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Rye Bread and Glucose and Insulin in Healthy Adults

Doctoral dissertation

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ABSTRACT

Dietary carbohydrate, per se, has not been associated with the risk of type 2 diabetes but the type and source of carbohydrate have acute and long-term effects on glucose metabolism. Although rye bread is a widely used cereal in Finland, its impact on glucose metabolism in healthy individuals is not fully understood.

The postprandial effects of conventional and experimental rye breads on glucose and insulin responses were compared to those of a refined wheat bread in healthy adults (10 males/10 females) and in postmenopausal women (n=19). All subjects with the exception of one woman had normal glucose tolerance. Since many physiological factors can influence postprandial glucose and insulin responses we also determined the release of incretin hormones (GIP, GLP-1), insulin secretion (C-peptide) and gastric emptying. To characterize food and dietary factors affecting the release of glucose from starch, the *in vitro* starch hydrolysis, the microscopic structure of breads and the effects of whole kernels and dietary fiber were studied. In addition, the effects of daily consumption of the high-fiber rye and the refined wheat breads on glucose metabolism were studied in 20 postmenopausal women using a 2 x 8 weeks randomized cross-over design. Frequently sampled intravenous glucose tolerance test was carried out at the run-in, and at the end of bread periods. Fasting plasma glucose and insulin concentrations were measured at the beginning and end of the bread periods.

Postprandial insulin and C-peptide responses of rye breads were decreased significantly as compared to wheat bread. There were no consistent differences in the early glucose response, but in contrast to rye bread, wheat bread displayed a steeper return of the plasma glucose to baseline and even below the fasting level. The presence of whole grains and the structure of rye bread, but not the quantity or quality of rye fiber, accounted for the reduced insulin response, the reduced starch hydrolysis *in vitro* and the decreased response of GIP. The GLP-1 response and gastric emptying rate were unaffected by the type of bread. The increase in the acute insulin response achieved by the daily consumption of rye bread was more enhanced than that of wheat bread ($9.9 \pm 24.2\%$ vs. $2.8 \pm 36.3\%$; $P=0.047$). However, no significant changes in any of the other measured variables (fasting plasma glucose and insulin, insulin sensitivity and glucose effectiveness indexes) were observed.

In conclusion, less insulin is needed and secreted to regulate the rise in postprandial glucose after ingestion of rye bread as compared to that of refined wheat bread in healthy adults. By enhancing the first-phase insulin secretion rye bread may contribute to the regulation of the postprandial glucose and the maintenance of normal insulin secretion. These observations in healthy subjects should be confirmed in risk groups of impaired glucose metabolism to determine whether rye bread can contribute to normal glucose tolerance and reduced risk of type 2 diabetes.

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Dedicated to my mother and
to the memory of my father

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ABBREVIATIONS

AIR	acute insulin response
AUC	area under the curve
AX	arabinoxylan
BMI	body mass index
CCK	cholecystokinin
CV	coefficient of variation
E %	percent of energy
FFA	free fatty acid
FSIGT	frequently sampled intravenous glucose tolerance test
GI	glycemic index
GIP	gastric inhibitory polypeptide, glucose-dependent insulintropic/insulin-releasing polypeptide
GL	glycemic load
GLP-1	glucagon-like peptide-1
GLUT	glucose transporter
HDL	high-density lipoprotein
HI	hydrolysis index
IFG	impaired fasting glucose
IGT	impaired glucose tolerance
IVGTT	intravenous glucose tolerance test
MINMOD	minimal model
NEFA	non-esterified fatty acid
OGTT	oral glucose tolerance test
RS	resistant starch
SCFA	short-chain fatty acid
S_G	glucose effectiveness index
S_I	insulin sensitivity index
WHO	World Health Organization

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications which will be referred to in the text by their Roman numerals I-IV.

- I Leinonen K, Liukkonen, K, Poutanen K, Uusitupa M, Mykkänen II. Rye bread decreases postprandial insulin response but does not alter glucose response in healthy Finnish subjects. *Eur J Clin Nutr* 1999;53:262-267.

- II Juntunen KS, Niskanen LK, Liukkonen KH, Poutanen KS, Holst JJ, Mykkänen HM. Postprandial glucose, insulin, and incretin responses to grain products in healthy subjects. *Am J Clin Nutr* 2002;75:254-262.

- III Juntunen KS, Laaksonen DE, Autio K, Niskanen LK, Holst JJ, Savolainen KE, Liukkonen KH, Poutanen KS, Mykkänen HM. Structural differences between rye and wheat bread but not total fiber content may explain the lower postprandial insulin response to rye bread. *Am J Clin Nutr* (in press).

- IV Juntunen KS, Laaksonen DE, Poutanen KS, Niskanen LK, Mykkänen HM. High-fiber rye bread and insulin secretion and sensitivity in healthy postmenopausal women. *Am J Clin Nutr* 2003;77:385-391.

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1. INTRODUCTION

It is estimated that the global prevalence of diabetes will increase from around 150 million cases in 2000 to 300 million by 2025, and almost 90 % of these cases will have type 2 diabetes (1). In Finland, the current number of 150 000 type 2 diabetic patients is expected to increase by 70 % until 2010 (2). Two large randomized intervention studies have shown that the risk of developing type 2 diabetes can be markedly reduced by lifestyle changes (3, 4). The most important risk factors which should be modified are obesity, inactivity and increased intake of fat, especially saturated fat and reduced intake of dietary fiber. Epidemiological studies have detected an association between the increased intake of whole-grain cereals, total and cereal fiber, as well as low glycemic index (GI) and glycemic load (GL) of the diet and reduced risk of type 2 diabetes (5-8).

Carbohydrates provide the main source of energy in the diet. They directly influence the rise of blood glucose, but the content of carbohydrate in the diet has not found to be related to the risk of developing diabetes (6, 7, 9). Since cereals, especially bread, are an essential part of the daily meals in western societies, they are a good source of dietary carbohydrate. The glycemic response to most of the conventional breads is known to be high (10), but it can be modified by the choice of raw material, baking process and conditions (11). Minimally processed whole grain products (12), and in some cases also a high content of dietary fiber in the bread (13), have been shown to reduce glucose and insulin responses. Rye bread, which is conventionally baked with sourdough from wholemeal flour, in some cases also from whole or cracked grains, and which contains an abundance of dietary fiber and associated bioactive substances (14), is a good candidate to seek and develop novel products aimed at regulating glucose metabolism. A series of postprandial studies were initiated to explore the physiological factors which affect the acute effects of conventional and experimental rye breads on glucose and insulin responses, and to investigate the dietary and food factors of rye breads which determine these responses. The factors regulating glucose metabolism are presented in Figure 1. The impact of high-fiber rye bread consumption on acute insulin response (AIR), insulin sensitivity and glucose effectiveness was also studied in an intervention study.

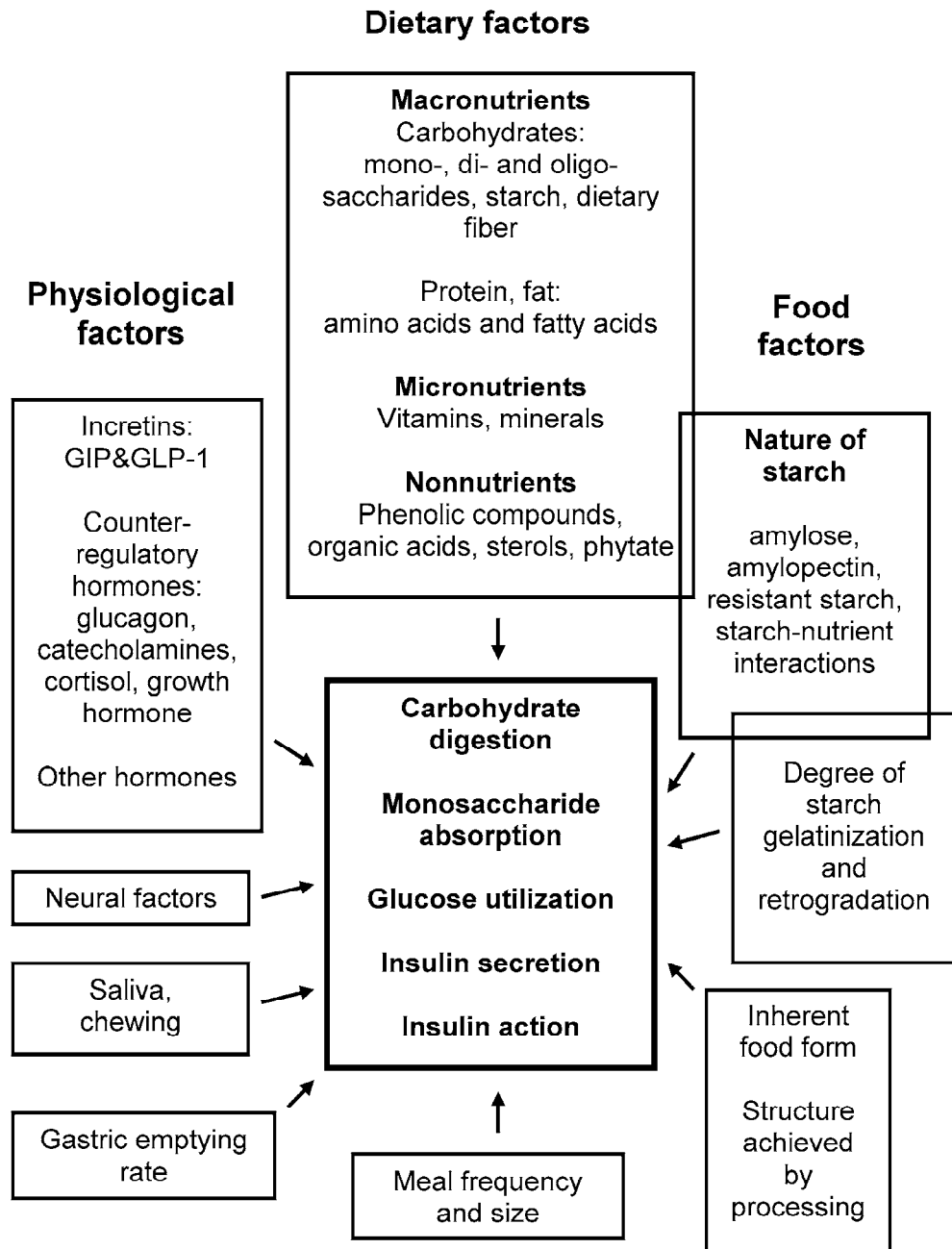


Figure 1. Factors affecting glucose metabolism

2. REVIEW OF THE LITERATURE

2.1 Glucose metabolism

2.1.1 Carbohydrate digestion and absorption

Dietary carbohydrate is classified as sugars (1-2 monosaccharide units), oligosaccharides (3-9 monosaccharide units) and polysaccharides (>9 monosaccharide units) based on the degree of polymerization (15). Most dietary carbohydrate is digested within the upper gastrointestinal tract to form monosaccharides which are then absorbed to the circulation. Insulin is subsequently released from pancreatic β cells in response to the rise in the blood glucose level. Indigestible carbohydrates such as dietary fiber and resistant starch (RS) serve as a substrate in colon fermentation yielding 1-2 kcal energy per g carbohydrate.

