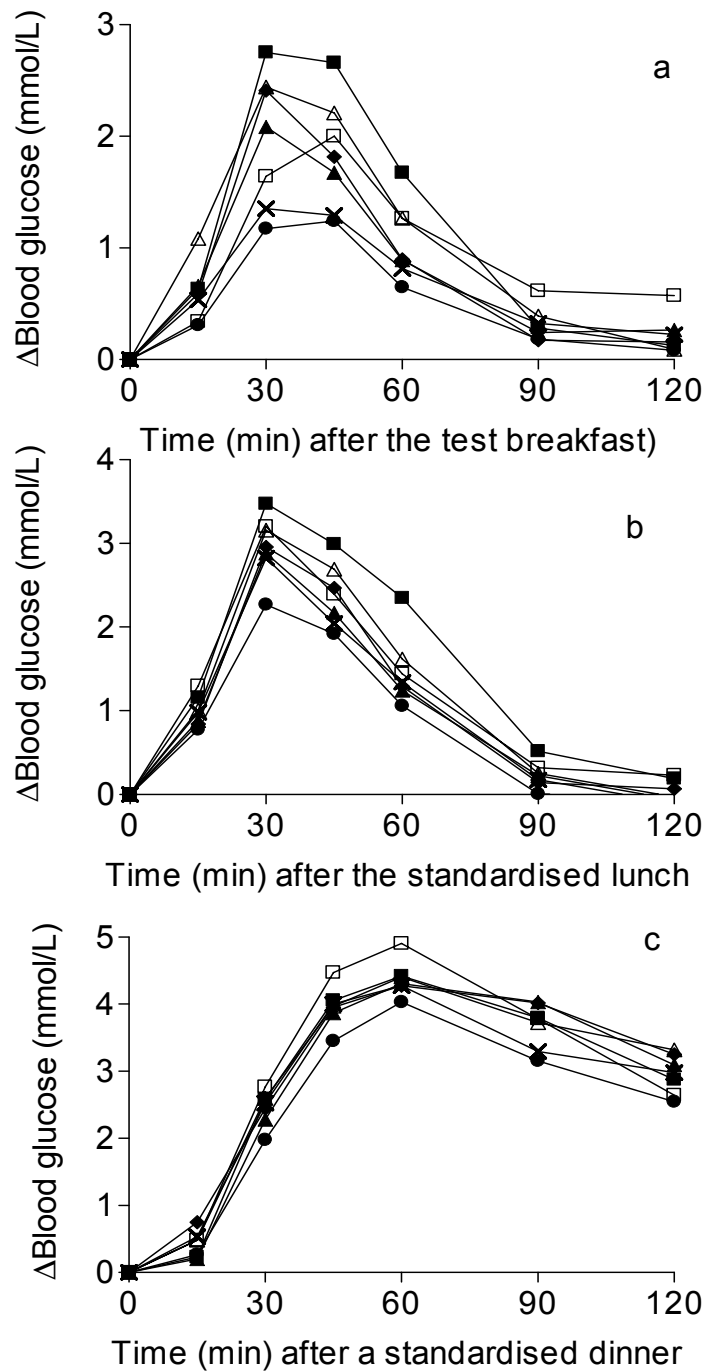


Figure 13. Mean incremental changes in blood glucose after: (a) the test breakfasts, (b) a standardised lunch and, (c) a standardised dinner meal, respectively. WWB (■), WMB porridge (Δ), wheat kernels (\blacktriangle), oat kernels (\blacklozenge), rye kernels (\times), barley kernels (\bullet), WWB+BDF (\square) (*Paper IV*).

Following the test breakfasts, the boiled barley- and rye kernels resulted in lower acute blood glucose responses (IAUC 0 - 120 min), compared with WWB ($P < 0.001$ and $P < 0.01$, respectively). A barley kernel breakfast also resulted in lower blood glucose response at breakfast compared with WMB porridge (IAUC 0 - 120 min, -49 %, $P < 0.01$). Similarly, in the early postprandial phase after breakfast (0 - 60 min) the blood glucose response (IAUC) was lower after the barley kernels (-55 %) and rye kernels (-48 %), compared with the WWB ($P < 0.001$), and also lower (-51 % and -44 %, respectively) compared with barley porridge ($P < 0.001$ and $P < 0.01$, respectively). In addition the early postprandial glucose response (IAUC 0 - 60 min) was 33 % lower also after the WWB+BDF test breakfast, compared with the WWB ($P < 0.05$). The barley DF used in the present thesis contained a substantial fraction of soluble DF (28 % dry weight basis), which was made up almost exclusively of β -glucans, yielding approximately 4 g β -glucans/serving. The beneficial effect of β -glucans on glucose tolerance is in line with previous studies with oat β -glucans, showing that 4 g β -glucans seems to be a critical level to elicit a significant decrease in glucose response to a breakfast based on β -glucan enriched muesli served with yoghurt and WWB (Granfeldt, Nyberg et al. 2007). Obviously, β -glucans from oats and barley appears to have similar benefits on acute glycaemia. The lack of effect when calculating the 0 - 120 min blood glucose IAUC was due to a higher blood glucose increment in the later (60 - 120 min) postprandial phase in the case of the meal enriched with barley DF. A prolonged net increment in blood glucose concentrations in the late postprandial phase was observed also after enrichment of the meal with 4 g oat β -glucans (Granfeldt, Nyberg et al. 2007). This effect probably relates to a prolonged digestive- and absorptive phase, and can be considered beneficial in that a hypoglycaemic state in between meals is avoided. Regarding the lack of difference in acute glycaemic response (IAUC) following a breakfast consisting of WMB porridge compared with WWB, indicates that the amounts of β -glucans in the whole grain flour porridge is too small (2.6 g). Thus, the low-GI features observed with the barley kernels are probably not related to the presence of cereal DF (2.9 g soluble DF) *per se*. This supports previous observations, showing that disruption of the botanical structure of cereal kernels prior to heat-treatment, e.g. by grinding to a whole grain flour, renders the starch highly susceptible to digestive enzymes (Liljeberg, Granfeldt et al. 1992; Björck, Granfeldt et al. 1994; Granfeldt, Liljeberg et al. 1994).

The breakfasts consisting of barley kernels, rye kernels, or wheat kernels resulted in a lower postprandial blood glucose response post lunch compared with the WWB breakfast ($P < 0.001$, $P < 0.05$, and $P < 0.05$, respectively, Table 10). The magnitude of the glycaemic response to the breakfast products seemed to be an important factor in predicting the glucose response at the subsequent standardised lunch. Thus, a significant positive correlation was observed between the blood glucose IAUC (0 – 120 min and 0 – 60 min) after the test breakfasts; and the glucose responses (IAUC 0 – 120 min) after the subsequent standardised lunch ($r = 0.30$, $P = 0.001$ and $r = 0.32$, $P < 0.01$, respectively). This finding agrees with previous studies showing that a low-GI breakfast with “lente” carbohydrates, i.e. carbohydrates capable of maintaining a low but sustained net increment in blood glucose, may significantly reduce postprandial glycaemia following a standardised lunch meal (Jenkins, Wolever et al. 1982; Liljeberg, Åkerberg et al. 1999; Liljeberg and Björck 2000).

The cumulative postprandial glucose areas (IAUC 0 - 120 min) after the three consecutive meals (breakfast, lunch, and dinner) are presented in **Figure 14**. The postprandial glucose IAUCs after breakfast + lunch were lower after all kernel based breakfasts compared with the WWB breakfast ($P < 0.05$). In addition, the barley kernel breakfast resulted in a lower cumulative blood glucose IAUC (breakfast + lunch) compared with WMB porridge or WWB+BDF, respectively ($P < 0.05$). No significant effects of breakfasts were seen following the standardised dinner meal 9.5 – 11.5 h after the test breakfasts (**Figure 13**). However, the cumulative postprandial glucose IAUC (0 – 120 min) over the course of the day (breakfast + lunch + dinner) was approximately 30 % or 20 % lower after the barley kernel- or the rye kernel breakfasts, respectively, compared with the WWB breakfast ($P < 0.05$). The cumulative glucose IAUC (breakfast + lunch + dinner) after the barley kernel breakfast was lower also compared with breakfasts consisting of WMB porridge or WWB+BDF, respectively ($P < 0.05$). In addition, when comparing only the barley products, i.e. barley kernels, WWB+barley DF and barley porridge in an ANOVA analysis, a significantly lower glucose IAUC 0 – 120 min was seen after the standardised dinner following the barley kernel breakfast compared with the WWB+barley DF breakfast (-18 %, $P < 0.05$). Glycaemia post dinner (IAUC 0 – 120 min) tended to be lower (16%) after the barley kernels compared with WMB porridge breakfast, but the difference did not reach significance ($P = 0.08$).

