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Glycaemic Response in Relation to Gastric Emptying and Satiety in Health and Disease

A study in Healthy Subjects and Patients with Diabetes Mellitus

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Academic thesis which, by due permission of the Faculty of Medicine at Lund University, will be publicly defended on Friday 4th of April, 2008, at 9.15 am in lecture hall at the Department of Internal Medicine, Entrance 35, Malmö University Hospital, Malmö, for degree of PhD.

Faculty Opponent: Professor Per M Hellström, Karolinska Institute, Department of Gastroenterology and Hepatology, Karolinska University Hospital, Stockholm, Sweden.

Glycaemic Response in Relation to Gastric Emptying and Satiety in Health and Disease

A study in Healthy Subjects and Patients with Diabetes Mellitus

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To

My Mother and Father

Den kloka kvinnan blir glad när hon anses vacker.

Den vackra kvinnan blir glad då hon anses klok.

Selma Lagerlöf

List of Publications

This thesis is based on five studies, reported in the following five papers, which are appended at the end of this thesis. The will be referred to in the text by their Roman numerals.

I. Effect of cinnamon on postprandial blood glucose, gastric emptying and satiety in healthy subjects
Hlebowicz J, Darwiche G, Björgell O, Almér LO
American Journal of Clinical Nutrition. 2007, Jun 85(6):1552-1556

II. Effect of commercial breakfast fibre cereals compared with corn flakes on postprandial blood glucose, gastric emptying and satiety in healthy subjects: A randomized blinded crossover trial
Hlebowicz J, Wickenberg J, Fahlström R, Darwiche G, Björgell O, Almér LO
Nutrition Journal. 2007, Sept 17;6:22

III. Effect of muesli with 4 g oat ß-glucan on postprandial blood glucose, gastric emptying and satiety in healthy subjects: A randomized crossover trial
Hlebowicz J, Darwiche G, Björgell O, Almér LO
Journal of the American College of Nutrition. 2008 (In press)

IV. The botanical integrity of wheat products in association with acetic acid influences the gastric distension and satiety in healthy subjects
Hlebowicz J, Lindstedt S, Höglund P, Björgell O, Almér LO, Darwiche G
Nutrition Journal. 2007 (Submitted for publication)

V. Effect of apple cider vinegar on delayed gastric emptying in patients with type 1 diabetes mellitus: A pilot study
Hlebowicz J, Darwiche G, Björgell O, Almér LO
BMC Gastroenterology. 2007, 7**:**46

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Summary

Dietary fibre and whole grains are recommended to prevent the development of type 2 diabetes. Low glycaemic index foods that are rich in fibre are recommended to control blood glucose levels. Gastric emptying, together with other factors, regulate the postprandial blood glucose response. A delay in the gastric emptying rate (GER) leads to a lower postprandial blood glucose concentration. However, 30-50% of diabetes patients have delayed gastric emptying. The aims of these studies were to evaluate the effect of different food factors on the GER, the postprandial blood glucose response, and satiety in healthy subjects and those with diabetes mellitus. The results show that inclusion of 6 g cinnamon in the diet lowers the postprandial blood glucose response, a change that is at least partially explained by delayed GER. Neither bran flakes nor wholemeal oat flakes has any effect on the total postprandial blood glucose response, GER or satiety compared with cornflakes. Muesli with 4 g oat β-glucan does not affect the GER or satiety, but lowers the postprandial blood glucose response, indicating that the GER is not involved in the blood glucose lowering mechanism. Whole-kernel wheat bread served with vinegar leads to higher satiety than wholemeal wheat bread with vinegar, or white wheat bread with or without vinegar in healthy subjects. This may be explained by increased antral distension caused by intact cereal kernels, but not by changes in GER or postprandial blood glucose responses. Vinegar affects insulin-dependent diabetes mellitus patients with diabetic gastroparesis by reducing the GER even further.

Populärvetenskaplig sammanfattning

(Summary in Swedish)

Ett intag av fibrer och hela korn rekommenderas för att förhindra utvecklingen av typ 2 diabetes. Kost med långsamma kolhydrater med fibrer rekommenderas till diabetiker för att kontrollera blodsockret. Magsäckens tömning, tillsammans med en mängd andra faktorer, reglerar blodsockernivåerna efter en måltid. En långsam magsäckstömning av mat till tolvfingertarmen leder till ett lägre blodsocker. Störd magsäckstömning är dock en vanligt förekommande komplikation bland diabetiker. Fördröjd magsäckstömning (gastropares) kan leda till tidig mättnadskänsla, fyllnadskänsla, buksmärta, illamående, kräkningar efter en måltid men även endast bristande blodsockerkontroll hos diabetiker utan andra symtom.

Inom ramen för avhandlingen har det ingått att utvärdera vilken effekt kanel, olika fiber sorter och vinäger har på magsäckens tömning, blodsocker svar samt mättnad hos friska försökspersoner. Det har även ingått att utvärdera effekten av vinäger hos diabetiker med en redan fördröjd tömning av magsäcken. Efter en provfrukost mättes magsäckens nedre tvärsnittsyta (antrum) med hjälp av en standardiserad ultraljudsmetod. Magsäckstömningen beräknades som den procentuella minskningen av antrums tvärsnittsyta mellan 15 och 90 minuter efter måltiden.

Ett intag av 6 g kanel har visat sig sänka blodsockersvaret vilket delvis kan förklaras av en fördröjd tömning av magsäcken. En frukost bestående av

fullkornsflingor av vete eller havre påverkade varken blodsockersvaret, mättnaden eller magsäckens tömning jämfört med cornflakes. Däremot sänkte müsli med 4 g havre β-glukan fibrer blodsockret utan att påverka magsäckens tömning eller mättnaden jämfört med cornflakes. Bröd med hela vetekorn doppat i vinäger har visat sig öka mättnaden jämfört med vitt bröd, vitt bröd med vinäger och grahams bröd med vinäger hos friska försökspersoner. Detta kan förklaras med uttöjning av nedre delen av magsäcken efter ett intag av bröd med hela vetekorn men inte av en fördröjd tömning av magsäcken eller förändring av blodsockersvar. Däremot fördröjde ett intag av vinäger hos patienter med insulinbehandlad diabetes en redan fördröjd tömning av magsäcken ytterligare.

Abbreviations

ADA	American Diabetes Association
AUC	area under the curve
BMI	body mass index
CCK	cholecystokinin
ENS	enteric nervous system
GER	gastric emptying rate
GIP	glucose-dependent insulinotropic polypeptide
GLP-1	glucagon-like peptide-1
HbA1c	haemoglobin A1c
HDL	high-density lipoprotein
IFG	impaired fasting glucose
IGT	impaired glucose tolerance
LDL	low-density lipoprotein
MMC	migrating motor complex
PYY	peptide YY
SEM	standard error of the mean
WHO	World Health Organization

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Chapter 1

Introduction

Changes in lifestyle throughout the world, such as increased energy intake and decreased physical activity, are causing overweight and obesity, leading to an epidemic increase in type 2 diabetes. The American Diabetes Association (ADA) recommends an intake of food containing 14 g dietary fibre per 1000 kcal, and that half of the grain intake be composed of whole grains, based on the recommendations of the U.S. Department of Agriculture (1-4). An increased intake of dietary fibre and whole grains has been shown to reduce the risk of type 2 diabetes (5-7). Intake of whole grain has been associated with increased insulin sensitivity (8) and inversely associated with cardiovascular risk factors, atherosclerosis, and incident cardiovascular disease (9-10). It is still unclear, according to the ADA, whether low-glycaemic-index food can in fact prevent diabetes mellitus (1-4). However, a low-glycaemic-index diet that is rich in fibre is recommended by the ADA to prevent the development of type 2 diabetes (1-4). The glycaemic index is defined as the increase in the area under the curve over 2 hours, above the fasting blood glucose, after the ingestion of a test meal containing 50 g carbohydrate, divided by the response to a reference meal containing 50 g carbohydrate such as white bread (3). The postprandial blood glucose concentration is determined by the amount and type of carbohydrate ingested (3). The specific type of food and macronutrients, the type of starch,

ripeness, method of preparation and degree of processing also influence postprandial blood glucose levels (3). This leads to considerable variability in the glycaemic-index of carbohydrate-containing products (11). Patients with diabetes are recommended by the ADA to consume carbohydrates in the form of fruit, vegetables, whole grains, legumes and low-fat milk to control their blood glucose levels (1-4). Some studies have shown that a low-glycaemic-index diet can reduce glycaemia in diabetic subjects (12-18), while others did not confirm this effect (19-22). Two recent meta-analyses of low-glycaemic-index studies in diabetic subjects showed a modest benefit in the haemoglobin A1c (HbA1C) values (23, 24). Therefore, the use of glycaemic index is considered by the ADA to provide only a modest additional benefit in controlling postprandial blood glucose levels (1-4).

Gastric emptying, together with other factors, regulates the postprandial blood glucose response, and a delay in the gastric emptying rate (GER) leads to a lower postprandial blood glucose concentration. Studies have shown that 30-50% of diabetes patients have delayed gastric emptying, and this is believed to be, at least partially, due to vagal denervation caused by autonomic neuropathy (25-29). Delayed gastric emptying may cause poor glycaemic control, especially in those receiving preprandial antidiabetic treatment, causing postprandial hypoglycaemia and gastrointestinal symptoms such as postprandial nausea, vomiting, bloating and early satiety (30, 31). The relationship between the symptoms of gastroparesis and the rate of gastric emptying is weak, and patients with delayed gastric emptying may not have any, or few, gastrointestinal symptoms (32-34). Abnormal gastric emptying has been associated with an increased frequency of hypoglycaemic events in insulin-treated diabetic patients, despite the lack of upper gastrointestinal symptoms (35).

Commercial products that reduce the changes in postprandial blood glucose levels, in order to improve glycaemic control in people with diabetes, may delay gastric emptying. Bearing this in mind, as well as the high prevalence of delay in

gastric emptying in patients with diabetes, knowledge is needed on the effect of different food factors on gastric emptying, postprandial blood glucose response and satiety, in both healthy subjects and patients with diabetes, in order to improve glycaemic control in people with diabetes.

Chapter 2

Background

2.1 The Stomach

The stomach is divided into the proximal part, the fundus, and the distal parts, the corpus and the antrum. The limitations of the stomach are the lower oesophageal sphincter and the pylorus. The inner layer of the stomach wall is called the mucosa. In the corpus the mucosa is composed of mucous cells, parietal cells that produce hydrochloric acid, and intrinsic factor and chief cells that produce pepsinogen. This is covered by the submucosa with the submucosal (Meissner's) plexus, and the muscular layer, with an inner circular layer and a longitudinal muscular layer. Only the proximal part of the stomach has an additional layer of oblique muscles. The thickness of the muscular layer increases towards the pylorus. The myenteric (Auerbach's) plexus is located between the longitudinal muscle and the circular muscular layer. The outer layer, the serosa, connects with the omentum (36).

The extrinsic, autonomic, innervation of the stomach is divided into the parasympathetic and sympathetic systems (36). The parasympathetic innervation derives from the vagus nerve, whose cell bodies (the nucleus ambiguous, nucleus dorsalis and tractus solitarius) are found in the brainstem (37). The vagus nerve consists of 20% efferent fibres and 80% afferent sensory fibres, which transmit

information to the brain (38). The afferent fibres in the gastric mucosa, muscularis and serosa respond to stretching, motility and chemical stimuli (39). The efferent fibres have both excitatory and inhibitory effects on gastric motor function (39). The sympathetic innervation derives from the thoracolumbar part of the spinal cord (36).

The intrinsic innervation is provided by the enteric nervous system (ENS), which is composed of the myenteric plexus, located between the circular and longitudinal muscle layers, and the submucosal plexus in the submucosa of the stomach wall. Many stimulating (acetylcholine, serotonin, histamine, cholecystokinin, angiotensin, motilin and gastrin) and inhibiting (dopamine, noradrenalin, glucagon, vasoactive intestinal polypeptide, somatostatin and enkephalin) neurotransmitters and hormones have been found in the ENS. These messengers can be secreted by neurocrine, paracrine and endocrine secretion (36).

2.2 Gastric motility

The stomach can be divided into two functional regions, the proximal and distal stomach. The proximal stomach is considered to be the fundus, and the proximal part of the corpus. The distal stomach is considered to be the distal part of the body and the antrum. The "pacemaker" cells of the stomach, called the interstitial cells of Cajal, are located in the greater curvature, and govern the motility of the smooth muscle cells (36).

During the fasting state, called phase I, which lasts for about 40 minutes, the stomach is inactive. Phase II also lasts for about 40 minutes, during which time the stomach's peristaltic contractions are irregular and increase in frequency and amplitude. In phase III the stomach reaches maximum contraction force and rate, three peristaltic waves per minute, which lasts for about 10 minutes. During this phase the lower oesophageal sphincter has a maximum pressure and the pylorus is

wide open allowing large food particles to leave the stomach. The stomach then returns to phase I and the 90-minute cycle starts again. This cycle is called the interdigestive migrating motor complex (MMC) and is generated by the enteric nervous system. (36)

The main function of the stomach is to break down ingested food by mechanical and chemical means, and to deliver the chyme to the duodenum at a suitable rate. The postprandial phase begins with relaxation of the proximal part of the stomach for about 20 seconds when food is received (called receptive relaxation), and when the food enters the stomach adaptive relaxation begins. The stomach is stretched by ingested food, leading to adaptive relaxation and postprandial gastric motility, mediated by the sensory and motor fibres of the vagus nerve. In this way, the pressure in the stomach is regulated and prevents the food from flowing back into the oesophagus. The distal stomach starts to contract after eating, first irregularly and then with a frequency of 3 waves per minute from the corpus to the antrum, and transporting the food into the duodenum. There is antroduodenal co-ordination of the peristaltic waves. The peristaltic waves from the antrum reach the pylorus which then closes. Liquids and small particles pass the pylorus by means of a pressure gradient between the stomach and the duodenum. Only 1.4 ml chyme passes the pylorus per wave. The pylorus opens slightly and then contracts at a rate of 3-12 times per minute (36).

2.3 Gastric emptying

A liquid meal begins to leave the stomach immediately at an exponential rate. However, semi-solid and solid meals start to leave the stomach after a few minutes, and at a linear rate. The volume of the food stimulates the stretch sensors in the stomach initiating gastric emptying, while osmosensors and chemosensors for amino acids, sugars, fats and pH in the duodenum regulate the rate of gastric emptying. Hypertonic liquids are emptied at a slower rate than

hypotonic liquids. The particle size of the food also affects the gastric emptying rate. Particle smaller than 0.5-1.5 mm can pass through the pylorus, while larger, indigestible particles leave the stomach in phase III of the MMC. Chyme leaves the stomach and enters the duodenum at a rate of about 2 kcal per minute (36). Meal volume and energy density thus also affect the gastric emptying rate (40).

It has been shown that hypoglycaemia increases the rate of gastric emptying in both healthy subjects and patients with type 1 diabetes mellitus (41-43). Hyperglycaemia, on the other hand, has been shown to decrease the GER (44-46). Physiological changes in blood glucose, from 4 to 8 mmol/l have also been shown to affect gastric emptying in healthy subjects and in insulin-dependent, diabetes mellitus patients (47). Hyperinsulinaemia (48, 49), body weight (50, 51), smoking (52-56), gender (57-59) and various drugs also affect the GER (36).

After vagotomy, the absence of adaptive relaxation of the proximal stomach after a meal leads to high intraventricular pressure and the rapid gastric emptying of liquids. The peristaltic contractions of the distal stomach are perturbed, and this will slow down the grinding, mixing and transport of solid food, leading to delayed gastric emptying of solid meals (36). The vagus nerve mediates the fundic–antral co-ordination by controlled delivery of the food from the fundus into the antrum. This ability is lost after vagotomy, leading to delayed antral filling (60).

Fats in the small intestine stimulate the secretion of the peptide CCK (cholecystokinin) from the mucosa in the duodenum and jejunum (36). CCK stimulates the postprandial bile secretion into the duodenum (36) and inhibits postprandial gastric emptying (61).

The gut peptide motilin, produced by the proximal small intestine, rises in concentration in the plasma at the start of phase II of the MMC and reaches

maximal concentration during phase III when it stimulates gastric emptying. The concentration of motilin has been shown to be related to gall bladder contractions. It is not clear what stimulates the release of motilin (36). However, it has been suggested that duodenal bile may be responsible for the release of motilin (62).

The first published study on gastric emptying in healthy humans using paracetamol absorption revealed no effect of the hormone grehlin (63). However, in a recent study using scintigraphy ghrelin was shown to stimulate gastric emptying in healthy subjects (64) and in patients with gastroparesis (65-67). Gastrointestinal hormones, such as glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are secreted by the K- and L-cells of the intestinal wall (68). GLP-1 inhibits gastric emptying, which leads to lower insulin and glucagons levels after a meal, resulting in lower blood glucose response (69-73). The gastrointestinal hormone peptide YY (PYY) has also been shown to delay gastric emptying (74).

2.4 Diabetic gastropathy

Neuropathy of peripheral autonomic and enteric nerve cells causes the gastroparesis associated with diabetes. This leads to a lack of postprandial relaxation, as well as weak contractions of the proximal stomach and hypomotility of the antrum. The lack of phase III of the MMC leads to fasting antral hypomotility, and indigestible particles can not leave the stomach causing the accumulation large ingestible particles. The contractions of the pylorus are too strong and all these factors lead to delayed gastric emptying of solid and liquid meals (36). A number of other factors may affect gastric emptying in diabetes, such as acute and chronic changes in blood glucose, abnormalities in the secretion of gastrointestinal hormones and neurotransmitters, changes in the myenteric plexus, and gastric smooth muscle degeneration (75).

Symptoms of delayed gastric emptying include nausea, vomiting, belching, bloating or full feeling, early satiety, upper abdominal pain, heartburn, anorexia and weight loss (36). Unexplained hypoglycaemic events are common and are associated with a delayed gastric emptying rate in insulin-treated diabetes patients (35).

Cross-sectional studies have shown that 30-50% of patients with type 1 and type 2 diabetes exhibit slow gastric emptying of solid and liquid meals (25-29). However, in patients with type 2 diabetes the gastric emptying rate may be a rapid in the early phase of the disease (76-77).

2.5 Blood glucose regulation

According to the latest report from the World Health Organization (WHO) normal fasting plasma blood glucose levels in healthy subjects are below 6.1 mmol/l (78). During the fasting state blood glucose is regulated by glucagon secretion by the α-cells of the islets of Langerhans in the pancreas, which leads to the release of glucose from the liver. Glucose in formed by the breaking down of glycogen in the liver, by the process of glycogenolysis. Basal insulin secretion during the fasting state suppresses excessive glycogenolysis. During a long period of fasting, gluconeogesis leads to the release of glucose from the liver by breaking down amino acids, glycerol and lactate (68).

Postprandial plasma blood glucose levels in healthy subjects are below 7.8 mmol/l (78). The secretion of peptide GLP-1, insulin and amylin is increased postprandially. Insulin mediates glucose uptake in the peripheral tissues, stimulates glycogenesis when glucose is stored as glycogen in the liver, and inhibits glucagon secretion, leading to the suppression of glycogenolysis and glyconeogenesis. Amylin is synthesized and secreted with insulin in the β-cells of

the islets of Langerhans. Amylin slows gastric emptying and prevents glucagon secretion. Gastrointestinal hormones such as GIP and GLP-1 are secreted by the K-cells and L-cells, respectively, of the intestinal wall. GLP-1 stimulates the glucose-dependent release of insulin, inhibits glucagon secretion and slows gastric emptying (68). GIP stimulates the glucose-dependent insulin secretion (79).

Diabetes mellitus is diagnosed as fasting plasma blood glucose ≥7.0 mmol/l, or symptoms of diabetes and postprandial plasma glucose levels ≥11.1 mmol/l, or plasma glucose ≥11.1 mmol/l, two hours after an oral glucose tolerance test (75 g glucose), according to the WHO (78). The American Diabetes Association recommends lowering the level of fasting plasma blood glucose to below 5.6 mmol/l (80). However, the WHO recommends that the current diagnostic criteria for diabetes be maintained due to a lack of evidence of the benefit of lowering the level of fasting plasma blood glucose level (78). Impaired fasting glucose level is defined as fasting plasma glucose values <7.0 but ≥6.1 mmol/l, and impared glucose tolerance (IGT) is defined as ≥7.8 but <11.1 mmol/l two hours after the glucose test, and a fasting plasma glucose <7.0 mmol/l (78). Subjects with IFG levels or IGT are considered at risk of developing diabetes and cardiovascular diseases (80, 81).

Type 1 diabetes (5-10% of those with diabetes) results from the slow or rapid autoimmune destruction of the β-cells of the pancreas (80, 81). Later in type 1 diabetes, the secretion of insulin and amylin almost ceases (68). Type 2 diabetes, accounting for about 90% of those with diabetes, is associated with peripheral insulin resistance that is compensated by a hyperinsulinaemia (80, 81). However, later in the disease β-cells release less true insulin and more proinsulin which has a lower effect, and this leads to decreasing levels of insulin and amylin. GLP-1 secretion is also decreased, which leads to increased glucagon secretion leading in turn to an increase in glucogenesis. The reduced uptake of blood glucose in the

peripheral tissues, caused by insulin resistance, contributes to postprandial hyperglycaemia (68).

2.6 Satiety and satiation

When considering the gastrointestinal mechanisms involved in the regulation of appetite, it is important to make the distinction between satiation and satiety. Satiation refers to the process that controls the size of a meal by terminating the period of eating, whereas satiety can be described as the state after a meal, during which hunger is dampened and the urge to consume food is inhibited. Appetite refers to the desire to eat, independent of the body's energy situation, whereas hunger can be described as the sensation that arises when metabolic signals indicate the need to provide the body with energy through food (82).

Food intake is regulated by the central nervous system, adrenal glands, the pancreas, the gastrointestinal tract and adipose tissue. Gastric distension signals are transmitted by the vagal afferent neurons, and the release of gastrointestinal hormones stimulates the sensory nucleus tractus solitarius in the brainstem. The arcuate nucleus in the hypothalamus receives input from the vagal nuclei in the brainstem, tractus solitarius, or directly by circulating hormones. Other regions of the hypothalamus and higher centres are involved in the control of food intake (83). Gastrointestinal hormones such as CCK (84, 85), PYY (86) and GLP-1 (72, 73, 87-91) have been shown to be important in the control of satiety and inhibition of food intake. The gastrointestinal hormone ghrelin has been shown to stimulate the appetite and increase food intake in humans (63, 92). The effects of the gastrointestinal hormones are not only mediated through the vagus nerve, but the gastrointestinal hormones are also released into the blood stream and have direct effects in the brain (83). Gastric distension has been shown to cause postprandial fullness and satiety (93-100). However, the inverse correlation between gastric emptying and fullness seems weak (99). The reduction of hunger

after a meal seems to be caused by the nutritional composition of a meal, but not gastric distension (94-97, 99).

2.7 Cinnamon and blood glucose control

Different herbs and medical plants have been tested and cinnamon has been shown to be the most effective in the regulation of blood glucose (101). A water-soluble polyphenol type-A polymer, isolated from cinnamon, has been shown to enhance the activity of insulin (102). Different species of cinnamon have been investigated, but no differences were found in the insulin-enhancing biological activity (102). Cinnamon has been shown *in vitro* to stimulate the insulin receptor by activating the insulin receptor kinase and inhibiting the insulin receptor phosphatase, which increases insulin sensitivity (103). *In vivo*, cinnamon has been shown to enhance glucose utilization in rats in a dose-dependent way by potentiating the insulin-stimulated tyrosine phosphorylation of the insulin receptor (IR)-β and the insulin receptor substrate (IRS)-1 and its association with phosphatidylinositol (PI) 3 kinase (104). When insulin-resistant rats were fed cinnamon for 3 weeks the insulin resistance decreased, as measured by the euglycaemic clamp; the explanation given was the improved insulin signalling pathway described above (105).

A study in Pakistan by Khan et al. showed that the ingestion of 1, 3 and 6 g cinnamon daily for 40 days lowered the levels of fasting glucose, triglyceride, low-density lipoprotein (LDL) cholesterol and total cholesterol in women and men with type 2 diabetes receiving oral blood-glucose-lowering treatment (106). In women and men with type 2 diabetes that were treated with oral blood-glucose-lowering therapy or diet and/or physical activity a 3 g cinnamon supplementation daily for 4 months resulted in a reduction in fasting plasma glucose levels, while no difference was observed in HbA1c, total cholesterol, LDL, high-density lipoprotein (HDL) or triacylglycerol concentration after cinnamon

supplementation (107). In overweight, postmenopausal women with type 2 diabetes on either oral blood-glucose-lowering medication or controlled diets supplementation with 1.5 g cinnamon a day for 6 weeks no improvement was seen in HbA1c, fasting glucose or insulin concentration, triacylglycerol, LDL, HDL, total cholesterol, or insulin resistance and sensitivity (108). In a study performed in the United States, no significant changes were seen in fasting glucose, lipid, HbA1c, or insulin levels in people with type 2 diabetes when 1 g cinnamon was consumed daily for three months (109). These results suggest that those with poorly controlled diabetes may benefit from cinnamon intake more than those receiving good treatment.

Patients with type 1 diabetes treated with 1 g cinnamon a day for 3 months showed no difference in HbA1c, total daily insulin intake, or number of hypoglycaemic episodes compared with the placebo group (110). This may be explained by the fact that cinnamon stimulates endogenous insulin production, which is lacking in type 1 diabetes patients. However, a recent meta-analysis of the above-mentioned five randomized, placebo-controlled trials did not show any significant changes in HbA1c, fasting blood glucose, or lipid levels (111). However, in women with polycystic ovary syndrome without diabetes a supplementation of 333 mg of cinnamon extract, three times a day, for 8 weeks lowered insulin resistance and fasting glucose (112). It was recently shown that 5 g cinnamon ingested 12 h prior to or in conjunction with an oral glucose test in healthy men reduced the blood glucose response and improved insulin sensitivity, but no difference was observed in the insulin response (113).