The digestion of food starts in the mouth by chewing and then degradation of released carbohydrates by salivary α -amylase (16). This enzyme is probably only partially inactivated in the stomach, and may contribute to the gastric hydrolysis of carbohydrates together with gastric acid. The rate of gastric emptying regulates the carbohydrate digestion and absorption, and the subsequent blood glucose rise and insulin release (17, 18). In healthy subjects, hypoglycemia (blood glucose 2.0 mmol/L) increases the rate of gastric emptying for both liquid and solid food (59 and 62 %, respectively) (19), whereas hyperglycemia (serum glucose 15.0 mmol/L) has an opposite effect (26 % increase in the recovery of gastric content) (20) as compared to normoglycemia (blood glucose 4-7 mmol/L and serum glucose 5.0 mmol/L, respectively). Gastric emptying of the meal begins as soon as any considerable part of the gastric content becomes fluid enough to pass through the pylorus (21), and it is modified by gastrointestinal hormones, such as cholecystokinin (CCK) (22) and glucagon-like peptide-1 (GLP-1) (23). Water and dilute fluids leave the stomach rapidly, followed by fluid meals with a high nutrient content, and finally solid particles (21). Also alkaline and hypotonic solutions empty faster than acid and isotonic solutions. Carbohydrates exit stomach the fastest followed by protein, whereas fat slows gastric emptying. With respect to other food factors, dietary fiber (24) and intact food structure and big food particles reduce the rate of gastric emptying (25).

In the upper small intestine, pancreatic α -amylase continues the digestion of carbohydrates (16). Both salivary and pancreatic amylases act only on the (1 \rightarrow 4) links of starch, and cannot hydrolyze the (1 \rightarrow 6) branching links of amylopectin. Therefore amylase action produces maltose, some glucose and large oligosaccharides (dextrins)

with (1→6) links. Further hydrolysis occurs in the brush border surface of the enterocytes by glycosidases, and hydrolysis is terminated by disaccharidases. The brush border is formed by invaginations (crypts) and villi surrounded by microvilli which guarantee efficient digestion and absorption because of the enormous surface area. Fluid layers immediately adjacent to the enterocyte membrane and the glycocalyx of the microvilli, i.e. unstirred water layer, differ from the fluid in the middle of the lumen, and prevents the peristaltic movement inside this layer. Therefore that layer also regulates the absorption of digested monosaccharides, glucose, galactose and fructose. Carbohydrate digestion and absorption can also be delayed by extrinsic factors such as viscous dietary fiber which may alter motility, decrease intraluminal mixing, and the surface area of the food particles available for digestion, as well as an increase in the thickness of the unstirred water layer (26, 27). The hydrolysis step is thought to be the rate-limiting step in the total process of converting raw starch to carbon dioxide (28). After that stage, glucose and galactose are actively transported into the intestinal mucosa via a specific, sodium-linked glucose transporter 1 (SGLT1) whereas fructose is absorbed by a facilitated transport system via the glucose transporter (GLUT) 5 (15).

Non-starch polysaccharides like cellulose, hemicellulose and pectin as well as lignin, RS and oligosaccharides are not digested in the small intestine (29). They are fermented in the large intestine by anaerobic bacteria releasing energy, gases and short-chain fatty acids (SCFA), i.e. acetate, propionate and butyrate in molar ratios of 57:22:21, respectively. Acetate is mainly found in peripheral blood and taken up by muscles. Butyrate is metabolized by the colonic mucosa and it is also taken up by the liver together with propionate. Even though propionate is known to be a major glucose precursor in ruminants, it is not an important substrate in human hepatocytes (15). In humans, SCFAs produced after colonic fermentation have been shown to improve glucose tolerance by suppressing hepatic glucose production and glucose and free fatty acid (FFA) responses after an oral glucose load (30). Inhibition of glucose production by propionate has also been confirmed in isolated rat hepatocytes (31).

2.1.2 Insulin biosynthesis and secretion

Insulin is a 51-amino acid peptide hormone which consists of two peptide chains linked by two disulphide bonds (32). The normal adult pancreas contains about 1 million islets of Langerhans which consist mainly of the insulin producing β cells (around 70 % of the total islet cell population) (33). β cells constitute about 1 % of the total gland mass (34). After proinsulin is synthesized in the cytoplasm of the β cells (35) it is converted to insulin and C-peptide until being secreted into the portal vein in

equimolar concentrations (36). Approximately 50 % of insulin is extracted during its first pass through the liver, and the rest is diluted in the systemic circulation. Glucose stimulation, which occurs in the range of glucose concentrations from 3.3 to 16.7 mmol/L, has been shown to regulate the synthesis of insulin messenger ribonucleic acid (mRNA) in isolated rat islets (37), suggesting that glucose itself is a factor which can regulate insulin biosynthesis both in the fasting and postprandial states.

Elevation of blood glucose concentration leads to glucose transport to the β cell through the GLUT 2 (38). Thereafter glucose is phosphorylated to glucose-6-phosphate, this being catalyzed by glucokinase, which is known to be the rate-limiting step in pancreatic glucose metabolism (39). Insulin secretion is evoked by a sequence of events involving an increase in the adenosine triphosphate (ATP): adenosine diphosphate (ADP) ratio, closure of the ATP-sensitive K^+ channels and activation of L-type voltage-gated Ca^{2+} channels and mobilization of calcium from intracellular stores.

The mean basal insulin secretion is around 110-120 pmol/L according to small studies measuring *in vivo* human insulin secretion (40, 41). At basal state, insulin is secreted in a pulsatile manner, with oscillations of 13 minutes (42). Pulsatile insulin secretion has a greater hypoglycemic effect as compared to steadily infused insulin based on greater insulin receptor binding (43). The postprandial insulin secretion is biphasic, as shown theoretically after the intravenous glucose load (Figure 2) (44). The first-phase (acute) insulin response to glucose begins within one minute, peaks between 3 and 5 minutes and lasts 10 minutes. The second-phase begins at 2 minutes but is not evident until 10 minutes have passed. It increases slowly for at least 60 minutes or until the stimulus ceases.

The intravenous glucose tolerance test (IVGTT) is a better evaluator of glucose-regulated insulin secretion than the oral glucose tolerance test (OGTT), because intravenous glucose administration bypasses several gastrointestinal factors which determine the magnitude of the insulin response such as gut hormones, neural stimulation and the rate of entry of ingested glucose into the circulation (44). The IVGTT also appears to have a reasonable reproducibility, the mean interday coefficient of variation (CV) for first-phase insulin secretion being 20 % with the variation for total insulin secretion being 21 % (45). In the case of OGTT, the test has been shown to be able to repeatedly classify around 66 % of the subjects to be normoglycemic, have impaired glucose tolerance (IGT) or diabetes (46). Despite the limitations, the OGTT has been used to evaluate insulin sensitivity and release because it is less costly and labour-intensive than IVGTT. The insulin area under the curve (AUC) which is formed during the first 30 minutes after an oral glucose ingestion divided by the corresponding

glucose AUC, also called insulinogenic index, has been used to represent the first-phase insulin secretion (47).

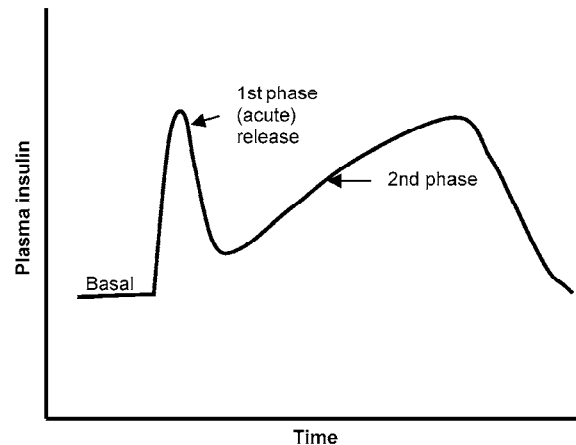


Figure 2. The theoretical biphasic insulin response to a constant intravenous glucose stimulus in healthy subjects. The peak of the first-phase is between 3 and 5 min and lasts 10 min. The second-phase begins at 2 min and increases slowly for at least 60 min. Modified from Ward et al, 1984 (44).

The plasma insulin half-life is around 6 minutes in healthy adults (48). Insulin is mainly degraded in the liver which accounts for 60-80 % of the body's total insulin disposal (49). The remaining degrading activity is located in the kidneys (10-20 %) and peripheral tissues, mainly skeletal muscle and adipose tissue.

2.1.3 Regulation of insulin biosynthesis and secretion

The connection between the gut and the pancreatic islets is called the "enteroinsular axis" (50). It comprises of hormonal and neural activation originating from gut, and of absorbed nutrients and their metabolites, which stimulate the islets of Langerhans. In addition to that axis, also local factors in the pancreas (51, 52) as well as other hormonal (53-55) and neural (56) factors are involved in the regulation of insulin secretion.

Thought, sight, smell, taste and chewing of food

The body is primed for the metabolic handling of ingested food via the cephalic phase of insulin secretion which is activated by thought, sight, smell, taste and chewing of food (57). Even though cephalic phase insulin secretion has been estimated to account for less than 1 % of the total insulin release in lean individuals following a

mixed nutrient meal, a failure in the normal cephalic phase insulin release has been proposed to lead over time to increased postprandial hyperglycemia and hyperinsulinemia (56). Therefore insulin secretion during the cephalic phase may crucially control the fluctuations in postprandial plasma glucose and insulin. In addition, gastrointestinal motility, gallbladder emptying, and gastric acid as well as secretions of pancreatic enzymes and gastrointestinal hormones are stimulated during the cephalic phase.

Nutrients

In mixed meals, many different dietary components influence insulin secretion at the same time. With respect to the individual nutrients, glucose, as well as to a lesser extent mannose and fructose, are the most potent nutrients which alone can stimulate the release of insulin (39). Of the non-carbohydrate metabolites, amino acids, fatty acids and ketoacids can acutely stimulate insulin secretion (58). The most insulinotropic amino acid is arginine followed by lysine, leucine and phenylalanine (59). One cannot detect any stimulation by protein and lipid metabolites when glucose is acutely present at high concentrations, but it is observed during chronic hyperglycemia as well as at low glucose concentrations and in the absence of glucose (58). This latter finding suggests that these compounds also have some role in the maintenance of some secretory stimuli for islets of Langerhans under conditions of hypoglycemia. Also alcohol augments the rise in plasma insulin in response to glucose even though it does not stimulate insulin secretion (60).

Hormones

Incretins

The term "incretin" was introduced at 1929 by Zunz and La Barre to describe the humoral part of the enteroinsular axis (61). These gut peptides potentiate the insulin releasing effect of glucose by up to 45-55 % after an oral glucose load (62), and therefore only 45-55 % of the insulin secretion of the β cells is directly stimulated by glucose. Several gastrointestinal peptides, such as gastrin, secretin and CCK have been suggested as possible incretins but their insulinotropic effects occur only at supraphysiologic doses (63-65). According to current knowledge, there are, however, only two peptides which do fulfill the criteria of evoking insulinotropic activity at physiologic doses, glucose-dependent insulinotropic polypeptide (GIP) and GLP-1 (66, 67). Although GLP-1 has been suggested to be a more potent stimulator of insulin

secretion than GIP, both peptides may contribute equally to the incretin effect (68) through the specific receptors in the β cells (69, 70).

GIP is called by several names, i.e. gastric inhibitory polypeptide, glucose-dependent insulinotropic/ insulin-releasing polypeptide. It is a 42-amino acid peptide hormone (molecular weight 4984) which is secreted by the epithelial K cells located primarily in the duodenum and jejunum (71, 72). The main form in plasma is GIP 1-42 which possesses insulinotropic activity in pancreatic β cells (73). GIP release is stimulated by the ingested carbohydrate and fat (74). The prerequisite for the insulinotropic effect of GIP is that there should be nutrient absorption in the gut, and the blood glucose elevation in plasma must be above 6 mmol/L (75). Since the first GIP increments are seen already 15-30 min after the ingestion of food, that is before the food reaches the gut (76), neural stimulation may be involved in GIP secretion (77). The plasma GIP concentration increases normally from the fasting concentration of below 20 pmol/L and varies from around 250 to 500 picomoles per liter after a meal (78, 79). The minimum concentration affecting insulin release is around 150 pmol/L (66, 78). GIP is metabolized rapidly (plasma half-life around 7 minutes) by the dipeptidyl-peptidase IV enzyme (79). The metabolic clearance rate is 4-8 ml/kg/min (78) and GIP is mainly degraded by the kidneys (80). In addition to insulinotropic activity, GIP may affect insulin secretion by stimulating β cell proliferation (81) and by reducing insulin extraction (82).