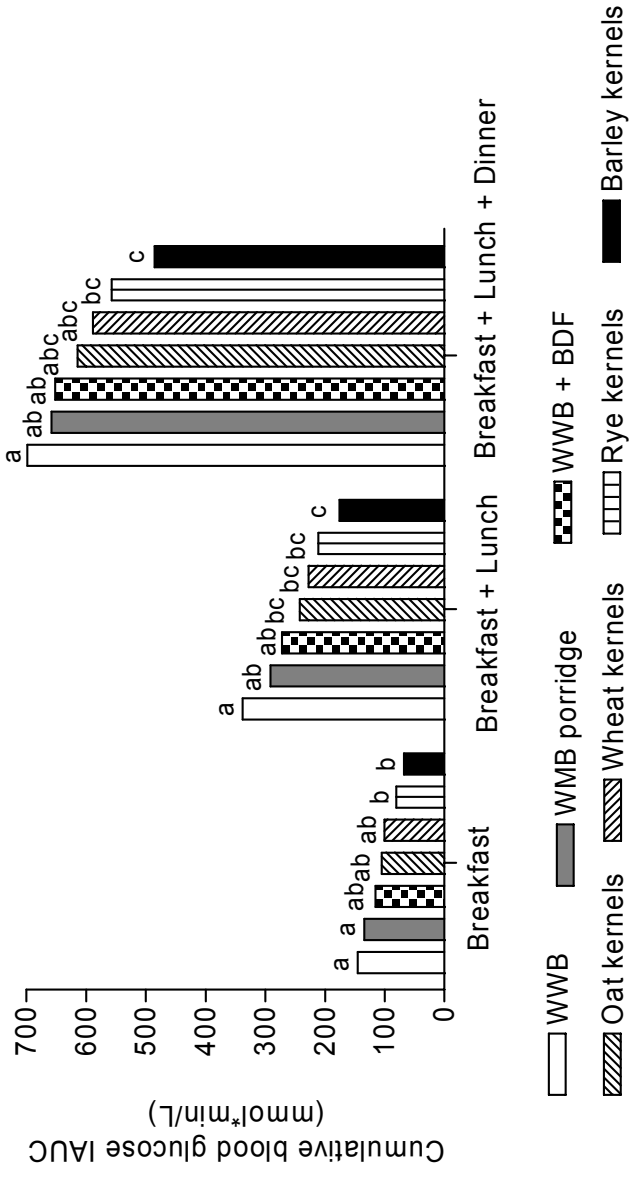


Figure 14. Cumulative blood glucose response (IAUCs 0 – 120 min) after breakfast + lunch + dinner. Bars in a group not sharing the same letters are significantly different, $P \leq 0.05$ (*Paper IV*).

There were no differences in fasting blood glucose values prior to the test breakfasts. The mean baseline blood glucose level showed a tendency to fall during the course of the experimental day. The mean glucose concentrations immediately prior to the lunch (4.4 ± 0.1 mmol/L) and dinner meals (4.0 ± 0.1 mmol/L) were lower ($P < 0.001$ and $P < 0.0001$, respectively) compared with the mean fasting glucose concentration prior to breakfast (4.6 ± 0.0 mmol/L). The mean blood glucose concentration prior to dinner was also lower than the corresponding value prior to lunch ($P < 0.0001$). The decline in baseline blood glucose concentration was however less pronounced after the barley kernel breakfast. Prior to lunch, the barley kernel breakfast maintained a significantly higher blood glucose concentration compared with the WWB breakfast (4.7 ± 0.1 and 4.2 ± 0.2 mmol/L, respectively, $P < 0.05$). Furthermore, prior to the dinner meal, the blood glucose concentration was higher following the barley kernel breakfast compared with the breakfast with boiled oat kernels (4.3 ± 0.1 and 3.8 ± 0.1 mmol/L, respectively, $P < 0.05$). The less decrease in baseline glucose concentrations with barley kernels might be associated with benefits on insulin sensitivity through a mechanism related to suppression of FFA concentrations in the blood.

The mean breath H_2 excretions after the different test breakfasts are shown in **Figure 15**. At the standardised lunch, 4 – 6 h after the test breakfast, the mean breath H_2 excretion was higher ($P < 0.05$) after the rye kernel breakfast compared with all the other breakfasts, except for the barley kernel breakfast. The barley kernel breakfast resulted in higher breath H_2 excretion at lunch, compared with WWB ($P < 0.05$). At the standardised dinner (9.5 – 11.5 h), barley kernels resulted in higher mean H_2 excretion compared with all the other breakfast meals ($P < 0.05$), with the exception of oat kernels. The mean H_2 excretion at dinner was higher after the oat kernel breakfast compared with WWB- or WMB porridge breakfasts, respectively ($P < 0.05$).

Breath H_2 excretion was negatively correlated to the blood glucose IAUCs (0 – 120 min) post lunch ($r = -0.33$, $P < 0.05$) and dinner ($r = -0.22$, $P < 0.05$), respectively. This indicates that the glucose response to the standardised lunch and dinner meals, were lower when markers of the colonic fermentation activity was higher.

Using breath H_2 excretion as an indicator, there was an elevated colonic fermentation activity already at the time of the lunch meal, especially after the rye kernel breakfast. Although low-GI features *per se* probably is the most important factor for the benefits on glucose tolerance seen in a shorter in-between meal period (from breakfast to lunch) with cereal products, additional mechanisms deriving

from colonic fermentation cannot be excluded in the case of presence of very rapidly fermentable carbohydrates. This has been reported in the literature in the case of e.g. lactulose, (Brighenti, Benini et al. 2006). However, the conclusive results from previous studies showing the importance of a low-GI feature of the breakfast in improving the glycaemic response at lunch (Jenkins, Wolever et al. 1982; Liljeberg, Åkerberg et al. 1999; Liljeberg and Björck 2000), and the correlation between glycaemia at breakfast and glycaemia at lunch found in the present thesis, make it possible to conclude that the glycaemic features of a cereal based breakfast is the major determinant of glycaemia at the subsequent lunch. On the other hand, the fact that a significant negative correlation between breath H₂ excretion (9.5 – 11.5 h) and glucose response (IAUC 9.5 – 11.5 h) were observed at the standardised dinner support a mechanism related to gut fermentation of indigestible carbohydrates and release of associated metabolites at this time point. Such a mechanism is supported by data in this thesis as discussed above; and by data in the literature showing an increased H₂ excretion accompanied by an improved glucose tolerance (OGTT) in the morning following a evening meal with pearled barley, compared with a evening meal with e.g. brown rice (Thorburn, Muir et al. 1993). Considering the results that the WMB porridge and WWB+BDF were devoid of effects, it can be suggested that the maintenance of a more intact botanical structure, and the concomitant elevation in RS content, may have contributed to the benefits on blood glucose regulation in the case of the boiled barley- and rye kernel breakfast.

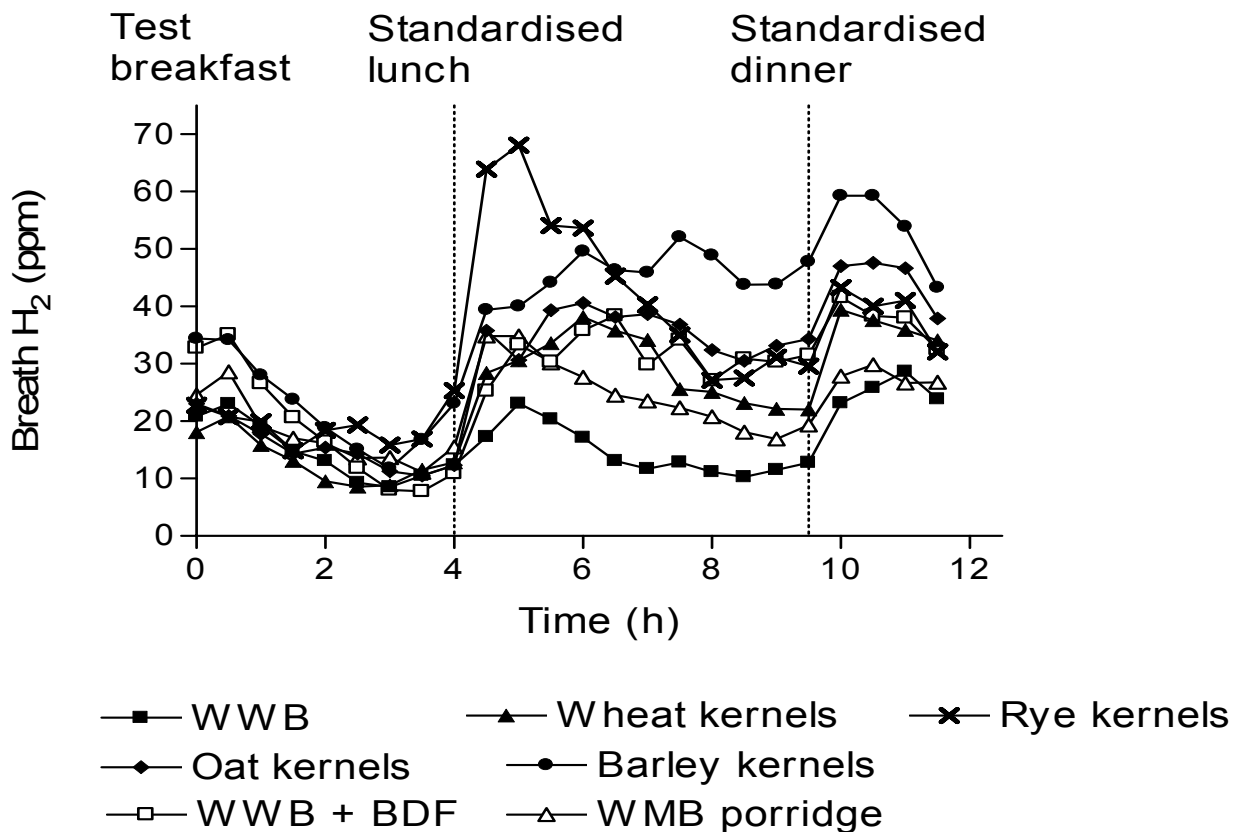


Figure 15. Mean breath H_2 concentrations during the course of the experimental day after different cereal test breakfasts (*Paper IV*).