2.8 Dietary fibre and gastric emptying

There are many definitions of dietary fibre, including a range of non-starch polysaccharides and lignin derived from cell walls that are poorly digested in the upper intestine. Non-starch polysaccharides can be divided into two groups,

soluble and insoluble. Soluble fibres are considered partially water soluble but not entirely, and include pectin, guar gum (galactomannan) and glucomannan (also known as konjac mannan), psyllium, β-glucan and arabinoxylans. Some soluble fibres such as guar gum and glucomannan have gel-forming properties, forming gels with high viscosity, and this has been suggested as the mechanism by which these fibres reduce postprandial glycaemia (114). It has been suggested that oat β-glucan may delay gastric emptying by the viscosity of the fibre, causing distension of the stomach. Only one study has previously been performed on the effect of β-glucan on GER, using rye bread, and paracetamol as a marker for gastric emptying (115). In that study, it was found that the GER in healthy subjects was not affected after a meal consisting of rye bread with β-glucan.

Previous studies on different combinations of dietary fibres show divergent effects on GER. A high-dietary-fibre meal composed of wholemeal products and legumes was found to delay gastric emptying in healthy subjects compared to a low-fibre meal composed of white products and legume juice when measured by ultrasonography (116). The addition of wheat bran and soluble fibre guar gum to a meal was shown to delay gastric emptying in patients with type 2 diabetes, using scintigraphy (117). However, it was found in another study that the GER following a high-fibre meal consisting of whole wheat grain and rye bread did not different from that following a low-fibre meal (118). Only one study has been conducted previously on the effect of whole kernels on gastric emptying, and it was found that the gastric emptying in healthy subjects was not affected after meals composed of whole-kernel rye bread or wholemeal rye bread compared with white wheat bread, when measured indirectly with paracetamol (110).

2.9 Oat β-glucan and postprandial blood glucose

Soluble fibre has generated considerable interest because of its cholesterol-lowering effect (119). β-glucan is a soluble dietary fibre mainly found in oat and

barley. It has previously been shown that oat β-glucan reduces postprandial glucose and insulinaemic responses in type 2 diabetics and in non-diabetic subjects (120-129). A dose–response relation has also been observed between the amount of oat β-glucan and the decrease in glucose and insulin levels in healthy subjects (122, 124, 127) and in type 2 diabetics (121).

The oat β-glucan effect is, however, not fully understood. One hypothesis is that oat β-glucan increases the viscosity in the small intestine and delays the digestion of food, leading to lower blood glucose and insulin response (114, 130). The viscosity of oat β-glucan has been shown to cause a reduction in plasma glucose and insulin (131). Furthermore, a relationship between glycaemic response and the concentration and molecular weight of oat β-glucan has also been observed (132). Another mechanism that has been proposed is that oat β-glucan may delay gastric emptying by the viscosity of the fibre, causing distension of the stomach. However, this has not been studied experimentally. Another hypothesis is that β-glucan is fermented in the colon by the bacterial flora leading to the release of short-chain fatty acids, lowering postprandial glucose levels (133) and serum lipids (134).

2.10 Whole grain and postprandial blood glucose

The term "whole grain" is often used to describe wholemeal products in which the structure of the cereal grain has been destroyed in the flour containing the original dietary fibre, but is also applied to cereal products in which a large portion of the cereal grain is intact. However, there seems to be a major difference in the metabolic response to whole grain and wholemeal products. No difference has been found in the glycaemic response to bread made of finely ground wholemeal flour compared to white wheat bread (135). The wheat germ of the whole grain acts as a natural amylase inhibitor, which can be destroyed during the processing of milling wheat into wholemeal flour (136). Bread containing whole or cracked

wheat kernels has been shown to induce a lower glycaemic response than white wheat bread (135). The glycaemic index was also decreased when increased portions of whole grain barley or bulgur (cracked wheat) were substituted for milled flour in bread (137). The preparation, cooking and particle size of the grain structures might also affect the metabolic response (138). However, the effects of the size of the grain or product seem to be complex. The glycaemic responses of bulgur (cracked wheat) and whole wheat kernels did not differ (139). This was explained by the smaller particle size of the bulgur, making crushing by chewing more difficult compared with whole kernels of wheat. This would result in similarly sized particles entering the small intestine leading to the same postprandial glycaemic response.

2.11 Vinegar and blood glucose control

The first reported antiglycaemic effect of vinegar was made by Ebihara and Nakajima who observed a significantly reduced blood glucose response in rats after a meal containing 2% acetic acid solution (140). However, in healthy human subjects the blood glucose response was not reduced, while the insulin response was reduced (140). Another study showed that the addition of 20 g white vinegar (5% acetic acid) to olive oil on lettuce and white bread containing 50 g carbohydrates significantly lowered the postprandial blood glucose response in healthy subjects, and this could not be explained by a delayed gastric emptying, measured with ultrasonography (141). The decreased postprandial blood glucose response was explained by a mechanism related to the inhibition of digestive amylases (141). When vinegar was neutralized to pH 6.0 with sodium bicarbonate no effect was seen on the postprandial blood glucose response (141). It has been demonstrated that the addition of 20 g white vinegar to a white wheat bread meal containing 50 g carbohydrates significantly lowered the postprandial blood glucose and insulin response in healthy subjects, and this was explained by delayed

gastric emptying (142). However, the gastric emptying was measured indirectly by paracetamol, which is regarded as an unreliable method.

In Japan the glycaemic index of common Japanese foods, each containing 50 g carbohydrates, has been evaluated and it was found that vinegar decreases the glycaemic index of, for example, rice in sushi by about 20 to 35% (143). The addition of pickled cucumber (1.6 g acetic acid) and yoghurt to a white wheat bread meal lowered the glycaemic index and the postprandial blood glucose and insulin response in healthy subjects compared to a white wheat bread meal (144). The glycaemic index of the meal was also lowered when 28 g white vinegar (6% or 28 mmol acetic acid) was added as a vinaigrette sauce to cold storage potatoes containing 50 g carbohydrates (145). Dose-response relations have been observed for blood glucose and insulin levels in healthy subjects after a white wheat bread meal with vinegar (146). Three levels of vinegar (18, 23 and 28 mmol acetic acid) were ingested with white wheat bread containing 50 g carbohydrates; the higher the amount of acetic, the lower the metabolic response (146). The rating of satiety was also related to the acetic acid level, but only the highest level of acetic acid resulted in significantly higher satiety scores (146). Ingestion of 20 g apple cider vinegar (5% acetic acid) prior to a high glycaemic meal, composed of bagel, butter and orange juice containing a total of 87 g carbohydrates, reduced the postprandial blood glucose and insulin response in healthy subjects (147). However, ingestion of 20 g apple cider vinegar prior a low glycaemic meal composed of chicken, cooked rice and vegetables containing 52 g carbohydrates, reduced the postprandial insulin response but did not affect the blood glucose response in healthy subjects (147).

It was recently demonstrated that both the postprandial insulin and glucose responses were reduced in insulin-resistant subjects when a drink containing 20 g apple cider vinegar was given prior to a meal consisting of white bagel, butter and orange juice containing 87 g total carbohydrates (148). However, in type 2

diabetes patients neither the postprandial glucose nor insulin responses were affected, while in healthy subjects the postprandial insulin level was reduced, but the blood glucose level was not affected when apple cider vinegar was consumed prior to the meal (148). A study on patients with diabetes mellitus type 2 not taking insulin showed a reduced fasting glucose level when 2 tablespoons of apple cider vinegar were ingested at bedtime with 1 oz ~28 g cheese (149). It has been proposed that the antihyperglycaemic effect of acetic acid may be mediated by enhanced glycogen repletion in the liver and skeletal muscle (150), and the suppression of disaccharidase activity in human intestinal cells (151).

Chapter 3

Aims

The aims of this work were:

- to study the effect of cinnamon on the rate of gastric emptying, the postprandial blood glucose response, and satiety in healthy subjects,
- to evaluate the effect of commercial fibre cereals on the gastric emptying rate, postprandial glucose response and satiety in healthy subjects,
- to evaluate the effect of an extruded muesli product based on oat β-glucan on the gastric emptying rate, postprandial blood glucose and satiety in healthy subjects,
- to evaluate the possible influence of maintained botanical integrity of cereals and the ingestion of acetic acid on gastric emptying rate and satiety in healthy subjects, and
- to investigate the effect of apple cider vinegar on gastric emptying rate in diabetes mellitus patients with diabetic gastroparesis.

Chapter 4

Subjects and Methods

4.1 Subjects

The healthy subjects were recruited from the population in southern Sweden (Skåne). None of the healthy subjects had symptoms or a prior history of gastrointestinal disease, abdominal surgery (except for appendectomy) or diabetes mellitus. These subjects had no connective tissue or endocrine or cerebrovascular disease, nor were they receiving any drugs except for oral contraceptives. Each subject was required to have a normal fasting blood glucose level on the day of the study. The subjects gave their informed consent before the study began and knew that they could withdraw from the study at any time.

The study described in **Paper I** included fourteen healthy subjects [eight men, six women; mean age 26 ± 5 years (range 20-38 years); mean body mass index (BMI) 23 ± 2 kg/m^2 (range 18-26 kg/m^2)]. Two subjects were smokers and two were snuff users. Four women were taking oral contraceptives.

The study presented in **Paper II** included twelve healthy subjects [six men and six women; mean age 28 ± 4 years (range 23-36 years); mean BMI 22 ± 2 kg/m^2 (range 19-24 kg/m^2)]. Four subjects were smokers and two were snuff users. Three of the women, including one with polycystic ovary syndrome, were taking oral contraceptives. The subject with the polycystic ovary syndrome had a BMI of

21 kg/m² and had previously undergone a glucose tolerance test which gave normal results.

The study described in **Paper III** included twelve healthy subjects [eight men and four women; mean age 27 ± 5 years (range 22-35 years); mean BMI 22 ± 3 kg/m² (range 17-27 kg/m²)]. Four of the subjects were smokers and two were snuff users. One of the women was taking oral contraceptives.

The study presented in **Paper IV** included thirteen healthy subjects [six men and seven women; mean age 25 ± 4 years (range 22-35 years); mean BMI 23 ± 3 kg/m² (range 18-30 kg/m²)]. Two women were taking oral contraceptives. One subject was a smoker and none was a snuff user.

Patients with type 1 diabetes were recruited from those previously diagnosed with gastroparesis, confirmed by scintigraphic and ultrasound methods at the Malmö University Hospital (152). A GER lower than 45%, determined with a standardized ultrasound method, indicates delayed gastric emptying, and has previously been shown to be strongly correlated to scintigraphic half-time values (153). Those having previously undergone major abdominal surgery, or had signs of renal failure (microalbuminuria > 20 μg/min), history of severe cardiovascular disease, hepatic disease, evidence of prior gastric outlet obstruction or connective tissue diseases were excluded from the study. The patients had symptoms typical of diabetic gastroparesis (postprandial abdominal fullness or nausea, vomiting, postprandial early satiety or early postprandial hypoglycaemia, despite the ingestion of food and correctly taken doses of insulin). All the diabetic patients were being treated with a multiple-dose regime, consisting of rapid- or short-acting insulin before meals and intermediate-acting insulin once or twice daily. No medication was changed, and no subject used any medication with known major gastrointestinal side effects during the study. None of the subjects used any

prokinetic treatment before or during the study. The subjects gave their informed consent before the study began and knew that they could withdraw from the study at any time.

In the study described in **Paper V** ten patients with type 1 diabetes mellitus and with diagnosed diabetic gastroparesis [five men and five women; mean age 68 ± 8 years (range 57-79 years); mean BMI 25.4 ± 2.9 kg/m² (range 21.2-30.9 kg/m²); mean duration of diabetes 41 ± 13 years (range 18-57 years), mean value of HbA1c 8.3 ± 0.7 % (range 7.5-9.5)] were included and completed the crossover study. Seven of the subjects had a history of peripheral neuropathy, eight had retinopathy and four had nephropathy. One of the patients had had his gall bladder removed, and two patients had had their uterus and ovaries removed; three patients had been appendectomized, and one had undergone vagotomy 16 years previously. Two of the subjects were snuff users but none smoked.

4.2 Determination of gastric emptying rate

The subjects were examined in the morning between 7:30 and 10:00 after an 8-h fast. Smoking and snuff-taking were prohibited for 8 h before and during the test. The fasting blood glucose concentration of each healthy subject was checked on the day of the examination to ensure that it was normal.

The measurements of GER in the diabetic subjects were performed providing that their fasting blood glucose level was between 3.5 and 9.0 mmol/L. If the subjects reported gastrointestinal symptoms (diarrhoea, constipation, nausea or vomiting) on the day of the study the examination was postponed. Diabetes patients with chronic constipation (in our study defined as symptoms for 1 year or longer) were not excluded, as this was assumed to be their basal state, possibly owing to autonomic neuropathy. The diabetic patients were asked not to consume any drugs or insulin on the day of the examination. Before each meal the diabetic

patients took their normal daily insulin dose, which was not changed during the study.

The subjects were examined in a supine position with the ultrasound transducer applied with minimal abdominal compression. Between the examinations all subjects were seated. The measurements of the gastric antrum were performed by the same radiologist, who was blinded with respect to the meals and the diagnosis of the subjects.

The sonographic examinations were performed with a 3.5-MHz abdominal transducer and an imaging system (**Papers I & III**; Acuson 128 XP 10, Siemens Medical Solutions, Mountain View, CA, USA), (**Papers II & IV**; Acuson Sequoa 512, Siemens Medical Solutions, Mountain View, CA, USA; Aloka Prof. Sound, Tokyo, Japan), (**Paper V**; Acusone Sequioa 512, Mountain View, CA, USA; Aloka ProSound SSD 5500, Tokyo, Japan; Siemens Elegra, Siemens Medical Solutions, Mountain View, CA, US; B-K Medical 2102 Hawk, Gentofte, Denmark). In each calculation of the GER, the antrum diameters had been measured using only one of the above machines. The same ultrasound equipment was not used in all the paired studies, but the same equipment was used for a particular pair of measurements. The GER was estimated using a previously described standardized ultrasound method (152). The abdominal aorta and the left lobe of the liver were used as internal landmarks when examining the gastric antrum. The measurements were made 15 and 90 min after the end of meal consumption. Gastric emptying was expressed as the percentage change in the antral cross-sectional area between 15 and 90 min. At each examination, 3 measurements of the longitudinal (d1) and anteroposterior (d2) diameters were made, and mean values were used to calculate the cross-sectional area of the gastric antrum using the following equation:

Antrum area = $\pi \times d1/2 \times d2/2 = \pi \times d1 \times d2/4$.

The gastric emptying rate was calculated using the following formula:

GER = [1- (Antrum area 90 min/Antrum area 15 min)] × 100

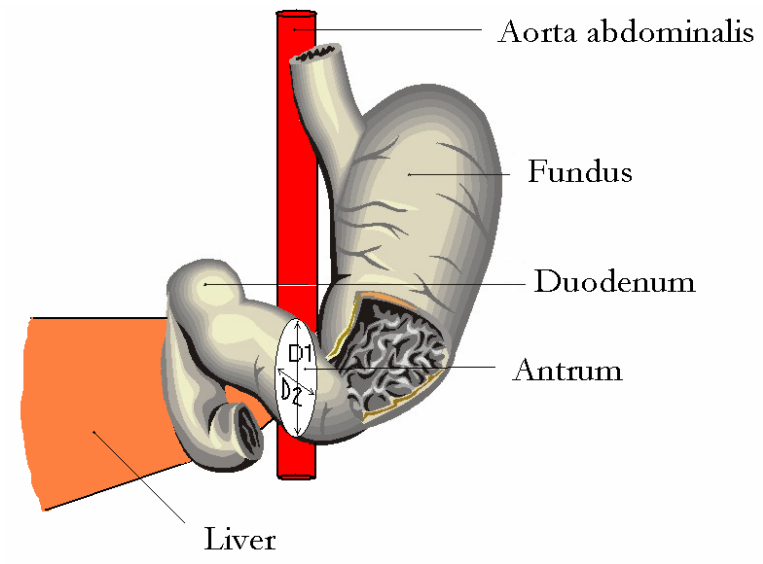

Figure 1. Illustration of the stomach showing the gastric antrum. The longitudinal (d1) and anteroposterior (d2) diameters of the antrum are shown.

4.3 Test meals

The test meal used in the study described in **Paper I** consisted of 300 g rice pudding (Axa Goda Gröten Risgrynsgröt; Lantmännen AXA, Järna, Sweden) mixed with 6 g cinnamon (Santa Maria AB, Mölndal, Sweden). The total caloric value was 330 kcal: 10% of the energy being derived from protein (3 g), 58% from carbohydrate (16 g), and 32% from fat (4 g). The reference meal consisted of 300 g rice pudding (Axa Goda Gröten Risgrynsgröt). The meals were served in a random order and ingested within 5 min.

The test meals used in the study presented in **Paper II** consisted of 300 g sour milk (Skånemejerier, 205 03 Malmö, Sweden) (caloric value 135 kcal) and 50 g bran flakes (Kellogg's All-Bran, Nordisk Kellogg's, Sverige Upplands Väsby, Sweden) (caloric value 163 kcal) or wholemeal oat flakes (Frebaco Fullkorns Havreringar, Frebaco Kvarn AB, Lidköping, Sweden) (caloric value 185 kcal). The reference meal consisted of 50 g cornflakes (Kellogg's Corn Flakes, Nordisk Kellogg's, Sverige Upplands Väsby, Sweden) (caloric value 185 kcal) and the same brand and quantity of sour milk as the test meal (see Table 1). The meals were served in a random order more than one week apart. Each meal was ingested within 5 minutes.

Table 1. Nutrient composition of the test meals (containing wholemeal oat flakes or All-Bran Regular) and the reference meal (containing cornflakes). Nutrient composition according to product information.

	Sour milk	Frebaco Wholemeal Oat flakes	Kellogg's All-Bran Regular Flakes	Kellogg's Cornflakes
Amount	300 g	50 g	50 g	50 g
Total energy (kcal)	135	185	163	185
Total protein (g)	12	6	5	3.5
Total fat (g)	1.5	2	1	0.35
Total Carbohydrate (g)	18	35.5	33.5	42
Sugar (g)	15	0.75	11	4
Total fibre (g)		4	7.5	1.5
β-glucan (g)		0.5		

The test meal used in the study described in **Paper III** consisted of 200 g vanilla yoghurt (caloric value 137 kcal) and 26.5 g Primaliv muesli (Skånemejerier,

Malmö, Sweden) (caloric value 72 kcal) (total caloric value 209 kcal). The Primaliv muesli was composed of 24.5 g flakes made from oat bran (OatWell, Swedish Oat Fibre/Crea Nutrition, Väröbacka, Sweden) containing 4 g oat β-glucan (caloric value 65 kcal), 0.8 g mini-cornflakes (caloric value 3 kcal), 0.6 g freeze-dried apple (caloric value 2 kcal) and 0.6 g freeze-dried strawberries (caloric value 2 kcal). The reference meal included the same brand and quantity of vanilla yoghurt, mini-cornflakes, apple and strawberries as the test meal, but the oat bran flakes were replaced with 17.5 g Kellogg's cornflakes (caloric value 65 kcal) (see Table 2). The test meal and the reference meal had the same total caloric value. Water (200 ml) was served with each meal and was ingested gradually during the meal, which lasted for 10 min. The meals were served in a random order.

Table 2. Nutrient composition of the test meal (containing oat bran flakes with β-glucan) and the reference meal (containing Kellogg's cornflakes). Nutrient composition according to product information.

	Vanilla yoghurt	Oat flakes (β-glucan)	Kellogg's cornflakes
Amount	200 g	24.5 g	17.5 g
Total energy (kcal)	137	65	65
Total protein (g)	8	4.4	1.2
Total fat (g)	1	1.5	0.1
Total carbohydrate (g)	24	8.7	14.7
Total fibre (g)	0	9	0.5
β-glucan (g)	0	4	0

Bread was served in the meals described in **Paper IV**. White wheat bread was made from 3700 g white wheat flour, 2000 g water and 200 g yeast. The dough was allowed to rise for 20 min at 28 °C. The dough was then divided into 440 g

pieces and left to prove for 35 min at 40 °C (RH: 80%). Loaves were baked at 210 °C for 22 min with the addition of steam during the first 30 s. The loaves were stored in a freezer at -20 °C until used.

Whole-kernel wheat bread was made from 3076 g wheat kernels that had been boiled for 20 min in 3076 g water and then cooled at room temperature, after which 1200 g water, 624 g white wheat flour and 200 g yeast were added. The dough was left to rise for 30 min and then divided into 580 g pieces. These pieces were then allowed to prove for 45 min at 40 °C (RH: 80%). Loaves were baked at 200 °C for 45 min.

Wholemeal wheat bread was made from 3076 g milled wheat kernels, (500 g of the flour was scalded with 1000 g boiling water) 1200 g water, 624 g white wheat flour and 200 g yeast. The dough was left to rise for 30 min and then divided into 580 g pieces. These were then allowed to prove for 45 min at 40 °C (RH: 80%). Loaves were baked at 200 °C for 45 min.

The reference and test meals contained 50 g available carbohydrates from bread products. The content of available carbohydrates was analysed according to the method of Holm et al. (154). The portion size of the white wheat bread was 106.34 g and, apart from the 50 g available carbohydrates, contained 2.1 g dietary fibre, 1.8 g fat and 8.3 g protein. The portion size of the whole-kernel wheat bread was 132.66 g and contained 7.2 g dietary fibre, 2.9 g fat and 9.2 protein, besides the 50 g available carbohydrates. The portion size of the wholemeal wheat bread was 107.62 g, and contained 7.2 g dietary fibre, 2.9 g fat and 9.2 g proteins, besides the 50 g available carbohydrates. The same baking recipes and baking process were used as described by Liljeberg et al. (155) for the white wheat reference bread and whole-kernel wheat bread; the contents of dietary fibre, fat and proteins were thus assumed to be the same. The wholemeal wheat bread was made from the same recipe as whole-kernel wheat bread but with milled wheat

kernels. The three test meals contained one of the three kinds of test bread dipped in 28 g white wine vinegar (5% acetic acid, pH 2.8-3 Druvan, DR Persfood AB, Eslöv, Sweden), which is equivalent to 23 mmol acetic acid in each test meal. Drinking water, 200 ml, was also served. The reference meal consisted of white wheat bread and water, without white wine vinegar. The test meals and the reference meal were served in random order during intervals of 1 week. Each meal was ingested within 10 minutes.

The reference meal used in the study described in **Paper V** consisted of 300 g rice pudding (BOB, Scan Foods AB, Johanneshov, Sweden, 100 g caloric value 110 kilocalories, 3.5 g protein, 17 g carbohydrates and 3 g fat). Each meal had to be consumed within 10 min and 200 ml water was consumed before ingestion of the rice pudding. One week before the first examination, the patients drank 200 ml water every morning before their breakfast. After the first examination the patients were given a 450 ml bottle of commercially available apple cider vinegar with honey flavouring (Druvan, DR Persfood AB, Eslöv, Sweden). The caloric value of 100 ml of this apple cider vinegar was 65 kcal; it also contained 16 g carbohydrates, < 0.5 g protein and 0 g fat. The apple cider vinegar was composed of 5% acetic acid and had a pH value of 2.8-3. The patients were asked to drink 30 ml apple cider vinegar mixed with 200 ml water (total volume 230 ml) every morning for two weeks before their breakfast. Each meal had to be consumed within 10 min and the water, with vinegar, was consumed before ingestion of the rice pudding. Ultrasound measurements of the cross-sectional antral areas then made after the ingestion of the rice pudding. One of the subjects who participated in the study was already consuming apple cider vinegar daily, and was therefore examined for the first time after drinking apple cider vinegar mixed with water before ingestion of the rice pudding. After a ten-day wash-out period with water before the ingestion of breakfast, the same subject was examined after the ingestion of the reference meal. The subjects were not prevented from consuming other vinegar or acetic-acid-containing products during the study. The order of

the two different meals was, unfortunately, not randomized.

4.4 Blood glucose measurements

Blood glucose concentrations were measured with the HemoCue Glucose system (HemoCue AB, Ängelholm, Sweden).

- In the study described in **Paper I** finger-prick capillary blood samples were collected before ingestion of the meals and at 15, 30, 45, 60, 90, and 120 min, after the start of the meals to determine the blood glucose levels.
- In the study described in **Paper II** finger-prick capillary samples were collected before ingestion of the meals and at 0, 20, 30, 40, 60, 80, 100 and 120, min after the end of the meals to determine the blood glucose levels.
- In the study described in **Paper III** finger-prick capillary blood samples were collected before the ingestion of the meals 30 and 60 min, after the end of the meals to determine the blood glucose levels.
- In the study described in **Paper IV** finger-prick capillary samples were collected before the ingestion of the meals and 15, 30, 45, 60, 90 and 120 min, after the start of the meal to determine the blood glucose levels.
- In the study described in **Paper V** finger-prick capillary blood samples were collected before the ingestion of the meals to determine the blood glucose levels.

4.5 Satiety score

The validated satiety score described by Haber et al. (156) was used to assess satiety. This scale ranges from –10 cm (extreme hunger) to +10 cm (extreme satiety).

- In the study described in **Paper I** satiety scores were assessed before the meals and 15, 30, 45, 60, 90, and 120 min after the start of the meals.
- In the study described in **Paper II** satiety scores were assessed before the meals and 0, 20, 30, 40, 60, 80, 100 and 120 min after the end of the meals.
- In the study described in **Paper III** satiety scores were assessed 15 and 90 min after the end of the meals.
- In the study described in **Paper IV** satiety scores were assessed before the meals and 15, 30, 45, 60, 90 and 120 min after the start of the meals.

4.6 Statistical analysis

Median values and quartiles (q1 and q3) are presented for the antral cross-sectional areas and the GER. The areas under the curves (AUCs) of each subject were measured for blood glucose and satiety by using GRAPH PAD PRISM software (version 4; GraphPad, San Diego, CA, USA). The AUC was calculated above zero. The AUC values are presented as means ± SEMs.

The statistical calculations in the studies described in **Papers I, III** and **V** were performed with SPSS for WINDOWS software (version 14.0; SPSS Institute, Chicago, IL, USA). Significant differences in GER, gastric antral cross-sectional area, and AUCs were evaluated using Wilcoxon's t-test. $P < 0.05$ was considered statistically significant.