GLP-1 (7-36) is a 30-amino acid peptide hormone with a molecular weight of 3298 (72). GLP-1, GLP-2 and glicentin, also called enteroglucagon, are synthesized from proglucagon by post-translational processing in L cells which are located mainly in the ileum and colon (83). Two forms of GLP-1 have identical insulin-releasing potencies at equimolar concentrations (84). These are GLP-1(7-37), and from that form a further C-terminal truncated and amidated form, GLP-1(7-36), which comprises 80 % of the circulating GLP-1 (85). Oral glucose and mixed meals stimulate GLP-1 secretion within 5 min after ingestion, and the secretion lasts for approximately 60 minutes (66, 68). Absorption of nutrients in the gut and the increase of plasma glucose concentration above 6 mmol/L are needed for GLP-1 to be released indicating that the presence of nutrients in ileal mucosa is not a prerequisite for the GLP-1 release (86, 87). Therefore, there is probably an indirect releasing mechanism between proximal and distal gut controlled by a neuronal and/ or endocrine loop (88-90) even though the exact mechanism in humans is still unknown. The GLP-1 concentration in plasma varies from below 20 pmol/L in fasting to a maximum of approximately 30-50 pmol/L after a glucose load and mixed meals under normal circumstances (66). The minimum

concentration affecting insulin release is above 30 pmol/L. After the reduction of glucose concentration in plasma by approximately 20%, GLP-1 loses its ability to stimulate insulin secretion in the β cells (91). After release, bioactive GLP-1 is quickly inactivated by the dipeptidyl-peptidase IV (plasma half-life around 1-1½ min) (92) and probably also by the dipeptidyl-peptidase-IV-independent membrane-bound endopeptidase (93). The metabolic clearance rate of GLP-1 is around 12 ml/kg/min (66) and it is catabolized by the kidney (94). In pancreatic islets, GLP-1 may stimulate insulin biosynthesis and secretion (95), increase β cell proliferation and neogenesis (96), participate in islet cell differentiation (97) and suppress glucagon secretion (91). GLP-1 may also slow the digestive functions by retarding gastric emptying (98), and preventing the secretion of gastric acid (99), as well as regulating food intake through enhanced satiety (100).

Pancreatic islet cell and other hormones

From intra-islet cell hormones glucagon, a 29-amino acid peptide, is secreted by the islet A cells (51). It is a major counter-regulatory hormone for insulin, stimulating hepatic glucose output in response to insulin-induced hypoglycemia. In spite of its hyperglycemic effect, glucagon can also stimulate insulin secretion (53). Somatostatin is a 14-amino acid peptide which is secreted by the pancreatic D cells (101). It locally inhibits both basal and stimulated secretions of insulin and glucagon (52). Amylin, a 37-amino acid peptide hormone, is co-secreted with insulin from the β cell (102). It has been shown to reduce gastric emptying and inhibit glucagon secretion. Recent data also indicate that amylin may modulate or restrain insulin secretion (103).

The secretion of adrenal catecholamines (epinephrine), cortisol, and growth hormone are stimulated by hypoglycemia (104). Catecholamines predominantly inhibit insulin secretion through the activation of α_2 -receptors, reduce insulin biosynthesis and stimulate glucagon secretion (53, 105). In the case of prolonged catecholamine stimulation with sustained inhibition of insulin secretion, the activation of β_2 -receptors can also transiently stimulate insulin secretion (106). The counter-regulatory effects of cortisol and growth hormone to insulin-induced hypoglycemia are induced later (2-3 hours) than the effects of glucagon and catecholamines (54, 55). The effects of cortisol and growth hormone on insulin secretion are indirect because they reduce the tissue sensitivity to insulin by increasing hepatic glucose production, decreasing glucose utilization and accelerating lipolysis. Growth hormone may also lead to increased insulin synthesis and secretion through complex interactions with insulin-like growth factor-1 (IGF-1) (107, 108). Glucocorticoids and estrogen have an insulinotropic effect

on β cells and they potentiate basal and glucose-induced insulin secretion (109). In addition, estrogen can suppress glucagon secretion from pancreatic A cells.

CCK was originally isolated from the gut as a 33-amino acid peptide hormone and it has been shown to reduce glucose absorption via the inhibition of gastric emptying (22). It also potentiates amino acid- and fat-induced insulin secretion. Ghrelin is a recently discovered, 28 amino acids containing peptide hormone that is produced in the stomach (110). It has been shown to initiate and increase food consumption in response to negative energy balance (111). In addition, ghrelin may promote hyperglycemia and inhibit insulin release (112) and have some effects on gastric motor function (113) though the effects of ghrelin on insulin and glucose homeostasis are not yet well understood.

Leptin is a 146-amino acid peptide hormone which is secreted by adipose tissue (114). It possibly decreases insulin secretion through a direct effect on β cells. The results thus far are, however, conflicting and many have been found only at supraphysiological concentrations of leptin. Leptin levels have also been associated with insulin resistance, which has indirect effect on insulin secretion.

Neurotransmitters

Insulin secretion is enhanced by vagal (parasympathetic) stimulation, and attenuated by the activation of the sympathetic nervous system (56). Parasympathetic nerves of vagus release acetylcholine, vasoactive intestinal polypeptide (VIP) and gastrin-releasing polypeptide (GRP) following the ingestion of nutrients (115). These neurotransmitters might augment insulin output via either cephalic or gastropancreatic and enteropancreatic vagovagal reflexes (116). However, the contribution of extrinsic pancreatic nerves to cephalic insulin secretion is still being debated, because patients with a completely denervated pancreas have a normal incretin effect after an oral glucose load. Insulin secretion during fasting and stress is inhibited by the activation of sympathetic neurons that release norepinephrine, galanin and neuropeptide Y (NPY) (115).

2.1.4 Glucose utilization and its connections to insulin metabolism

After the dietary carbohydrate is digested and absorbed, it enters the circulation as monosaccharides (117). With respect to the absorbed monosaccharides, glucose is readily metabolisable energy whereas fructose and galactose are taken up by the liver, and converted to glucose. Therefore their direct blood glucose raising effect is less than that of glucose. Since fructose does not stimulate secretion of insulin, excess

consumption of fructose may also induce hyperglycemia (118). If large amounts of fructose are consumed, hepatic lipogenesis begins to predominate at the expense of glucose production. During the interdigestive phases, the blood glucose concentration is maintained by endogenous hepatic and renal glucose production from lactate, glycerol and glucogenic amino acids (119, 120). The kidneys have a minor role in gluconeogenesis since they are estimated to account for 5-25 % of total glucose production after an overnight fast (121). Glucose can also be mobilized from skeletal muscle and liver where carbohydrate is stored as glycogen (122). From that reservoir around 70 g exists in the liver, and is easily mobilized after any decrease in the exogenous supply, whereas muscle glycogen (around 250 g) can only be used by muscles. The kidneys can also completely reabsorb the filtered glucose during hypoglycemia (123). In the postabsorptive state, gluconeogenesis and glycogenolysis each contribute approximately 50 % of the glucose output into the systemic circulation (120).

Most organs use glucose as a source of energy, but the brain and erythrocytes are especially dependent on this substrate (120). After an overnight fast, hepatic glucose production is 1.8-2.2 mg/min/kg body weight (124). The brain accounts for around 50 % of that postabsorptive glucose disposal, splanchnic organs (liver and gut) for around 25 % and insulin-dependent tissues, primarily muscle, for the remaining 25 %. Glucose transport across the plasma membrane is the rate-limiting step in glucose utilization in muscle and fat cells (38).

Six structurally related GLUT proteins have been identified (38). These specialized transport proteins transfer glucose molecules through the cell membrane into the cytoplasm of the cell through a facilitated diffusion mechanism. The entry of glucose to the brain across the blood-brain-barrier, and to the erythrocytes via tissue-specific GLUT 1 is independent of insulin whereas transport into muscle and adipose tissue via GLUT 4 is insulin-dependent (123). Glucose transport into the skeletal muscle is also stimulated by an insulin-independent mechanism activated by contractions, hypoxia and nitric oxide, and mediated by Ca^{2+} (125). Uptake to the liver is mediated by GLUT 2, similarly as in the pancreatic β cell, and it is not dependent on insulin (38).

Insulin-dependent glucose uptake

A prerequisite for the glucose uptake into the insulin-dependent tissues is binding of insulin to the transmembrane insulin receptor which is a protein with two extracellularly localized α -subunits and two transmembraneous β -subunits linked by disulfide bonds (126). Insulin activates the tyrosine kinases of the β -subunits which

phosphorylate the different insulin receptor substrates (IRS-1, -2, -3 and -4) (127). The branching of insulin signaling pathways in insulin sensitive cells is regulated by the specific IRS which is activated, leading to the activation of phosphatidylinositol 3-kinase (PI 3-kinase) or mitogen-activated protein kinase (MAP-kinase) pathways. The activation of the PI 3-kinase pathway causes metabolic insulin effects such as substrate uptake and utilisation including GLUT4 translocation and glucose transport into the cell, glycogen, protein and fatty acid synthesis, and inhibition of lipolysis (128). MAP-kinase activation is related to the mitogenic effects such as cell growth and proliferation. In addition to insulin-dependent muscle and adipose tissues, insulin receptors are widely found in various tissues such as the liver, brain, pancreatic β cells, kidney, heart, monocytes, and endothelial and vascular smooth muscle cells (126).

Insulin-independent glucose uptake

Binding of insulin to its hepatic membrane receptors does not contribute to glucose uptake into the liver cells through GLUT 2 (38). However, insulin reduces hepatic glucose production by the inhibition of gluconeogenesis and glycogenolysis both directly and indirectly (121). The direct effect of insulin on glucose production occurs acutely after the binding of insulin to its hepatocyte receptors which leads to the rapid inhibition of glycogen breakdown. The indirect pathway is suggested to involve a cross-talk between the liver and peripheral tissues in the maintenance of normal glucose tolerance after carbohydrate ingestion (129). One explanation is that the reduced fatty acid oxidation results in a compensatory increase in glucose oxidation, and a consequent reduction in hepatic gluconeogenesis (130). Indirect effects of insulin may also partly be mediated by the inhibition of glucagon secretion, a reduction in the output of gluconeogenic amino acids from muscle, and by an alteration of renal glucose production (121).

2.1.5 Impaired glucose metabolism

The World Health Organization (WHO) classifies the increases in plasma glucose intermediate between normal and diabetic states as IGT and impaired fasting glucose (IFG) (131). The diagnostic criteria for IGT are fasting plasma glucose concentration <7.0 mmol/L (whole blood <6.1 mmol/L), and 2 hours after a 75 g oral glucose load 7.8 mmol/L or above to <11.1 mmol/L in plasma (6.7 mmol/L or above to <10.0 mmol/L in whole venous blood). The corresponding values for IFG are 6.1 mmol/L or above to <7.0 mmol/L in plasma (5.6 mmol/L or above to <6.1 in whole blood), and <7.8 mmol/L in plasma (<6.7 mmol/L in whole venous blood). WHO recommends the use of

both fasting and 2 h values for the diagnosis of individual cases, because these two measurements have been shown partly to identify different patients. Middle-aged, more obese people are more likely to have diagnostic fasting values whereas elderly and less obese individuals are more likely to have diagnostic 2 h values. According to etiological classification, the diabetes mellitus is classified to type 1 and type 2, gestational and a group of other specific types of diabetes. Type 1 diabetics require insulin for survival due to β cell destruction whereas in type 2 diabetes, insulin is needed only in part of the cases for control of the disease. The presentation of this section will concentrate on illustrating the disturbances in type 2 diabetes, because it is the most common type.