5.2.2. Blood glucose and breath H_2 concentrations 9.5 h after a test breakfast (daylong) or test dinner (overnight)

The postprandial mean blood glucose responses to the standardised breakfast post the different test evening meals are shown in **Figure 16** (*Paper IV, Series 2*). When expressed as 0 - 120 min IAUC, the postprandial blood glucose response after the standardised breakfast was lower following an evening meal with barley kernels compared with a corresponding evening meal with WWB (IAUC 80.9 ± 12.4 and 121.6 ± 10.7 mmol*min/L, respectively, $P < 0.05$). On the other hand, an evening meal with WWB+BDF did not affect breakfast glucose tolerance (IAUC 0 – 120 min: 115.5 ± 15.6 mmol*min/L). The mean breath H_2 concentration after the standardised breakfast (0 – 120 min) was higher following an evening meal with barley kernels compared with an evening meal with WWB (54.9 ± 9.9 and 18.4 ± 2.9 ppm, respectively, $P < 0.001$, **Figure 17**).

Accordingly, the results in *Paper IV* are thus consistent with findings in *Papers I–III*, and support a relation between breath H₂ excretion and reduced glycaemia.

The blood glucose response (0 – 120 min IAUC) to the standardised WWB evening meal (9.5 h post test breakfast) was significantly higher compared with the corresponding area obtained at the standardised WWB breakfast (9.5 h post the test evening meals) ($P < 0.0001$) (**Figure 16**). There were, however, no corresponding differences in mean H₂ concentrations after the test meals depending on whether the test period was from evening meal to breakfast, or from breakfast to evening meal (**Figure 17**). This indicates a lower glucose tolerance in the evening compared with in the morning. The decrease in glucose tolerance seen in healthy subjects over the course of the day in the present thesis has been reported also by others (Van Cauter, Polonsky et al. 1997; Dos Santos, Aragon et al. 2006), and is the opposite to the situation in diabetic- (NIDDM and IDDM) or obese subjects (Van Cauter, Polonsky et al. 1997) The knowledge about potential differences in glucose tolerance during the course of a day is of importance and may have implications for e.g. diagnosis based on OGTT, and measurement of GI.

Taken together, the data obtained in *Papers I – IV* suggests that certain cereal products, with low GI and/or high contents of specific indigestible carbohydrates (RS + DF) have the capacity to modulate the glucose response not only in the acute phase, but also during the course of a whole day, and in the perspective from a late evening meal to the subsequent breakfast. This information provides support for additional metabolic benefits of low-GI foods with optimal contents of indigestible carbohydrates and a new dimension for the design of cereal foods with magnified metabolic benefits on blood glucose regulation.

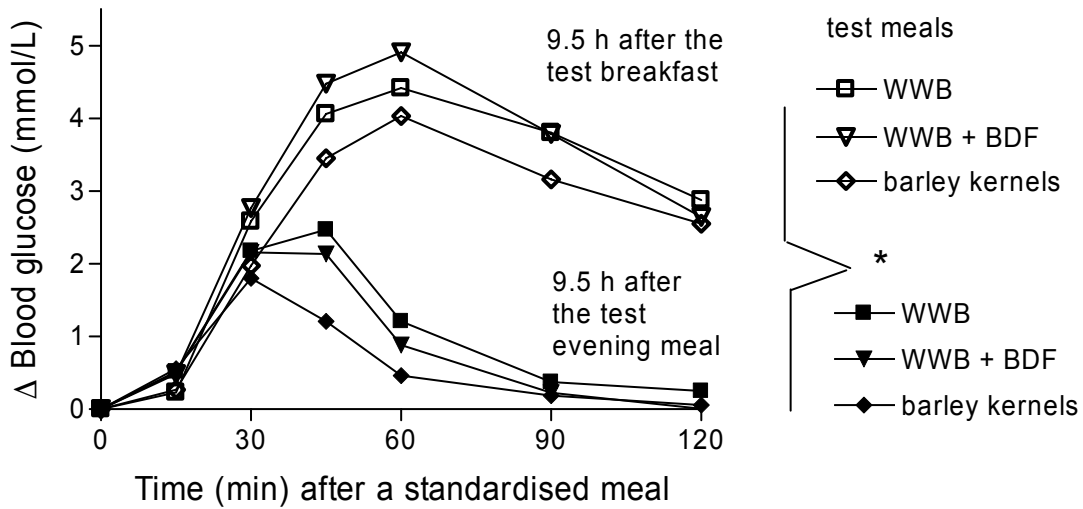


Figure 16. Blood glucose responses to a standardised WWB meal consumed 9.5 hours after test meals consumed either at dinner (filled symbols) or at breakfast (open symbols). *; $P < 0.0001$, the blood glucose responses to all test meals were higher 9.5 h after a breakfast compared with 9.5 h after an evening meal.

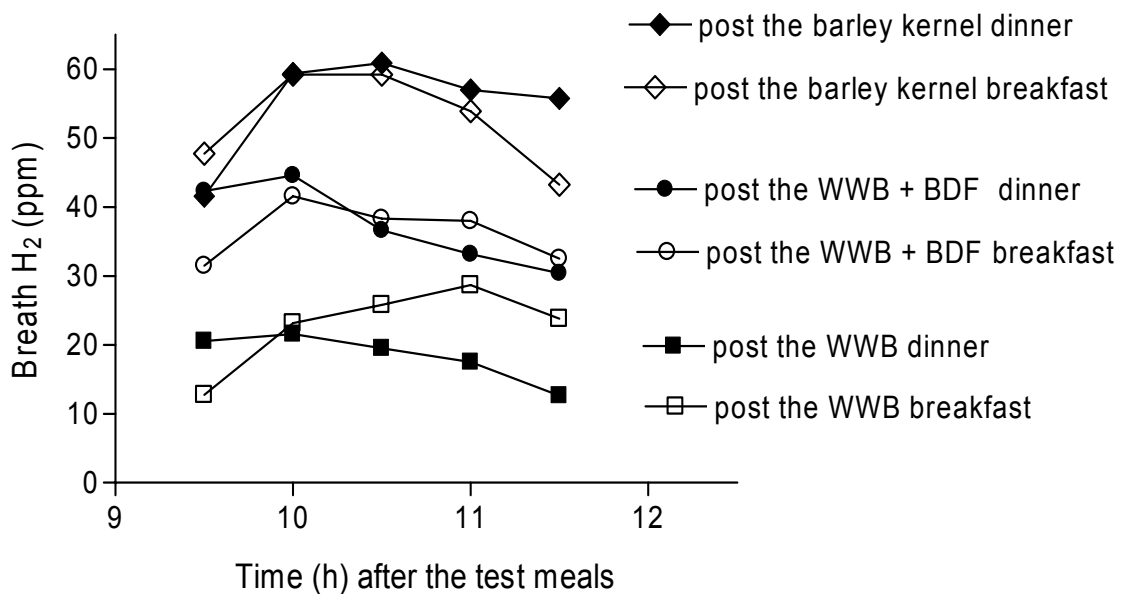


Figure 17. Breath H_2 concentrations after a standardised WWB meal consumed 9.5 hours after test meals consumed either at dinner (filled symbols) or at breakfast (empty symbols). There were no significant differences depending on when the test meals were consumed.

5.3. Cognitive performance in relation to postprandial glycaemia and glucose tolerance

To simulate a low-GI or a high-GI breakfast, respectively, healthy middle-aged volunteers, (20 women and 20 men) consumed a 50 g glucose solution through either a bolus or sipping regimen (*Paper V*). The blood glucose responses following the sipping or bolus load of glucose are shown in Figure 5. During the first half of the test period (0 – 90 min) the blood glucose IAUC was larger after the bolus regimen compared with sipping ($P<0.0001$), whereas during the last half of the experimental period (90 - 180 min), the blood glucose IAUC was larger after during the sip breakfast ($P<0.0001$). Consequently, with respect to blood glucose concentrations, it was possible to simulate typical behaviour of high-GI- versus low-GI meals by administrating glucose as a bolus dose or in a sipping manner, respectively.