In the study described in **Paper IV**, changes between pre-ingestion values of blood glucose and satiety and values after the different meals were presented as means ± SEMs, and were tested globally in a repeated-measures, linear mixed model using the interaction of time and treatment as fixed effects and subjects as random effects (SAS, version 8.2, SAS Institute, Cary, NC, USA). For the

covariance structure of the repeated measures within a series a spatial exponential model was used. The AUCs above zero for changes in blood glucose and satiety were tested globally in a mixed model where the meals were entered as fixed effects and subjects were entered as random effects. Tukey's multiple comparisons test was applied after the mixed models when appropriate. In addition, BMI was included as a covariate in the mixed model for glucose, and also possible correlations between satiety and antral areas or GER were also investigated. Antral cross-sectional areas and the GER were tested globally using the Friedman rank sum test, and when the null hypothesis was rejected, this was followed by pair-wise comparisons using Wilcoxon's rank sum test with the Holm sequential procedure for P-value adjustment (R, version 2.6, The R foundation for statistical computing, http://www.r-project.org/). Statistical significance was defined as $P<0.05$.

Chapter 5

Results

5.1 Cinnamon and postprandial blood glucose response

Ingestion of rice pudding with cinnamon resulted in a significantly lower blood glucose response in the postprandial phase (15-45 min) than did the reference meal ($P<0.01$) (**Paper I**) (Figure 2). The blood glucose AUCs after 15 minutes were significantly lower after the ingestion of rice pudding with cinnamon than after ingestion of the reference meal (Table 3). However, the AUCs at 0-15 min did not differ significantly between the test and reference meals.

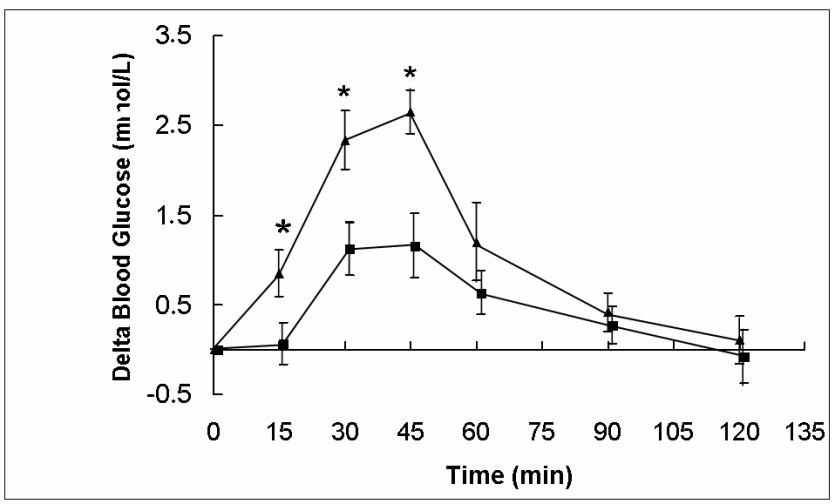

Figure 2. Mean (± SEM) incremental blood glucose concentrations in 14 healthy subjects after ingestion of meals consisting of rice pudding with (■) and without (▲) cinnamon. *Significantly different, $P<0.01$.

Table 3. Postprandial blood glucose areas under the curve (AUCs) in healthy subjects after ingestion of meals consisting of rice pudding with and without cinnamon (mean ± SEM; n=14).

AUC	Rice pudding without cinnamon (mmol * min/L)	Rice pudding with cinnamon (mmol * min/L)
0 - 15 min	6.8 ± 1.8	3.6 ± 1.0
0 - 30 min	30.7 ± 5.1	13.7 ± 3.4**
0 - 45 min	68.1 ± 8.2	32.4 ± 6.6*
0 - 60 min	97.2 ± 11.0	47.3 ± 9.2*
0 - 90 min	125.0 ± 16.8	63.3 ± 11.7*
0 -120 min	139.1 ± 19.6	75.0 ± 13.7**

* Significantly different from rice pudding without cinnamon, *P*= 0.001

** Significantly different from rice pudding without cinnamon, *P*= 0.003

5.2 Cinnamon and gastric emptying rate

The median values of the antral cross-sectional area after the ingestion of the cinnamon meal were 595 ± 234 mm^2 (range: 283–1181 mm^2; q1 = 458 mm^2, q3 = 809 mm^2) and 372 ± 366 mm^2 (range: 83–1525 mm^2; q1 = 282 mm^2, q3 = 593 mm^2) 15 and 90 min, respectively, after the end of the study meal. In the same subjects, the median values of the antral cross-sectional area after the ingestion of the reference meal were 531 ± 386 mm^2 (range: 262–1626 mm^2; q1 = 319 mm^2, q3 = 891 mm^2) and 317 ± 338 mm^2 (range: 50–1389 mm^2; q1 = 195 mm^2, q3 = 546 mm^2) 15 and 90 min, respectively, after the end of the meal. The median gastric antral cross-sectional areas were significantly larger 90 min after ingestion of rice pudding with cinnamon than 90 min after ingestion of rice pudding only (*P*=0.022) (**Paper I**). No significant differences were seen between gastric antral cross-sectional areas at 15 min. The median value of GER after the cinnamon meal was estimated to be 35% (range: –29% to 74%; q1 = 7%, q3 = 52%) (Figure 3), and that after the reference meal was estimated to be 37%; the difference was

statistically significant (range: 15 to 87%; q1 = 29%, q3 = 54%) (*P*=0.025) (Figure 3).

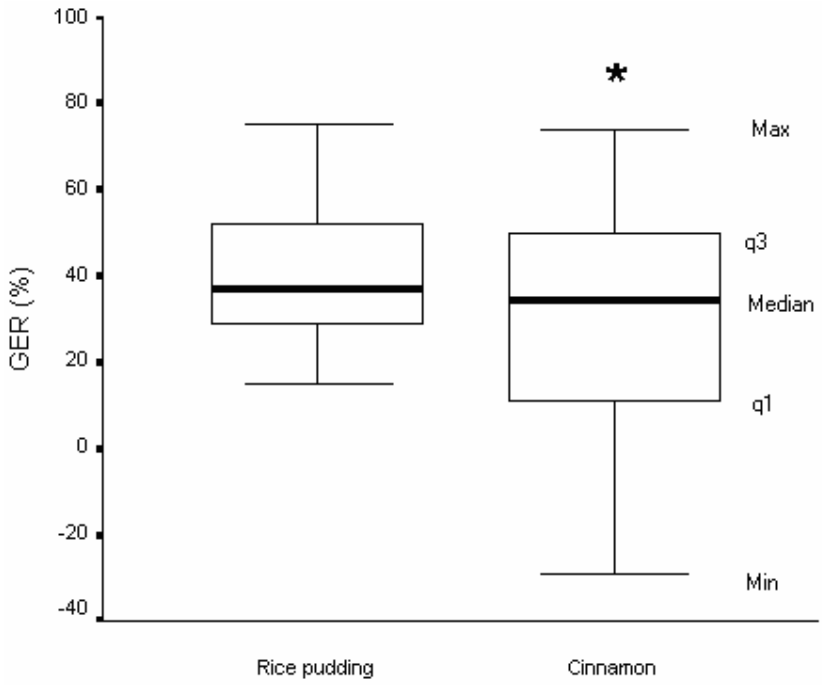

Figure 3. Gastric emptying of rice pudding with and without cinnamon, estimated as gastric emptying rate (GER), in 14 healthy subjects. The median, minimum (Min), and maximum (Max) values and the values of the first (q1) and the third (q3) quartiles are shown. * Significantly different from rice pudding without cinnamon, *P*= 0.025.

5.3 Cinnamon and satiety

Ingestion of rice pudding with cinnamon did not result in a significantly longer period of satiety than that following the reference meal of rice pudding (**Paper I**) (Figure 4). The AUCs for satiety were not significantly different after ingestion of rice pudding with cinnamon from those after ingestion of rice pudding only (Table 4).

Table 4. Areas under the curve for satiety in healthy subjects after ingestion of meals consisting of rice pudding with or without cinnamon (mean ± SEM; n=14). Significant differences in satiety scores were evaluated with the Wilcoxon t-test. There were no significant differences between the satiety scores area under the curves.

AUC	Rice pudding without cinnamon (cm* min)	Rice pudding with cinnamon (cm* min)
0 - 15 min	43.4 ± 7.2	48.3 ± 7.3
0 - 30 min	126.9 ± 21.5	140.8 ± 22.2
0 - 45 min	207.0 ± 35.3	225.9 ± 35.6
0 - 60 min	282.6 ± 47.4	302.7 ± 47.2
0 - 90 min	390.0 ± 68.7	430.7 ± 70.2
0 - 120 min	466.4 ± 89.8	538.7 ± 92.9

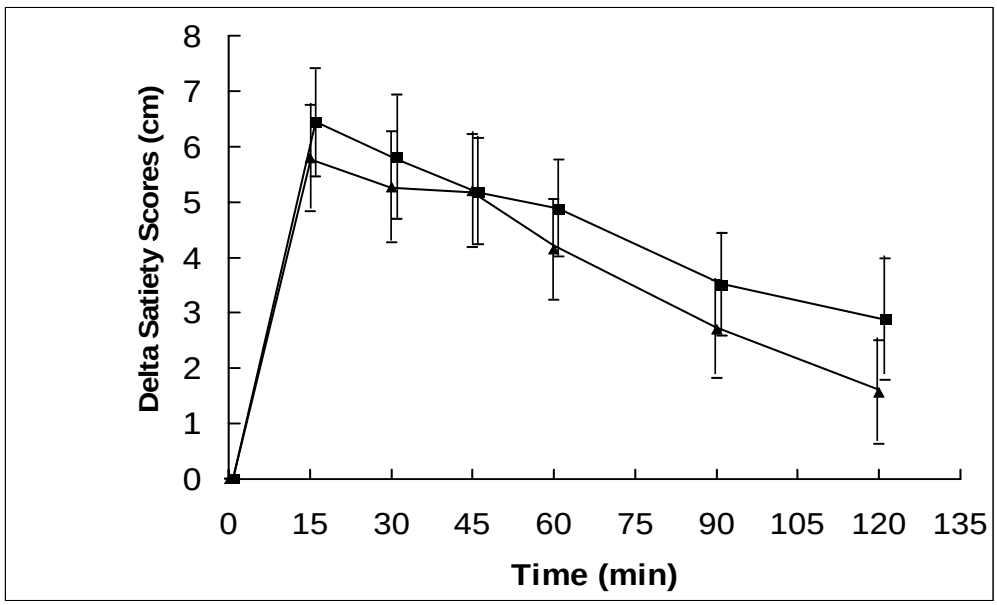

Figure 4. The mean (± SEM) incremental satiety scores in fourteen healthy subjects after ingestion of meals consisting of rice pudding with (■) and without (▲) cinnamon. There were no significant differences between the mean incremental satiety scores.

5.4 Fibre-rich cereals and postprandial blood glucose response

Ingestion of cereal bran flakes resulted in a significantly lower blood glucose response in the initial postprandial phase (40 min) than did the reference meal of corn flakes (*P*=0.045) (Figure 5). Ingestion of bran flakes resulted in a significantly lower blood glucose response in the late postprandial phase (120 min) than did wholemeal oat flakes (*P*=0.023) (Figure 5). However, the blood glucose AUCs did not differ significantly between bran flakes, wholemeal oat flakes and corn flakes (**Paper II**). The results are summarized in Table 5.

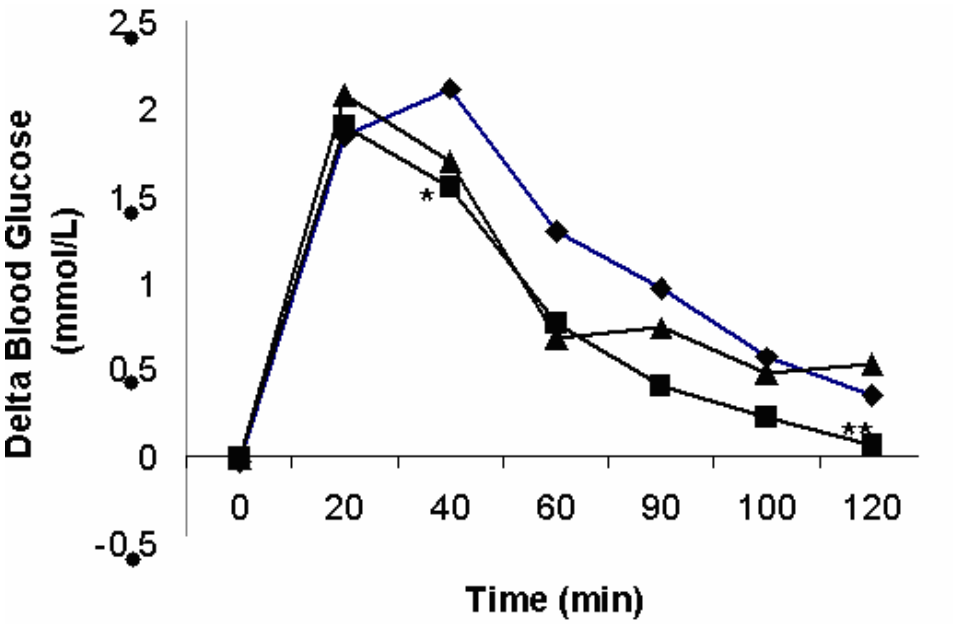

Figure 5. Mean of incremental blood glucose concentrations in 12 healthy subjects after ingesting meals consisting of sour milk with bran flakes (■), cornflakes (♦) or wholemeal oat flakes (▲). Significant differences were calculated with the Wilcoxon t-test. * Response to bran flakes significantly different from that to cornflakes. ** Response to bran flakes significantly different from that to wholemeal oat flakes.

Table 5. Postprandial blood glucose areas under the curve (AUCs) after ingestion of meals consisting of wholemeal oat flakes, bran flakes or cornflakes in 12 healthy subjects (mean ± SEM; n= 12). There were no significant differences between the AUCs.

AUC	Wholemeal oat Flakes (mmol * min/L)	Bran flakes (mmol * min/L)	Cornflakes (mmol * min/L)
0 – 5 min	0.3 ± 0.1	1.3 ± 0.1	0.3 ± 0.1
0 - 25 min	21.2 ± 2.8	19.3 ± 1.4	19.3 ± 3.4
0 – 45 min	58.9 ± 7.1	53.8 ± 3.9	59.2 ± 10.2
0 - 65 min	83.0 ± 12.4	76.9 ± 7.7	93.8 ± 16.9
0 - 85 min	97.8 ± 16.3	88.7 ± 9.9	116.8 ± 21.8
0-105 min	110.1 ± 18.9	96.0 ± 10.4	124.4 ± 26.4
0 - 125 min	120.6 ± 21.5	106.8 ± 12.9	143.0 ± 26.3

5.5 Fibre-rich cereals and gastric emptying rate

The median values of the antral cross-sectional area after ingestion of the cereal bran flakes meal were 641 ± 197 mm² (range 418 to 1035 mm²) (q1= 524 mm², q3= 824 mm²) and 331 ± 253 mm² (range 137 to 924 mm²) at 15 and 90 min, respectively, after the end of the meal. In the same subjects the median values of the antral cross-sectional area after the ingestion of the wholemeal oat flakes meal were 743 ± 240 mm² (range 498 to 1188 mm²) (q1= 568 mm², q3= 1003 mm²) and 331 ± 226 mm² (range 149 to 852 mm²) (q1= 205 mm², q3= 626 mm²) at 15 and 90 min after the end of the meal. In the same subjects the median values of the antral cross-sectional area after the ingestion of the cornflakes meal were 716 ± 187 mm² (range 170 to 740 mm²) (q1= 436 mm², q3= 905 mm²) and 481 ± 227 mm² (range 380 to 1008 mm²) (q1= 251 mm², q3= 495 mm²) at 15 and 90 min after the end of the meal. There were no significant differences between gastric antral cross-sectional areas at 15 and 90 min between the different meals consisting of bran flakes, wholemeal oat flakes and cornflakes (**Paper II**).

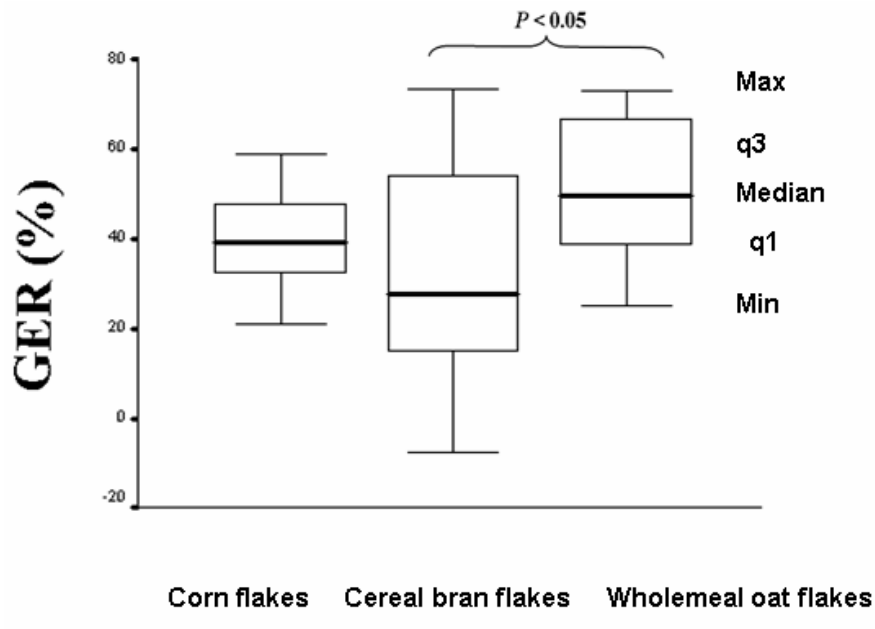

Figure 6. Gastric emptying following meals consisting of sour milk with bran flakes, wholemeal oat flakes or cornflakes, expressed as gastric emptying rate (GER), in 12 healthy subjects. The median, minimum (Min), and maximum (Max) values and the values of the first (q1) and the third (q3) quartiles are shown. The response following the meal with bran flakes was significantly different from that following wholemeal oat flakes.

The median value of GER after the bran flakes meal was estimated to be 28% (range -8% to 73%) (q1= 15%, q3= 56%) compared with the value after the wholemeal oat flakes meal, which was estimated to be 50 % (range 25% to 73 %) (q1= 38%, q3= 70%). The median value of GER after the cornflakes meal was estimated to be 39 % (range 21% to 73 %) (q1= 31%, q3= 49%). The meal containing bran flakes induced a significantly lower GER than that with wholemeal oat flakes meal (P=0.023) (Figure 6). There were no significant differences between cereal bran flakes or wholemeal oat flakes compared to cornflakes with regard to GER.

5.6 Fibre-rich cereals and satiety

Ingestion of cereal bran flakes or wholemeal oat flakes did not result in a significantly higher satiety compared to the reference corn flakes meal (**Paper II**) (Table 6, Figure 7).

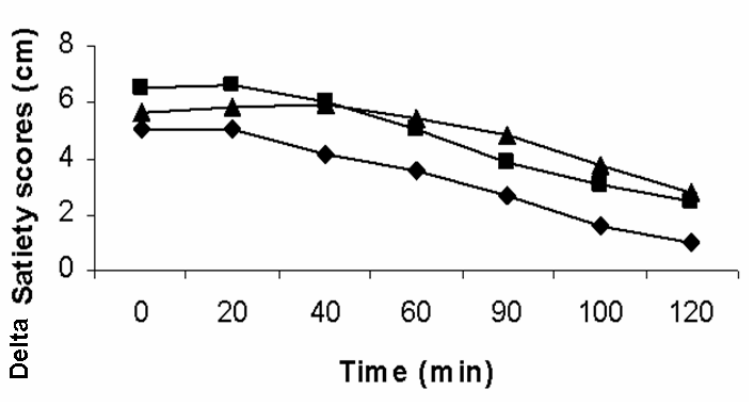

Figure 7. Mean of incremental satiety scores in 12 healthy subjects after ingesting meals consisting of sour milk with bran flakes (■), cornflakes (♦) or wholemeal oat flakes (▲). There were no significant differences between the meals.

Table 6. Areas under the satiety curve after ingestion of meals consisting of wholemeal oat flakes, bran flakes or cornflakes in 12 healthy subjects (mean ± SEM; n= 12). There were no significant differences between the meals.

AUC	Wholemeal oat flakes (cm^2)	Bran Flakes (cm^2)	Cornflakes (cm^2)
0 – 5 min	14.0 ± 2.1	16.4 ± 2.4	12.5 ± 2.5
0 - 25 min	128.1 ± 18.7	148.5 ± 19.4	112.9 ± 21.1
0 – 45 min	244.0 ± 34.7	275.6 ± 34.2	206.2 ± 38.0
0 - 65 min	359.8 ± 45.8	386.4 ± 46.9	289.4 ± 52.6
0 - 85 min	459.8 ± 56.6	466.6 ± 56.1	363.5 ± 65.8
0-105 min	542.3 ± 66.1	544.9 ± 64.9	418.1 ± 75.0
0 - 125 min	602.4 ± 74.6	600.8 ± 12.9	454.6 ± 80.1

5.7 Oat ß-glucan and postprandial blood glucose response

The blood glucose levels at 30 min and the AUC (0-30) min were significantly lower after the ingestion of muesli with 4 g oat β-glucan than muesli with cornflakes (**Paper III**) (*P*=0.045) (Figure 8). However, the glucose levels at 60 min and the AUC (0-60) min did not differ significantly between the muesli with oat β-glucan and the muesli with cornflakes (Table 7).

Table 7. Postprandial blood glucose levels (expressed as the area under the curve, AUC) after ingestion of meals consisting of yoghurt and muesli with 4 g oat β-glucan or cornflakes in 12 healthy subjects (mean ± SEM; n= 12).

AUC	Oat flakes (β-glucan) mmol * min/L	Cornflakes mmol * min/L
0- 30 min	17.1 ± 3.7*	25.0 ± 4.3
0- 60 min	39.3 ± 7.8	54.8 ± 14.9

*Significantly different from cornflakes, *P*=0.045.

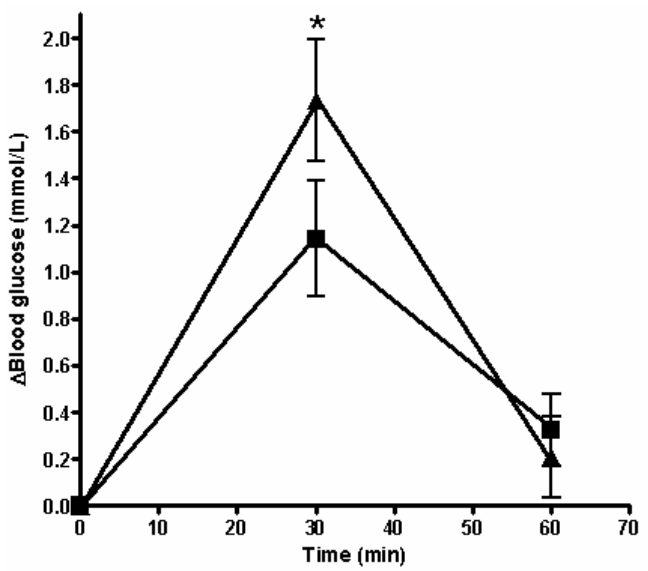

Figure 8. Mean values (±SEM) of the incremental blood glucose concentrations in 12 healthy subjects after ingesting meals consisting of yoghurt with muesli containing oat β-glucan (■) or cornflakes (▲). * Significantly different, *P*=0.045.

5.8 Oat ß-glucan and gastric emptying rate

The median values of the antral cross-sectional area after ingestion of the muesli with β-glucan were 577 mm² (range 368 to 963 mm²) (q1= 415 mm², q3= 859 mm²) and 213 mm² (range 71 to 585 mm²) (q1= 185 mm², q3= 326 mm²) 15 and 90 min after the end of the study meal, respectively. The median values of the antral cross-sectional area in the same subjects after the ingestion of the meal containing cornflakes were 660 mm² (range 376 to 1289 mm²) (q1= 507 mm², q2= 960 mm²) and 382 mm² (range 198 to 1149 mm²) (q1= 208 mm², q3= 593 mm²) 15 and 90 min after the end of the meal, respectively. The median gastric antral cross-sectional areas were significantly larger after ingestion of the muesli with cornflakes than after ingestion of the muesli with oat bran flakes containing 4 g β-glucan at 15 min (P=0.004) (**Paper III**). However, there was no significant difference between gastric antral cross-sectional areas at 90 min. The median value of the GER after the meal with 4 g β-glucan was estimated to be 60% (range 80% to 30%) (q1= 48%, q3= 72%), while the corresponding values after the meal with cornflakes were 44% (range 74% to -8 %) (q1= 5%, q3= 67%). This difference was not significant (Figure 9).

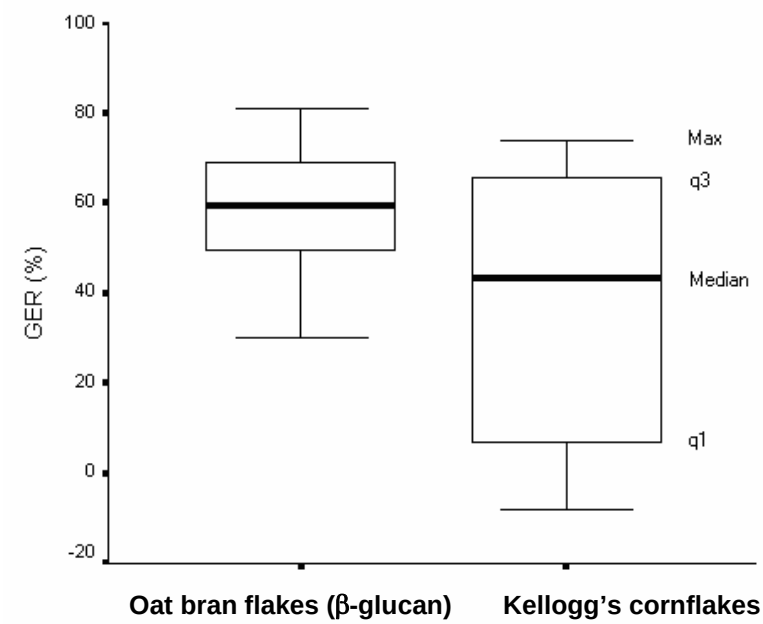

Figure 9. Gastric emptying of meals consisting of yoghurt with muesli containing oat flakes (β-glucan) or cornflakes, estimated as gastric emptying rate (GER), in 12 healthy subjects. The GER is expressed as the percentage change in the antral cross-sectional area between 15 and 90 min. The median, minimum (Min), and maximum (Max) values and the values of the first (q1) and the third (q3) quartiles are shown. There were no significant differences between the GERs.