Type 2 diabetes is characterized by insulin resistance and impaired insulin secretion (132). The inability to compensate for a decrease in insulin action by increased insulin secretion distinguishes individuals who develop diabetes from those who remain normoglycemic. Abnormal pulsatile insulin secretion is an early marker for β cell dysfunction, involving impaired rapid pulsatile secretion (133) and ultradian (134) and diurnal oscillations (135). Also occurring early in the development of β cell dysfunction is a decrease in first-phase insulin secretion (132). The first-phase has a crucial role for the normalization of postprandial blood glucose because it enhances glucose clearance and reduces hepatic gluconeogenesis (136). The blunted first-phase insulin secretion is accompanied by a prolonged and exaggerated second-phase insulin response to glucose (135) and by increased fasting insulin concentrations (137). The latter may predispose the patient over time to an increased risk of coronary heart disease (138). In addition, increased β cell mass may contribute to insulin oversecretion in response to insulin resistance (139) and a disproportionate increase in the proinsulin to insulin ratio has been proposed as a marker of defect in insulin secretion (140). With time, compensatory second-phase insulin secretion cannot be maintained and in established type 2 diabetes, the second-phase is also attenuated and marked hyperglycemia is manifested (141). During the worsening of the diabetic state, the β cells lose glucose-induced insulin secretion entirely, this being accompanied also by a partial loss of non-glucose-induced secretion (142). Glycogen, lipid, and amyloid deposits are suggested to induce fibrosis and cell apoptosis leading to structural damage of β cells in the final stage of the development of β cell degeneration. These states are also known as glucose and lipid toxicity.

Postprandial glucose excursions have a clear connection to the disorders of type 2 diabetes. If glucose rises rapidly it leads to an enhanced incretin effect and insulin-induced hypoglycemia (143). Subsequent release of counter-regulatory hormones restore euglycemia and elevate FFA levels, thus inducing insulin resistance (130).

Repeated postprandial hyperinsulinemia may also overstimulate β cells (144), and contribute to insulin resistance through a reduction of insulin efficiency by downregulation of insulin receptors (145), and through induction of obesity by episodes of overeating after hypoglycemia (143).

Insulin resistance is defined as the inability of a known quantity of exogenous or endogenous insulin to increase glucose uptake and utilization in an individual as much as it does in the normal population (146). It can develop slowly and remain undiagnosed for years (147). At least 25 % of the non-obese healthy population with normal oral glucose tolerance are estimated to have reduced glucose uptake at least to the same degree as in subjects with IGT and type 2 diabetes (148). The cause of insulin resistance is multifactorial including factors like genetic abnormalities, fetal malnutrition, obesity, and reduced physical activity (146). The state of the metabolic syndrome is involved in the etiology of type 2 diabetes in the majority of cases (149). The typical features are glucose intolerance, insulin resistance, raised plasma triglycerides, low high-density lipoprotein (HDL)-cholesterol, hypertension, abdominal obesity, and impaired prothrombotic and proinflammatory states (131, 148). These components of the syndrome convey also an increased risk for cardiovascular diseases.

Even though muscle is the major site for insulin-dependent glucose metabolism, insulin resistance is suggested to be initiated in the adipose tissue, and induce the insulin resistance in other tissues (150). Inadequately suppressed lipolysis in adipose tissue leads to elevated circulating non-esterified fatty acid (NEFA) concentrations both postprandially and in the postabsorptive state (151). Preferential oxidation of NEFA rather than glucose in skeletal and cardiac muscle results in reduced glucose utilization, the development of hyperglycemia, and further impairment of insulin resistance (130). In the liver, suppression of hepatic glucose production is reduced (152) and the release of triglyceride-rich very-low-density lipoprotein (VLDL) particles is enhanced (153). The former is suggested to be the main reason for the postprandial hyperglycemia (154), and the latter results in further impairment of postprandial hypertriglyceridemia. Increased delivery of NEFA to the liver inhibits also hepatic insulin clearance by reducing insulin binding to hepatocytes, thus aggravating the hyperinsulinemia (151).

Insulin resistance is also associated with impaired vascular function, chronic activation of the innate immune system, and the increased thrombotic risk by decreased fibrinolysis (155-157). Hyperglycemia induces the production of reactive oxygen species which leads to increased oxidative stress in a variety of tissues (158). This may contribute to late diabetic complications as well as to insulin resistance and impaired insulin secretion.

2.2 Glycemic index, glycemic load and hydrolysis index

GI was initially introduced by Jenkins in 1981 for identifying carbohydrate-containing foodstuffs which minimize the postprandial glucose rise, and are therefore especially suitable for diabetics (159). It is defined as the incremental area under the blood glucose response curve of the same amount of carbohydrate portion of a test food expressed as a percent of the response to the same amount of carbohydrate from a standard food taken by the same subject. The dose of available carbohydrate used per test portion has usually been 50 g with white wheat bread or glucose being used as the standard. The highest GIs have been obtained for potatoes, breakfast cereals and conventional breads, and the lowest for legumes and pasta products, respectively (10). The term "lente carbohydrate" refers to a foodstuff which releases slowly its carbohydrates (160) studied also by *in vitro* starch hydrolysis method (161, 162). Since *in vitro* data has been found to positively correlate with *in vivo* results (162), it has been used for ranking of carbohydrate-containing foods, and as a preliminary study before *in vivo* studies. The results are expressed as percentage of digested sugars released after grinding or chewing of food, and subsequent incubation with digestive enzymes, or as the hydrolysis index (HI) calculated analogously to GI (162, 163).

The use of GI is continuously a matter of intense debate because of multiple reasons. It has been argued that GIs of food components cannot predict the response to a whole meal (164), though this has been rejected by others (165). There exists a variation in glycemic responses between subjects with different regulatory capacity of glucose metabolism (CV of the GI values 10 % in type 2 diabetics and 18 % in type 1 diabetics), as well as day-to-day variability within individuals (166). The intraindividual variation is shown to be greater (CV of the GI values 16 % in type 2 diabetics and 25 % in type 1 diabetics) (166), and it can be reduced by repeated tests with the same test food (167). Since glycemic responses differ on a relative rather than an absolute basis, the interindividual variation can be overcome by expressing responses as GI values rather than as response areas (CV of the glycemic response areas 25 % and of the GI values 10 % in type 2 diabetics) (166). The methods for calculating response areas have also been criticized, because the higher the fasting blood glucose value, the smaller the contribution of the postprandial rise to the total glycemia (168).

Since GI takes into account the quality of carbohydrate but does not consider the edible portion of food, the GL was proposed to represent both the quality and quantity, and to serve as a measure of dietary insulin demand (7, 9). It is calculated as the GI of food multiplied by grams of carbohydrate per serving size and divided by 100 (10). Since the glycemic response has been estimated to predict only 23 % of the variability

in insulin responses, the use of insulin indexes has been proposed (169). The indexes were calculated as being analogous to GI for 1000 kJ test portions.

It has been claimed that a low-GI diet may reduce the risk of type 2 diabetes (7, 9), and would have beneficial effects both on postprandial glucose metabolism (170) and longer-term (2 x 24-day cross-over) regulation of glucose and insulin (171). Improvements in glucose tolerance at the subsequent meal after the consumption of low-GI foods have been demonstrated both after the dinner (172) and the breakfast (173), and the low-GI breakfast has been suggested to determine the glycemic control over the whole day regardless of the composition of other meals (174). Support for the benefits of low-GI diets come also from studies on isocaloric diets with varying meal frequency (nibbling vs. gorging) (175), and the use of synthetic (176) or natural (177) α -glucosidase inhibitors in foods, all of which have been used as models for the reduced rate of carbohydrate absorption. Although the clinical usefulness of the GI concept remains controversial, some health organizations currently recommend consuming low-GI foods in the management of type 2 diabetes (178), and as a part of a healthy diet in the general population (15).

2.3 Dietary carbohydrate with focus on rye bread

2.3.1 Health effects of carbohydrate consumption

A diet containing more than half of the energy from carbohydrates is recommended both for healthy individuals (55-60 percent of energy, E %) (179) and for diabetic patients (45-60 E %) (178, 180). The current intake of carbohydrates among Finnish men is 45.6 E % with the corresponding value for women being 49.6 E %, respectively (181). The primary sources are cereals, dairy products, fruits, berries, vegetables and sugar. The proportion of cereals and potatoes as a source of carbohydrates has decreased at the expense of fruits and vegetables in Finland since the 1970's (182). However, cereals are still the predominant source, providing almost half (46-48 %) of the total intake of carbohydrates, and 32-34 % of the total intake of energy (181). Bread accounts for 24-28 % carbohydrates and 15-16 % energy, respectively.

Both the quantity and quality of carbohydrates affect glucose and insulin metabolism. Healthy, moderately active adults require daily at least 200 g carbohydrates (117). The greater the amount of carbohydrates, the quicker it is converted to blood glucose and the higher is the rise in postprandial glucose (183). The rate of conversion is affected by the source of carbohydrate, and the extent and type of processing of food (11). To achieve desirable effects on glucose and insulin responses, and to lower the risk of type 2 diabetes, priority in the diet should be given to the quality of carbohydrates rather than quantity alone (184). According to epidemiological studies the total intake of carbohydrates is not associated with increased risk of type 2 diabetes (6, 7, 9, 185). On the contrary, the intake of whole grain cereal products, such as dark bread, has been shown to reduce (6, 8, 186), and that of highly refined grain products, such as white wheat bread and white rice, to increase the risk of type 2 diabetes (7, 9).

An increase of the content of carbohydrates in the diet above 50 % of the daily intake of energy has been shown to induce a reduction in total plasma cholesterol and an adverse elevation of plasma triglycerides together with a reduction in HDL-cholesterol concentrations (187). Hypertriglyceridemia may further over time predispose the individual to insulin resistance. However, if high-carbohydrate diets are rich in unrefined and whole structure carbohydrate sources, and in some cases also restricted in simple sugars, they do not elevate triglyceride concentrations as compared to high-fat low-carbohydrate diets (188-190). In these studies also indicators of insulin sensitivity were improved (188-190). Beneficial effects on glucose and lipid metabolism have also been found in studies in which wholegrain pumpernickel and sourdough rye breads have served as an essential source of dietary carbohydrates (21-36 % from total

carbohydrates) as compared to diets containing wheat bread (191, 192). These findings indicate that by using slowly-digestible carbohydrate foods in a high-carbohydrate diet, the adverse changes in glucose and lipid metabolism can be overcome. That is also in line with the results of a meta-analysis which found that the low-GI diets reduce triglycerides and total cholesterol on an average by 9 and 6 %, respectively, together with significant improvements in the glycemic control and insulin sensitivity as compared to the high-GI diets (193). However, the long-term benefits of low-GI food consumption at the currently recommended consumption level of carbohydrates, and the compliance of the subjects need to be confirmed in further studies.

2.3.2 Bread in the diet

Bread is an essential part of the daily meals in western societies including Finland. There are many different types of breads on sale, e.g. rye- and wheat-based products together with oat- and barley-based breads, or different combinations of these flours. Rye and wheat are, however, quantitatively the most commonly used cereals in Finnish breads (181). Since the Second World War the proportion of bread in the diet has decreased and wheat bread has partly replaced rye bread (14). Rye (*Secale cereale L.*) is traditionally used as a raw material in bread baking in the northern and eastern parts of Europe, even if it is predominantly cultivated and consumed as animal feed. On the global scale, 95 % of rye is produced in the area between the North Sea and the Ural Mountains in Russia due to modest demands for soil, fertilization and climate. In Finland, the yearly consumption of rye is 17 kg per capita and that amount is predominantly eaten as bread. In addition to bread, there are different types of rye flours, groats and flakes available for baking, cooking porridges and as breakfast cereals (194). Traditionally eaten rye products are Karelian pastries, "Mämmi" (Finnish Easter pudding), rye-berry pies and "Kalakukko" (rye-dough-covered baked dish containing fish and meat). Novel rye products are rye-containing pasta products, mixture of rice and steel cut rye, rye hamburgers and rye-based snack products. Since all the studies included in this thesis were performed by using rye breads, this presentation will specifically concentrate on rye. Wheat is discussed to some extent because it was used as a reference grain, and is another main ingredient of breads.