The 50 g bolus glucose load resulted in significant negative correlations ($P\leq 0.01$) between blood glucose concentrations in the early (35 - 45 min) and the late postprandial period (150 – 180 min), i.e. a high blood glucose increment in the early postprandial period resulted in low blood glucose concentrations in the late postprandial period. This relation was probably due to a more elevated insulin release, resulting in over-regulation with an accompanying under-shoot in blood glucose concentrations.

Glucose tolerance (GT) was shown to importantly affect the cognitive tests (discussed below). Consequently, the difference between the highest and the lowest individual blood glucose concentrations (mmol/L) after the “high-GI” breakfast was taken as a measure of glucose tolerance and entered in ANOVAs as covariate when evaluating out-come of the cognitive tests. The median difference in mmol/L between the highest and the lowest postprandial (0 – 180 min) glucose concentrations after the bolus glucose drink was 5.4 mmol/L. Subjects with a larger difference than the median value were classified as with lower GT, and those with a smaller difference were classified as with higher GT.

The results from the cognitive tests after the bolus and sip glucose breakfast, respectively, are presented in **Table 11 - 12**. The results displayed significant differences in cognitive performance depending on glycaemic properties of the test breakfast in both the WM-test at 90 min (scores: bolus 32.8 ± 0.8 and sip 33.2 ± 1.0 , $P < 0.05$) and the SA-test at 170 min (scores: bolus 48.6 ± 2.4 and sip 49.5 ± 2.1 , $P < 0.05$). At 90 min, the sip regimen maintained a constant and positive net increment in blood glucose, whereas the bolus load caused a rapid decline in blood glucose. The benefits with sipping over bolus at 90 min may indicate that a rapid decline in blood glucose level in the later postprandial phase, frequently associated with high-GI starchy foods, is less advantageous from a cognition perspective. The SA-test was performed in the late postprandial period (170 min), and during this particular time period the increments in blood glucose concentrations was significantly higher during sipping- compared with the bolus condition. The better performance in the SA-test during the sipping condition could be interpreted as if low-GI foods by virtue of their low but sustained net increment in blood glucose offer cognitive advantages in the later postprandial period. The finding of improved cognitive performance in the late postprandial period after a breakfast with low-GI properties compared with high-GI properties in the present thesis are well in line with previous findings by Benton *et al.* (Benton, Ruffin et al. 2003), showing that certain measures of cognitive functions in healthy students (mean age of 21 years) was enhanced by a low-GI compared with a high-GI breakfast in the later postprandial phase i.e. 150 min and at 210 min after the breakfast. However, in the study referred to above, an enhancement of cognitive function was seen despite differences in blood glucose concentrations, nor in the slope of the glycaemic curve at these specific time points.

Table 11. Scores in the WM-test following bolus- and sip breakfast, respectively.

WM-test	Bolus breakfast	Sip breakfast
35 min	32.3 ± 0.7 ^a	33.2 ± 0.8 ^a
90 min	32.8 ± 0.8 ^a	33.2 ± 1.0 ^b
120 min	32.3 ± 0.8 ^a	32.2 ± 0.9 ^a
150 min	33.9 ± 0.9 ^a	34.3 ± 0.8 ^a

WM: working memory. Max scores 48 credits. Results within a row not followed by the same letter are significantly different, $P < 0.05$.

Table 12. Scores in the test SA-test following bolus- and sip breakfast, respectively

SA-test	Bolus breakfast	Sip breakfast
Correct response	48.6 ± 2.4 ^a	49.5 ± 2.1 ^b
Reaction time (ms)	1322 ± 24 ^a	1316 ± 26 ^a

SA: selective attention. Max scores (correct response) 72 credits. The SA-test was performed 170 min after commencing the breakfasts. Results within a row not followed by the same letter are significantly different, $P < 0.05$.

An additional finding in the present thesis was that, independently of test meal, the middle aged subjects performed significantly better at the second experimental day compared with the first day in the WM-test (35 min) as well as in the SA-test (170 min). It seems plausible that the improved performance at the second experimental day was due to a learning effect. Interestingly, a significantly better performance in the WM-test was found during the course of the first experimental day (0 – 120 min) after the bolus glucose breakfast compared with the sipping condition. The enhanced outcome in the repeated tests following a bolus glucose drink is suggested to be due to an advantageous effect of a higher postprandial glycaemia if occurring simultaneously with the first exposure to the mental challenge in a series of repeated tests. This can be interpreted as if a higher blood glucose concentration at the start of a series of repeated tests may promote learning. There may be a critical blood glucose level which needs to be reached after a breakfast meal to achieve optimal conditions for such a learning effect as measured with repeated WM-tests.

Although all subjects displayed glycaemic responses to the 50 g bolus glucose drink within a normal range; significant differences were found in cognitive performance depending on whether the subject was characterised as with higher or lower GT, respectively. As a general feature, the subjects with lower GT (as judged from the glycaemic response to the 50 g bolus glucose drink) performed less well in the cognitive tests compared with the subjects with higher GT, whether evaluated in the early or late postprandial phase and whether performed during bolus or sipping conditions. The results are displayed in **Table 13 - 14**. This finding is in line with previous results in healthy subjects showing impaired cognitive capacity in young- (Donohoe and Benton 1999) and middle-aged (Kaplan, Greenwood et al. 2000) subjects with less efficient glucose regulation, or subjects with type II diabetes (Strachan, Deary et al. 1997; Ryan and Geckle 2000; Gallacher, Pickering et al. 2005). The findings from the present thesis suggest that impaired cognitive function may be an early manifestation of the metabolic syndrome, since differences in cognitive performance were displayed in subjects with lower GT albeit still with normal glucose tolerance.

Insulin resistance is suggested to be a main factor underlying the metabolic dysfunctions involved in the metabolic syndrome (DeFronzo and Ferrannini 1991; Ferrannini, Haffner et al. 1991). Impaired GT is one of the main features of this syndrome, and identifies subjects with increased risk of type 2 diabetes and cardiovascular disease. Since the introduction of the GI-concept, an accumulating body of data has shown beneficial effects of low-GI foods in treatment and prevention of diabetes, cardiovascular disease (Jenkins, Kendall et al. 2002; Brand-Miller 2003), and the metabolic syndrome (Frost and Dornhorst 2000; Brand-Miller 2004; McKeown, Meigs et al. 2004). The results from the present thesis may suggest that low-GI foods in addition also might prevent cognitive decline related to impaired glucose tolerance. The lowered learning effect associated with the simulated low-GI regimen in the present work may indicate that GI optimization should not aim at too low glycaemic excursions.

Table 13. Results of the WM-tests after the different test breakfasts in relation to glucose tolerance

<i>Bolus (B) or Sip (S)</i>	WM-test	
	Glucose tolerance	
	<i>higher (n=20)</i>	<i>lower (n=20)</i>
B-35 min	33.8 ± 1.0 ^a	30.8 ± 1.0 ^b
B-90 min	33.9 ± 1.1 ^a	31.6 ± 1.0 ^a
B-120 min	34.4 ± 1.0 ^a	30.6 ± 1.0 ^b
B-150 min	36.1 ± 1.2 ^a	31.6 ± 1.3 ^b
B-mean (35 – 150 min)	34.6 ± 0.9 ^a	31.2 ± 1.0 ^b
S-35 min	34.9 ± 1.0 ^a	31.6 ± 1.2 ^a
S-90 min	35.8 ± 1.0 ^a	30.6 ± 1.5 ^b
S-120 min	34.4 ± 1.0 ^a	30.1 ± 1.2 ^b
S-150 min	35.5 ± 1.1 ^a	33.0 ± 1.0 ^a
S-mean (35 – 150 min)	34.8 ± 0.9 ^a	31.3 ± 1.1 ^b

WM: working memory. Max scores 48 credits. Values within the same row not followed by the same letter are significantly different, $P < 0.05$.

Table 14. Results of the SA-test after the different test breakfasts in relation to glucose tolerance

<i>Bolus (B) or Sip (S)</i>	SA-test	
	Glucose tolerance	
	<i>higher (n=20)</i>	<i>lower (n=20)</i>
B-correct response (max 72 p)	51.2 ± 3.4 ^a	46.1 ± 3.3 ^a
S-correct response (max 72 p)	54.1 ± 2.3 ^a	44.8 ± 3.1 ^b
B-reaction time (ms)	1311 ± 30 ^a	1324 ± 35 ^a
S-reaction time (ms)	1298 ± 34 ^a	1344 ± 42 ^a

SA: selective attention. Values within the same row not followed by the same letter are significantly different, $P < 0.05$.