5.9 Oat ß-glucan and satiety

The mean change in satiety after the ingestion of muesli with 4 g β-glucan was 2.7 ± 0.9 cm (range 0-10 cm); the corresponding values after the ingestion of muesli with cornflakes were 3.2 ± 0.5 cm (range 0-6 cm). Thus, the ingestion of muesli with 4 g β-glucan did not result in a significantly longer period of satiety than the reference meal, i.e. muesli with cornflakes.

5.10 Fibre, vinegar and postprandial blood glucose

No significant differences were seen in blood glucose responses at different times, or in the incremental areas under the postprandial glucose curves between the different bread meals (**Paper IV**) (Figure 10). The mean blood glucose AUC 0-120 min after ingestion of the reference meal of white wheat bread was 147 ± 14 mmol min/L. The AUCs after ingestion of vinegar together with white wheat bread, wholemeal bread or whole-kernel bread were 114 ± 12, 110 ± 10 and 135 ± 13 mmol min/L, respectively. The blood glucose AUCs did not differ significantly between the meals, (P=0.13 in the test of the global hypothesis). The inclusion of BMI as a covariate in the analysis of the postprandial blood glucose did not improve the model.

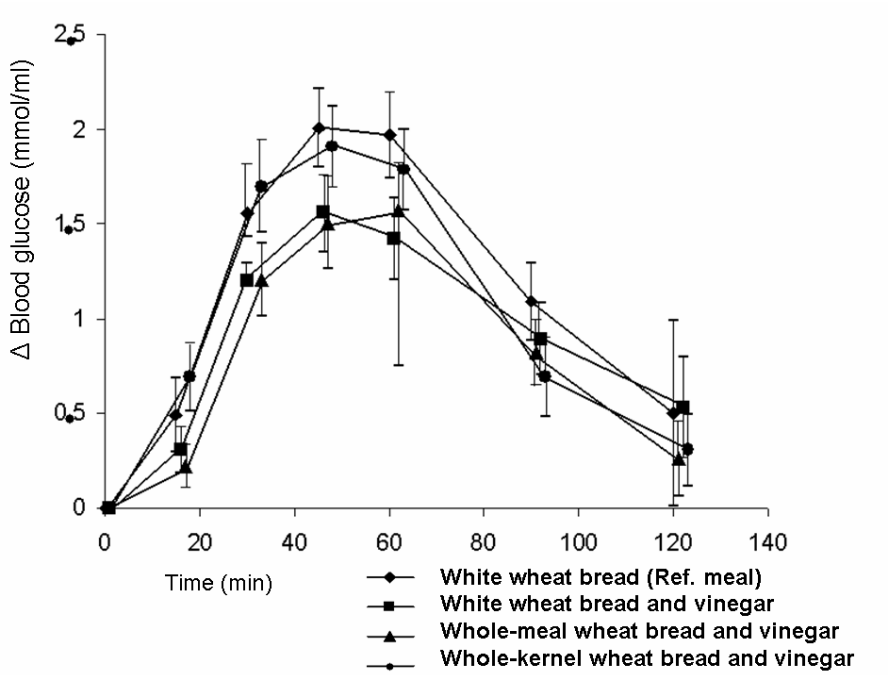

Figure 10. The mean (±SEM) incremental blood glucose concentration in 13 healthy subjects after the ingestion of meals consisting of white wheat bread only (reference) (♦), white wheat bread with vinegar (■), wholemeal wheat bread with vinegar (▲) and whole-kernel bread with vinegar (•). No significant differences were found following the various meals.

5.11 Fibre, vinegar and gastric emptying

No significant differences were observed between the meals with regard to gastric emptying rates (**Paper IV**) (Figure 3). The median value of the GER after the reference meal was estimated to be 51% (q1= 40%, q3= 61%) compared with the corresponding value after the reference meal with vinegar, which was estimated to be 47% (q1= 36%, q3= 56%). The median value of the GER after the wholemeal wheat bread with vinegar meal was estimated to be 62% (q1= 39%, q3= 74%), which can be compared with the median value of the GER after the whole-kernel wheat bread with vinegar meal, of 43% (q1= 39%, q3= 53%).

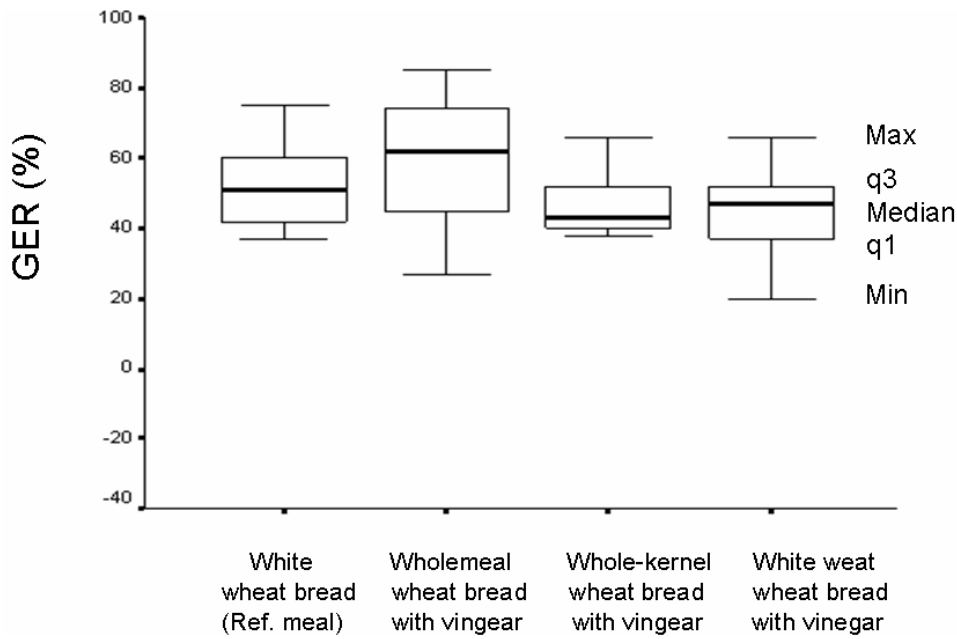

Figure 11. Gastric emptying rate following the ingestion of white wheat bread with vinegar, wholemeal bread with vinegar, whole-kernel wheat bread with vinegar and white wheat bread (reference), in 13 healthy subjects. The median, minimum (Min), and maximum (Max) values and the values of the first (q1) and the third (q3) quartiles are shown. No significant differences were found between the GERs.

The median values of the antral cross-sectional area after the ingestion of the reference meal were 525 mm^2 (q1= 431 mm^2, q3= 707 mm^2) and 295 mm^2 (q1=193 mm^2, q3=364 mm^2) 15 and 90 min, respectively, after the end of the meal. The median values of the antral cross-sectional area after the ingestion of the reference meal with vinegar were 607 mm^2 (q1=607 mm^2, q3= 1092 mm^2) and 317 mm^2 (q1=264 mm^2, q3=507 mm^2), 15 and 90 min, respectively, after the end of the meal. The median values of the antral cross-sectional area after the ingestion of the wholemeal wheat bread with vinegar were 660 mm^2 (q1= 531 mm^2, q3= 885 mm^2) and 266 mm^2 (q1= 166 mm^2, q3=422 mm^2), respectively, 15 and 90 min after the end of the meal. After the ingestion of the whole-kernel wheat bread with vinegar the median values of the antral cross-sectional area were 857 mm^2 (q1=657 mm^2, q3= 1057 mm^2) and 477 mm^2 (q1= 329 mm^2, q3= 558 mm^2), respectively, 15 and 90 min after the end of the meal. The median value of the early antral cross-sectional area after the whole-kernel wheat bread with vinegar (857 mm^2) was significantly larger ($P<0.05$ in a pairwise comparison using the Wilcoxon rank sum test after the global Friedman rank sum test being significant $P=0.0022$) than the corresponding area after ingestion of the reference meal (525 mm^2).

5.12 Fibre, vinegar and satiety

Ingestion of the whole-kernel wheat bread with vinegar resulted in significantly higher satiety scores at 15, 30, 45, 60 and 90 min than white wheat bread with vinegar and the reference meal, white wheat bread without vinegar (**Paper IV**) (Figure 12). Ingestion of whole-kernel wheat bread with vinegar resulted in significantly prolonged satiety, i.e. a higher AUC from 0-120 min, compared with the other bread meals (white wheat bread with vinegar, wholemeal wheat bread with vinegar and the reference white wheat bread). The mean AUC for satiety score after ingestion of the reference meal, i.e. white bread, (AUC from 0-120 min) was 333 ± 56 cm min. The corresponding values after ingestion of the test

meals with vinegar were higher: 393 ± 79 cm min for white wheat bread with vinegar, 501 ± 80 cm min for wholemeal bread with vinegar, and 795 ± 82 cm min for whole-kernel wheat bread with vinegar. There was no significant correlation between the satiety with antral areas or GER.

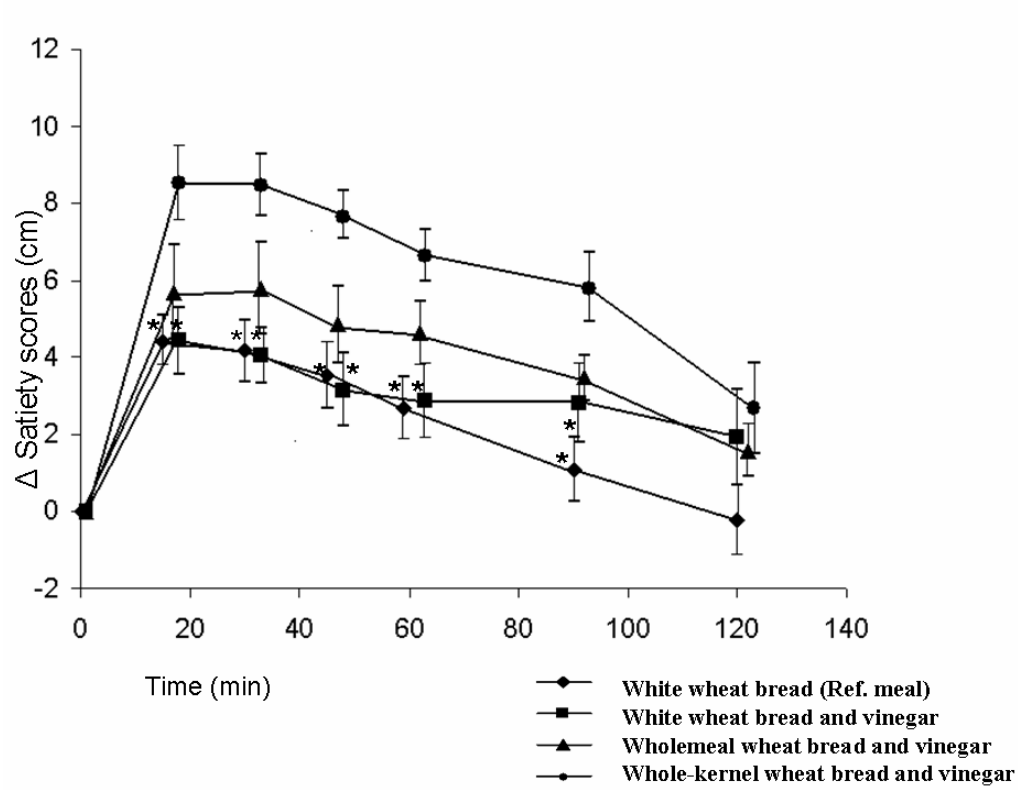

Figure 12. The mean (±SEM) incremental satiety scores reported by 13 healthy subjects after the ingestion of meals consisting of white wheat bread (reference) (♦), white wheat bread with vinegar (■), wholemeal bread with vinegar (▲), and whole-kernel wheat bread with vinegar (•). * Significantly different from the response to whole-kernel bread with vinegar, $P<0.05$.

5.13 Vinegar and fasting blood glucose levels

The mean fasting blood glucose level before the reference meal was 6.9 ± 0.6 mmol/L and before ingestion of the meal including vinegar 7.3 ± 0.5 mmol/L. The difference was not significant.

5.14 Vinegar and gastric emptying rate

The median values of the antral cross-sectional area after ingestion of the meal including vinegar were 888 mm^2 (range 654 to 1626 mm^2, q1= 694 mm^2, q3= 1230 mm^2) and 786 mm^2 (range 447 to 1851 mm^2, q1= 586 mm^2, q3= 959 mm^2) at 15 and 90 min, respectively, after the end of the meal, compared to 866 mm^2 (range, 602 to 1710 mm^2, q1= 725 mm^2, q3= 1071 mm^2) and 611 mm^2 (range, 295 to 1709 mm^2, q1= 561 mm^2, q3= 760 mm^2) at 15 and 90 min, respectively, after the end of the reference meal. The median gastric antral cross-sectional areas were significantly larger after ingestion of the rice pudding meal including vinegar than after the reference meal with water at 90 min (P=0.017), but there were no significant differences between gastric antral cross-sectional areas at 15 min (**Paper V**). The median value of the GER after the meal including vinegar was 17% (range –55% to 43%, q1= –9%, q3= 32%), while the median value of the GER after the reference meal was 27% (range –11% to 51%, q1= 5%, q3= 41%). Gastric emptying rates after the meal including vinegar were significantly lower than after the reference meal (P=0.047) (Figure 14). Individual values of GER indicated reduced values in all patients except two, after drinking apple cider vinegar.

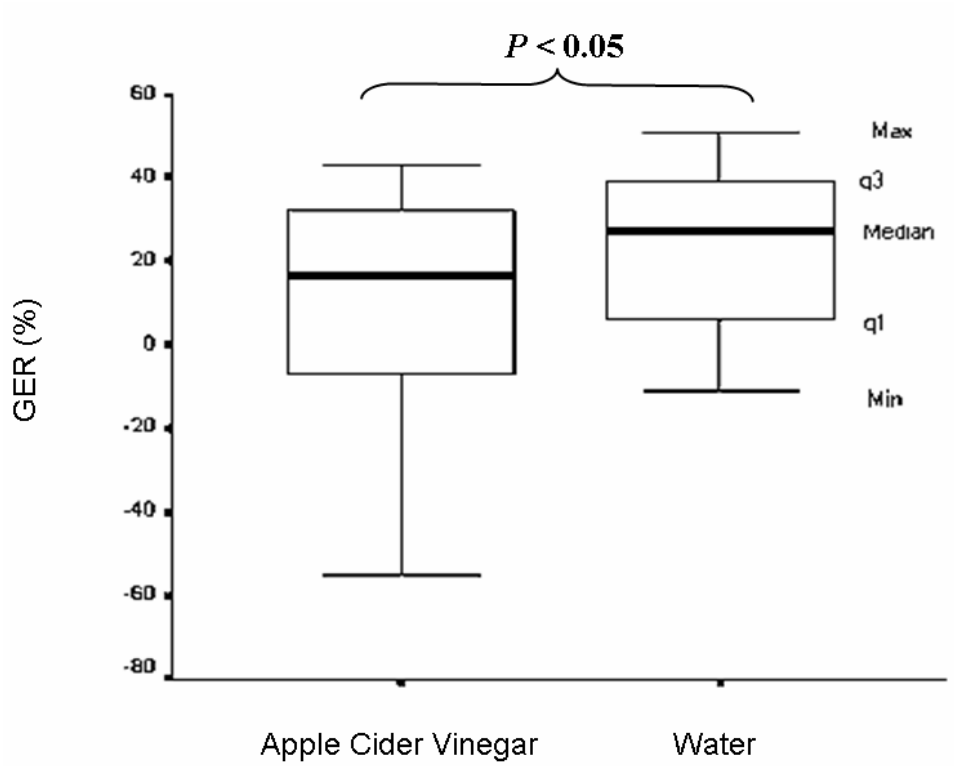

Figure 14. Gastric emptying of a rice pudding meal ingested with and without apple cider vinegar, expressed as the gastric emptying rate (GER), in 10 type 1 diabetics with clinically diagnosed diabetic gastroparesis. The median, minimum (Min), and maximum (Max) values and the values of the first (q1) and the third (q3) quartiles are shown.

Chapter 6

Discussion

The aim of the studies described in this thesis was to find products that reduce the change in postprandial blood glucose levels in order understand how to improve glycaemic control in people with diabetes. Based on the high prevalence of delay in gastric emptying in patients with diabetes in consideration, the effects on gastric emptying in relation to postprandial blood glucose were studied. The satiety was also evaluated in order to identify products that increased postprandial satiety. An understanding of food factors affecting postprandial satiety may lead to low-energy products that can help reduce overweight, obesity and type 2 diabetes.

6.1 The effects of cinnamon

Ingestion of 6 g cinnamon together with rice pudding reduces the postprandial blood glucose concentration and GER in healthy subjects. This could indicate that the reduction in the postprandial blood glucose response may be partly explained by an accompanying reduction in gastric emptying rate. However, the reduction in blood glucose concentration was much more pronounced in the present study than was the lowering of the GER, which was unexpected (**Paper I**). Therefore, the change in GER can not be the only reason for the lower blood glucose

response after the addition of cinnamon to the meal. In fact, cinnamon has been shown to improve insulin receptor function in rats, which leads to enhanced cellular glucose uptake (104, 105). A recent short-term study in healthy subjects revealed lower postprandial blood glucose levels and improved insulin sensitivity when 5 g cinnamon was taken 12 hours before, or ingested with, an oral glucose tolerance test (113). A long-term study of non-diabetic women with polycystic ovary syndrome also revealed a significant reduction in insulin resistance after the intake of 1 g cinnamon for 8 weeks before an oral glucose test was performed (112). However, a recent meta-analysis of five long-term studies of type 1 and type 2 diabetes subjects showed that the intake of cinnamon did not significantly improve HbA1c, fasting blood glucose, or lipid parameters (111). It is therefore still unknown whether the intake of cinnamon can help prevent diabetes.

The median gastric antral cross-sectional area was significantly larger 90 min after the ingestion of rice pudding with added cinnamon than after the ingestion of rice pudding without cinnamon (**Paper I**). Gastric distension has been shown to cause postprandial fullness and satiety (93-100). However, the data suggest slightly greater satiety at each time after ingestion of cinnamon, but no statistically significant differences were found, probably because of the small number of study subjects.

6.2 The effects of fibre-rich breakfast cereals

The study presented in **Paper II** shows that the presence of fibre in a semi-solid meal does not affect the total postprandial blood glucose or satiety responses in healthy subjects, despite the fact that a delay was observed in gastric emptying for the product containing the higher amount of fibre (bran flakes). This study was designed to evaluate the effect of commercial breakfast cereals on blood glucose, satiety and GER. The postprandial glucose response was reduced during the initial postprandial phase after the bran flakes meal compared to the cornflakes meal.

Similar results have been presented previously (157). It was shown in the same study that this lower postprandial blood glucose response was related to a higher initial postprandial plasma insulin response after a meal composed of 119.2 g cereal bran flakes compared to a meal of 60.9 g cornflakes. However, the total postprandial insulin AUC was identical for the two meals, and gastric emptying was not measured. The bran flakes meal contained a smaller amount of carbohydrates than the cornflakes meal in the study presented in **Paper II**. However, if the same amount of carbohydrates had been used in each meal in the present study, the difference in energy would have had a larger influenced on the GER results as, an increase in the caloric value of a meal can delay the GER (158). The bran flakes meal had the lowest total caloric value, 298 kcal, compared to the other meals, both with 320 kcal. Still, the difference in GER was only significant between the bran flakes meal and the wholemeal oat flakes, probably due to the higher amount of fibre in the bran flakes meal. The glucose response was also reduced at the end of the postprandial phase after the cereal bran flakes meal compared to the wholemeal oat flakes meal, which could be related to the lower GER.

According to previous studies, oat β-glucan reduces postprandial glucose and insulinaemic responses in type 2 diabetes and in non-diabetic subjects (120-129). A dose–response relation between the amount of oat β-glucan and the decrease in glucose and insulin levels has also been observed in healthy subjects (122, 124, 127) and in type 2 diabetes subjects (121). The wholemeal oat flakes meal contained only 0.5 g β-glucan. The amount of β-glucan was thus probably too small to affect the blood glucose response in the study presented in **Paper II**. However, the results presented in **Paper III** suggest that the presence of 4 g of oat β-glucan in a semi-solid meal does not significantly affect the GER or the satiety in healthy subjects. Thus, the reduction in postprandial glucose response after the 4 g oat β-glucan meal in healthy subjects could not be explained by

delayed GER. A significantly lower blood glucose level was found 30 min after the end of the oat β-glucan meal than after the cornflakes meal. However, no significant difference was found between the meals regarding blood glucose 60 min after the end of the meals. Unfortunately, the blood glucose levels were not measured more frequently, and the effect on the postprandial blood glucose was therefore not adequately evaluated in this study. The peak glucose concentration may have been missed in some of the subjects in this study. However, the same muesli as was used in this study, containing 4 g oat β-glucan, has previously been shown to reduce the postprandial glucose and insulin levels in healthy subjects (124).

No significant difference in satiety was observed despite delayed gastric emptying after the cereal bran flakes meal compared to the wholemeal oat flakes meal (**Paper II**). Neither were any significant differences found between gastric antral cross-sectional areas at 15 or 90 min between the different meals.

The median antrum area 15 min after the intake of the meal containing cornflakes was significantly larger than that after the intake of the meal containing β-glucan (**Paper III**). This is probably due to the larger volume of the cornflakes than the oat bran flakes. The antrum area at 90 min, the GER and satiety after the meals were not significantly different. However, a limitation of this study is that the satiety was not evaluated more frequently.

6.3 The effects of fibre and vinegar

The aim of the study presented in **Paper IV** was to evaluate the effect of maintaining the botanical structure and dietary fibre present in wheat-based bread products in combination with vinegar, on gastric emptying rate, glycaemic response and satiety in healthy subjects. The hypothesis was that an intake of

intact cereal kernels with or without vinegar would increase satiety and lower the postprandial blood glucose response due to delayed gastric emptying. This hypothesis could not be verified. The results showed a significant increase in satiety after ingestion of the whole-kernel wheat bread with vinegar compared to the other meals, but no statistically significant differences were seen in gastric emptying rate or postprandial blood glucose response.

The lack of difference in postprandial blood glucose response between the bread meals was most unexpected as, in a previous study using the same bread recipes but without vinegar, a significantly lower blood glucose response was observed after eating whole-kernel wheat bread than after white wheat bread (155). The botanical integrity of the grain kernels may have been destroyed unintentionally during the baking process, which would explain the observations in the present study. However, the structure of the bread was not investigated. The lack of difference in GER between the bread meals is in agreement with results obtained by Juntunen et al., who compared whole-kernel rye bread and wholemeal rye bread to white wheat bread, despite the known difference in insulin response between rye and wheat (115).

Another intention of the current study was to evaluate the effect of vinegar on blood glucose response and gastric emptying. However, the lowering effect of vinegar on blood glucose response previously reported with white wheat bread (142, 146) and potatoes (145) was not seen in the present study. The lack of differences in postprandial blood glucose after the ingestion of meals to which vinegar had been added is in agreement with the findings of a study performed by Johnston et al., who found reported that the postprandial blood glucose response was not affected in healthy subjects when apple cider vinegar was consumed prior to the meal (147). However, they demonstrated that the postprandial insulin response was reduced (147). When apple cider vinegar was ingested prior to a

low-glycaemic meal the postprandial insulin response was lower, but no effect was observed on the blood glucose response in healthy subjects (148). However, ingestion of apple cider vinegar prior to a high-glycaemic meal reduced the postprandial blood glucose and insulin response in healthy subjects (148).

The findings of the present study, namely, that there was no difference in gastric emptying rate after a meal including vinegar, agree with those of Brighenti et al., who found no difference in gastric emptying, measured by ultrasonography, after a meal with white vinegar, although the blood glucose response was reduced in healthy subjects (141). Another study showed that adding white vinegar to a bread meal lowered the postprandial blood glucose and insulin responses in healthy subject, and this was explained by delayed gastric emptying (142). However, gastric emptying was measured indirectly using paracetamol, which is a less reliable method (159, 160). The paracetamol method is dependent on the release and absorption of paracetamol across the small intestine which makes this method unreliable, as the pharmacokinetics of paracetamol vary within and between individuals (161, 162).

The antral cross-sectional area was significantly larger 15 min after the ingestion of whole-kernel wheat bread with vinegar than after the ingestion of the white wheat reference bread. Thus, the distension of the antrum may explain the increase in satiety scores reported after the whole-kernel wheat bread meal with vinegar compared to the other bread meals with vinegar. However, the antral cross-sectional area was not correlated to the satiety. The effects of whole-kernal bread without vinegar were not investigated, but as both the white wheat reference bread and the whole-kernal bread were given with vinegar, the differences can be assumed to be the result of the different kinds of bread. This study thus indicates that the botanical structure rather than the amount of fibre per se causes distension of the antrum and increased satiety. This relationship

between antral area and satiety in healthy subjects has been observed previously by others (93-100). Holt et al. have also reported an association between the particle size of wheat and satiety (163). The present finding that there was no difference in satiety after a meal including vinegar disagrees with those of a previous study showing that white wheat bread served with white vinegar not only increased but also prolonged satiety in healthy subjects (146). A dose–response relationship was also found between the amount of vinegar added and the level of satiety (146).

6.4 The effect of vinegar on gastric emptying rate

The primary aim of the study described in **Paper V** was to determine whether the ingestion of vinegar improved the gastric emptying rate of diabetes mellitus patients. Despite the fact that only ten patients with type 1 diabetes mellitus and diabetic gastroparesis were studied, a significant delay in the already delayed gastric emptying of these subjects was demonstrated after the ingestion of vinegar. The median gastric antral cross-sectional areas were significantly larger after the ingestion of the meal including vinegar than after the reference meal at 90 min. The subject that had undergone vagotomy responded in the same way as the others, showing a further reduction in the GER after drinking apple cider vinegar. The subject who was already consuming vinegar daily before the start of the study also showed a reduced GER after drinking apple cider vinegar.