In Finland, rye bread has been baked since the Iron Age (500 B.C.–1200 A.D.), and it is most commonly used as a whole grain product (14). The traditional rye bread in Finland, the Baltic countries, Poland, Belarus and the Russian Federation was made with sourdough containing wholemeal rye flour, water and salt, but nowadays commercial bakeries use also wheat flour and yeast. Lactic acid fermentation gives a

rich flavor and aroma, serves as a rising agent, and prolongs the shelf life by preventing the bread from growing stale and inhibiting the growth of mould (195). There have been some differences in the traditional ways of baking and the extraction rate of the flours in the western regions where rye bread is eaten as compared to the eastern regions (194). In Finland and Denmark, flours have been typically made of whole grains with an extraction rate of 100 in contrast to the extraction rate of 80 in Sweden and Norway. While the Finnish rye breads have typically been various types of soft loafs and crispbreads, also pumpernickel-type whole grain sourdough rye breads have been popular in northern Europe, especially in Germany for centuries (196). Pumpernickel rye bread is made of rye kernels, white wheat flour and rye malt and slowly baked at a low temperature (up to 24 h). The Finnish bread selection contains also one type of bread, “Jälkiuunileipä”, which is made of wholemeal flour like the soft rye breads but baked longer at a low temperature (14). Therefore the moisture content of the bread is lower and it can be stored longer than conventional soft rye breads. Traditionally Finland has also been divided to the western dry rye bread, and to the eastern soft rye bread regions (14).

Although the consumption of rye bread has declined over the past decades, rye is still the most commonly eaten bread in Finland (181). There are also regional, educational, age and gender differences in the intake of rye and wheat breads (197). Rye bread is eaten most frequently in the rural parts of the eastern and northern Finland and among lower educated groups. The consumption has decreased especially among the younger age groups and well-educated population in urbanized southern Finland. There is, however, a trend toward increased use among the urban well-educated women. Men eat on an average 100 g of rye bread per day, women eat about 66 g (181). The respective values for mixed flour breads are 54 g for men, and 39 g for women. Since rye breads contain completely or partly wholemeal flour, they contribute considerably to the intake of dietary fiber, and provide also many vitamins, minerals and bioactive substances (see 2.3.3.1 Cereal fiber and 2.3.3.5 Other factors). Rye bread is the most important individual foodstuff which contributes to the intake of dietary fiber in Finland (198). Rye products provide 45 % of the total intake of fiber in men and 36 % in women, respectively.

2.3.3 Food and dietary factors of bread affecting glucose metabolism

2.3.3.1 Cereal fiber

Definition and composition

The term “dietary fiber” was first introduced by Hipsley in 1953 as the nondigestible constituents that make up the plant cell wall (199). Since then, different definitions based on different analytical procedures and physiological effects have been published (15). The currently used definition was published in 2001 by the American Association of Cereal Chemists (AACC) (199). It characterizes fiber as “the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fiber includes polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fiber promotes beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation.” The overall improvement to earlier definitions has been to characterize the whole dietary fiber complex by adequate methods. Therefore the updated definition also more clearly delineates the fructo-oligosaccharides, fructans, which is also one component of dietary fiber in rye (200). Analytically dietary fiber can be divided on the basis of water solubility into insoluble and soluble fibers (201). The former include cellulose, lignin and many hemicelluloses. The latter consists of the pectins, some hemicelluloses, gums, mucilages and storage polysaccharides.

Rye and wheat, as with other cereals, contain the same dietary fiber fractions but the total dietary fiber content of rye grain is higher as compared to wheat (15-17 % vs. 10-13 %) (202) because of the higher content of fiber polysaccharides within the endosperm in rye than wheat (203). Arabinoxylans (AX) are the most abundant part of dietary fiber in rye (8-10 %), followed by linear polysaccharide (1→3), (1→4)-β-D-glucan, i.e. β-glucan (1.5-2.2 %), cellulose (1.3-2.5 %), Klason lignin (1.1-1.6 %), and fructans (2.5-2.7 %) (200, 202, 204, 205). AX's belong to hemicelluloses and heteroxylans, and it is formed by a linear backbone of (1,4)-β-D-xylopyranosyl units and a varying degree of substitution with mainly terminal α-L-arabinofuranosyl substituents attached to carbon 2 or carbons 2 and 3 (in some cases only to carbon 3) (199, 205). AX is also sometimes regarded and studied as pentosan, which is formed by AX and arabinogalactan (206). Since the content of galactose in rye is very low, the pentosan content of rye consists mainly of AX (207). About 20-38 % of AX in rye is water-extractable (202), and it forms mainly the soluble fiber part of rye and wheat

together with β -glucan (203, 208). The soluble fraction from β -glucan in rye is, however, smaller than that in oats (209). The dietary fiber components of rye are concentrated in the outer layers of the kernel (70-80 % of the total amount) (204, 205), but the content of soluble AX in endosperm is higher than in the outer bran layers where AX is mainly insoluble (210). This is explained by the branched structure of AX in endosperm due to the arabinose residues attachments to xylose backbone (206).

Fiber and glucose metabolism

Numerous studies of glycemic and insulinemic responses have been performed especially with soluble fibers such as gums, psyllium and pectin, and with insoluble fibers such as wheat bran as reviewed by Jenkins (160), and Wursch and Pi-Sunyer (211). In many studies, purified fiber components have been added to the test meal or glucose solution, given in a capsule, taken before a meal, or in other cases been incorporated into foods or naturally existing in real foods. In conclusion, meals or glucose loads containing viscous soluble fibers have been shown to flatten postprandial glycemia more consistently than wheat bran and other insoluble fiber components. However, the focus in this thesis will be on the effects of cereal fiber components of rye in bread matrix either endogenously existing in bread or enriched with some fraction or with flours from high-fiber genotype. Therefore also studies with cereals other than rye will be discussed if they contain the same fiber fractions as found in rye. From diet intervention studies, the focus will be on studies where diet has been modified only with bread or together with other cereals.

Both the quantity and quality of cereal fiber in bread may affect glucose metabolism. However, postprandial studies on rye bread have not revealed the effect of total content of fiber on postprandial glucose response in breads baked with milled flours (12, 212-214) with one exception (215) (Table 1). The ineffectiveness of the quantity of fiber on postprandial glucose (12, 216-221) and insulin (216, 220, 221) responses has also been shown among wheat (12, 216-219, 221) and barley (220) breads. On the contrary, the enrichment of bread with soluble AX with doses of 3.7-7.4 g/ meal (13) or with β -glucan with doses of 8-14 g/meal from high-fiber barley genotype (220) has been found to reduce postprandial glycemia and insulinemia whereas the insoluble cereal fiber in the form of wheat bran enriched wheat bread was ineffective (221).

Over time the acutely lowered glucose responses after soluble fiber ingestion followed by reduced need for insulin may lead to improved insulin sensitivity, because the consumption of soluble fiber may increase the translocation of GLUT 4 receptors to

First author year (ref)	Number of subjects, sex ¹	Age, glucose tolerance status	Study length/ blood sample	Available carbohydrate in portion	Control/ test breads	Outcome measure	Results
Brand, 1990 (212)	7 M&F from the pool of 5M and 7F	20-30 y, normal glucose tolerance	120 min/ capillary blood	50 g, analyzed	glucose/ WM wheat bread, rye Schinkenbröt, rye Riga Black Bread	GI II ¹³ <i>In vitro</i> starch digestibility, %	78±16,86±15,76±14 121±30,57±14,89±17 (analysis of II not reported) 34 ^a , 19 ^b , 22 ^b
Rasmussen, 1991 (219)	6 M, 1 F	21-41 y, DM1	180 min/ not reported	53-54 g, analyzed	RWB/ 75% WM rye; 25% cracked rye grains	glucose AUC	↓
Liljeberg, 1992 (163)	5 M, 5 F	26-50 y, non-diabetics, glucose tolerance status not reported	180 min/ capillary blood	50 g, analyzed	RWB/ 80% WRK; 20% RW	GI II HI ¹⁴	↓ ↓ ↓
Liljeberg, 1994 (222)	5 M, 3 F	25-47 y, non-diabetics, glucose tolerance status not reported	180 min/ capillary blood	50 g, analyzed	RWB/ commercial pumpernickel	GI II HI resistant starch, % (dry weight basis)	↓ ↓ NS 0.6±0.00 vs. 4.5±0.09
Bimbacher, 1995 (214)	5 boys, 9 girls	12.8 ± 1.4 y, DM1	150 min/ capillary blood	0.9 ± 0.2 g carbohydrate/ kg body weight	RWB/ dark rye bread	glucose AUC	NS

¹ M=males, F=females; ² DM1=type 1 diabetes mellitus; ³ DM2=type 2 diabetes mellitus; ⁴ RWB=refined wheat bread; ⁵ WM=wholemeal flour; ⁶ RW=refined wheat flour; ⁷ AUC=area under the curve; ⁸ NS=not statistically significantly different; ⁹ WRK=whole rye kernels; ¹⁰ GI= glycemic index; ¹¹ GIP=glucose-dependent insulinotropic polypeptide; ¹² significantly decreased as compared to refined wheat bread; ¹³ II=insulin index; ¹⁴ HI=hydrolysis index

the muscle cell membrane, thus increasing glucose disposal (223). The effect of soluble fiber in the small intestine is thought to be based on the viscosity of the intestinal contents (see 2.1.1 Carbohydrate digestion and absorption) and therefore the presence of fiber in a test meal seems to be a prerequisite to achieve a reduction in postprandial glucose and insulin responses (224, 225). In rye bread, the viscosity has been shown to be lower than in the raw material extracts of rye, thus highlighting the impact of baking on viscosity (205). In addition, considerably large amounts of soluble fiber are needed to be effective (3.7-14 g/meal) (13, 220). It is claimed that at least 4-6 g β -glucan/meal is needed if it is to have any impact on glucose metabolism (208). Moreover, a 50 % reduction in the glycemic peak is to be expected with a cereal product containing approximately 8-10 % β -glucan (211). Such huge amounts seldom exist in conventional breads. It is likely that soluble fiber in bread contributes to the glucose metabolism but it is not the sole, and obviously not the main explanation. This is further emphasized by the finding that total dietary fiber was significantly related to GI, but the variation was only partly explained by the uronic acids in soluble fiber (34 %) and total dietary fiber (21 %) (226).

The findings of epidemiological studies may seem totally contradictory to the acute postprandial studies, since the intake of cereal fiber, which is mainly insoluble, has been shown to be inversely related to fasting insulin levels (227, 228) and to protection against type 2 diabetes (5, 6, 9, 229). Since the beneficial effects are related to whole-grain cereals (6, 8, 186), these beneficial effects of cereal fiber may be attributable to the preserved structure of the grains (163, 230) or processing modified structure of cereal products (231) which reduce the rate of starch hydrolysis in the small intestine. Insoluble fiber may also reduce carbohydrate absorption by shortening the transit-time in the small intestine (232), and consequently reduce the time for digestion and absorption. Both the dietary fiber and food form may have improved insulin sensitivity after the consumption of wheat-based (80%) unrefined grain products in comparison to refined ones in a recent 2 x 6 weeks cross-over study (233). In addition, the most marked reduction in the risk of diabetes has been found in the combination of a high intake of cereal fiber and a low GL in the diet (7, 9).

Only two earlier intervention studies on rye bread have investigated glucose metabolism (234, 235) (Table 2). They found no differences in fasting glucose and/or insulin responses although the daytime glucose response and daily insulin dose were reduced in type 1 diabetics. Similarly no improvement in insulin sensitivity either after the consumption of fiber-enriched wheat (225, 236-238), oats (225, 239), or barley

Table 2. Human intervention studies on rye bread and effects on glucose metabolism.