6. CONCLUSIONS

The data obtained in the present thesis provide evidence that certain cereal products facilitates blood glucose regulation, not only in the acute phase, but also during the course of a whole day and in the perspective from a late evening meal to a subsequent breakfast. These effects were seen also in the absence of effects on insulin response, suggesting an improvement in glucose tolerance.

The degree of food processing, e.g. prolonged cooking time or disruption of the food matrix by milling, increased GI features of cereal test breakfasts (measured and/or predicted). Thus, whereas boiled barley- and rye kernels lowered the acute glucose response (0 – 120 min) compared with WWB, a whole grain barley flour porridge did not. In contrast, supplementation of a breakfast meal (WWB) with a β -glucan rich DF fraction from barley (approx. 4 g β -glucan/serving) lowered the acute blood glucose response (IAUC 0 – 60 min).

A breakfast with low-GI characteristics enhanced performance in cognitive test of working memory and selective attention in the late postprandial phase (90 and 170 min respectively), compared with a breakfast with high-GI characteristics. This was probably mediated by avoidance of a rapid decline in blood glucose concentrations, and maintenance of a net increment in blood glucose in the late postprandial phase.

Although the influence of colonically derived metabolites cannot be excluded, the glycaemic features of the cereal based breakfasts were probably the major determinant of glycaemia at the subsequent standardised lunch. Thus, a low blood glucose response at breakfast resulted in a low blood glucose response at lunch.

Increased levels of colonically derived metabolites (plasma SCFAs and breath H_2) were associated with improved glucose regulation within a 9.5 – 12.5 h second-meal perspective (from the evening test meal to breakfast). A low GI of the test meal *per se* did not significantly improve glucose tolerance at a second standardised meal ingested after 10.5. Instead, a specific prebiotic mixture (DF and RS), as present in e.g. boiled barley kernels, was a prerequisite for improved glucose tolerance.

Boiled barley- and rye kernel breakfasts had the capacity to lower the incremental blood glucose responses during the course of a whole day. These benefits on

glycaemic regulation were probably mediated by the combined effect of the low-GI properties, and the metabolites formed upon colonic fermentation of intrinsic prebiotic carbohydrates.

Suppression of circulating FFAs was a major factor involved in the improvements seen in glucose tolerance in the perspective from a test evening meal to breakfast. Serum IL-6 was reduced and plasma adiponectin increased at breakfast following an evening meal with bread made of barley kernels, compared with a WWB evening meal. This may have suppressed lipolysis, hence reducing FFA levels and increasing insulin sensitivity. Since it has been reported that SCFAs have anti-inflammatory properties and lower FFAs, it can be suggested that colonic fermentation of indigestible carbohydrates may have been involved in suppressing FFAs and IL-6, and improving glucose tolerance after certain cereal products.

Markers of colonic fermentation (breath H_2) following the evening test meals were negatively correlated to GER and positively correlated to satiety at the standardised breakfast. A negative correlation was seen between GER and satiety. In addition to plasma SCFAs, also plasma GLP-1 was negatively associated with glycaemic response at breakfast. It is put forward that SCFAs and GLP-1 influence glucose regulation and satiety through factors that involve GER. Since SCFAs have been reported to stimulate GLP-1 response, it can be suggested that the increased satiety and facilitated blood glucose regulation seen with certain evening test meals in the present thesis are both mediated through a mechanism involving colonic fermentation.

Taken together the findings in the thesis provide evidence for a link between the gut microbial metabolism and key factors associated with improved insulin sensitivity. The findings in an acute and semi-acute perspective provide mechanisms whereby low-GI food and whole grain protect against development of type 2 diabetes, coronary heart disease, and the metabolic syndrome. The data in the thesis also indicates that impaired cognitive function may be an early manifestation of the metabolic syndrome, since a decline in cognitive performance were displayed in subjects with lower glucose tolerance albeit still with normal glucose tolerance. The results provide information to be used for tailoring of new foods which facilitate glycaemic regulation and related parameters over the course of several meals, which may have beneficial implications on metabolic risk factors, weight control, and cognitive function.

7. FUTURE WORK

The results found in the thesis open for further studies of different combinations of prebiotic mixtures on glucose tolerance and other risk markers for the metabolic syndrome. In particular, it is relevant to further exploit the potential additive benefits of low GI cereal products containing specific prebiotic and/or whole grain fractions. This needs to be exploited in meal studies, but also in longer term interventions in healthy lean population and in risk subjects. More knowledge in this area will make possible to tailor cereal products with magnified benefits on glucose tolerance based on a cautious design of carbohydrate release features in the upper-gut; and of prebiotic carbohydrates capable of improving glucose tolerance at subsequent meals.

8. ACKNOWLEDGEMENTS

Jag vill tacka alla trevliga människor på våning 4 för att ni har bidragit till en trivsamt doktorandtid. Det har varit jättekul!

Några vill jag tacka speciellt;

Mina handledare Inger och Elin vill jag tacka för helhjärtad support och positiv inställning, och för den stora kunskap och erfarenhet ni har bidragit med. Det är härligt med en sådan uppbackning!

Micke, du ska ha ett jättetack, speciellt för all hjälp med mina datorproblem.

Yvonne som bland annat hjälpte mig att komma igång med mina försök.

Kalle, det har varit ett stort nöje att få samarbeta med dig! Tack för ditt engagemang och stora tålamod. Det tog ett tag, men nu har jag nog fått kläm på er kaffemaskin!

Jag vill tacka Lisbeth för hennes skickliga hjälp i labbet och inte minst för den ordning och reda hon skapar.

Ett stort tack till alla försökspersoner, utan er insats hade det inte gått.

Min LIFT mentor Bengt Jakobsson på Lyckeby Stärkelsen ska ha ett stort tack som ställt upp till 100 %.

9. REFERENCES

- Anderson, J. W. (2003). "Whole grains protect against atherosclerotic cardiovascular disease." Proceedings of the Nutrition Society **62**(1): 135-42.
- Anderson, J. W. and S. R. Bridges (1984). "Short-chain fatty acid fermentation products of plant fiber affect glucose metabolism of isolated rat hepatocytes." Proceedings of the Society for Experimental Biology and Medicine **177**(2): 372-6.
- Anderson, J. W., T. J. Hanna, et al. (2000). "Whole grain foods and heart disease risk." Journal of the American College of Nutrition **19**(3 Suppl): 291S-299S.
- Arner, P. (2001). "Free fatty acids--do they play a central role in type 2 diabetes?" Diabetes, obesity & metabolism **3 Suppl 1**: S11-9.
- Arner, P. (2002). "Insulin resistance in type 2 diabetes: role of fatty acids." Diabetes/Metabolism Research and Reviews **18 Suppl 2**: S5-9.
- Asp, N.-G., C.-G. Johansson, et al. (1983). "Rapid enzymatic assay of insoluble and soluble dietary fibre." Journal of Agricultural and Food Chemistry **31**: 476-482.
- Behall, K. M., D. J. Scholfield, et al. (2006). "Consumption of both resistant starch and beta-glucan improves postprandial plasma glucose and insulin in women." Diabetes Care **29**(5): 976-81.
- Belfort, R., L. Mandarino, et al. (2005). "Dose-response effect of elevated plasma free fatty acid on insulin signaling." Diabetes **54**(6): 1640-8.
- Benton, D. and P. Y. Parker (1998). "Breakfast, blood glucose, and cognition." Am J Clin Nutr **67**(4): 772S-778S.
- Benton, D., M. P. Ruffin, et al. (2003). "The delivery rate of dietary carbohydrates affects cognitive performance in both rats and humans." Psychopharmacology (Berl) **166**(1): 86-90.
- Berggren, A. M., E. M. Nyman, et al. (1996). "Influence of orally and rectally administered propionate on cholesterol and glucose metabolism in obese rats." Br J Nutr **76**(2): 287-94.
- Björck, I., Y. Granfeldt, et al. (1994). "Food properties affecting the digestion and absorption of carbohydrates." Am J Clin Nutr **59**(3 Suppl): 699S-705S.
- Björck, I. M. E. and M. Siljeström (1992). "In-Vivo and In-Vitro Digestibility of Starch in Autoclaved Pea and Potato Products." J Sci Food Agric **58**: 541-553.
- Boillot, J., C. Alamowitch, et al. (1995). "Effects of dietary propionate on hepatic glucose production, whole-body glucose utilization, carbohydrate and lipid metabolism in normal rats." Br J Nutr **73**(2): 241-51.
- Bourre, J. M. (2006). "Effects of nutrients (in food) on the structure and function of the nervous system: update on dietary requirements for brain. Part 1: micronutrients." J Nutr Health Aging **10**(5): 377-85.
- Brand-Miller, J. C. (2003). "Glycemic load and chronic disease." Nutrition Reviews **61**(5 Pt 2): S49-55.
- Brand-Miller, J. C. (2004). "Glycemic index in relation to coronary disease." Asia Pac J Clin Nutr **13**(Suppl): S3.
- Brighenti, F., L. Benini, et al. (2006). "Colonic fermentation of indigestible carbohydrates contributes to the second-meal effect." Am J Clin Nutr **83**(4): 817-22.
- Brouns, F., I. Björck, et al. (2005). "Glycaemic index methodology." Nutrition Research Reviews **18**(1): 145-171.
- Burdge, G. C. and P. C. Calder (2005). "Plasma cytokine response during the postprandial period: a potential causal process in vascular disease?" Br J Nutr **93**(1): 3-9.
- Båvenholm, P. N. and S. Efendic (2006). "Postprandial hyperglycaemia and vascular damage--the benefits of acarbose." Diab Vasc Dis Res **3**(2): 72-9.