It was hypothesized that daily consumption of vinegar would affect diabetes patients with gastroparesis by increasing the gastric emptying rate. However, the effect observed in this study was the opposite. A limitation of this study is that it was not randomized and, unfortunately, the postprandial blood glucose levels were not measured. The examination was performed only if the fasting blood glucose level was in the range 3.5 to 9.0 mmol/L; none of the subjects reported any symptoms of hypoglycaemia on the day of the ultrasonography examination.

The mean fasting blood glucose level before the reference meal was not significantly different from that before ingestion of the meal including vinegar.

The mechanism by which vinegar reduces postprandial blood glucose levels has been suggested to be a delay in gastric emptying, measured indirectly with paracetamol (142), or by the inhibition of amylases (141). It has been suggested that non-specific acid or pH receptors in the small intestine could reduce the GER (164). The postprandial blood glucose level in healthy subjects was reduced but the GER, measured with ultrasonography, was not affected after a meal including white vinegar, compared with a meal including neutralized vinegar (141).

There were some differences between the results reported in **Papers IV** and **V** that may have influenced the effect of vinegar on GER. The subjects with insulin-dependent diabetes mellitus ingested the vinegar with water before the study meal, while the healthy subjects ingested the vinegar with the meal. It is known that emptying of liquids from the stomach begins immediately and that there is a lag phase before a semi-solid meal starts to leave the stomach. In diabetic gastroparesis both liquids and solids are subject to delayed gastric emptying. However, after a vagotomy the liquids empty rapidly but the solids empty slowly. Therefore, there may be a difference in the time taken for the vinegar to reach the duodenum in the studies presented in **Papers IV** and **V.** The gastric emptying rate may also be modified in the study described in **Paper IV** by the daily intake of vinegar. A number of studies have shown that adaptive diet-induced changes may affect gastric emptying (165-168). There may also be a difference in the way in which vinegar affects the pH in the stomach in healthy persons and in patients with type 1 diabetes, as the latter often suffer from achlorhydria (169). The vinegar used in the two studies described in **Papers IV** and **V** were not the same. White vinegar was used in the former and apple cider vinegar in the latter. The healthy subjects thus ingested white vinegar and the subjects with diabetes

mellitus ingested apple cider vinegar. However, these two vinegars and the amounts ingested can be considered equal.

The various limitations of the studies have already been discussed. However, there are some general limitations of the present work. We did not monitor the subjects' choice of food or amount of exercise the night before the tests. This may have affected the postprandial blood glucose responses. Another limitation of the present work is that the sample size was small. Clearly, larger trials involving a greater number of subjects are needed to validate the findings of these studies.

Chapter 7

Conclusions

The presence of 6 g cinnamon in a semi-solid meal was found to reduce postprandial glucose response in healthy subjects, and this reduction could be partly due to delayed GER.

The intake of equal amounts of either bran flakes or wholemeal oat flakes containing 0.5 g oat β-glucan had no effect on the total postprandial blood glucose response, GER or satiety compared with cornflakes. Eating muesli containing 4 g oat β-glucan in a semi-solid meal did not affect the GER or satiety in healthy subjects compared to cornflakes. A reduction in postprandial glucose response was seen in healthy subjects after the meal containing 4 g oat β-glucan muesli, compared to muesli with cornflakes, but this cannot not be explained by a slower GER.

Ingesting vinegar does not affect the GER or postprandial blood glucose response in healthy subjects. However, vinegar delays gastric emptying in insulin-dependent diabetes mellitus patients with diabetic gastroparesis.

The postprandial ratings of satiety were higher after the whole-kernel wheat bread meal with vinegar than after meals of wholemeal wheat bread with vinegar, white wheat bread with vinegar or a reference meal of white wheat bread without

vinegar. This may be explained by increased antral distension after the ingestion of intact cereal kernels.

The amount of fibre or botanical structure in whole-kernel wheat bread or wholemeal wheat bread does not affect the GER or postprandial blood glucose response in healthy subjects compared with white wheat bread.

These studies show the complexity of satiety. Not only does gastric distension by food play a role, but there is probably an interaction between the nutritional components of the meal, hormonal responses and receptors in the upper gut and the brain leading to the sensation of satiety. The results in this thesis open for further studies of the effect of different food factors effect on gastric emptying, postprandial blood glucose response and satiety, in both healthy subjects and patients with diabetes, in order to improve glycaemic control in people with diabetes.

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I

Effect of cinnamon on postprandial blood glucose, gastric emptying, and satiety in healthy subjects[1–3]

Joanna Hlebowicz, Gassan Darwiche, Ola Björgell, and Lars-Olof Almér

ABSTRACT

Background: Previous studies of patients with type 2 diabetes showed that cinnamon lowers fasting serum glucose, triacylglycerol, and LDL- and total cholesterol concentrations.

Objective: We aimed to study the effect of cinnamon on the rate of gastric emptying, the postprandial blood glucose response, and satiety in healthy subjects.

Design: The gastric emptying rate (GER) was measured by using standardized real-time ultrasonography. Fourteen healthy subjects were assessed by using a crossover trial. The subjects were examined after an 8-h fast if they had normal fasting blood glucose concentrations. GER was calculated as the percentage change in the antral cross-sectional area 15–90 min after ingestion of 300 g rice pudding (GER1) or 300 g rice pudding and 6 g cinnamon (GER2).

Results: The median value of GER1 was 37%, and that of GER2 was 34.5%. The addition of cinnamon to the rice pudding significantly delayed gastric emptying and lowered the postprandial glucose response ($P < 0.05$ for both). The reduction in the postprandial blood glucose concentration was much more noticeable and pronounced than was the lowering of the GER. The effect of cinnamon on satiety was not significant.

Conclusions: The intake of 6 g cinnamon with rice pudding reduces postprandial blood glucose and delays gastric emptying without affecting satiety. Inclusion of cinnamon in the diet lowers the postprandial glucose response, a change that is at least partially explained by a delayed GER. *Am J Clin Nutr* 2007;85:1552–6.

KEY WORDS Gastric emptying, blood glucose, healthy subjects, cinnamon, diabetes, satiety

INTRODUCTION

Around the world, the incidence of type 2 diabetes mellitus is increasing rapidly. Changing the diet helps to prevent development of type 2 diabetes and to control blood glucose concentrations. Traditional herbs and spices also can be used to control blood glucose concentrations. Allspice, cinnamon, bay leaf, cloves, nutmeg, witch hazel, oregano, and black and green tea have been shown to have an insulin-like biological activity (1). Of these substances, cinnamon has been shown to have the highest bioactivity (1). A water-soluble polyphenol typ-A polymer from cinnamon has been isolated and shown in vitro to have insulin-like activity as well as an antioxidant effect (2). Cinnamon has been shown to reduce fasting serum glucose, triacylglycerol, and total and LDL-cholesterol concentrations in patients with type 2 diabetes when it is added to the diet for 40 d in doses of 1, 3, or 6 g (3). The same study showed that, after the consumption of cinnamon for 40 d, the serum concentrations of glucose and triacylglycerol remained lower, even after a 20-d washout period (3), which indicated that it is not necessary to consume cinnamon every day (3).

The effect of cinnamon on gastric emptying has not previously been studied. Gastric emptying, along with other factors, regulates the postprandial blood glucose response, and a delay in the gastric emptying rate (GER) leads to a lower postprandial blood glucose concentration. Therefore, this study was designed to determine whether there is a delay in gastric emptying that affects postprandial blood glucose concentrations and satiety in healthy subjects after cinnamon consumption.

SUBJECTS AND METHODS

Fourteen healthy subjects [8 M, 6 F; $\bar{x} \pm$ SD age: 25.6 ± 4.8 y (range: 20–38 y); body mass index (BMI; in kg/m^2): 22.6 ± 2.2 (range: 18.4–26.0)] without symptoms or a history of gastrointestinal disease, abdominal surgery, or diabetes mellitus were included in the crossover study. The subjects had no connective tissue disease or cerebrovascular or endocrine disease, and only the 4 women who took birth control medication were receiving any drugs. Two subjects were smokers and 2 were snuff users. All subjects were recruited from the population in southern Sweden.

The subjects were examined between 0730 and 1000 after an 8-h fast. Smoking and snuff-taking were prohibited for 8 h before and during the test. Each subject was checked for a normal fasting blood glucose concentration on the day of the examination. If the subjects reported symptoms from the gastrointestinal tract (diarrhea or constipation) on the study day, the examination was postponed. The test meal consisted of 300 g rice pudding (Axa Goda Gröten Risgrynsgröt; Lantmännen AXA, Järna, Sweden) mixed with 6 g cinnamon (Santa Maria AB, Mölndal, Sweden). The total caloric value was 330 kcal: 10% of energy from protein (3 g), 58% of energy from carbohydrate (16 g), and 32% of energy from fat (4 g). The reference meal consisted of 300 g rice

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pudding (Axa Goda Gröten Risgrynsgröt). The meals were served in a random order and ingested within 5 min.

GER was estimated by using a previously described, standardized ultrasound method (4) The sonographic examination was performed with a 3.5-MHz abdominal transducer and an imaging system (Acuson 128 XP 10; Siemens Medical Solutions, Mountain View, CA). The measurements of the gastric antrum were performed by the same radiologist, who was blinded with respect to the meals. At each observation of the gastric antrum, the abdominal aorta and the left lobe of the liver were used as internal landmarks. The subjects were examined in a supine position, but, between examinations, they were seated. The measurements were taken 15 and 90 min after the end of meal consumption. Gastric emptying was expressed as the percentage change of the antral cross-sectional area from 15 to 90 min. At each examination, 3 measurements of the longitudinal (d1) and anteroposterior (d2) diameters were taken, and mean values were used to calculate the cross-sectional area of the gastric antrum by using the following equation:

$$\text{Antral area} = \pi \times r^2 = \pi \times d1/2 \times d2/2 = (\pi \times d1 \times d2)/4 \quad (1)$$

The GER was calculated by using the following equation:

$$\text{GER} = [1 - (\text{antral area at 90 min}/\text{antral area at 15 min})] \times 100 \quad (2)$$

Finger-prick capillary blood samples were taken 15, 30, 45, 60, 90, and 120 min after the start of the meal to measure glucose. Blood glucose concentrations were measured with the HemoCue Glucose system (HemoCue AB, Ángelholm, Sweden). A validated satiety score scale was used according to the method of Hauber et al (5) on the basis of a scoring system with grades from −10 (extreme hunger) to 10 (extreme satiety). Satiety scores were estimated before the meal (0 min) and 15, 30, 45, 60, 90, and 120 min after the start of the meal by using scoring that was graded from 0 for extreme hunger to 20 for extreme satiety.

All subjects provided written informed consent. The study was approved by the Ethics Committee at Lund University and performed according to the Helsinki Declaration.

Median values and quartiles (q1 and q3) are presented for the antral cross-sectional areas and the GER. The areas under the curves (AUCs) of each subject were measured for blood glucose and satiety by using GRAPH PAD PRISM software (version 4; GraphPad, San Diego, CA). The AUC was calculated above zero. The AUC values are presented as means ± SEMs. All statistical calculations were performed with SPSS for WINDOWS software (version 14.0; SPSS Institute, Chicago, IL). Significant differences in GER, gastric antral cross-sectional areas, and AUCs were evaluated with the use of Wilcoxon's *t* test. $P < 0.05$ was considered significant.

RESULTS

Postprandial blood glucose response

Ingestion of rice pudding with cinnamon resulted in a significantly ($P < 0.05$) lower blood glucose response in the postprandial phase (15, 30, and 45 min) than did the reference meal

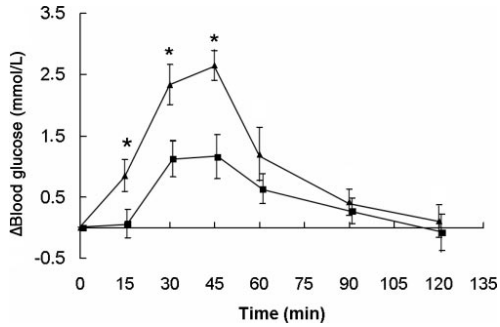

FIGURE 1. Mean (± SEM) incremental blood glucose concentrations in 14 healthy subjects after ingestion of meals consisting of rice pudding with (■) and without (▲) cinnamon. Δ, change. *Significantly different from the response to rice pudding with cinnamon, $P < 0.05$.

(**Figure 1**). The blood glucose AUCs at 0–30, 0–45, 0–60, 0–90, and 0–120 min were significantly ($P < 0.05$) lower after ingestion of rice pudding with cinnamon than after ingestion of the reference meal (**Table 1**). However, the AUCs at 0–15 min did not differ significantly between the test and reference meals (Table 1).

Gastric emptying rate

The median values of the antral cross-sectional area after the ingestion of the cinnamon meal were 595 ± 234 mm^2 (range: 283–1181 mm^2; q1 = 458 mm^2, q3 = 809 mm^2) and 372 ± 366 mm^2 (range: 83–1525 mm^2; q1 = 282 mm^2, q3 = 593 mm^2) 15 and 90 min, respectively, after the end of the study meal. In the same subjects, the median values of the antral cross-sectional area after the ingestion of the reference meal were 531 ± 386 mm^2 (range: 262–1626 mm^2; q1 = 319 mm^2, q3 = 891 mm^2) and 317 ± 338 mm^2 (range: 50–1389 mm^2; q1 = 195 mm^2, q3 = 546 mm^2) 15 and 90 min, respectively, after the end of the meal. The median gastric antral cross-sectional areas were significantly ($P < 0.05$) larger 90 min after ingestion of rice pudding with added cinnamon than 90 min after ingestion of rice pudding. There were no significant differences between gastric antral cross-sectional areas at 15 min. The median value of GER after

TABLE 1
Postprandial blood glucose areas under the curve (AUCs) in healthy subjects after ingestion of meals consisting of rice pudding with or without added cinnamon[1]

AUC	Rice pudding without cinnamon	Rice pudding with cinnamon
	mmol · min/L	
0–15 min	6.8 ± 1.8	3.6 ± 1.0
0–30 min	30.7 ± 5.1	13.7 ± 3.4[2]
0–45 min	68.1 ± 8.2	32.4 ± 6.6[2]
0–60 min	97.2 ± 11.0	47.3 ± 9.2[2]
0–90 min	125.0 ± 16.8	63.3 ± 11.7[2]
0–120 min	139.1 ± 19.6	75.0 ± 13.7[2]

[1] All values are $\bar{x}$ ± SEM; $n = 14$. Significant differences in postprandial blood glucose AUCs were evaluated with Wilcoxon's *t* test.

[2] Significantly different from rice pudding without cinnamon, $P < 0.05$.

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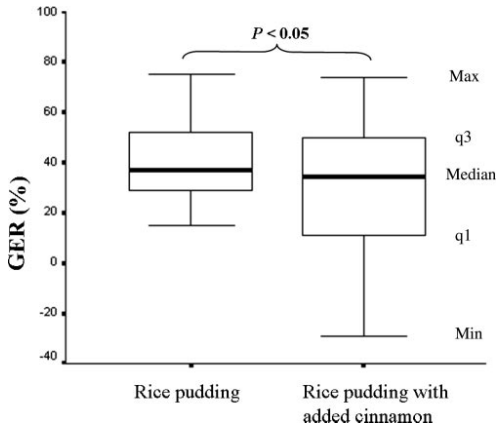

FIGURE 2. Gastric emptying of rice pudding with and without cinnamon, estimated as the gastric emptying rate (GER), in 14 healthy subjects. The median, minimum (Min), and maximum (Max) values and the values of the first (q1) and third (q3) quartiles are shown. Values of $P < 0.05$ (Wilcoxon's *t* test) were considered significant.

the cinnamon meal was estimated at 34.5% (range: −29% to 74%; q1 = 7%, q3 = 52%) (**Figure 2**), whereas that after the reference meal was estimated at 37.0% (range: 15–87%; q1 = 28.8%, q3 = 54%) (Figure 2). The ingestion of cinnamon resulted in significantly ($P < 0.05$) lower GERs.

Satiety

Ingestion of rice pudding with cinnamon did not result in significantly longer satiety (15, 30, 45, 60, 90, and 120 min) than that seen with the reference meal of rice pudding (**Figure 3**). The AUCs at 0–15, 0–30, 0–45, 0–60, 0–90, and 0–120 min for satiety were not significantly longer after ingestion of rice pudding with cinnamon than after ingestion of rice pudding only (**Table 2**).

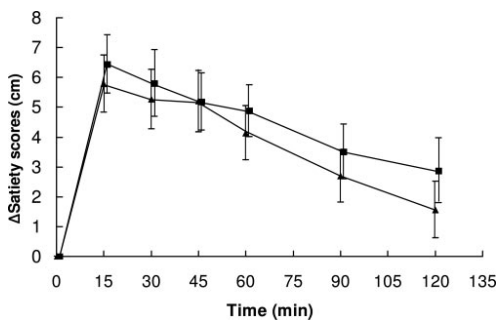

FIGURE 3. Mean (± SEM) incremental satiety scores in 14 healthy subjects after ingestion of meals consisting of rice pudding with (■) and without (▲) cinnamon. Δ, change. There were no significant differences between the mean incremental satiety scores.

TABLE 2
Satiety score areas under the curve (AUCs) in healthy subjects after ingestion of meals consisting of rice pudding with or without cinnamon[1]

AUC	Rice pudding without cinnamon	Rice pudding with cinnamon
	cm · min	
0–15 min	43.4 ± 7.2	48.3 ± 7.3
0–30 min	126.9 ± 21.5	140.8 ± 22.2
0–45 min	207.0 ± 35.3	225.9 ± 35.6
0–60 min	282.6 ± 47.4	302.7 ± 47.2
0–90 min	390.0 ± 68.7	430.7 ± 70.2
0–120 min	466.4 ± 89.8	538.7 ± 92.9

[1] All values are $\bar{x}$ ± SEM; $n = 14$. Significant differences in satiety score AUCs were evaluated with Wilcoxon's *t* test. No significant differences were found between the satiety score AUCs.

DISCUSSION

This study shows that ingestion of 6 g cinnamon reduces postprandial blood glucose concentrations and GER in healthy subjects. This finding could indicate that the reduction in the postprandial blood glucose response seen after the ingestion of cinnamon could be partly explained by an accompanying reduction in gastric emptying, because the rate of gastric emptying acts as a major factor in blood glucose homeostasis in normal subjects by controlling the delivery of carbohydrate to the small intestine (6, 7). However, the reduction in the blood glucose concentrations, unexpectedly, was much more noticeable and pronounced in the present study than was the lowering of the GER. Therefore, it should be assumed that the change in GER itself could not be the only reason for the lower blood glucose response after the addition of cinnamon to the meal. In fact, cinnamon has been shown to improve insulin receptor function by activating insulin receptor PI 3-kinase and inhibiting tyrosine phosphates (8). Cinnamon has also been shown to stimulate the insulin receptor activity by increasing the concentrations of the phosphorylated intracellular protein IRS-1 and increasing the binding to PI 3-kinase, which leads to enhanced cellular glucose uptake (9). It has been shown that cinnamon prevents the development of insulin resistance in rats fed a high-fructose diet by enhancing the insulin signaling, possibly via the nitric oxide pathway in skeletal muscle (10). Essential oils composed of pumpkinseed oil, extra virgin olive oil, oregano, cinnamon, fenugreek, cumin, fennel, myrtle, allspice, and ginger lowered blood glucose concentrations and increased insulin sensitivity in rats (11). There was no significant difference in the concentrations of circulating insulin after the intake of the different essential oils (11). A new study shows that rats given cinnamon and then administered a glucose tolerance test had decreased blood glucose concentrations (12). The same study shows that cinnamon has a direct antidiabetic effect by increasing insulin concentrations in plasma (12).

After meal ingestion, the food initially remains in the proximal part of the stomach (fundus) (13) and is then delivered in portions to the distal stomach (eg, antrum) (14, 15). If there is a substantial inhibition of gastric emptying, one could expect a 90-min antral area wider than a 15-min antral area and thereby a negative GER, which was the case in 3 of the participants.

Prolonged postmeal satiety is in accordance with a reduced GER, because distension of the stomach is one factor that

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promotes a feeling of satiety. The data suggest slightly greater satiety at each timepoint after ingestion of cinnamon, but there were no significant differences in satiety, probably because of the low number of study subjects. Consequently, it is not clear why cinnamon ingestion did not result in a prolongation of postmeal satiety in the present study. An elevation of blood glucose concentrations, even including concentrations within the physiologic range, reduces the GER in healthy subjects (16), and hypoglycemia increases the GER (17, 18). It has also been shown that insulin-induced hypoglycemia increases botht he gastric emptying and the drinking capacity of meat soup in healthy volunteers (19). Accordingly, because the reduction in postprandial blood glucose concentrations was prominent, it may well have influenced the results of the GER, giving a less pronounced reduction in gastric emptying and less postmeal satiety than would otherwise be expected.

To prevent development of diabetes mellitus, the American Diabetes Association recommends a reduction in calories and consumption of dietary fiber and foods containing whole grains (20). Low-glycemic-index food that reduces postprandial hyperglycemia is recommended, but it is not clear whether it prevents diabetes mellitus (21–27). Glycemic index, glycemic load, and carbohydrate consumption are probably not associated with insulin sensitivity, insulin secretion, or adiposity (28). A low-glycemic-index diet can be recommended to control glycemia (29, 30). Patients with diabetes mellitus (type 2 or type 1) are recommended to have 60–70% of energy intake provided by carbohydrates and monosaturated fat (20). There are no recommendations for the intake of herbs, but they may be an important unexplored source of glycemia control in patients with diabetes mellitus type 2. In the present study, the rice pudding with added cinnamon was well tolerated, and none of the subjects reported any discomfort or postprandial nausea. The question of whether cinnamon could be a cause of contact stomatitis was discussed previously (31). However, only a few cases were reported during past years, and this possibility should be evaluated in the future. Whether a continuous daily intake of cinnamon can be recommended also should be further evaluated. The present study was designed to determine the mechanism of cinnamon's effect on postprandial blood glucose concentrations and on gastric emptying.

The present study shows that the presence of cinnamon in a semisolid meal reduces postprandial glucose responses in healthy subjects and that the cause of this reduction could at least partly be a delayed GER. Further investigation of the effect of cinnamon on the insulin resistance in patients with type 2 diabetes mellitus is needed.

The authors' responsibilities were as follows—JH: the design of the study, recruitment of subjects, performance of statistical calculations and creation of the graphs, and drafting of the manuscript; GD: the design of the study, performance of the statistical calculations and creation of the graphs, securing study funding, and drafting of the manuscript; OB: the design of the study and performance of the ultrasound examinations; and LOA: the design of the study and drafting of the manuscript. All authors read and approved the final manuscript. None of the authors had any personal or financial conflict of interest.

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II

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Effect of commercial breakfast fibre cereals compared with corn flakes on postprandial blood glucose, gastric emptying and satiety in healthy subjects: a randomized blinded crossover trial

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Abstract

Background: Dietary fibre food intake is related to a reduced risk of developing diabetes mellitus. However, the mechanism of this effect is still not clear. The aim of this study was to evaluate the effect of commercial fibre cereals on the rate of gastric emptying, postprandial glucose response and satiety in healthy subjects.

Methods: Gastric emptying rate (GER) was measured by standardized real time ultrasonography. Twelve healthy subjects were assessed using a randomized crossover blinded trial. The subjects were examined after an 8 hour fast and after assessment of normal fasting blood glucose level. Satiety scores were estimated and blood glucose measurements were taken before and at 0, 20, 30, 40, 60, 80, 100 and 120 min after the end of the meal. GER was calculated as the percentage change in the antral cross-sectional area 15 and 90 min after ingestion of sour milk with corn flakes (GER1), cereal bran flakes (GER2) or wholemeal oat flakes (GER3).

Results: The median value was, respectively, 42% for GER1, 33 % for GER2 and 51% for GER3. The difference between the GER after ingestion of bran flakes compared to wholemeal oat flakes was statistically significant ($p = 0.023$). The postprandial delta blood glucose level was statistically significantly lower at 40 min ($p = 0.045$) and 120 min ($p = 0.023$) after the cereal bran flakes meal. There was no statistical significance between the areas under the curve (AUCs) of the cereals as far as blood glucose and satiety were concerned.

Conclusion: The result of this study demonstrates that the intake of either bran flakes or wholemeal oat flakes has no effect on the total postprandial blood glucose response or satiety when compared to corn flakes. However, the study does show that the intake of cereal bran flakes slows the GER when compared to oat flakes and corn flakes, probably due to a higher fibre content. Since these products do not differ in terms of glucose response and satiety on healthy subjects, they should be considered equivalent in this respect.

Trial registration: ISRCTN90535566

Background

In Sweden and worldwide the incidence of type 2 diabetes mellitus is increasing rapidly. To prevent the development of diabetes mellitus, the American Diabetes Association recommends a reduction in caloric intake and increased consumption of dietary fibre and food containing whole grain [1]. An increased intake of fibre has been shown to reduce the risk of diabetes [2,3]. Whether low-glycemic-index food in fact prevents diabetes mellitus is still unclear [2,4-7]. However, a low-glycemic-index diet that reduces postprandial hyperglycemia is recommended by the American Diabetes Association (ADA) to control glycemia in patients with diabetes [8,9].

Fibre has been shown to delay gastric emptying rate, reduce the glycemic response and delay the return of hunger in healthy subjects [10]. It has been assumed that fibre fermented in the colon by the bacterial flora releases short chain fatty acids, thus lowering postprandial glucose levels [11-14]. Moreover, this fermentation has shown to result in a suppression of the hepatic glucose production and serum-free fatty acids [15]. Colonic fermentation, measured by breath hydrogen test, has been observed – after a meal consisting of ingestible carbohydrates – to reduce the insulin and glucose response at the following meal. This effect is called a second meal effect. [16]. However, another study showed that meals with what was assumed to be fermentable carbohydrates did not improve glucose or insulin response at the second meal [17]. A recently published study shows that an increased 3-day intake of insoluble fibre in obese subjects improved whole-body insulin sensitivity [18].