First author year (ref)	Number of subjects, sex ¹	Age, glucose tolerance status	Design	Intervention	Outcome measure	Results
Nygren, 1984 (234)	5 F	32 ± 5 y, DM1 ²	2-wk control + 4-wk intervention periods	low-bran rye bread (5 % DF ³) vs. high- bran rye bread (18 % DF)	fasting glucose	NS
					daytime glucose curve insulin dose body weight	NS ↓ NS
Leinonen, 2000 (235)	2 M, 5 F	41 ± 13 y, DM1	2-wk control + 2-wk intervention periods	usual bread (4 % DF) vs. high-bran rye bread (18 % DF)	fasting glucose	NS
					daytime glucose curve insulin dose body weight	↓ NS NS
	18 M, 22 F	43 ± 2 y, non-diabetics	cross-over, 4-wk + 4-wk	conventional refined wheat breads (provided 3.6-4.7 g DF/day) vs. conventional rye breads (provided 16.4-22.1 g DF/day)	fasting glucose	NS
					fasting insulin	NS

¹ M=males, F=females; ² DM1=type 1 diabetes mellitus; ³ DF=dietary fiber; ⁴ significantly decreased as compared to control diet

(240) breads were found with one exception (241). The diet was modified only with bread (236, 237) or together with other cereal products (225, 238-240). Glucose metabolism was measured by fasting glucose (225, 236, 238-240) and insulin (225, 239) concentrations and/or by OGTT (237, 239). These methods may, however, be too crude measures of insulin sensitivity (242) because the improvement was able to be characterized by a clamp study in the abovementioned study (233). In addition, longer intervention periods may be needed, and high-fiber bread and cereal products may serve as a marker for other components of cereals which offer health benefits (see 2.3.3.5 Other factors).

Consumption of wholemeal rye bread increases also the fermentable load entering the colon (243). Rye bread fermentation as compared to wheat bread produces significantly more propionate (244) which is related to the inhibition of glucose production in hepatocytes and improved glucose tolerance (31). Human trials are, however, needed to evaluate the impact of colonic events on glucose and insulin metabolism.

2.3.3.2 Starch

Starch is the major carbohydrate source in the human diet and in bread (245). The total starch content of rye and wheat is 64 and 68 % on a dry matter basis, respectively (246), and it consists of amylose and amylopectin (247). Amylose is a linear glucose polymer containing (1→4) links whereas amylopectin is highly branched and contains also (1→6) links (248). These glucose polymers are insoluble in cold water and located in starch granules which are formed by concentric rings of alternating amorphous and semi-crystalline composition (247). Starch in food is classified as rapidly digestible, slowly digestible and RS (249). Conventional white and wholemeal breads are among the most rapidly digested foodstuffs, in which the contents of rapidly digestible starch have been estimated to vary between 90-92 % (249), thus resulting also in high glucose and insulin responses in humans (12, 222).

Cereal starches contain approximately 25 % amylose and 75 % amylopectin (250). There are only minor differences between grain species among naturally existing types of cereals i.e. the contents of amylose for rye, wheat, barley and oats are 27, 26, 22 and 23-24%, respectively. Therefore also the metabolic effects of these glucose polymers in the conventional diet are expected to be rather similar. There are, however, some genetically modified varieties of barley (251), rice (252) and corn (253) in which amylose is virtually absent, i.e. referred to as waxy, and varieties in which amylose accounts for as much as 70 % of total starch (253). It has been found that the higher the

amylose-amylopectin ratio in the cereal product, the slower the rate of *in vitro* starch hydrolysis (251), and the lower the postprandial insulin response as well as a slower decline of blood glucose levels (252, 254), and in some studies also the glucose response (253) in humans. This has been claimed to be due to differential enzymatic hydrolysis of the amylose and amylopectin and lipid-amylose complexes. Although the gluten network may prevent enzymatic access to encapsulated starch in wheat breads (255), the starch-protein interaction is likely to have minor importance on glucose and insulin responses because the network is disrupted during chewing (230). The use of an amylose-rich diet for four weeks as compared to an amylose-poor diet has not altered glucose and insulin metabolism as determined by fasting values (254) or by OGTT (256) in humans, although studies in rats have indicated that diets high in amylopectin may induce insulin resistance (257) and decrease insulin stimulated glucose oxidation (258).

RS is determined as the sum of starch and products of starch hydrolysis not absorbed in the small intestine of healthy individuals (259), and it consists of three RS subclasses, RS1, RS2 and RS3 (249). RS1 is based on physically inaccessible starch granules such as those present in whole and partly milled grains where the food matrix delays or prevents the action of digestive enzymes. The second subclass, RS2, includes native starch granules like raw potato and banana starches which are not relevant in breads. The most abundant form of RS, RS3, in bread is formed during baking and other food processes (see 2.3.3.4 Processing and baking). It occurs mainly as retrograded amylose (75-85 % of RS) (222), which has been shown to be a poor substrate for the amylolysis (245). However it does serve as a substrate for fermentation in the large intestine (28), and therefore the SCFAs produced after fermentation may influence glucose and insulin metabolism (30). RS elicits a similar postprandial glucose and insulin response as insoluble fiber (260). However, RS can be used to partly substitute for the content of available starch in food and this can then blunt the blood glucose and insulin responses due to the reduced amount of digestible carbohydrates (259).

Since bread is commonly eaten at daily meals, it may contribute to the intake of RS. The intake of RS depends, however, on the quantity to be eaten, and the type of breads to be consumed (261). Higher contents of RS has been found in kernel breads based on wheat (1.7 %) (230) or rye (6-8 %) (222, 261), and in wholemeal rye breads with 100 % rye flour (1.4 %) or mixed with white wheat flour (1.9-2.5 %) (261) than in breads baked with white wheat flour (0.6-1.0 %) (230, 261) expressed on a starch basis. Recent studies in Sweden and in Italy have estimated the average daily intake of RS from bread to be 41 % (1.3 g) and 31 % (2.6 g) from the total intake of RS, respectively (261, 262).

2.3.3.3 Food form

Structure of food is probably the most important individual factor affecting glucose and insulin responses. The structure can be present in a food as a botanical structure or it can be achieved by processing. The particle size of cereal grain is inversely associated with postprandial glucose and insulin responses as shown in whole grains, cracked grains, coarse and fine wholemeal flours (263). In breads, the addition of whole and cracked kernels originating from wheat, rye and barley has been shown to reduce the rate of *in vitro* starch hydrolysis (163, 230), and to decrease the postprandial glycemic and insulinemic responses as compared to breads baked with flours (163, 230, 264). The greater the proportion of kernels in bread, the nearer the response has lowered to those obtainable with whole kernels (264), and pasta which are known to be foodstuffs with low postprandial responses (10). One exception is oat kernel bread, which decreased only the insulin response probably because the integrity of oat kernels is more easily disrupted by heat treatment (163). These clear differences in the metabolic responses of breads with different particle sizes have led to the suggestion that one should use the term “wholegrain” for breads containing whole kernels in distinction to “wholemeal” breads made from milled flour (264). On the other hand, the importance of the structure achieved by processing has been demonstrated in a study where pasta made from the same durum wheat flour as bread produced lower postprandial glucose (-35 %) and insulin (-65 %) responses, and prevented the drop of glucose response below the fasting level in the late postprandial phase (231). This was associated with the slower rate of digestion in pasta as indicated by *in vitro* starch hydrolysis.

It has also been demonstrated that compositional differences in cereals can have an impact on bread texture, which affects the ability of dough to retain gas. During conventional wheat bread baking, wheat gluten forms an extensible viscoelastic network in which starch granules are entrapped (265). Gluten is known to be the main flour component which slows down the rate of diffusion of carbon dioxide out of the dough (206). Since rye contains only minimal amounts of gluten, the continuous phase in rye bread is formed by a less extensive protein-starch matrix where the starch granules are stuck to each other causing a firm and less porous structure (265). The gas retention of rye dough is based on the high viscosity of soluble AX which behaves somewhat similarly to gluten but not so effectively (206). Water extractable AX determines mainly the viscosity in rye dough, this being related to improved bread structure, whereas water unextractable AX has deleterious effects by destabilizing the structure. Since the cell walls of starchy endosperm are rich in water extractable AX, the flours containing endosperm have a higher viscosity than wholegrain rye flours (210). While wheat bread

is more porous and softer than rye bread (265), it is probably easier and more rapidly chewed into smaller pieces, and therefore more accessible to hydrolysis. Loaf volume, and more porous and softer crumb have been associated with increased glucose and insulin responses between white and coarse wheat breads (230), and might be associated to the more rapid *in vitro* starch hydrolysis in commercial as compared to homemade white and wholemeal breads (266). Swallowing carbohydrate foods without chewing has also demonstrated the importance of particle size in this respect (267).

2.3.3.4 Processing and baking

There are two typical processes, gelatinization and retrogradation, which modify starch granule structure during baking (248). Gelatinization occurs during heat-treatment when starch granules absorb water, swell irreversibly to many times their original size, lose their native crystalline structure and become more easily hydrolyzed. Therefore one reason for the high content of rapidly digestible starch in conventional breads is that they exhibit effective gelatinization of the starch (249). The degree of gelatinization can, however, be modified by baking conditions. In general, it can be enhanced by increasing water (268), moisture (268, 269), temperature (268, 270), baking time (268), by the choice of ingredients (268), and by decreasing the crust-to-crumb ratio of the bread (269). The extent of gelatinization has been found to be associated with increased *in vitro* starch hydrolysis (268, 270, 271), and may also affect *in vivo* glucose and/ or insulin responses after ingestion of bread (270, 271). Due to its ingredients, rye dough is gelatinized more easily than wheat dough because of the lower gelatinization temperature (below 60°C and over 62°C, respectively) and higher moisture content of rye dough (272, 273).

Retrogradation occurs when bread is cooled to room temperature. During this process starch granules recrystallize changing to RS (248). Amylose retrogrades immediately after baking and the process is terminated within a few hours. In contrast, with amylopectin this process takes days, even weeks before it reaches a plateau level. The formation of retrograded amylopectin may be the reason for reduced postprandial glucose response to stored as compared to fresh bread (274) and can result also in firmer crumb, deterioration of crust characteristics, and the loss or change of aroma during the storage of bread, i.e. the bread becomes stale (206). However, freezing and storage of bread after drying does not seem to increase the formation of RS (275).

When baking bread, starch granules in rye behave in a different way from wheat starch granules (265). In a typical wheat bread baking, starch remains inside the starch granule, and these granules are swallowed and become gelatinized and thus accessible

to the digestion of amylase. On the contrary, in baking rye bread, starch granules become more swollen due to the higher water content and the presence of α -amylase. In addition, amylose leaches out of the starch granule, coats the surface of the granule, and subsequently changes to RS during cooling (222). Retrograded amylose near the surface of the granule has also been shown to retard the hydrolysis of amylopectin (276). Leaching of amylose is also enhanced by the endogenous AX-degrading enzyme, xylanase, in rye flour (277). It can form pinholes from which amylose leaches out of the starch granules. Soluble fiber has also been suggested to surround the starch granules which could further slow down the hydrolysis of starch (278).

As indicated in previous sections, gelatinization and retrogradation have contradictory effects on the rate of starch hydrolysis, and therefore also on the expected postprandial glucose and insulin responses. These processes and the overall bioavailability of carbohydrate can be modified by different pretreatments of flour and grains, and the methods of processing the bread. Sourdough fermentation has a long history in bread-making, and it is still commonly used in many countries especially with rye breads (see 2.3.2. Bread in the diet). Lactic acid (279, 280) and acetic acid (281) are produced during spontaneous sourdough fermentation. These acids are thought to slow down the rate of starch hydrolysis and to delay gastric emptying, and thereby reduce glucose and insulin responses. The long baking time at low temperatures is found to promote the formation of retrograded amylose in pumpernickel-type rye breads (222). Puffing (271) and extrusion cooking (216) of crispbread is found to favor the complete gelatinization, and hence the digestibility of starch. In puffing, high-moisture dough is cooked under pressure, extruded through a die and puffed with a sudden release of pressure. The sudden expansion and release of water vapor expands the grains to several times their original volume.