- Cani, P. D., C. A. Daubioul, et al. (2005). "Involvement of endogenous glucagon-like peptide-1(7-36) amide on glycaemia-lowering effect of oligofructose in streptozotocin-treated rats." J Endocrinol **185**(3): 457-65.
- Cani, P. D., C. Knauf, et al. (2006). "Improvement of glucose tolerance and hepatic insulin sensitivity by oligofructose requires a functional glucagon-like peptide 1 receptor." Diabetes **55**(5): 1484-90.
- Carpentier, Y. A., L. Portois, et al. (2006). "n-3 fatty acids and the metabolic syndrome." Am J Clin Nutr **83**(6 Suppl): 1499S-1504S.
- Ceriello, A. (2006). "Oxidative stress and diabetes-associated complications." Endocr Pract **12 Suppl 1**: 60-2.
- Chen, Y. T., J. J. Mukherjee, et al. (2002). "Comparing fasting plasma glucose against two-hour post-load glucose concentrations for the diagnosis of diabetes mellitus and glucose intolerance in Singaporean hospital patients." Ann Acad Med Singapore **31**(2): 189-94.
- Cherbut, C. (2003). "Motor effects of short-chain fatty acids and lactate in the gastrointestinal tract." Proceedings of the Nutrition Society **62**(1): 95-9.
- Chew, G. T., S. K. Gan, et al. (2006). "Revisiting the metabolic syndrome." Med J Aust **185**(8): 445-9.
- Chiasson, J. L., R. G. Josse, et al. (2003). "Acarbose treatment and the risk of cardiovascular disease and hypertension in patients with impaired glucose tolerance: the STOP-NIDDM trial." Jama **290**(4): 486-94.
- Colonna, P., J.-L. Barry, et al. (1990). "Enzymic susceptibility of starch from pasta." Journal of Cereal Science **11**: 59-70.
- Convit, A., O. T. Wolf, et al. (2003). "Reduced glucose tolerance is associated with poor memory performance and hippocampal atrophy among normal elderly." Proc Natl Acad Sci U S A **100**(4): 2019-22.
- Cuche, G., J. C. Cuber, et al. (2000). "Ileal short-chain fatty acids inhibit gastric motility by a humoral pathway." Am J Physiol Gastrointest Liver Physiol **279**(5): G925-30.
- Cummings, J. H., G. T. Macfarlane, et al. (2001). "Prebiotic digestion and fermentation." American Journal of Clinical Nutrition **73**(2 Suppl): 415S-420S.
- Cummings, J. H., E. W. Pomare, et al. (1987). "Short chain fatty acids in human large intestine, portal, hepatic and venous blood." Gut **28**(10): 1221-7.
- Deacon, C. F., L. Pridal, et al. (1996). "Glucagon-like peptide 1 undergoes differential tissue-specific metabolism in the anesthetized pig." Am J Physiol **271**(3 Pt 1): E458-64.
- Deepa, R., K. Velmurugan, et al. (2006). "Serum levels of interleukin 6, C-reactive protein, vascular cell adhesion molecule 1, and monocyte chemoattractant protein 1 in relation to insulin resistance and glucose intolerance--the Chennai Urban Rural Epidemiology Study (CURES)." Metabolism **55**(9): 1232-8.
- DeFronzo, R. A. and E. Ferrannini (1991). "Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease." Diabetes Care **14**(3): 173-94.
- Delorme, S. and J. L. Chiasson (2005). "Acarbose in the prevention of cardiovascular disease in subjects with impaired glucose tolerance and type 2 diabetes mellitus." Curr Opin Pharmacol **5**(2): 184-9.
- Donohoe, R. T. and D. Benton (1999). "Cognitive functioning is susceptible to the level of blood glucose." Psychopharmacology (Berl) **145**(4): 378-85.
- Dos Santos, M. L., F. F. Aragon, et al. (2006). "Daytime variations in glucose tolerance in people with impaired glucose tolerance." Diabetes Res Clin Pract **74**(3): 257-62.

- Ebbesson, S. O., P. M. Risica, et al. (2005). "Omega-3 fatty acids improve glucose tolerance and components of the metabolic syndrome in Alaskan Eskimos: the Alaska Siberia project." *Int J Circumpolar Health* **64**(4): 396-408.
- Eckel, R. H., S. M. Grundy, et al. (2005). "The metabolic syndrome." *Lancet* **365**(9468): 1415-28.
- Esmailzadeh, A., M. Kimiagar, et al. (2006). "Fruit and vegetable intakes, C-reactive protein, and the metabolic syndrome." *Am J Clin Nutr* **84**(6): 1489-97.
- Esposito, K., F. Nappo, et al. (2002). "Inflammatory cytokine concentrations are acutely increased by hyperglycemia in humans: role of oxidative stress." *Circulation* **106**(16): 2067-72.
- Evans, J. L., I. D. Goldfine, et al. (2003). "Are oxidative stress-activated signaling pathways mediators of insulin resistance and beta-cell dysfunction?" *Diabetes* **52**(1): 1-8.
- Ferrannini, E., S. M. Haffner, et al. (1991). "Hyperinsulinaemia: the key feature of a cardiovascular and metabolic syndrome." *Diabetologia* **34**(6): 416-22.
- Festa, A., R. D'Agostino, Jr., et al. (2001). "The relation of body fat mass and distribution to markers of chronic inflammation." *Int J Obes Relat Metab Disord* **25**(10): 1407-15.
- Fontvieille, A. M., S. W. Rizkalla, et al. (1992). "The use of low glycaemic index foods improves metabolic control of diabetic patients over five weeks." *Diabetes Medicine* **9**(5): 444-50.
- Ford, E. S. (2005). "Prevalence of the metabolic syndrome defined by the International Diabetes Federation among adults in the U.S." *Diabetes Care* **28**(11): 2745-9.
- Ford, E. S., A. H. Mokdad, et al. (2003). "The metabolic syndrome and antioxidant concentrations: findings from the Third National Health and Nutrition Examination Survey." *Diabetes* **52**(9): 2346-52.
- Frost, G. and A. Dornhorst (2000). "The relevance of the glycaemic index to our understanding of dietary carbohydrates." *Diabet Med* **17**(5): 336-45.
- Frost, G., B. Keogh, et al. (1996). "The effect of low-glycemic carbohydrate on insulin and glucose response in vivo and in vitro in patients with coronary heart disease." *Metabolism* **45**(6): 669-72.
- Frost, G., A. Leeds, et al. (1998). "Insulin sensitivity in women at risk of coronary heart disease and the effect of a low glycemic diet." *Metabolism* **47**(10): 1245-51.
- Fukagawa, N. K., J. W. Anderson, et al. (1990). "High-carbohydrate, high-fiber diets increase peripheral insulin sensitivity in healthy young and old adults." *Am J Clin Nutr* **52**(3): 524-8.
- Gallacher, J. E., J. Pickering, et al. (2005). "Glucoregulation has Greater Impact on Cognitive Performance than Macro-vascular Disease in Men with type 2 Diabetes: Data from the Caerphilly Study." *Eur J Epidemiol* **20**(9): 761-8.
- Granfeldt, Y., I. Björck, et al. (1992). "An in vitro procedure based on chewing to predict metabolic response to starch in cereal and legume products." *European Journal of Clinical Nutrition* **46**(9): 649-60.
- Granfeldt, Y. and I. Björck (1990). "Glycemic response to starch in pasta: a study of mechanism of limited enzyme availability." *Journal of Cereal Science* **14**: 47-61.
- Granfeldt, Y., H. Liljeberg, et al. (1994). "Glucose and insulin responses to barley products: influence of food structure and amylose-amylopectin ratio." *Am J Clin Nutr* **59**(5): 1075-82.
- Granfeldt, Y., L. Nyberg, et al. (2007). "Muesli with 4 g oat beta-glucans lowers glucose and insulin responses after a bread meal in healthy subjects." *Eur J Clin Nutr*.
- Granfeldt, Y., X. Wu, et al. (2006). "Determination of glycaemic index; some methodological aspects related to the analysis of carbohydrate load and characteristics of the previous evening meal." *Eur J Clin Nutr* **60**(1): 104-12.