The β-glucan effect is not fully understood. Products enriched with β-glucan have been shown to reduce postprandial glucose and insulinemic responses in healthy subjects [19-21] and in type 2 diabetes patients [22-24]. Reduced postprandial glucose and insulin concentrations after consumption of viscous types of fibre have been discussed to be caused by delayed mouth-to caecum transit and delayed absorption of glucose in the small intestine [25]. The viscosity of oat gum, an oat extract composed of β-glucan, has been shown to cause a reduction in plasma glucose and insulin levels [26]. However, lower postprandial glucose and insulin concentrations have not been shown to be caused by the fermentation of β-glucan in the colon [27]. Another mechanism discussed is that β-glucan delays gastric emptying.

Healthy subjects are recommended to consume products with fibre to prevent the development of diabetes mellitus. Also, patients with diabetes consume commercial products with fibre and low glycemic food to control the blood glucose levels. This study was designed to determine whether there is a delay in gastric emptying in healthy subjects, thus affecting postprandial blood glucose levels and satiety, after consumption of commercially popular fibre cereals.

Methods

Twelve healthy subjects (six men and six women; mean age 28 ± 4 years [range 23–36 years]; mean BMI 22 ± 2 kg/m^2 [range 19–24 kg/m^2], without symptoms or a prior history of gastrointestinal disease, abdominal surgery or diabetes mellitus were included in the study. One subject had been appendectomized. None of the subjects used any drugs on the examination day. Three of the women, including one with polycystic ovary syndrome, had birth control medication. The subject with the polycystic ovary syndrome had a BMI 21 kg/m^2 and previously underwent a glucose tolerance test which proved normal. All subjects were recruited from the population of a southern county of Sweden. Four of the subjects were smokers and two were snuff users. The subjects were examined between 8:00 and 10:00 am after an 8 hour fast. Smoking and snuff-taking were prohibited for 8 h before the test as well as during the test. Each subject was checked for normal fasting blood glucose concentration on the day of the examination. For subjects with symptoms from the gastrointestinal tract (diarrhoea or constipation) on the examination day, the examination was postponed. The test meals consisted of 300 g sour milk (Skånemejerier, 205 03 Malmö, Sweden) (caloric value 135 kcal) and 50 g cereal bran flakes (Kellogg's All-Bran, Kellogg's, Sverige Konsumentkontakt, Box 742, 194 27 Upplands Väsby, Sweden) (caloric value 163 kcal) or wholemeal oat flakes (Frebaco Fullkorns Havreringar, Frebaco Kvarn AB, Box 878, 531 18 Lidköping, Sweden) (caloric value 185 kcal). The reference meal, consisting of 50 g corn flakes (Kellogg's Corn Flakes, Nordisk Kellogg's, Sverige Konsumentkontakt, Box 742, 194 27 Upplands Väsby, Sweden) (caloric value 185 kcal), had the same brand and quantity of sour milk as the test meal (Table 1). The meals were served in a random order more than one week apart. Each meal was ingested within 5 minutes.

GER was estimated using a previously described standardised ultrasound method [28]. The sonographic examination was performed using two different ultrasound machines (Siemens Acuson Sequoa 512 and Aloka Prof. Sound) with an abdominal transducer multi-MHz. For every single calculation of GER the same machine was used. The measurements of the gastric antrum were performed by the same radiologist who was blinded with regard to the meals. At each observation of the gastric antrum the abdominal aorta and the left lobe of the liver were used as internal landmarks. The subjects were examined lying down, but they were in upright position between examinations. Measurements were taken 15 and 90 min after the end of meal ingestion. Gastric emptying

Table 1: Nutrient composition of the test product portions.

	Sour Milk	Frebaco Wholemeal Oatflakes	Kellogg's All-Bran Regular Flakes	Kellogg's Cornflakes
	300 g	50 g	50 g	50 g
Total energy (kcal)	135	185	163	185
Total protein (g)	12	6	5	3.5
Total fat (g)	1.5	2	1	0.35
Total Carbohydrate (g)	18	35.5	33.5	42
Sugar (g)	15	0.75	11	4
Total Fibre (g)		4	7.5	1.5
β-glucan (g)		0.5		

was expressed as the percentage change of the antral cross-sectional area from 15 to 90 min. At each examination three measurements of the longitudinal (d1) and antero-posterior (d2) diameters were performed and mean values were used to calculate the cross-section area of the gastric antrum using the following formula:

$$\text{Antrum area} = \pi \times r^2 = \pi \times d1/2 \times d2/2 = \pi \times d1 \times d2/4$$

The gastric emptying (GER) was calculated using the following formula:

$$\text{GER} = [1 - (\text{Antrum area 90 min}/\text{Antrum area 15 min})] \times 100$$

Finger-prick capillary samples were taken before and at 0, 20, 30, 40, 60, 80, 100 and 120 min after the end of the meal to measure glucose. Blood glucose concentrations were measured with HemoCue Glucose system (HemoCue AB, Ängelholm, Sweden). A validated satiety score numerical scale was used according to the method of Haber et al on the basis of a scoring system with grades from -10 cm (extreme hunger) to 10 cm (extreme satiety) [29]. Satiety score was estimated before the meal and at 0, 20, 30, 40, 60, 80, 100 and 120 min after the end of the meal.

All subjects provided written informed consent. The study was approved by the Ethics Committee at Lund University and performed according to the Helsinki Declaration.

Median values with quartiles (q1 to q3) are presented for the antral cross-sectional areas and the GER. Delta values of blood glucose and satiety scores are calculated as changes at 0, 20, 30, 40, 60, 80, 100 and 120 min after the end of the meal from a fasting value. The AUCs for each subject were determined for the delta blood glucose and satiety (Graph Pad PRISM, version 4, San Diego). The AUCs were calculated above zero. The AUCs values are presented as means ± SEMs. All statistical calculations were performed in SPSS for Windows. Significant differences of GER, gastric antral cross-sectional areas, delta blood glucose and AUCs were evaluated with the Wilcoxon t-test. Values of $P < 0.05$ were considered significant.

Results

Postprandial blood glucose response

Ingestion of cereal bran flakes resulted in a significantly lower blood glucose response in the initial postprandial phase (40 min) than did the reference meal of corn flakes ($p = 0.045$) (Figure 1). Ingestion of cereal bran flakes resulted in a significantly lower blood glucose response in the late postprandial phase (120 min) than did wholemeal oat flakes ($p = 0.023$) (Figure 1). However, the blood glucose AUCs did not differ significantly between cereal bran flakes, wholemeal oat flakes and corn flakes (Table 2).

Gastric emptying rate

The median values of the antral cross-sectional area after ingestion of the cereal bran flakes meal were 641 ± 197

Table 2: Postprandial blood glucose areas under the curve (AUCs) after ingestion of meals consisting of wholemeal oat flakes, cereal bran flakes or corn flakes in twelve healthy subjects[1]. Significant differences of postprandial blood glucose AUCs were calculated with the Wilcoxon t-test. There were no significant differences between the AUCs.

AUC	Wholemeal Oat Flakes *mmol * min/L*	Cereal Bran Flakes *mmol * min/L*	Corn Flakes *mmol * min/L*
0 – 5 min	0.3 ± 0.1	1.3 ± 0.1	0.3 ± 0.1
0 – 25 min	21.2 ± 2.8	19.3 ± 1.4	19.3 ± 3.4
0 – 45 min	58.9 ± 7.1	53.8 ± 3.9	59.2 ± 10.2
0 – 65 min	83.0 ± 12.4	76.9 ± 7.7	93.8 ± 16.9
0 – 85 min	97.8 ± 16.3	88.7 ± 9.9	116.8 ± 21.8
0–105 min	110.1 ± 18.9	96.0 ± 10.4	124.4 ± 26.4
0 – 125 min	120.6 ± 21.5	106.8 ± 12.9	143.0 ± 26.3

[1]Mean ± SEM; n = 12

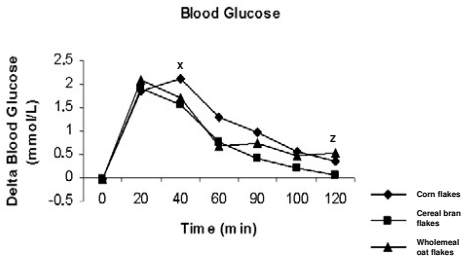

Figure 1
Means (± SEM) incremental blood glucose concentrations in twelve healthy subjects after ingesting meals consisting of sour milk with cereal bran flakes, corn flakes or wholemeal oat flakes. Significant differences calculated with the Wilcoxon t-test. X Cereal bran flakes significantly different in response compared to corn flakes ($p < 0.05$). Z Cereal bran flakes significantly different from response compared to wholemeal oat flakes ($p < 0.05$).

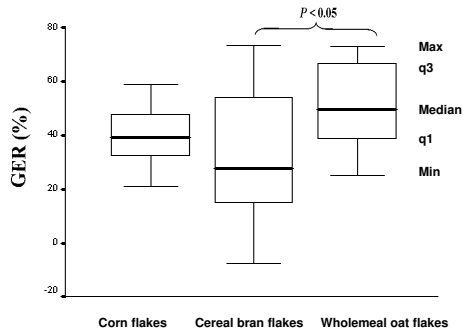

Figure 2
Gastric emptying of sour milk with cereal bran flakes, wholemeal oat flakes or corn flakes, estimated as gastric emptying rate (GER), in twelve healthy subjects. The median, minimum (Min), and maximum (Max) values and the values of the first (q1) and the third (q3) quartiles are shown. Significant differences were calculated with the Wilcoxon t-test. Cereal bran flakes significantly different in response compared to wholemeal oat flakes ($p < 0.05$)

mm^2 (range 418 to 1035 mm^2) (q1 = 524 mm^2, q3 = 824 mm^2) and 331 ± 253 mm^2 (range 137 to 924 mm^2) at 15 and 90 min, respectively, after the end of the meal. In the same subjects the median values of the antral cross-sectional area after the ingestion of the wholemeal oat flakes meal were 743 ± 240 mm^2 (range 498 to 1188 mm^2) (q1 = 568 mm^2, q3 = 1003 mm^2) and 331 ± 226 mm^2 (range 149 to 852 mm^2) (q1 = 205 mm^2, q3 = 626 mm^2) at 15 and 90 min after the end of the meal. In the same subjects the median values of the antral cross-sectional area after the ingestion of the corn flakes meal were 716 ± 187 mm^2 (range 170 to 740 mm^2) (q1 = 436 mm^2, q3 = 905 mm^2) and 481 ± 227 mm^2 (range 380 to 1008 mm^2) (q1 = 251 mm^2, q3 = 495 mm^2) at 15 and 90 min after the end of the meal. There were no significant differences between gastric antral cross-sectional at 15 or 90 min between the different meals. The median value of GER after the cereal bran flakes meal was estimated at 28% (range -8% to 73%) (q1 = 15%, q3 = 56%) compared to the median value of GER after the wholemeal oat flakes meal which was estimated at 50 % (range 25% to 73 %) (q1 = 38%, q3 = 70%). The median value of GER after the corn flakes meal was estimated at 39 % (range 21% to 73 %) (q1 = 31%, q3 = 49%). The cereal bran flakes meal had a significantly lower GER compared to wholemeal oat flakes meal ($p = 0.023$) (Figure 2). There were no significant differences between cereal bran flakes or wholemeal oat flakes compared to corn flakes with regard to GERs (Figure 2).

Satiety

Ingestion of cereal bran flakes or wholemeal oat flakes did not result in a significantly higher satiety compared to the reference corn flakes meal (Table 3, Figure 3).

Discussion

The results of this study show that the presence of fibre in a semisolid meal does not affect total postprandial blood glucose or satiety responses in healthy subjects, despite the delay in gastric emptying for the product containing the higher amount of fibre (cereal bran flakes). This study was designed to evaluate the effect of commercial cereals on blood glucose, satiety and GER. The postprandial glucose response was reduced at the initial postprandial phase after the cereal bran flakes meal compared to the corn flakes meal. Similar results have previously been presented showing a lower early postprandial blood glucose response after the intake of cereal bran flakes when compared to corn flakes [30]. In the same study it was shown that this lower postprandial blood glucose response was related to a higher initial postprandial plasma insulin response after a meal composed of 119.2 g cereal bran flakes compared to a meal of 60.9 g corn flakes [30]. However, the total postprandial insulin AUC was identical for the two meals and gastric emptying was not measured [30]. It is obvious that healthy subjects have a sufficient insulin response, thus giving a normal blood glucose response. Consequently, we have had similar total postprandial blood glucose AUCs for the products in our

Table 3: Satiety areas under the curve (AUCs) after ingestion of meals consisting of wholemeal oat flakes, cereal bran flakes or corn flakes in twelve healthy subjects[1]. Significant differences of satiety AUCs were calculated with the Wilcoxon t-test. There were no significant differences between the AUCs.

AUC	Wholemeal Oat Flakes *cm²*	Cereal Bran Flakes *cm²*	Corn Flakes *cm²*
0 – 5 min	14.0 ± 2.1	16.4 ± 2.4	12.5 ± 2.5
0 – 25 min	128.1 ± 18.7	148.5 ± 19.4	112.9 ± 21.1
0 – 45 min	244.0 ± 34.7	275.6 ± 34.2	206.2 ± 38.0
0 – 65 min	359.8 ± 45.8	386.4 ± 46.9	289.4 ± 52.6
0 – 85 min	459.8 ± 56.6	466.6 ± 56.1	363.5 ± 65.8
0–105 min	542.3 ± 66.1	544.9 ± 64.9	418.1 ± 75.0
0 – 125 min	602.4 ± 74.6	600.8 ± 12.9	454.6 ± 80.1

[1] Mean ± SEM; n = 12

study. Moreover, the cereal bran flakes meal had a smaller amount of carbohydrates than that of the corn flakes meal. However, if we had used the same amount of carbohydrates from each cereal brand in our study, we would have had a larger difference in energy, which, in turn, could potentially have influenced the GER results. An increased caloric value of a meal can delay the GER [31]. The cereal bran flakes meal had the lowest total caloric value, 298 kcal, compared to the other meals with 320 kcal, respectively. Still, the difference in GER was only significant between the cereal bran flakes meal and the wholemeal oat flakes, probably due to the higher amount of fibre in the cereal bran flakes meal. Also, the glucose response was reduced at the end of the postprandial phase after the cereal bran flakes meal compared to the wholemeal oat flakes meal (Figure 1), which could be related to the lower GER (Figure 3). However, in patients with type 2 diabetes, oat bran flour containing 9.4 g β-glucan lowered the postprandial glycemia [24]. In the same study on type 2 diabetes patients using oat bran crisps containing 3.0 g β-glucan, the postprandial glycemia was also reduced, although the reduction was only half as large as compared to oat bran flour containing 9.4 g β-glucan [24]. It has previously been shown in type 2 diabetes patients that each gram of β-glucan in food can lower the GI by four GI units [23]. The wholemeal oat flakes meal contained only 0.5 g β-glucan. Probably the amount of β-glucan was too small to affect the blood glucose response. In this study we could not show any significant difference in satiety despite a delayed gastric emptying after the cereal bran flakes meal compared to the wholemeal oat flakes meal. A delay in GER has previously been shown to increase satiety [32]. However, despite a difference in postprandial blood glucose and satiety hormones – such as ghrelin and plasma peptide YY (PYY) – after consumption of oat and wheat fibre, no difference was found with regard to satiety in healthy subjects [33].

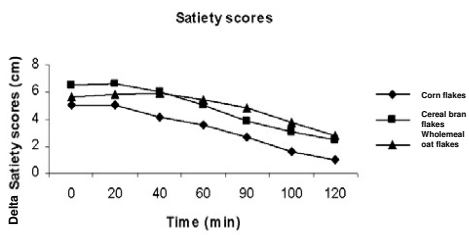

Figure 3
Means (± SEM) incremental satiety scores in twelve healthy subjects after ingesting meals consisting of sour milk with cereal bran flakes, corn flakes or wholemeal oat flakes. Significant differences calculated with the Wilcoxon t-test. There were no significant differences between the mean incremental satiety scores.

The American Diabetes Association (ADA) recommends a daily intake of 14 g fibre/1.000 kcal and foods with whole grains to prevent diabetes [1]. The intake of total dietary fibre, particularly insoluble and cereal fibre, has been shown to have an inverse association with diabetes type 2 [2]. Insoluble fibre and fibre from fruit, vegetables, or legumes have been shown to be unrelated to diabetes [2]. It is unclear whether low glycemic index food prevents diabetes mellitus. Still, the ADA recommends low glycemic index foods that are rich in fibre [1]. However, the composition of the commercial product should be more important than the fibre content alone. Several studies have shown that there was no difference in postprandial blood glucose response directly after the intake of fibre, whereas a beneficial effect on glucose metabolism was observed on the second meal test due to colonic fermentation [14,34,35].

Conclusion

The result of this study demonstrates that the intake of equal amounts of either cereal bran flakes and wholemeal oat flakes has no effect on the total postprandial blood

glucose response or satiety when compared to corn flakes. Furthermore, this study shows that the intake of cereal bran flakes slows the GER when compared to wholemeal oat flakes and corn flakes, probably due to a higher content of fibre. Since these products do not differ in terms of glucose response and satiety on healthy subjects, they should be considered equivalent in this respect. However, this study does not exclude a potential difference between the meals with regard to a delayed second meal effect.

Competing interests

The author(s) declare that they have no competing interests.

Authors' contributions

JH participated in the design of the study, performed the statistical calculations and the graphs and drafted the manuscript. RF and JW participated in the design of the study, recruited subjects, collected the data and drafted the manuscript. GD participated in the design of the study, performed the statistical calculations and the graphs, paid for the study and participated in drafting the manuscript. OB participated in the design of the study and performed the ultrasound examinations. LOA participated in the design of the study and in drafting the manuscript. All authors read and approved the final manuscript. All authors lacked any conflict of interest.

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III

Effect of muesli with 4 g oat β-glucan on postprandial blood glucose, gastric emptying and satiety in healthy subjects: A randomized crossover trial

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Abstract

Objective: Products enriched with oat β-glucan have been shown to reduce postprandial glucose and insulinemic responses. The aim of this study was to evaluate the effect of an extruded muesli product based on oat β-glucan on the rate of gastric emptying, postprandial blood glucose and satiety in healthy subjects.

Methods: Gastric emptying rate (GER) was measured by standardized real-time ultrasonography. Twelve healthy subjects were assessed using a randomized crossover double blind trial. The meals were administered after 8 hours' fasting after measuring the subject's normal fasting blood glucose level. Blood glucose measurements were made before, 30 and 60 min after the end of the meal. Satiety scores were estimated 15 and 90 min after the end of the meal. The GER was calculated as the percentage change in the antral cross-sectional area 15 and 90 minutes after ingestion of vanilla yoghurt with muesli containing 4 g oat β-glucan (GER1) or vanilla yoghurt with muesli containing cornflakes (GER2).

Results: The median values were 60% for GER1 and 44% for GER2. The effect of 4 g oat β-glucan on the rate of gastric emptying was not statistically significant compared with corn flakes. Muesli with 4 g oat β-glucan lowered the postprandial glucose response significantly compared to the cornflakes meal ($p=0.045$). The effect of oat β-glucan on satiety was not statistically significantly.

Conclusions: The results of this study suggest that intake of muesli with 4 g oat β-glucan does not affect the gastric emptying rate or satiety but lowers the postprandial blood glucose response, indicating that the GER does not regulate the blood glucose level.

Key words: gastric emptying, blood glucose, healthy subjects, oat, β-glucan, satiety

Founding source: This study was supported by a grant from Skånemejerier, Malmö, Sweden.

Abbreviations: AUC= areas under the curve, GER= gastric emptying rate, GI= glycemic-index, Min= minimum, Max= maximum, q1= the first quartile, q3= the third quartile.

Introduction

The incidence of type 2 diabetes mellitus is increasing rapidly worldwide. To prevent the development of diabetes mellitus, the American Diabetes Association recommends a reduction in caloric intake and increased consumption of dietary fiber and food containing whole grain [1]. Low-glycemic-index (GI) food that reduces postprandial hyperglycemia is recommended, but it is unclear whether it prevents diabetes mellitus [2-8]. A low-GI diet can be recommended to control glycemia in patients with diabetes [9-10]. Products enriched with oat β-glucan have been shown to reduce postprandial glucose and insulinemic responses in healthy subjects [11-14] and in type 2 diabetes patients [15-17]. Another advantageous effect of oat β-glucans is a reduction in total cholesterol [12].

The oat β-glucan effect is, however, not fully understood. One hypothesis is that oat β-glucan increases the viscosity in the small intestine and delays the digestion of food, leading to lower blood glucose and insulin response. Another mechanism that has been discussed is that oat β-glucan may delay gastric emptying caused by the viscosity of the fibre causing distention of the stomach. Only one study has been performed previously on the effect of β-glucan on gastric emptying rate (GER), using rye bread, and paracetamol as a marker for gastric emptying [18]. In that study, it was found that the GER in healthy subjects was not affected after a meal consisting of rye bread with β-glucan. However, the individual pharmacokinetics of paracetamol varies [19], which may reduce the agreement between serum paracetamol and rate of GER [20]. Gastric emptying, among other factors, regulates the postprandial blood glucose response, and a delay leads to a lower postprandial blood glucose level [21, 22]. Therefore, this study was designed to determine whether there is a delay in gastric emptying in healthy subjects, affecting postprandial blood glucose levels and satiety, after consumption of oat β-glucan.

Material and Methods

Twelve healthy subjects, eight men and four women; mean age 27 ± 5 years [range 22-35 years]; mean BMI 22 ± 3 kg/m² [range 17.0-27.0 kg/m²], without symptoms or a prior history of gastrointestinal disease, abdominal surgery or diabetes mellitus, were included in the study. The subjects were not taking any medication, except for one of the women who was taking oral contraceptives. All subjects were recruited from the population

in a southern county of Sweden. The study started 2003-04-01 and ended 2004-01-01. Four of the subjects were smokers and two were snuff users. The subjects were examined between 8:00 and 10:00 a.m. after 8 hours' fasting. Smoking and snuff-taking were prohibited for 8 h before the test as well as during the test. Each subject was required to have a normal fasting blood glucose level on the day of the study. The mean fasting blood glucose levels before ingestion of the muesli with β-glucan were 4.6 ± 0.5 mmol/l [range 4.0-5.4 mmol/l] and were 4.6 ± 0.4 mmol/l [range 4.0-5.0 mmol/l] before ingestion of the reference meal, respectively. If a subject showed gastrointestinal tract symptoms (diarrhea or constipation) the study was postponed.

The test meal (total caloric value 209 kcal) consisted of 200 g vanilla yoghurt (caloric value 137 kcal) and 26.5 g Primaliv muesli (Skånemejerier, Malmö, Sweden) (caloric value 72 kcal). The Primaliv muesli was composed of 24.5 g flakes made from oat bran (OatWell, Swedish Oat Fibre/Crea Nutrition, Väröbacka, Sweden) containing 4 g oat β-glucan (caloric value 65 kcal), 0.8 g mini-cornflakes (caloric value 3 kcal), 0.6 g freeze-dried apple (caloric value 2 kcal) and 0.6 g freeze-dried strawberries (caloric value 2 kcal), (Table 1). The reference meal included the same brand and quantity of vanilla yoghurt, mini-cornflakes, apple and strawberries as the test meal, but the oat bran flakes were replaced with 17.5 g Kellogg's cornflakes (Nordisk Kellogg's, Upplands Väsby, Sweden) (caloric value 65 kcal) (Table 1). The test meal and the reference meal had the same total caloric value. Water (200 ml) was served with each meal and was ingested gradually during the meal, which lasted for 10 min. The meals were served in a random order. A randomization techniques such as random number drawing was used.

Table 1. Nutrient composition of the test meal (containing oat bran flakes with β-glucan) and the reference meal (containing Kellogg's cornflakes). Nutrient composition according to product information.

Amount	Vanilla yoghurt 200 g	Oat flakes (β-glucan) 24.5 g	Kellogg's cornflakes 17.5 g
Total energy (kcal)	137	65	65
Total protein (g)	8	4.4	1.2
Total fat (g)	1	1.5	0.1
Total carbohydrate (g)	24	8.7	14.7
Total fiber (g)	0	9	0.5
β-glucan (g)	0	4	0

The GER was estimated using a previously described standardized ultrasound method [23]. The sonographic examination was performed using a 3.5 MHz abdominal transducer and an Acuson 128 XP 10 ultrasound system (Siemens Medical Solutions, Mountain View, CA, USA). Measurements of the gastric antrum were performed by the same radiologist who was blinded with regard to the meals. At each observation of the gastric antrum the abdominal aorta and the left lobe of the liver were used as internal landmarks. The subjects were examined in supine position, but sat between examinations. Measurements were made 15 and 90 minutes after the end of the meal. The GER was expressed as the percentage change in the antral cross-sectional area between 15 and 90 min. Three measurements of the longitudinal (d1) and anteroposterior (d2) diameters were made at each examination, and the mean value was used to calculate the cross-sectional area of the gastric antrum, using the following equation:

$$\text{Antrum area} = \pi \times d1/2 \times d2/2 = \pi \times d1 \times d2/4.$$

The GER was calculated using the following expression:

$$\text{GER} = [1 - (\text{Antrum area 90 min} / \text{Antrum area 15 min})] \times 100$$

Finger-prick capillary blood samples were taken before, 30 and 60 min after the end of the meal to measure blood glucose. Blood glucose concentrations were measured with a HemoCue Glucose system (HemoCue AB, Ängelholm, Sweden). Satiety was estimated using a validated numerical satiety scale, according to the method of Haber et al., based on a scoring system with grades from -10 cm (extreme hunger) to +10 cm (extreme satiety) [24]. Satiety scores were estimated 15 and 90 min after the end of the meal. The study was performed according to the Helsinki declaration and approved by the Ethics Committee of Lund University. All subjects provided written informed consent. Power calculation was required by the Ethics Committee of Lund University and done before the study. Paired t-test power calculations showed a 71% power to detect a 20% change in GER.

Median values with quartiles (q1 to q3) are presented for the antral cross-sectional areas and the GER. Delta values of blood glucose levels were calculated as the difference between blood glucose levels before the meal (fasting value) and 30 and 60 min after the end of the meal. The change in satiety was calculated 15 and 90 min after the end of the meal. The change in satiety was determined for each subject, and the results are presented as the mean ± SEMs for the whole group (n=12). The blood glucose was determined from the areas under the curves (AUCs), using the area above zero, for each subject (Graph

Pad PRISM, version 4, San Diego). These values are presented as the mean ± SEM for the whole group. All statistical calculations were performed using SPSS for Windows (version 14.0, 2005). Differences in the GER, gastric antral cross-sectional area, change satiety scores and blood glucose levels were evaluated with the Wilcoxon signed rank test. Values of $p<0.05$ were considered significant.