Also the use of additives and alterations in the inherent properties of flour can modify the structure of the bread and hence may influence its glycemic response. The addition of monoglycerides (230) or xylanases (206) are found to increase loaf volume, improve oven raising properties, and make the crumb softer and more porous. High endogenous enzyme activities of amylase and pentosanase in rye flour have been shown to decrease viscosity, hence improving crumb properties (202). Propionate is also commonly used in the baking industry to inhibit mould and bacterial growth, and its sodium salt has been observed to reduce postprandial glycemia and insulinemia by slowing down the gastric emptying (279, 280), or by reducing the rate of starch digestion (282). Several pretreatment processes such as the germination (206) and malting (283) of grains can improve the hydrolysis of starch by amylases. When the

particle size is decreased by milling, the increase of the surface area and the disruption of botanical structure are associated with enhanced enzyme access. Even though scalding, i.e. traditional treatment of grains or flours with boiling water, has been found to increase the RS content of bread (261), it has not influenced glucose and insulin responses (222).

2.3.3.5 Other factors

Rye contains many different bioactive substances such as plant sterols (284), phenolic compounds including lignans (204), phenolic acids (285), tannins (286), and alk(en)ylresorcinols (287) as well as antinutrients like phytic acid (288), and protein inhibitors (289). Rye is also a valuable source of B-vitamins, vitamin E, magnesium, iron, zinc, copper and manganese (290-292). These substances are mainly concentrated in the outer bran layers of the rye grain (293).

To the author's knowledge, there are no published studies of the effects of these bioactive substances on glucose and insulin metabolism in rye grain or rye bread matrix. The impact of these substances has, however, been studied in this respect either in their purified form or after their incorporation into other foods. There is evidence that phytic acids (294), tannins (295), wheat amylase inhibitors (177) and phenolic compounds (296) are able to reduce the postprandial glucose response in humans. *In vitro* studies have revealed a reduced rate of starch digestibility as a result of enzyme or starch interaction with the bioactive substances (294, 297, 298), inhibited glucose absorption (299, 300), and enhanced insulin activity (301). Rats treated with phenolic acid (302) and β -sitosterol (303) have shown a reduction in the plasma glucose response after an oral glucose load. These effects have been explained by enhanced glucose utilization (302) and by increased insulin secretion (303).

Many vitamins and minerals have a direct impact on glucose and insulin metabolism since they are cofactors for signaling of insulin action or part of key enzymes of glucose metabolism. Deficiencies of magnesium and vitamin E have been suggested to be related to insulin resistance (304) and accelerated free radical stress (305), respectively. Both of these states are associated with impaired insulin secretion and late diabetic complications in type 2 diabetics as a result of tissue damage (158). Since the majority of the bioactive substances present in rye are found to be natural antioxidants (304, 306-311) and the antioxidant activity in rye bread remains unchanged regardless of baking as compared to wholemeal rye flour (293), the consumption of rye products could offer a mean to enhance and supplement the endogenous antioxidant defense.

2.3.3.6 Summary of the studies with rye bread

The first experimental studies on the effects of rye bread on glucose metabolism were carried out in rats at the beginning of the 1980's (215, 312-314). These findings pointed to improvements in glycemic control postprandially (215) and after the consumption of high-fiber rye bread for 2-17 weeks as compared to refined flour rye (312-314) or white wheat bread (215) in normal and diabetic rats.

The first intervention study in humans was performed in 1984. This revealed that rye bran-enriched rye crispbread for 2-4 weeks could reduce the insulin requirement and lower daytime blood glucose curve in type 1 diabetics without having any effect on fasting glucose (234) (Table 2, page 39). This nonsignificant effect on fasting glucose together with the unchanged fasting insulin was confirmed in another intervention study, where hypercholesterolemic subjects ate conventional rye and wheat breads for four weeks in a random order (235).

The acute effects of rye bread have also been studied in eight postprandial studies (Table 1, pages 36-37). They have been performed both in diabetics (12, 213-215, 219) and non-diabetics (163, 212, 222). In general, in both subject groups, breads baked with milled flours have not affected glucose response (12, 212-214) except in one study with type 2 diabetics (215), whereas breads with cracked or whole grains have lowered glucose response (12, 163, 219, 222). Except for one report (215), these studies with diabetics have usually measured only the glucose response. In healthy subjects, incorporation of whole kernels to the bread has induced a parallel reduction of insulin response as that seen in the glucose curve (163, 222), whereas an unchanged glucose response has accompanied the lowered insulin response with breads baked with flours (212).

The physiological factors as well as the food and dietary components of rye breads which can affect glucose and insulin responses are poorly known. Some of the studies have claimed that the high content of RS (222) or the reduced rate of *in vitro* starch hydrolysis (163, 212) with rye breads account for the reduced postprandial glucose (163, 222) and/ or the insulin (163, 212, 222) response. In rats, starch remains longer in the stomach after the ingestion of rye than wheat bread (215). There is only a single rye bread study in humans (215) in which biochemical measurements other than glucose and insulin have been performed either in postprandial or intervention studies. It seems, however, likely that numerous inherent properties of rye grains and rye breads baked with wholemeal rye flours may possess an ability to modify glucose and insulin metabolism in humans.

3. AIMS OF THE STUDY

This thesis is part of a larger project aiming to examine the beneficial effects of rye bread on human health. The aim of this thesis is to evaluate the impact of rye bread on glucose metabolism in healthy subjects, and thereby to determine whether regular consumption of rye bread could be used to reduce the risk of type 2 diabetes. The thesis has concentrated on the following questions:

1. Do the conventional and experimental rye breads reduce postprandial glucose and insulin responses (I-III)?
2. Which physiological factors affect the postprandial glucose and insulin responses to rye bread (II-III)?
3. Which characteristics in the composition and structure of the rye breads modify the postprandial glucose and insulin responses (II-III)?
4. Does daily consumption of rye bread alter glucose metabolism by enhancing insulin sensitivity, glucose effectiveness and acute insulin response (IV)?

4. SUBJECTS AND METHODS

4.1 Study designs

Postprandial studies

In *Studies I-III* the effect of rye breads on postprandial glucose metabolism was compared to that of refined wheat bread. *Study I* included commercial rye breads whereas in *Studies II-III* experimental rye breads were studied. The effect of whole kernels was studied in one, and the content of soluble fiber in another bread in *Study II*. In *Study III*, the quantity of total dietary fiber in rye bread was investigated.

The postprandial studies were performed in the morning after the subjects had fasted for 12-15 hours. After the body weight measurement and the insertion of the intravenous catheter in the antecubital vein of the arm, the fasting blood sample was taken and the test food portion was eaten within 15 minutes. From the time when the subjects started to eat, altogether 8 postprandial blood samples were drawn (15, 30, 45, 60, 90, 120, 150 and 180 min) for the determination of blood glucose and insulin (*Studies I-III*), GIP and GLP-1 (*Studies II-III*), and paracetamol (*Study II*) concentrations. In addition, in *Study III* four blood samples (0, 30, 60 and 120 min) were taken for the determination of serum C-peptide concentrations. Each subject consumed all of the study products in a random order with the minimum of a one week interval. β -glucan rye bread in *Study II* was exceptionally served at the last study visit in order to avoid the effect of freezing on β -glucan. Control wheat bread was eaten twice in *Studies II-III*, and the means of these two measurements were used as the values for control wheat bread.

The subjects were given both oral and written instructions to maintain their diet and body weight unchanged throughout the study, and to avoid heavy exercise one day before the tests. Alcohol consumption was not allowed two days before the test, nor was smoking permitted on the study morning. In addition, they were asked to arrive to the study visits by car or by bus if possible, or in any some way to avoid extra physical stress. The use of paracetamol containing analgesic drugs was also forbidden during *Study II* because their use could have confounded the measurement of paracetamol which was baked into the study breads in order to measure gastric emptying. The compliance with the diet was checked by a food record which was kept for one day before each study visit.

Intervention study

Study IV was carried out as a randomized cross-over study (Figure 3). The effects of high-fiber rye bread consumption were compared to those of refined wheat bread consumption over 8 weeks in a random order. The first test bread period was preceded by a 2-3-week run-in period during which the subjects ate their conventional diet, and kept a 4-day food record in order to determine their usual energy intake. Four-day food records were also kept during weeks 4-6 in both bread periods. The lifestyle habits, consumption of regularly used medication, and body weight were advised to be kept unchanged throughout the study. The body weight was measured every two weeks, and the subjects recorded daily the frequency of exercise at the run-in as well as during the experimental periods.

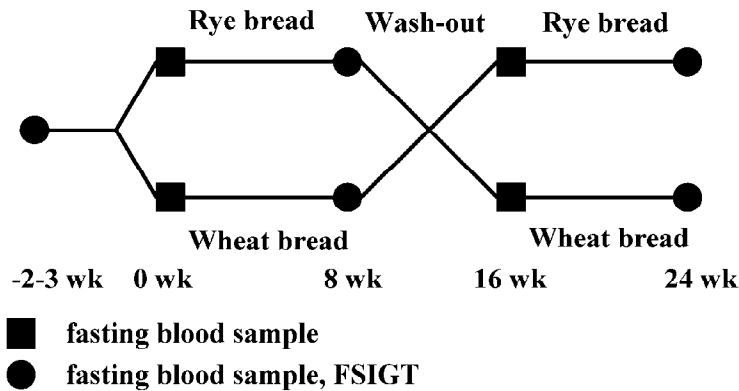


Figure 3. Cross-over study design used in *Study IV*.

The frequently sampled intravenous glucose tolerance test (FSIGT) was performed at the run-in, and at the end of both of bread consumption periods (Figure 3). In addition, the fasting blood samples were drawn at the beginning of both periods to determine the plasma glucose and insulin concentrations. The mean of two measurements (-5 and 0 min) before the FSIGT was used as the fasting value for the plasma glucose and insulin at the end of both experimental periods. The instructions to be prepared for FSIGT were similar as those given for postprandial *Studies I-III*. Four women used oral low-dose postmenopausal medication, and consequently had menstrual flow. Therefore in those women, the duration of the test bread periods and the washout period was adjusted to their cycle length. Their blood samples were collected at the same phase of the menstrual cycle, except in one woman where we encountered scheduling problems.

The protocols for the *Studies I-IV* were approved by the Ethics Committee of the Kuopio University and Kuopio University Hospital.

4.2 Subjects

Healthy men and women for the *Studies I-II* were recruited mainly from the students and staff of the University of Kuopio, and Kuopio University Hospital by internal mail and announcements in staff newsletters. Postmenopausal women for *Study IV* were sought by advertisement in a local paper. The subjects in *Study III* were recruited from the larger study group which included the subjects of *Study IV*. All experiments were carried out in the Department of Clinical Nutrition, University of Kuopio.

The primary inclusion criterion for the postprandial studies (*Studies I-III*) was normal glucose tolerance. It was confirmed by OGTT (WHO criteria) (315), and screened also for the intervention study (*Study IV*). One woman in *Study III* had IFG and three women in *Study IV* had IGT (Table 3). Since one of the objectives of *Study IV* was to investigate lipid metabolism, the inclusion criteria were a serum total cholesterol concentration of 5.0-8.5 mmol/L, a non-HDL-cholesterol concentration of 3.5-6.5 mmol/L (calculated as serum total cholesterol – serum HDL-cholesterol), a serum total triglyceride concentration < 2.5 mmol/L, and body mass index (BMI) of 20-33 kg/m². Use of lipid-lowering drugs, laxatives, or corticosteroid medication was not allowed, and the subjects should not have been diagnosed with diabetes mellitus. Postmenopausal status was confirmed by measuring serum follicle-stimulating hormone concentrations.