- Haidara, M. A., H. Z. Yassin, et al. (2006). "Role of oxidative stress in development of cardiovascular complications in diabetes mellitus." Curr Vasc Pharmacol **4**(3): 215-27.
- Harris, P. J. and L. R. Ferguson (1993). "Dietary fibre: its composition and role in protection against colorectal cancer." Mutat Res **290**(1): 97-110.
- Heliövaara, M. K., A. M. Teppo, et al. (2005). "Plasma IL-6 concentration is inversely related to insulin sensitivity, and acute-phase proteins associate with glucose and lipid metabolism in healthy subjects." Diabetes Obes Metab **7**(6): 729-36.
- Henningsson, A. M., I. M. Bjorck, et al. (2002). "Combinations of indigestible carbohydrates affect short-chain fatty acid formation in the hindgut of rats." J Nutr **132**(10): 3098-104.
- Holm, J., I. Björck, et al. (1986). "A Rapid Method for the Analysis of Starch." Starch/Stärke **38**(7): 224-226.
- Holst, J. J. and C. Orskov (2001). "Incretin hormones--an update." Scand J Clin Lab Invest Suppl(234): 75-85.
- Jazet, I. M., H. Pijl, et al. (2003). "Adipose tissue as an endocrine organ: impact on insulin resistance." Neth J Med **61**(6): 194-212.
- Jenkins, A. L., D. J. Jenkins, et al. (2002). "Depression of the glycemic index by high levels of beta-glucan fiber in two functional foods tested in type 2 diabetes." Eur J Clin Nutr **56**(7): 622-8.
- Jenkins, D. J., M. Axelsen, et al. (2000). "Dietary fibre, lente carbohydrates and the insulin-resistant diseases." British Journal of Nutrition **83 Suppl 1**: S157-63.
- Jenkins, D. J., C. W. Kendall, et al. (2002). "Glycemic index: overview of implications in health and disease." American Journal of Clinical Nutrition **76**(1): 266S-73S.
- Jenkins, D. J., C. W. Kendall, et al. (2002). "Soluble fiber intake at a dose approved by the US Food and Drug Administration for a claim of health benefits: serum lipid risk factors for cardiovascular disease assessed in a randomized controlled crossover trial." Am J Clin Nutr **75**(5): 834-9.
- Jenkins, D. J., T. M. Wolever, et al. (1978). "Dietary fibres, fibre analogues, and glucose tolerance: importance of viscosity." British Medical Journal **1**(6124): 1392-4.
- Jenkins, D. J., T. M. Wolever, et al. (1982). "Slow release dietary carbohydrate improves second meal tolerance." American Journal of Clinical Nutrition **35**(6): 1339-46.
- Jenkins, D. J. A., C. W. Kendall, et al. (1998). "dietary fiber, the evolution of the human diet and coronary heart disease." Nutrition Research **18**(4): 633-652.
- Järvi, A. E., B. E. Karlström, et al. (1999). "Improved glycemic control and lipid profile and normalized fibrinolytic activity on a low-glycemic index diet in type 2 diabetic patients." Diabetes Care **22**(1): 10-8.
- Kaplan, R. J., C. E. Greenwood, et al. (2000). "Cognitive performance is associated with glucose regulation in healthy elderly persons and can be enhanced with glucose and dietary carbohydrates." Am J Clin Nutr **72**(3): 825-36.
- King, G. L. and M. R. Loeken (2004). "Hyperglycemia-induced oxidative stress in diabetic complications." Histochem Cell Biol **122**(4): 333-8.
- Krarup, T., S. Madsbad, et al. (1983). "Diminished immunoreactive gastric inhibitory polypeptide response to a meal in newly diagnosed type I (insulin-dependent) diabetics." J Clin Endocrinol Metab **56**(6): 1306-12.
- Kruger, D. F., C. L. Martin, et al. (2006). "New insights into glucose regulation." Diabetes Educ **32**(2): 221-8.
- Laurent, C., C. Simoneau, et al. (1995). "Effect of acetate and propionate on fasting hepatic glucose production in humans." Eur J Clin Nutr **49**(7): 484-91.

- Leeman, M., M. Bårström, et al. (2005). "In vitro availability of starch in heat-treated potatoes as related to genotype, weight and storage time." Journal of the Science of Food and Agriculture **85**(5): 751-756.
- Levy, E., S. Spahis, et al. (2006). "Overproduction of intestinal lipoprotein containing apolipoprotein B-48 in *Psammomys obesus*: impact of dietary n-3 fatty acids." Diabetologia **49**(8): 1937-45.
- Liljeberg, H. and I. Björck (2000). "Effects of a low-glycaemic index spaghetti meal on glucose tolerance and lipaemia at a subsequent meal in healthy subjects." European Journal of Clinical Nutrition **54**(1): 24-8.
- Liljeberg, H., Y. Granfeldt, et al. (1992). "Metabolic responses to starch in bread containing intact kernels versus milled flour." European Journal of Clinical Nutrition **46**(8): 561-75.
- Liljeberg, H. G., Y. E. Granfeldt, et al. (1996). "Products based on a high fiber barley genotype, but not on common barley or oats, lower postprandial glucose and insulin responses in healthy humans." Journal of Nutrition **126**(2): 458-66.
- Liljeberg, H. G., A. K. Åkerberg, et al. (1999). "Effect of the glycemic index and content of indigestible carbohydrates of cereal-based breakfast meals on glucose tolerance at lunch in healthy subjects." American Journal of Clinical Nutrition **69**(4): 647-55.
- Liljeberg, H. G. M., C. H. Lönner, et al. (1995). "Sourdough fermentation or addition of organic acids or corresponding salts to bread improves nutritional properties of starch in healthy humans." Journal of Nutrition **125**: 1503-1511.
- Liu, S., W. C. Willett, et al. (2000). "A prospective study of dietary glycemic load, carbohydrate intake, and risk of coronary heart disease in US women." American Journal of Clinical Nutrition **71**(6): 1455-61.
- Lopez-Garcia, E., M. B. Schulze, et al. (2004). "Major dietary patterns are related to plasma concentrations of markers of inflammation and endothelial dysfunction." Am J Clin Nutr **80**(4): 1029-35.
- Martin, P. Y. and D. Benton (1999). "The influence of a glucose drink on a demanding working memory task." Physiol Behav **67**(1): 69-74.
- McKeown, N. M., J. B. Meigs, et al. (2004). "Carbohydrate nutrition, insulin resistance, and the prevalence of the metabolic syndrome in the Framingham Offspring Cohort." Diabetes Care **27**(2): 538-46.
- McKeown, N. M., J. B. Meigs, et al. (2002). "Whole-grain intake is favorably associated with metabolic risk factors for type 2 diabetes and cardiovascular disease in the Framingham Offspring Study." American Journal of Clinical Nutrition **76**(2): 390-8.
- Messier, C. (2004). "Glucose improvement of memory: a review." Eur J Pharmacol **490**(1-3): 33-57.
- Messier, C., M. Gagnon, et al. (1997). "Effect of glucose and peripheral glucose regulation on memory in the elderly." Neurobiol Aging **18**(3): 297-304.
- Messier, C., M. Tsiakas, et al. (2003). "Effect of age and glucoregulation on cognitive performance." Neurobiol Aging **24**(7): 985-1003.
- Miyazaki, Y., H. Kawano, et al. (2007). "Pancreatic B-cell function is altered by oxidative stress induced by acute hyperglycaemia." Diabet Med **24**(2): 154-60.
- Morrison, D. J., K. Cooper, et al. (2004). "A streamlined approach to the analysis of volatile fatty acids and its application to the measurement of whole-body flux." Rapid Communications in Mass Spectrometry **18**(21): 2593-600.
- Nauck, M. A., U. Niedereichholz, et al. (1997). "Glucagon-like peptide 1 inhibition of gastric emptying outweighs its insulinotropic effects in healthy humans." Am J Physiol **273**(5 Pt 1): E981-8.