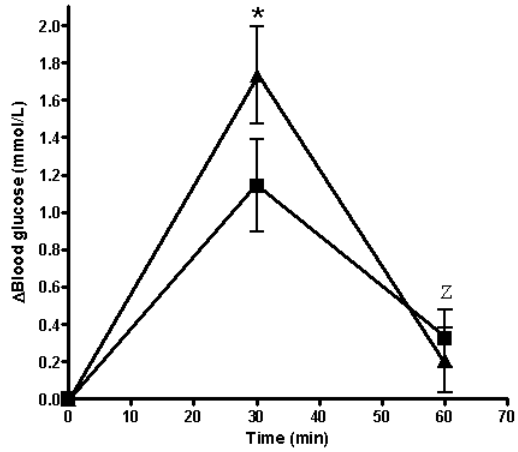

Figure 1. Mean values (± SEM) of the difference in blood glucose concentrations (relative to fasting values) in twelve healthy subjects after ingesting meals consisting of yoghurt with muesli containing 4 g oat β-glucan (■) or cornflakes (▲). * Significantly different from response to oat β-glucan muesli ($p<0.05$). Z Not significantly different from response to oat β-glucan muesli ($p=0.045$).

Results

Postprandial blood glucose response

The blood glucose levels at 30 min and the AUC (0-30) min were significantly lower after the ingestion of muesli with 4 g oat β-glucan than muesli with cornflakes ($p=0.045$) (Figure 1, Table 2). However, the glucose levels at 60 min and the AUC (0-60) min did not differ significantly between the muesli with oat β-glucan and the muesli with cornflakes (Table 2).

Table 2. Postprandial blood glucose levels (expressed as the area under the curve, AUC) after ingestion of meals consisting of yoghurt and muesli with 4 g oat β-glucan or cornflakes in twelve healthy subjects[1]. Significant differences in postprandial blood glucose levels were evaluated with the Wilcoxon t-test.

AUC	Oat flakes (β-glucan) mmol * min/ L	Cornflakes mmol * min/ L
0- 30 min	17.1 ± 3.7[2]	25.0 ± 4.3
0- 60 min	39.3 ± 7.8	54.8 ± 14.9

[1] Mean ± SEM; n= 12
[2] Significantly different from cornflakes, $p=0.045$
AUC= area under the curve

GER

The median values of the antral cross-sectional area after ingestion of the muesli with β-glucan were 577 mm² (q1= 415 mm², q3= 859 mm²) and 213 mm² (q1= 185 mm², q3= 326 mm²) 15 and 90 min after the end of the study meal, respectively. The median values of the antral cross-sectional area in the same subjects after the ingestion of the meal containing

cornflakes were 660 mm² (q1= 507 mm², q2= 960 mm²) and 382 mm² (q1= 208 mm², q3= 593 mm²) 15 and 90 min after the end of the meal, respectively. The median gastric antral cross-sectional areas were significantly larger after ingestion of the muesli with cornflakes than after the ingestion of the muesli with oat bran flakes containing 4 g β-glucan at 15 min (p=0.004). However, there was no significant difference between gastric antral cross-sectional areas at 90 min. The median value of the GER after the meal with 4 g β-glucan was estimated to be 60% (q1= 48%, q3= 72%), while the corresponding values after the meal with cornflakes was estimated to be 44% (q1= 5%, q3= 67%). This difference was not significant (Figure 2).

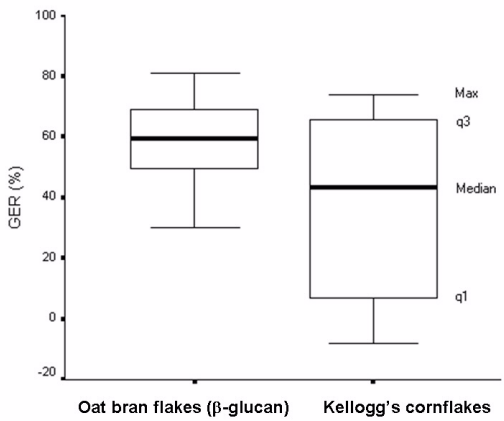

Figure 2. Gastric emptying of yoghurt with muesli containing oat flakes (β-glucan) or cornflakes, estimated as gastric emptying rate (GER), in twelve healthy subjects. The GER is expressed as the percentage change in the antral cross-sectional area between 15 and 90 min. The median, minimum (Min), and maximum (Max) values and the values of the first (q1) and the third (q3) quartiles are shown. There were no significant differences between the GERs.

Satiety

The mean change in satiety after the ingestion of muesli with 4 g β-glucan was 2.7 ± 0.9 cm (range 0-10 cm); the corresponding values after the ingestion of muesli with cornflakes were 3.2 ± 0.5 (range 0-6 cm). Thus, the ingestion of muesli with 4 g β-glucan did not result in a significantly prolonged period of satiety compared to the reference meal, i.e. muesli with cornflakes.

Discussion

This study was designed to evaluate the effect of oat β-glucan on the GER. The results suggest that the presence of 4 g of oat β-glucan in a semisolid meal does not significantly affect the GER or the satiety in healthy subjects. Thus, the reduction in postprandial glucose response after the 4 g oat β-glucan meal in healthy subjects could not be explained by delayed GER. The median antrum area 15 min after the intake of the meal containing Kellogg's cornflakes was significantly larger than the 15 min antrum area after the intake of the meal containing β-glucan. This is probably due to the larger volume of the cornflakes than the oat bran flakes. The antrum area at 90 min and the GER after the meals were not significantly different. The negative value of GER is caused by an increased gastric antral cross-sectional area at 90 min and this could be due to increased amount of gastric juices and saliva in the stomach. It is known that

an increased caloric value of a meal can delay the GER [25]. Therefore, the total caloric values of the meals were the same, but not the carbohydrate load. The meals were composed of commercial products and not our own manufactured test meals.

We found a significantly lower blood glucose level 30 min after the end of the of the oat β-glucan meal than after the cornflakes meal. However, we found no significant difference between the meals regarding blood glucose 60 min after the end of the meals. The reduced postprandial blood glucose seen after ingesting the muesli containing 4 g oat β-glucan could therefore be due to a reduced glycemic load compared to the muesli with cornflakes. Unfortunately, we did not measure the blood glucose levels more frequently, and the effect on the postprandial blood glucose was not adequately evaluated in this study. The glucose peak concentration may have been missed for some of the subjects in this study. However, the same muesli as was used in this study, containing 4 g oat β-glucan, has previously been shown to reduce the postprandial glucose and insulin levels in healthy subjects [11]. Another limitation of this study is that we did not evaluate the satiety more frequently.

It might be that 4 g oat β-glucan is needed to decrease glucose and insulin levels in healthy subjects, however this is only based on an observation that 3 g oat β-glucan did not affect the glycemic response [11]. It has previously been shown that oat β-glucan reduces postprandial glucose and insulinemic responses in healthy subjects [11-14] and in type 2 diabetes patients [15-17]. In patients with type 2 diabetes it has been shown that oat bran flour containing 9.4 g β-glucan lowered postprandial glycemia [17]. In the same study, oat bran crisps containing 3.0 g β-glucan reduced postprandial glycemia but the reduction was only half that observed with the oat bran flour [17]. Another study on type 2 diabetes patients showed that each gram of oat β-glucan in food could lower the GI by four units [16].

It has been suggested that reduced postprandial glucose and insulin concentrations after the consumption of viscous types of fiber could be caused by delayed mouth-to-cecum transit and delayed absorption of glucose in the small intestine [26]. The viscosity of oat gum, an oat extract composed of β-glucan, has been shown to cause a reduction in plasma glucose and insulin [27]. A relationship between glycemic response and the concentration and molecular weight of oat β-glucan has also been observed [28]. Another hypothesis that has been proposed is that β-glucan is

fermented in the colon by the bacterial flora and leads to a release of short-chain fatty acids, lowering postprandial glucose levels [29] and serum lipids [30].

It has been found, using paracetamol as a marker for gastric emptying, that the GER was not affected in healthy subjects after a meal consisting of rye bread containing β-glucan [18]. The paracetamol method is dependent on the release and absorption of paracetamol in the small intestine, which makes this method unreliable; also, the study was designed to evaluate the effect of rye and not oat β-glucan. In the same study the postprandial blood glucose response to the β-glucan rye bread containing 5.4 g β-glucan did not differ from that to white wheat bread. However, the same study showed that postprandial insulin and glucose-dependent insulinotropic polypeptide responses were lower after the β-glucan rye bread meal [18]. When 5 g or 10 g of oat β-glucan were consumed this led to lower postprandial blood glucose levels, but no effect was observed after consumption of the same amount of barley β-glucan in hypercholesterolemic subjects [12]. The effect on postprandial blood glucose may, therefore, depend on the cereal source of β-glucan.

Conclusions

The results of the present study suggest that the muesli containing 4 g oat β-glucan in a semisolid meal does not affect the GER or satiety in healthy subjects compared to cornflakes. The reduction in postprandial glucose response in healthy subjects after the meal containing 4 g oat β-glucan muesli, compared to muesli with cornflakes, could not be explained by delayed GER. Clearly, a larger trial involving a greater number of patients would be needed to validate the findings of this small study.

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IV

The botanical integrity of wheat products in association with acetic acid influences the gastric distention and satiety in healthy subjects

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Manuscript

Abstract

Background: Maintenance of the botanical integrity of cereal kernels and the addition of acetic acid (as vinegar) in the product or meal has been shown to lower the postprandial blood glucose and insulin response and to increase satiety. However, the mechanism behind the benefits of acetic acid on blood glucose and satiety is not clear. We hypothesized that the gastric emptying rate could be involved. Thus, the aim of this study was to evaluate the possible influence of maintained botanical integrity of cereals and the presence of acetic acid (vinegar) on gastric emptying rate (GER) and satiety.

Methods: Fifteen healthy subjects were included in a blinded crossover trial, and thirteen of the subjects completed the study. Equicarbohydrate amounts of the following wheat-based meals were studied: white wheat bread, whole-kernel wheat bread or wholemeal wheat bread served with white wine vinegar. The results were compared with a reference meal consisting of white wheat bread without vinegar. The GER was measured with standardized real-time ultrasonography using normal fasting blood glucose <6.1 mmol/l or plasma glucose <7.0 mmol/l as an inclusion criterion. The GER was calculated as the percentage change in the antral cross-sectional area 15 and 90 minutes after ingestion of the various meals. Satiety scores were estimated and blood glucose was measured before and 15, 30, 45, 60, 90 and 120 min after the start of the meal.

Results: The whole-kernel wheat bread with vinegar resulted in significantly higher (<0.05) satiety than the wholemeal wheat bread and white wheat bread with vinegar and the reference bread. Wheat fiber present in the wholemeal wheat bread, or the presence of wheat kernels per se, did not affect the

postprandial blood glucose or GER significantly compared with white wheat bread, neither did the addition of vinegar to white bread affect these variables. There was no correlation found between the satiety with antral areas or GER

Conclusions: The present study shows higher satiety after a whole-kernel wheat bread meal served with vinegar than after wholemeal wheat bread with vinegar or white wheat bread with or without vinegar. This may be explained by increased antral distension after ingestion of intact cereal kernels but, in this study, not by a lower gastric emptying rate or higher postprandial blood glucose response.

Trial registration: NTR number 1116

Background

Changing the diet can control the blood glucose level and help prevent the development of type 2 diabetes. The American Diabetes Association recommends a reduced calorie intake and increased intake of dietary fiber and whole grains to prevent the development of type 2 diabetes (1). Foods with a low glycemic index that are rich in fiber are recommended (1).

The first reported antiglycemic effect of vinegar was by Ebihara and Nakajima (2). Vinegar decreases the glycemic index of, for example, rice in sushi by about 20-35% (3). Also, when white vinegar was added to cold storage potatoes as a vinaigrette sauce the glycemic index was lowered in healthy subjects (4). Vinegar in a salad dressing added to lettuce and ingested with white wheat bread has been shown to reduce the blood glucose response, but the gastric emptying rate (GER) measured by ultrasonography was not delayed (5). When vinegar was neutralized to pH 6.0 no effects were seen on the postprandial blood glucose response (5). The decreased postprandial blood glucose response was explained by a mechanism related to acidity and inhibition of digestive amylases (5). Further, vinaigrette sauce added to a white wheat bread meal has been shown to reduce postprandial blood glucose and insulin responses in healthy subjects; this was explained by delayed gastric emptying, measured indirectly with paracetamol (6). Insulin sensitivity was improved and postprandial insulin and glucose responses were reduced in insulin-resistant subjects after a meal containing vinegar (7). However, in healthy subjects, only the postprandial insulin response was reduced, not the blood glucose response, after ingestion of a white bagel with apple cider vinegar (7).

Satiety and eating behavior are complex, but play a key role in energy intake and metabolic control in healthy subjects and in patients with diabetes. Only one previous study on healthy

subjects has been conducted to evaluate the effect of vinegar on satiety. That study showed that white wheat bread ingested with vinegar increased and prolonged the feeling of satiety according to a dose-response relation (8).

The term "whole grain" is often used for wholemeal products in which the structure of the cereal grain is destroyed in the flour containing the original dietary fiber, but also for cereal products in which a large proportion of whole cereal grains is intact. However, there seems to be a major difference in metabolic response between whole grain and wholemeal products. The preparation, cooking and particle size of the grain structures may also affect the metabolic response. The glycemic index decreased in patients with type 2 diabetes when increasing proportions of whole grain bulgur (cracked wheat) were substituted for miller flour in bread (9). However, in another study, the glycemic response did not differ between bulgur and whole wheat kernels in patients with type 1 and type 2 diabetes (10). This can be explained by the similar particle sizes of bulgur and wheat after chewing. The particle size of wheat has been found to affect the digestion rate and metabolic response in healthy subjects (11). The wheat germ of the whole grain acts as a natural amylase inhibitor, which can be destroyed during the milling of wheat into wholemeal flour (12).

Only one study has been conducted previously on the effect of whole kernels on gastric emptying, which showed that in healthy subjects the gastric emptying, measured indirectly with paracetamol, was not affected after meals composed of whole-kernel rye bread or wholemeal rye bread compared to with white wheat bread (13). A high-dietary-fiber meal was found to delay the GER, measured by ultrasonography, in healthy subjects compared with a low-fiber meal (14). However, another study showed that the GER following a high-fiber meal consisting of whole wheat grain and rye bread, was not different from that following a low-fiber meal (15).

These divergent results indicate that not only glucose response, as was previously known, but also satiety and gastric emptying rate seem to be influenced by variables related to processing conditions and botanical structure. The effects of a combination of vinegar and different fiber structures on the postprandial blood glucose response, gastric emptying or satiety has, as far as we know, not been studied previously. Thus, the aim of this study was to evaluate the possible influence of maintaining the botanical integrity of cereals and the addition of acetic acid (as vinegar) on the GER and satiety.

The hypothesis was that products that delay gastric empting rate also lead to higher satiety.

Material and Method

Fifteen healthy subjects were included in the study. However, one male subject was excluded because he was found to have diabetes mellitus, and one female subject was excluded because celiac disease was diagnosed during the study. Thirteen healthy subjects (six men and seven women: mean age 25 ± 4 years [range 22-35 years]; mean BMI 22.8 ± 3.07 kg/m² [range 17.7-29.7 kg/m²]), without symptoms or a prior history of gastrointestinal disease, abdominal surgery or diabetes mellitus, completed the study. The subjects were not receiving any drugs, except two of the women who were taking birth control medication. One subject was a smoker and none was a snuff user. None of the subjects used any drugs on the day of the examination.

White wheat bread was made from 3700 g white wheat flour, 2000 g water and 200 g yeast. The dough was allowed to rise for 20 min at 28 °C. The dough was then divided into 440 g pieces and left to rise for a second time, for 35 min at 40 °C (RH: 80%). Loaves were baked at 210 °C for 22 min with the addition of steam during the first 30 s. The loaves were stored in a freezer at -20 °C until used.

The whole-kernel wheat bread was made from 3076 g wheat kernels that were boiled for 20 min in 3076 g water and then cooled at room temperature, after which 1200 g water, 624 g white wheat flour and 200 g yeast were added. The dough was left to rise for 30 min and then divided into 580 g pieces. These pieces were then allowed to rise for a second period of 45 min at 40 °C (RH: 80%). Loaves were baked at 200 °C for 45 min.

The wholemeal wheat bread was made from 3076 g milled wheat kernels, (500 g of the flour was scaled with 1000 g boiling water) 1200 g water, 624 g white wheat flour and 200 g yeast. The dough was left to rise for 30 min and then divided into 580 g pieces. These were then allowed to rise for a period of 45 min at 40 °C (RH: 80%). Loaves were baked at 200 °C for 45 min.

The reference and test meals contained 50 g available carbohydrates from bread products. The content of available carbohydrates was analyzed according to Holm et al (16). The portion size of the white wheat bread was 106.34 g and, besides 50 g available carbohydrates, contained 2.1 g dietary fiber, 1.8 g fat and 8.3 g protein. The portion size of the whole-kernel wheat bread was 132.66 g and

contained, besides 50 g available carbohydrates, 7.2 g dietary fiber, 2.9 g fat and 9.2 protein. The portion size of the wholemeal wheat bread was 107.62 g, and contained 7.2 g dietary fiber, 2.9 g fat and 9.2 g proteins, besides the 50 g available carbohydrates. We used the same baking recipes and baking process as Liljeberg et al (17) for the white wheat reference bread and whole-kernel wheat bread; the content of dietary fiber, fat and proteins were thus assumed to be the same as previously described by Liljeberg et al (17). The wholemeal wheat bread was made from the same recipe as whole-kernel wheat bread but with milled wheat kernels. The three test meals contained one of the three kinds of test bread dipped in 28 g white wine vinegar (5% acetic acid, pH 2.8-3 Druvan, DR Persfood AB, Eslöv, Sweden), which is equivalent to 23 mmol acetic acid in each test meal. Drinking water, 200 ml, was also served. The reference meal contained white wheat bread and water, but without white wine vinegar. The test products and the reference were served in random order during intervals of 1 week.

The subjects were examined between 8:00 and 10:00 am after an 8-h fast. Smoking was prohibited for 8 h before and during the test. The fasting blood glucose concentration of each subject was checked on the day of the examination to ensure that it was normal. The mean fasting blood glucose was 4.4 ± 0.2 mmol/l before the ingestion of the reference meal. The mean fasting blood glucose before the ingestion of vinegar together with white wheat bread, wholemeal bread and whole-kernel bread were 4.5 ± 0.1, 4.6 ± 0.1 and 4.5 ± 0.2 mmol min/l, respectively. If the subject reported gastrointestinal symptoms (diarrhea or constipation) on the study day, the examination was postponed. Each meal was ingested within 10 minutes.

The GER was estimated using a previously described standardized ultrasound method (18). The sonographic examination was performed using two different ultrasound machines (Siemens Acuson Sequoa 512 and Aloka Prof. Sound) with a multi-MHz abdominal transducer. The same machine was used to calculate values of GER. The measurements of the gastric antrum were performed by the same radiologist, who was blinded with regard to the meals. The measurements were made 15 and 90 minutes after the end of meal ingestion (25 and 100 min after the start of the meal). Gastric emptying was expressed as the percentage change of the antral cross-sectional area from 15 to 90 min. Paired t-test was performed before the beginning of the study and power calculations showed a 71% power to detect a 20% change in GER.

Finger-prick capillary samples were collected before and 15, 30, 45, 60, 90 and 120 min after the start of the meal to measure blood glucose levels. Blood glucose concentrations were measured with a HemoCue Glucose system (HemoCue AB, Ängelholm, Sweden). The validated satiety score scale was used according to the method of Haber et al on the basis of a scoring system with grades from -10 (extreme hunger) to 10 (extreme satiety) (19). Satiety scores were estimated before the meal and 15, 30, 45, 60, 90 and 120 min after the start of the meal, using the same scoring system.

The study was performed according to the Helsinki declaration and was approved by the Ethics Committee at Lund University, and participants provided written informed consent.

The changes from pre-ingestion values in blood glucose and satiety after the different treatments were presented as means ± SEMs and were tested globally in a repeated measures linear mixed model using the interaction of time and treatment as fixed effects and subjects as random effects (SAS, version 8.2, SAS Institute, Cary, NC). For the covariance structure of the repeated measures within a series a spatial exponential model was used. The areas under the curve (AUCs) above zero for delta blood glucose and satiety responses of the four treatments were determined for each subject (Graph Pad PRISM, version 4, San Diego, CA) and presented as means ± SEMs. These were tested globally in a mixed model where treatments were entered as fixed effects and subjects were entered as random effects. Tukey's multiple comparisons test was used as follow-up procedure after the mixed models when appropriate. In addition we tested the inclusion of BMI as covariate in the mixed model for glucose and also the possible correlation between satiety and antral areas or GER. Median values and quartiles are presented for the antral cross-sectional areas and the GER. These were tested globally using the Friedman rank sum test, and when the null hypothesis was rejected, followed by pair-wise comparisons using Wilcoxon rank sum test with the Holm sequential procedure for P-value adjustment (R, version 2.6, The R foundation for statistical computing, http://www.r-project.org/). Statistical significance was accepted at $p<0.05$.

Results

Postprandial blood glucose response

No significant differences were seen in blood glucose responses at different times, or in the incremental areas under the postprandial glucose curves between the different bread meals (Figure 1). The mean blood glucose AUC 0-120 min after ingestion of the reference meal of white wheat bread was 147 ± 14 mmol min/l. The AUCs after

ingestion of vinegar together with white wheat bread, wholemeal bread and whole-kernel bread were 114 ± 12, 110 ± 10 and 135 ± 13 mmol min/l, respectively. The blood glucose AUCs did not differ significantly between the meals, (p=0.13 in the test of the global hypothesis). The inclusion of BMI as a covariate in the analysis of postprandial blood glucose response did not improve the model.

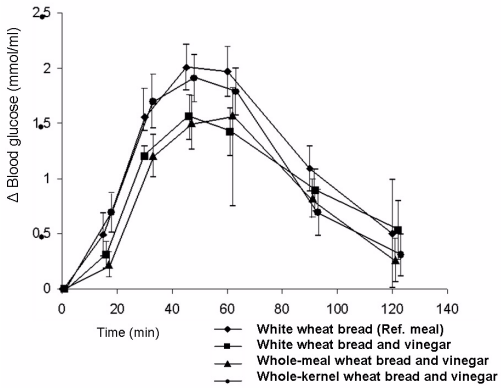

Figure 1. The mean (± SEM) incremental blood glucose concentration in thirteen healthy subjects 15-120 minutes after the ingestion of meals consisting of white wheat bread only (reference) (◆), white wheat bread with vinegar (■), wholemeal wheat bread with vinegar (▲) and whole-kernel bread with vinegar (•). No significant differences were found between the incremental blood glucose concentrations following the various meals.

Satiety

Ingestion of the whole-kernel wheat bread with vinegar resulted in significantly higher satiety scores at 15, 30, 45, 60 and 90 min than the white wheat bread with vinegar and the reference meal, white wheat bread without vinegar (p<0.05) (Figure 2). Ingestion of whole-kernel wheat bread with vinegar resulted in significantly prolonged satiety, i.e. a higher AUC from 0-120 min, compared with the other bread meals (white wheat bread with vinegar, wholemeal wheat bread with vinegar and the reference white wheat bread, p<0.05). The mean satiety score after ingestion of the reference meal, i.e. white bread, (AUC from 0-120 min) was 333 ± 56 cm min. The corresponding values after ingestion of the test meals with vinegar were higher: 393 ± 79 cm min for white wheat bread with vinegar, 501 ± 80 cm min for wholemeal bread with vinegar, and 795 ± 82 cm min for whole-kernel wheat bread with vinegar.

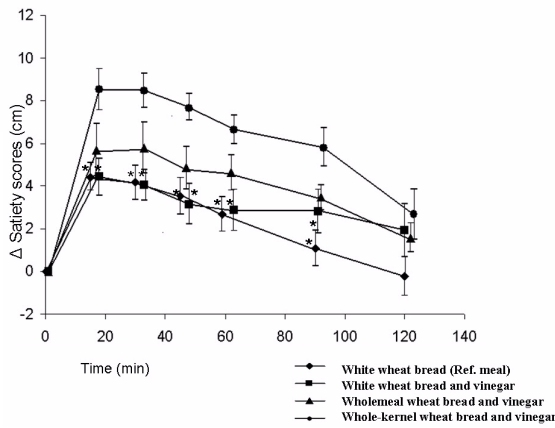

Figure 2. The mean (± SEM) incremental satiety scores reported by thirteen healthy subjects 15-120 minutes after the ingestion of meals consisting of white wheat bread (reference) (◆), white wheat bread with vinegar (■), wholemeal bread with vinegar (▲), and whole-kernel wheat bread with vinegar (•). * Significantly different from the response to whole-kernel bread with vinegar (p<0.05).

Gastric emptying rate

No significant differences were observed between the meals with regard to gastric emptying rates (Figure 3). The median value of the GER after the reference meal was estimated to be 51% (q1=40%, q3=61%) compared with the corresponding value after the reference meal with vinegar, which was estimated to be 47 % (q1=36%, q3=56%). The median value of the GER after the wholemeal wheat bread with vinegar meal was estimated to be 62% (q1= 39%, q3= 74%) which can be compared with the median value of the GER after the whole-kernel wheat bread with vinegar meal, of 43% (q1=39%, q3=53%).

The median values of the antral cross-sectional area after the ingestion of the reference meal were 525 mm^2 (q1= 431 mm^2, q3= 707 mm^2) and 295 mm^2 (q1=193 mm^2, q3=364 mm^2) 15 and 90 min, respectively, after the end of the meal. The median values of the antral cross-sectional area after the ingestion of the reference meal with vinegar were 607 mm^2 (q1=607 mm^2, q3= 1092 mm^2) and 317 mm^2 (q1=264 mm^2, q3=507 mm^2), 15 and 90 min, respectively after the end of the meal. The median values of the antral cross-sectional area after the ingestion of the wholemeal wheat bread with vinegar were 660 mm^2 (q1= 531 mm^2, q3= 885 mm^2) and 266 mm^2 (q1= 166 mm^2, q3=422 mm^2), respectively, 15 and 90 min after the end of the meal. After the ingestion of the whole-kernel wheat bread with vinegar the median values of the antral cross-sectional area were 857 mm^2 (q1=657 mm^2, q3= 1057 mm^2) and 477 mm^2 (q1= 329 mm^2, q3= 558 mm^2), respectively, 15 and 90 min after the end of the meal. The median value of the early antral cross-sectional area after the whole-kernel wheat bread with vinegar (857 mm^2) was significantly larger ($p<0.05$ in a pairwise comparison using Wilcoxon rank sum test after the global Friedman ranks sum test being significant $p=0.0022$) than the corresponding area after ingestion of the reference meal (525 mm^2) (Figure 4).

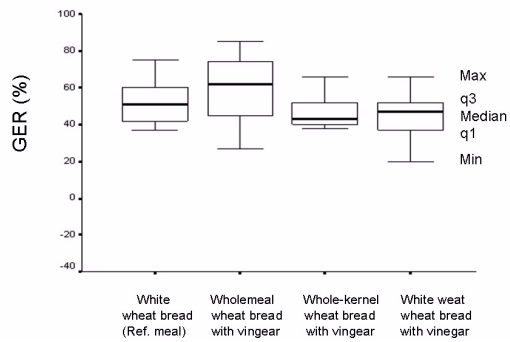

Figure 3. Gastric emptying rate following the ingestion of white wheat bread with vinegar, wholemeal bread with vinegar, whole-kernel wheat bread with vinegar and white wheat bread (reference), in thirteen healthy subjects. The median, minimum (Min), and maximum (Max) values and the values of the first (q1) and the third (q3) quartiles are shown. No significant differences were found between the GERs.

Relation of satiety to GER or antral area

There was no significant correlation between the satiety with antral areas or GER.

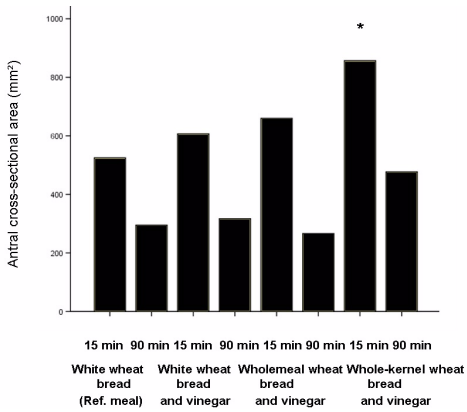

Figure 4. Median antral area in thirteen healthy subjects 15 and 90 minutes after the end of meal ingestion meals consisting of white wheat bread (reference), white wheat bread with vinegar, wholemeal bread with vinegar, and whole-kernel wheat bread with vinegar. * Significantly different from the response to the white wheat bread (reference) ($p<0.05$).

Discussion

The aim of this study was to elucidate the effect of maintained botanical structure and dietary fiber present in wheat-based bread products in combination with vinegar, on gastric emptying rate, glycemic response and satiety in healthy subjects. Our hypothesis was that an intake of intact cereal kernels with vinegar would increase satiety and lower the postprandial blood glucose response due to delayed gastric emptying. We were not able to verify this hypothesis. The results showed a significant increase in satiety after ingestion of the whole-kernel wheat bread with vinegar compared with the other meals, but no statistically significant differences were seen in gastric emptying rate or postprandial blood glucose response. However, the antral cross-sectional area was significantly larger 15 min after the ingestion of whole-kernel wheat bread with vinegar than after the white wheat reference bread. Thus, the distension of the antrum may explain the increase in satiety scores reported after the whole-kernel wheat bread meal with vinegar compared to the other bread meals with vinegar. However, the antral cross-sectional area did not correlate to the satiety scores. Clearly, a larger trial involving a greater number of subjects would be needed to validate the findings of this small study. A limitation of this study is that the sample size was small. Because of the lack of suitable control for the whole-kernel bread served with vinegar this study shows that the whole-kernel bread was more satiating than the other meals regardless of adding vinegar. This study thus shows that the botanical structure rather than the amount of fiber per se causes the distension of the antrum and increased satiety. This relationship between antral area and satiety in healthy subjects has been observed previously by others (20-24). Holt et al have also reported an association between the particle size of wheat and satiety (25).

Another intention of the current study was to evaluate the effect of whole kernels on blood glucose response and gastric emptying. This lack of difference in postprandial blood glucose response between the bread meals was most unexpected as, in a previous study using the same bread recipes but without vinegar, it was observed a significantly lower blood glucose response after whole-kernel wheat bread than after white wheat bread (17). The botanical integrity of the grain kernels may have been unintentionally destroyed during the baking process, which would explain the present observations. However, the structure of the bread was not investigated. The lack of difference in GER between the bread meals is in agreement with studied performed by Juntunen et al, who compared whole-kernel rye bread and wholemeal rye bread to white wheat bread, despite the known difference in insulin response between rye and wheat (13). Unfortunately, we did not control the subjects for exercise or food choice the night before of the testing. This may have affected the postprandial blood glucose responses.

Another intention of the current study was to evaluate the effect of vinegar on blood glucose response and gastric emptying. However, we did not observe the lowering effect of vinegar on blood glucose response reported with white wheat bread (8) and potatoes (4) in previous studies. However, when 20 g apple cider vinegar was ingested prior to a low-glycemic meal the postprandial insulin response was lower, but no effect was observed on the blood glucose response in healthy subjects (26). However, ingestion of 20 g apple cider vinegar prior to a high-glycemic meal composed of bagel, butter and orange juice, reduced the postprandial blood glucose and insulin response in healthy subjects (26). In a recent study, the postprandial glucose and insulin responses of type 2 diabetes patients were found not to be affected, and in healthy subjects the postprandial blood glucose levels were not affected, but the insulin levels were reduced when apple cider vinegar was consumed prior to the meal (27). However, it was demonstrated in insulin-resistant subjects that the postprandial insulin and glucose responses were reduced after drinking 20 g apple cider vinegar prior to a meal consisting of white bagel, butter and orange juice (27).

Our findings, that there was no difference in gastric emptying rate after a meal including vinegar, agree with those of Brighenti et al, who found no difference regarding gastric emptying, measured by ultrasonography, after a meal with 20 ml white vinegar (16 mmol acetic acid), although the blood glucose response was reduced in healthy subjects (5). They explained the lower postprandial blood glucose response

as being due to a mechanism related to acidity and the inhibition of digestive amylases. Another study showed that an addition of 20 g white vinegar (18 mmol acetic acid) to a bread meal lowered the postprandial blood glucose and insulin responses in healthy subject, and this was explained by delayed gastric emptying (6). However, gastric emptying was measured indirectly using paracetamol, which is a less reliable method.

Conclusions

The present study shows that the post-prandial ratings of satiety were higher after whole-kernel wheat bread meal with vinegar than after meals of wholemeal wheat bread with vinegar, white wheat bread with vinegar or a reference meal of white wheat bread without vinegar. This may be explained by increased antral distension after the ingestion of intact cereal kernels.

Competing interest

All authors declare that they have no competing interest.

Authors' contributions

JH participated in the design of the study, recruited the subjects, collected the data, performed the statistical calculations and drafted the manuscript. PH performed the statistical calculations and participated in drafting the manuscript. SL participated in drafting the manuscript. GD participated in the design of the study and participated in drafting the manuscript. OB participated in the design of the study and performed the ultrasound examinations. LOA participated in the design of the study and in drafting the manuscript. All authors read and approved the final manuscript. All authors lack any conflict of interest.

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Effect of apple cider vinegar on delayed gastric emptying in patients with type 1 diabetes mellitus: a pilot study

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Abstract

Background: Previous studies on healthy people show that vinegar delays gastric emptying and lowers postprandial blood glucose and insulin levels. The aim of this study was to investigate the effect of apple cider vinegar on delayed gastric emptying rate on diabetes mellitus patients.

Methods: Ten patients with type 1 diabetes and diabetic gastroparesis, including one patient who had undergone vagotomy, were included and completed the investigator blinded crossover trial. The gastric emptying rate (GER) was measured using standardized real-time ultrasonography. The GER was calculated as the percentage change in the antral cross-sectional area 15 and 90 minutes after ingestion of 300 g rice pudding and 200 ml water (GER1), or 300 g rice pudding and 200 ml water with 30 ml apple cider vinegar (GER2). The subjects drank 200 ml water daily before breakfast one week before the measurement of GER1. The same subjects drank 200 ml water with 30 ml vinegar daily before breakfast for two weeks before the measurement of GER2.

Results: The median values of GER1 and GER2 were 27% and 17%, respectively. The effect of vinegar on the rate of gastric emptying was statistically significant ($p < 0.05$).

Conclusion: This study shows that vinegar affects insulin-dependent diabetes mellitus patients with diabetic gastroparesis by reducing the gastric emptying rate even further, and this might be a disadvantage regarding to their glycaemic control.

Trial registration number: ISRCTN33841495.

Background

Diabetes mellitus is a growing problem globally. According to recent estimates there were over 171 million people living with diabetes worldwide in 2000, and the number is estimated to increase to 366 million by 2030 [1]. Studies have shown that 30–50% of diabetes patients have delayed gastric emptying and this is believed to be, at least partially, due to vagal denervation caused by autonomic neuropathy [2-6]. Delayed gastric emptying may cause poor glycaemic control, especially in those with preprandial antidiabetic treatment leading to/causing postprandial hypoglycaemia and gastrointestinal symptoms such as postprandial nausea, vomiting, bloating and early satiety [7]. The relationship between the symptoms of gas-

troparesis and the rate of gastric emptying is weak, and patients with delayed gastric emptying may not have any, or few, gastrointestinal symptoms [8-10]. An increased frequency of hypoglycaemic events in insulin-treated diabetic patients has been associated with abnormal gastric emptying, despite the lack of upper gastrointestinal symptoms [11]. However, diabetes mellitus has been associated with an increased prevalence of upper and lower gastrointestinal symptoms compared with healthy subjects, and these symptoms have been found to be associated with poor glycaemic control but not the duration of diabetes [12]. There is a significant, albeit weak, relationship between gastric emptying of solids and the presence of upper gastrointestinal symtoms; increased retention in the distal but not proximal stomach is associated with increased gastrointestinal [13].

Studies have shown that vinegar reduces postprandial blood glucose levels in healthy subjects [14-17], and it has been discussed whether this could be explained by delayed gastric emptying [14,15]. However, the effect of vinegar on gastric emptying in diabetic patients with gastroparesis has not been studied previously. In a recent study, apple cider vinegar was shown to improve insulin sensitivity, and lowered the postprandial blood glucose and insulin levels in insulin-resistant subjects [18]. Subjects with type 2 diabetes showed a slight improvement in insulin sensitivity, but postprandial blood glucose and insulin levels were not affected when apple cider vinegar was added to a meal [18]. In the Framingham offspring study a diet with high glycaemic index was found to be associated with metabolic syndrome [19]. A diet with low glycaemic index, such as a meal including vinegar, is favourable in healthy subjects and in insulin-resistant subjects [14-18]. In Sweden, and in other European countries, it is the custom to consume vinegar in salad dressing. It is common for patients with diabetes in Sweden to drink vinegar daily because of its positive effect on blood glucose. However, if vinegar delays the gastric emptying rate (GER), this may cause instability of the metabolic control and increase gastrointestinal symptoms or the frequency of hypoglycaemic events in insulin-dependent diabetes mellitus patients with gastroparesis, especially those injecting insulin preprandially. Insulin-dependent diabetes patients should perhaps also take into account the composition of the meal and its affects on the GER, as well as the carbohydrate content of the meal before the administration of short-acting insulin.

The aim of this study was therefore to evaluate the influence of vinegar on the GER in insulin-dependent diabetes mellitus patients with diabetic gastroparesis.

Methods

Ten patients with type 1 diabetes mellitus and with diagnosed diabetic gastroparesis (five men and five women; mean age 67.9 ± 8.0 years [range 57–79 years]; mean Body Mass Index 25.4 ± 2.9 kg/m^2 [range 21.2–30.9 kg/m^2]; mean duration of diabetes 41.3 ± 13.2 years [range 18–57 years], mean value of Hemoglobin A1c 8.3 ± 0.7 % [range 7.5–9.5]) were included in and completed the crossover study. Patients were recruited from those previously diagnosed with gastroparesis measured by a previously described and standardised scintigraphic and real-time ultrasound method [20] at the Malmö University Hospital. A GER lower than 45% indicates delayed gastric emptying, and has been shown to be strongly correlated to scintigraphic half-time values [21]. The patients had symptoms typical of diabetic gastroparesis (postprandial abdominal fullness or nausea, vomiting, postprandial early satiety or early postprandial hypoglycaemia, despite the ingestion of food and correctly taken doses of insulin).

Seven of the subjects had a history peripheral neuropathy, eight had retinopathy and four had nephropathy. Those with renal failure (microalbuminuria > 20 microg/min), previously major abdominal surgery, history of severe cardiovascular disease and hepatic disease were excluded from the study. The patients showed no evidence of prior gastric outlet obstruction or connective tissue diseases. One of the patients had had his gall bladder removed, and two patients had had their uterus and ovaries removed, three patients had been appendectomized, and one had undergone vagotomy 16 years previously. All of the patients were being treated with a multiple-dose regime, consisting of rapid- or short-acting insulin before meals and intermediate-acting insulin once or twice daily. No medication was changed, and no subject used any medication with known major gastrointestinal side effects during the study. None of the subjects used any prokinetic treatment before or during the study. Two of the subjects were snuff users but none smoked; snuff-taking was prohibited for 8 h before and during the test. The subjects were examined in the morning between 8:00 and 10:00 after fasting for 8 hours. The examination was conducted providing that the subject's fasting blood glucose level was in the range 3.5–9.0 mmol/l. The subjects were asked not to consume any drugs or insulin on the day of the examination. If the blood glucose level was lower than 3.5 mmol/l or higher than 9.0 mmol/l on the day of the study the examination was postponed. Blood glucose concentrations were measured with the HemoCue Glucose system (HemoCue AB, Ängelholm, Sweden). Before each meal the subjects took their normal daily insulin dose which was not changed during the study. If the subjects reported gastrointestinal symptoms (diarrhoea, constipation, nausea or vomiting) on the day of the study the examination was postponed. Patients with chronic consti-

pation (in our study defined as symptoms for 1 year or longer) were not excluded, as this was assumed to be their basal state, possibly owing to autonomic neuropathy. GER measurements were made on two different occasions.

One week before the first examination, the patients drank 200 ml water every morning before their breakfast. The reference meal consisted of 300 g rice pudding (BOB, Scan Foods AB, Johanneshov, Sweden, 100 g caloric value 110 kilocalories, 3.5 g protein, 17 g carbohydrates and 3 g fat). GER measurements were then made after drinking 200 ml water before the ingestion of the rice pudding. Each meal had to be consumed within 10 min and the water was consumed before ingestion of the rice pudding meal. After the first examination the patients were given a 450 ml bottle of commercial available apple cider vinegar with honey flavouring (Druvan, DR Persfood AB, Eslöv, Sweden). The caloric value of 100 ml apple cider vinegar was 65 kilocalories, 16 g carbohydrates, < 0.5 g protein and 0 g fat. The apple cider vinegar was composed of 5% acetic acid and had a pH value of 2.8–3. The patients drank 200 ml water mixed with 30 ml apple cider vinegar (total volume 230 ml) every morning for two weeks before their breakfast. GER measurements were then made after drinking 30 ml apple cider vinegar mixed with 200 ml water (total volume 230 ml) before the ingestion of the rice pudding. Each meal had to be consumed within 10 min and the water, with vinegar, was consumed before ingestion of the rice pudding meal. One of the subjects who participated in the study was already consuming apple cider vinegar daily, and was therefore examined for the first time after drinking apple cider vinegar mixed with water before ingestion of the rice pudding. After a ten-day wash-out period with water before the ingestion of breakfast, the same subject was examined after the ingestion of the reference meal. The subjects were not prohibited to consume any other vinegar or acetic-acid containing products during the study. The order of the two different meals were not randomized.

Gastric emptying was measured using ultrasonography employing a previously described method [20]. We did not have a specific room with only one machine and we used the available ultrasound machine at the moment. The sonographic examination was performed using four different ultrasound machines ((Acusone Sequioa 512, Mountain View, CA), (Aloka ProSound SSD 5500, Tokyo, Japan), (Siemens Elegra, Siemens Medical Solutions, Mountain View, CA), (B-K Medical 2102 Hawk, Gentofte, Denmark)) with an abdominal 2.5–4 MHz transducer. For each calculation of the GER, the antrum diameters had been measured using only one of the above machines. However, the same ultrasound machine was not used for the paired studies. Measurements of the gastric antrum were performed by the same radiologist who was blinded with regard to the meals. The radiologist applied minimal abdominal compression with the abdominal transducer during the measurements. The subjects were examined in the supine position, but sat between the examinations, 15 and 90 minutes after the end of meal ingestion. The GER was expressed as the percentage change in the antral cross-sectional area from 15 to 90 min. Three measurements were made of the longitudinal (d_1) and anteroposterior (d_2) diameters at each examination, and the mean value was used to calculate the cross-sectional area of the gastric antrum using the following formula:

$$\text{Antrum area} = \pi \times r^2 = \pi \times d_1/2 \times d_2/2 = \pi \times [d_1 \times d_2]/4$$

At each measurement of the gastric antrum the abdominal aorta and the left lobe of the liver were used as internal landmarks. The GER was calculated using the following formula:

$$\text{GER} = [1\text{- (Antrum area 90 min/Antrum area 15 min)}] \times 100$$

The study was performed according to the Helsinki declaration. All subjects gave written, informed consent before participating in the experiments.

Median values with quartiles (q1 to q3) are presented for the antral cross-sectional areas and the GER. All statistical calculations were performed in SPSS for Windows (SPSS (Version 14.0, 2005;Chicago IL, USA). The significance of differences in GER, antral cross-sectional areas and blood glucoe values were evaluated with the Wilcoxon t-test. P-values < 0.05 were considered significant.

Results

Blood glucose

The mean fasting blood glucose levels were before the reference meal 6.9 ± 0.6 mmol/l compared and not significant different to before ingestion of the meal including vinegar 7.3 ± 0.5 mmol/l.

GER

The median values of the antral cross-sectional area after ingestion of the meal including vinegar were 887.5 mm^2 (range 654 to 1626 mm^2, q1 = 694 mm^2, q3 = 1230 mm^2) and 786 mm^2 (range 447 to 1851 mm^2, q1 = 586 mm^2, q3 = 959 mm^2) at 15 and 90 min, respectively, after the end of the meal, compared to 866 mm^2 (range, 602 to 1710 mm^2, q1 = 725 mm^2, q3 = 1071 mm^2) and 611 mm^2 (range, 295 to 1709 mm^2, q1 = 561 mm^2, q3 = 760 mm^2) at 15 and 90 min, respectively, after the end of the reference meal. The median gastric antral cross-sectional areas were significantly larger after ingestion of the meal includ-

ing vinegar than after the reference meal including water at 90 min ($p < 0.05$), but there were no significant differences between gastric antral cross-sectional areas at 15 min. The median value of the GER after the meal including vinegar was 17% (range -55% to 43%, q1 = -9%, q3 = 32%), while the median value of the GER after the reference meal 27% (range -11% to 51%, q1 = 5%, q3 = 41%). These results are shown in Figure 1. Gastric emptying rates after the meal including vinegar were significantly lower ($p < 0.05$) than after the reference meal. Individual values of GER indicated reduced values in all patients except two, after drinking apple cider vinegar (Figure 1).

Discussion

The primary objective of this pilot study was to determine weather apple cider vinegar would improve a delayed gastric emptying rate on diabetes mellitus patients. Despite the fact that we only studied ten patients with type 1 diabetes mellitus and diabetic gastroparesis, we were able to demonstrate a significant delay in already delayed gastric emptying of these subjects after the ingestion of vinegar. The median gastric antral cross-sectional areas were significantly larger after ingestion of the meal including vinegar than after the reference meal at 90 min. This difference could be due to delayed gastric emptying and therefore increased amount of gastric juices and saliva in the stomach. The subject that had undergone vagotomy responded in the same way as the others, showing a further reduction in the GER after drinking apple cider vinegar. The subject that was already consuming vinegar daily before of the start of the study also showed a reduced GER after drinking apple cider vinegar. He reported fewer gastrointestinal symptoms after the consumption of vinegar for a long period, which prompted us to initiate this study.

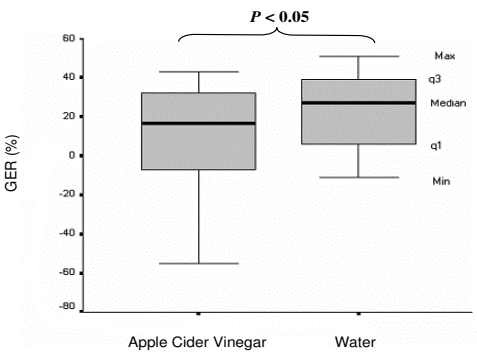

Figure 1
Gastric emptying of a rice pudding meal ingested with and without apple cider vinegar, expressed as the gastric emptying rate (GER), in ten type 1 diabetics with clinically diagnosed diabetic gastroparesis. The median (Md), minimum (Min), and maximum (Max) values and the values of the first (q1) and the third (q3) quartiles are shown. Values of $p < 0.05$ were considered significant.

We hypothesized that daily consumption of vinegar would affect diabetes patients with gastroparesis by increasing the gastric emptying rate. However, the effect found was the opposite in this study, namely the same as that observed by others in healthy subjects [15], i.e. a decrease in the rate of gastric emptying after the intake of vinegar. One of the subjects who already had a slow GER spontaneously reported a higher frequency of hypoglycaemic episodes during the two-week period of drinking apple cider vinegar. Neither the severity of the symptoms nor the frequency of hypoglycaemic episodes was recorded in this study. However, poor correlation has been observed between gastrointestinal tract symptoms and gastric autonomic neuropathy among subjects with diabetes mellitus [8]. Bloating and fullness have been associated with diabetic gastroparesis, whereas other upper gastrointestinal symptoms were found to be correlated weakly with solids emptying, and not at all with liquid emptying [9]. An increase in the frequency of unexplained hypoglycaemic episodes has, however, been found to be associated with an abnormal GER [11]. The measurements of gastric emptying were performed by the same radiologist who was blinded with regard to the meals. However, a limitation of this study is that it was not randomized and the subjects were not blinded. Another limitation of this study is that there may be a variation in measurments between the different machines used in the paired studies.

In a recent study, it was shown that white wheat bread served with white vinegar, reduced the postprandial blood glucose and insulin levels, and not only increased but also prolonged satiety in healthy subjects [17]. A dose-response relationship was also found between the amount of vinegar added and the levels of glucose, insulin and satiety [17]. Vinaigrette sauce added to cold boiled potatoes has also been found to reduce the postprandial blood glucose and insulin levels in healthy subjects [16]. The mechanism by which vinegar reduces postprandial blood glucose levels has been suggested to be a delay in gastric emptying [15], or the inhibition of amylases [14]. However, the organic acid sodium propionate has not been shown to affect starch hydrolysis *in vitro* [22]. In both healthy subjects and patients with type 1 diabetes mellitus it has been shown that hypoglycaemia increases the rate of gastric emptying [23,24]. Hyperglycaemia has also been shown to decrease gastric emptying in insulin-dependent diabetes mellitus patients [25]. Even physiological changes in blood glucose, from 4 to 8 mmol/l have been shown to affect gastric emptying in healthy subjects

and in insulin-dependent diabetes mellitus patients [26]. Unfortunately, did we not measure the postprandial blood glucose levels. However, the examination was performed only if the fasting blood glucose level was in the range 3.5 to 9.0 mmol/l and none of the subjects reported any symptoms of hypoglycaemia on the day of the ultrasonography examinations. The mean fasting blood glucose level before the reference meal was not significantly different from that before ingestion of the meal including vinegar.

It has been shown, using paracetamol as a marker for gastric emptying, that gastric emptying is delayed in healthy subjects after a meal including white vinegar [15]. The paracetamol method is dependent on the absorption of paracetamol across the small intestine which makes this method unreliable, as the pharmacokinetics of paracetamol vary within and between individuals [27,28]. The antihyperglycaemic effect of acetic acid has been shown to be mediated by enhanced glycogen repletion in liver and skeletal muscle [29], and in the suppression of disaccharidase activity in human intestinal cells [30]. In insulin-resistant subjects, apple cider vinegar has also been shown to improve postprandial insulin sensitivity [18]. It has been suggested that non-specific acid or pH receptors in the small intestine could reduce the GER [31]. The postprandial blood glucose level in healthy subjects was found to be reduced after a meal including white vinegar, compared with a meal including neutralised vinegar [14]. However, in the same study, gastric emptying measured using ultrasonography showed no difference in antral area after the reference meal compared with the meals including white vinegar or neutralised white vinegar [14].

Whether low-glycemic-index food in fact prevents diabetes mellitus is still unclear [32-36]. However, a low-glycemic-index diet that reduces postprandial hyperglycemia is recommended by the American Diabetes Association (ADA) to control glycemia in patients with diabetes [37,38]. Therefore the benefit findings of vinegar that has been shown to improve postprandial insulin sensitivity in insulin-resistant subjects [18] needs to be further studied. However, if vinegar reduces postprandial hyperglycemia and delays gastric emptying, the doses of preprandial injections of short-acting insulin may have to be adjusted. Furthermore, blood glucose must be monitored more frequently in patients with diabetes treated with insulin experiencing gastroparesis to prevent adverse hypoglycaemic episodes.

Conclusion

This small study show that vinegar delays gastric emptying in insulin-dependent diabetes mellitus patients with diabetic gastroparesis. Clearly, a larger, randomized trial involving a greater number of patients would be needed to validate the findings of this pilot study.

Competing interests

The author(s) declare that they have no competing interests.

Authors' contributions

JH participated in the design of the study, recruited the subjects, performed the statistical calculations and drafted the manuscript. GD participated in the design of the study, and participated in drafting the manuscript. OB participated in the design of the study and performed the ultrasound examinations. LOA participated in the design of the study and drafting of the manuscript. All authors read and approved the final manuscript.

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