REFERENCES

- Orskov, C., L. Rabenhoj, et al. (1994). "Tissue and plasma concentrations of amidated and glycine-extended glucagon-like peptide I in humans." *Diabetes* **43**(4): 535-9.
- Pereira, M. A., D. R. Jacobs, Jr., et al. (2002). "Dairy consumption, obesity, and the insulin resistance syndrome in young adults: the CARDIA Study." *Jama* **287**(16): 2081-9.
- Persson, J., I. Sjöberg, et al. (2004). Healthrelated habits of life 1980 - 2002. Stockholm, statistics Sweden: 81-89.
- Pischon, T., C. J. Girman, et al. (2005). "Association between dietary factors and plasma adiponectin concentrations in men." *Am J Clin Nutr* **81**(4): 780-6.
- Pollitt, E., S. Cueto, et al. (1998). "Fasting and cognition in well- and undernourished schoolchildren: a review of three experimental studies." *Am J Clin Nutr* **67**(4): 779S-784S.
- Qiao, Q., K. Pyorala, et al. (2002). "Two-hour glucose is a better risk predictor for incident coronary heart disease and cardiovascular mortality than fasting glucose." *Eur Heart J* **23**(16): 1267-75.
- Radeborg, K., V. Briem, et al. (1999). "The effect of concurrent task difficulty on working memory during simulated driving." *Ergonomics* **42**: 767-777.
- Rask-Madsen, C. and G. L. King (2007). "Mechanisms of Disease: endothelial dysfunction in insulin resistance and diabetes." *Nat Clin Pract Endocrinol Metab* **3**(1): 46-56.
- Reimer, R. A. and M. I. McBurney (1996). "Dietary fiber modulates intestinal proglucagon messenger ribonucleic acid and postprandial secretion of glucagon-like peptide-1 and insulin in rats." *Endocrinology* **137**(9): 3948-56.
- Rivellese, A. A., C. De Natale, et al. (2002). "Type of dietary fat and insulin resistance." *Ann N Y Acad Sci* **967**: 329-35.
- Robertson, M. D., A. S. Bickerton, et al. (2005). "Insulin-sensitizing effects of dietary resistant starch and effects on skeletal muscle and adipose tissue metabolism." *Am J Clin Nutr* **82**(3): 559-67.
- Robertson, M. D., J. M. Currie, et al. (2003). "Prior short-term consumption of resistant starch enhances postprandial insulin sensitivity in healthy subjects." *Diabetologia* **46**(5): 659-65.
- Robertson, R. P., J. Harmon, et al. (2003). "Glucose toxicity in beta-cells: type 2 diabetes, good radicals gone bad, and the glutathione connection." *Diabetes* **52**(3): 581-7.
- Rodriguez-Cabezas, M. E., J. Galvez, et al. (2003). "Intestinal anti-inflammatory activity of dietary fiber (*Plantago ovata* seeds) in HLA-B27 transgenic rats." *Clin Nutr* **22**(5): 463-71.
- Ross, R. (1999). "Atherosclerosis--an inflammatory disease." *N Engl J Med* **340**(2): 115-26.
- Rumessen, J. J. (1992). "Hydrogen and methane breath tests for evaluation of resistant carbohydrates." *European Journal of Clinical Nutrition* **46 Suppl 2**: S77-90.
- Ryan, C. M. and M. Geckle (2000). "Why is learning and memory dysfunction in Type 2 diabetes limited to older adults?" *Diabetes Metab Res Rev* **16**(5): 308-15.
- Salmeron, J., A. Ascherio, et al. (1997). "Dietary fiber, glycemic load, and risk of NIDDM in men." *Diabetes Care* **20**(4): 545-50.
- Salmeron, J., J. E. Manson, et al. (1997). "Dietary fiber, glycemic load, and risk of non-insulin-dependent diabetes mellitus in women." *Journal of the American Medical Association* **277**(6): 472-7.
- Santomauro, A. T., G. Boden, et al. (1999). "Overnight lowering of free fatty acids with Acipimox improves insulin resistance and glucose tolerance in obese diabetic and nondiabetic subjects." *Diabetes* **48**(9): 1836-41.
- Scholey, A. B., S. Harper, et al. (2001). "Cognitive demand and blood glucose." *Physiol Behav* **73**(4): 585-92.

- Scholey, A. B., S. Laing, et al. (2005). "Blood glucose changes and memory: Effects of manipulating emotionality and mental effort." Biol Psychol.
- Schulze, M. B., S. Liu, et al. (2004). "Glycemic index, glycemic load, and dietary fiber intake and incidence of type 2 diabetes in younger and middle-aged women." American Journal of Clinical Nutrition **80**(2): 348-56.
- Slater, C. and T. Preston (2004). "Automated breath hydrogen measurement at the part-per-million level on a continuous-flow isotope-ratio mass spectrometer." Rapid Communications in Mass Spectrometry **18**(20): 2502-4.
- Slavin, J. (2003). "Why whole grains are protective: biological mechanisms." Proc Nutr Soc **62**(1): 129-34.
- Strachan, M. W., I. J. Deary, et al. (1997). "Is type II diabetes associated with an increased risk of cognitive dysfunction? A critical review of published studies." Diabetes Care **20**(3): 438-45.
- Tappy, L., E. Gugolz, et al. (1996). "Effects of breakfast cereals containing various amounts of beta-glucan fibers on plasma glucose and insulin responses in NIDDM subjects." Diabetes Care **19**(8): 831-4.
- Thorburn, A., J. Muir, et al. (1993). "Carbohydrate fermentation decreases hepatic glucose output in healthy subjects." Metabolism **42**(6): 780-5.
- Trinick, T. R., M. F. Laker, et al. (1986). "Effect of guar on second-meal glucose tolerance in normal man." Clinical Science **71**(1): 49-55.
- Tschritter, O., A. Fritsche, et al. (2003). "Plasma adiponectin concentrations predict insulin sensitivity of both glucose and lipid metabolism." Diabetes **52**(2): 239-43.
- Van Cauter, E., K. S. Polonsky, et al. (1997). "Roles of circadian rhythmicity and sleep in human glucose regulation." Endocr Rev **18**(5): 716-38.
- van Hall, G., A. Steensberg, et al. (2003). "Interleukin-6 stimulates lipolysis and fat oxidation in humans." J Clin Endocrinol Metab **88**(7): 3005-10.
- Vander, A. J., J. H. Sherman, et al. (2001). Human Physiology. The Mechanisms of Body Function. Human Physiology. K. T. Kane. NY, McGraw-Hill 599-612.
- Wannamethee, S. G., J. Tchernova, et al. (2007). "Associations of adiponectin with metabolic and vascular risk parameters in the British Regional Heart Study reveal stronger links to insulin resistance-related than to coronary heart disease risk-related parameters." Int J Obes (Lond).
- Venter, C. S., H. H. Vorster, et al. (1990). "Effects of dietary propionate on carbohydrate and lipid metabolism in healthy volunteers." American Journal of Gastroenterology **85**(5): 549-53.
- Verdich, C., A. Flint, et al. (2001). "A meta-analysis of the effect of glucagon-like peptide-1 (7-36) amide on ad libitum energy intake in humans." J Clin Endocrinol Metab **86**(9): 4382-9.
- WHO (1999). Definition, diagnosis and classification of diabetes mellitus and its complications. Geneva, World Health Organisation, Department of Noncommunicable disease surveillance: Part 1.
- Wirfalt, E., B. Hedblad, et al. (2001). "Food patterns and components of the metabolic syndrome in men and women: a cross-sectional study within the Malmo Diet and Cancer cohort." Am J Epidemiol **154**(12): 1150-9.
- Wolever, T. M., F. Brighenti, et al. (1989). "Effect of rectal infusion of short chain fatty acids in human subjects." Am J Gastroenterol **84**(9): 1027-33.
- Wolever, T. M., D. J. Jenkins, et al. (1988). "Second-meal effect: low-glycemic-index foods eaten at dinner improve subsequent breakfast glycemic response." American Journal of Clinical Nutrition **48**(4): 1041-7.

REFERENCES

- Wolever, T. M., P. Spadafora, et al. (1991). "Interaction between colonic acetate and propionate in humans." *Am J Clin Nutr* **53**(3): 681-7.
- Wolever, T. M. S., A. Bentum-Williams, et al. (1995). "Physiological modulation of plasma free fatty acid concentrations by diet. Metabolic implications in nondiabetic subjects." *Diabetes Care* **18**(7): 962-970.
- Würsch, P. and E. X. Pi-Sunyer (1997). "The role of viscous soluble fibre in the metabolic control of diabetes." *Diabetes Care* **20**(11): 1774-1780.
- Yamauchi, T., J. Kamon, et al. (2002). "Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase." *Nat Med* **8**(11): 1288-95.
- Yip, R. G. and M. M. Wolfe (2000). "GIP biology and fat metabolism." *Life Sci* **66**(2): 91-103.
- Zimmet, P. (2005). "Epidemiology of diabetes mellitus and associated cardiovascular risk factors: focus on human immunodeficiency virus and psychiatric disorders." *Am J Med* **118 Suppl 2**: 3S-8S.
- Zimmet, P. Z. (1999). "Diabetes epidemiology as a tool to trigger diabetes research and care." *Diabetologia* **42**(5): 499-518.
- Åkerberg, A. K. E., H. G. M. Liljeberg, et al. (1998). "An in vitro method, based on chewing, to predict resistant starch content in foods allows parallel determination of potentially available starch and dietary fibre." *Journal of Nutrition* **128**: 651-659.
- Östman, E. M., A. H. Frid, et al. (2006). "A dietary exchange of common bread for tailored bread of low glycaemic index and rich in dietary fibre improved insulin economy in young women with impaired glucose tolerance." *Eur J Clin Nutr* **60**(3): 334-41.