BMI after measurements of height and weight was calculated for all study subjects. The subjects had to meet normal criteria for systolic and diastolic blood pressure, routine hematological measures, serum creatinine, thyroxine, and liver enzyme concentrations before entry into the study. One subject both in *Studies III* and *IV* discontinued because of health problems, and the results of one woman were rejected in *Study IV* as a result of technical difficulties with the FSIGT during the run-in. The final number and the basic characteristics of the subjects are shown in Table 3.

Table 3. Characteristics of the subjects¹.

	Study I	Study II	Study III	Study IV
Subject N (M/F) ²	20 (10/10)	20 (10/10)	19 F	20 F
Age, y	30 (5)	28 (6)	61 (5)	59 (6)
BMI ³ , kg/m ²	22.4 (2.7)	22.9 (2.9)	26.0 (2.5)	27.5 (2.9)
Glucose tolerance status ⁴	normal	normal	normal (n=18), and IFG ⁵ (n=1)	normal (n=17) and IGT ⁶ (n=3)

¹ mean (SD); ² M=males, F=females; ³ BMI=body mass index; ⁴ based on oral glucose tolerance test; ⁵ IFG=impaired fasting glucose; ⁶ IGT=impaired glucose tolerance

4.3 Methods

4.3.1 Study meals and diets

The study products and their nutrient composition in *Studies I-III* are shown in Table 4. The test food portions were planned to contain 50 g available carbohydrates, and served with 40 g cucumber (breads) or 19 g crushed tomatoes (pasta), and with 3 dl non-caloric orange drink. Because the determination of available carbohydrate content of test bread portions in *Study I* was based on calculated data, the analyzed content differed from 50 g. Therefore, the statistical analysis was performed between two pairs (white wheat vs. wholekernel rye, and wholemeal rye vs. rye crispbread, respectively), which nearly had the same carbohydrate content (61 vs. 55g, and 43 vs. 45g, respectively) (Table 4). However, in the results section, these four test breads are presented in the same figures.

The study breads in *Study I* and dark durum wheat pasta, which was used in *Study II* as a positive control, were conventional products which were available commercially. The experimental breads for *Studies II-III*, and the control wheat bread for *Study II*, were developed and baked at VTT Biotechnology (Espoo, Finland), whereas the control wheat bread for *Studies I* and *III* was a commercial refined wheat bread. One experimental bread in *Study II* contained 60 % autoclaved whole rye kernels, and 40 % rye flour. Another rye bread was baked with oat β -glucan concentrate (Swedish Oat Fiber, Väröbacka, Sweden) which was the main source of soluble fiber (5.3g/ portion). In *Study III*, the dietary fiber content of rye breads was varied but the structure was similar while all breads were baked by milled flours. In the endosperm rye bread the total dietary fiber content was 5.5 %, because the flours were milled from the

Table 4. Nutrient composition of the test bread portions in postprandial studies (*Studies I-III*)¹.

	Energy, kJ	Available CHO ² , g	Total DF ³ , g	Insoluble DF ³ , g	Soluble DF ³ , g	Protein, g	Fat, g	Moisture, g
Study I								
White wheat	1341	61	2.3	1.7	0.6	10.5	3.4	40.8
Wholekernel rye	1173	55	13.5	11.2	2.3	9.2	2.2	61.4
Wholemeal rye	974	43	10.1	8.3	1.8	9.3	2.3	25.4
Rye crispbread	994	45	12.1	9.7	2.4	8.7	2.2	2.5
Study II								
White wheat	1117	50	3.1	2.3	0.9	8.4	3.0	43.2
Wholekernel rye	1084	50	12.8	9.0	3.8	7.4	2.6	53.1
β -glucan rye	1134	50	17.1	10.3	6.8	10.5	2.4	80.6
Pasta	1250	50	5.6	4.2	1.3	12.1	4.7	102.4
Study III								
White wheat	1177	50	2.7	1.5	1.2	9.0	5.2	38.2
Endosperm rye	1056	50	6.1	3.1	3.0	4.9	3.4	46.7
Traditional rye	1295	50	15.2	10.9	4.3	11.1	7.8	58.2
High-fiber rye	1486	50	29.0	24.1	4.8	16.7	10.2	90.2

¹ based on analyzed data; ² CHO=carbohydrate; ³ DF=dietary fiber

inner fractions of rye kernel. The traditional rye bread contained 10.7 % dietary fiber which corresponded to that of the conventional rye breads. High-fiber rye bread (14.5 %) was baked by adding rye bran to the dough.

The experimental products in *Studies II-III* were studied at VTT Biotechnology (Espoo, Finland) by microscopy to determine the structure of the breads. The selection of the experimental products for clinical trials was based on the rate of *in vitro* starch hydrolysis (161) of experimental breads and pasta determined before *Studies II-III* at VTT Biotechnology (Espoo, Finland). In addition, starch hydrolysis was investigated in *Study I* after the clinical trial was carried out. The detailed descriptions of the microscopy and *in vitro* starch hydrolysis methods are given in original publication *I-III*.

In *Study IV*, the intention was to increase the intake of dietary fiber by baking a rye bread with rye bran enrichment which caused a major increase in insoluble fiber content. Rye bread made up 78 % (35.5 ± 7.3 g) of total, 91 % (29.7 ± 6.3 g) of insoluble and 64 % (5.8 ± 1.1 g) of soluble dietary fiber intake, respectively. The respective values for wheat bread were 33 % (4.7 ± 1.0 g), 53 % (3.1 ± 0.6 g), and 34 % (1.6 ± 0.4 g). The experimental rye bread was baked by the same recipe than that used in *Study III* for high-fiber rye bread. The high-fiber rye bread contained 17 % dietary fiber as compared to 2.8 % in the control wheat bread (Table 5).

Table 5. Nutrient composition of the test breads (g/100 g) in *Study IV*¹.

	High-fiber rye bread	Wheat bread
Energy, kJ	790	1107
Available carbohydrate, g	33.6	49.6
Total dietary fiber, g	17.0	2.8
Insoluble dietary fiber, g	14.2	1.8
Soluble dietary fiber, g	2.8	1.0
Protein, g	9.0	8.2
Fat, g	1.8	3.4
Ash, g	2.9	1.5
Moisture, g	35.7	34.5

¹ High-fiber rye bread values are average values of four rye breads baked with the same recipe, and wheat bread values are average values of seven commercial wheat breads based on analyzed data.

The subjects were advised to modify their consumption of cereal products by eating at least 20 % of their daily energy intake as experimental breads, and restricting the consumption of other cereal products to one portion a day. No maximum amount of

bread to be consumed was given, but the subjects were advised to eat the breads in amounts which corresponded to their habitual diet. Otherwise the subjects were asked to keep their diet unchanged. The subjects recorded daily the use of test bread portions as well as the consumption of other cereals. The aims of the diet were successfully fulfilled since the subjects ate the experimental breads more than the recommended amount of 20% of daily intake of energy (23.4 ± 4.3 E % during the rye, and 26.7 ± 8.2 E % during the wheat bread period, respectively). In addition, the consumption of other cereal products did not exceed one portion per day and the intakes of energy and nutrients with the exception of fat and protein remained constant throughout the study.

The same recipe of high-fiber rye bread was used to bake four rye breads which differed in shape and appearance in order to increase the variability in the consumption of bread. The rye breads were baked in two lots, frozed at the Department of Clinical Nutrition and given to the subjects at the study visits. Seven different commercial wheat breads were chosen for the wheat bread period, and fresh-baked breads were available at the Department of Clinical Nutrition once a week. All experimental breads in *Studies I-III*, with the exception of β -glucan rye bread in *Study II*, were baked before the clinical trial in the same batch, and stored frozen until being taken to room temperature on the afternoon prior to the study morning.

All calculations of energy and nutrient intakes were done by the Micro-Nutrica software package (version 2.0 in *Studies I, II and IV*, and version 2.5 in *Study III*, respectively; Finnish Social Insurance Institution, Turku, Finland). The nutrient compositions of the study breads and pasta were analyzed at VTT Biotechnology (Espoo, Finland) and added to the Micro-Nutrica database. The result on carbohydrate content was used for the determination of test food portions in postprandial studies. The energy intakes before the study visits (*Studies I-III*) and between the diet periods (*Study IV*), as well as body weight throughout all studies, remained unchanged indicating successful implementation of diet counseling.

4.3.2 Laboratory methods

The analyses of glucose, insulin and paracetamol were made at the University of Kuopio, Department of Clinical Nutrition; C-peptide at the Kuopio University Hospital, Department of Clinical Chemistry, and GIP and GLP-1 at the University of Copenhagen, Department of Medical Physiology in Denmark.

Plasma glucose, and plasma and serum insulin

Plasma glucose was analyzed by the glucose oxidase method (Glucose Auto & Stat, Model GA-110, Daiichi, Kyoto, Japan) in *Study I*, and by the enzymatic photometric method (Granutest 100 or 250, Merck, Darmstadt, Germany) in *Studies II-IV* by using the Kone Specific/Pro Clinical Analyzer (Kone Ltd, Espoo, Finland). All samples from the same study were analyzed by the same method and the comparisons were made within the study. The interassay CV's were 0.9-3.9 % and 0.6-3.7 %, in the concentration ranges of 4.9-5.2 mmol/L (n=13-149), and 10.2-17.8 mmol/L (n=13-149), respectively.

Insulin samples were analyzed by radioimmunoassay (Phadaseph Insulin RIA 100, Pharmacia Diagnostica, Uppsala, Sweden). The interassay CV's were 3.8-7.2 % (n=11-37), 3.2-5.7 % (n=10-37), and 4.7-5.8 % (n=11-37) for the lowest (72-93 pmol/L), middle (239-298 pmol/L) and highest (579-692 pmol/L) insulin controls, respectively.

Frequently sampled intravenous glucose tolerance test

The FSIGT was performed as described by Bergman (316). The test was carried out by a glucose dose of 330 mg/kg body weight followed by a bolus of 0.03 U insulin/kg body weight 20 min after glucose load and by frequent blood sampling during a 3-hour test period. Thereafter, the computer-assisted minimal model (MINMOD) program was used (317). This program utilizes physiological models of glucose use and insulin kinetics observed during the FSIGT to derive the measurements of insulin sensitivity (S_I) and glucose effectiveness (S_G) indexes. The former represents the insulin-dependent increase in the net glucose disappearance rate, and the latter the effect of glucose per se, at basal insulin, to normalize the glucose concentration within the extracellular glucose pool. FSIGT was also utilized to calculate the AIR (0-10 min) which has been used as a quantitative measure of first-phase insulin secretion and β cell function.

Serum C-peptide

C-peptide was analyzed by the AutoDELFIA time-resolved fluoroimmunoassay method (TR-FIA, Perkin Elmer Wallac, Turku, Finland). It is a solid phase, two-site immunofluorometric assay which is based on the direct sandwich technique in which two monoclonal antibodies derived from mice are directed against separate antigenic determinants on the C-peptide molecule (318). The detection of the formed sandwich complexes is based on the time-resolved fluorometry of the europium-label (319). The intra- and interassay CV's were 3.1-5.0 % and 1.9-3.0 %, respectively, in the concentration range of 0.5-2.9 nmol/L (n=27).

Plasma GIP and GLP-1

GIP and GLP-1 concentrations in plasma were measured by radioimmunoassay after the extraction of plasma with 70% ethanol. The total GIP measurement uses the C-terminal directed antiserum R65, which cross-reacts fully with human GIP, but not with the so-called GIP 8000 (320). Human GIP and ¹²⁵I human GIP (70 MBq/nmol) were used as standards and tracer. The plasma concentrations of GLP-1 were measured against standards of synthetic GLP-1(7-36) amide by using antiserum (code no. 89390), which is specific for the amidated C-terminus of GLP-1, and therefore mainly reacts with GLP-1 of intestinal origin (85). For these assays, the sensitivity was < 1 pmol/L, the intra-assay CV < 6% at 20 pmol/L, and the recovery of standard (added to plasma before extraction) was ~ 100% when corrected for losses inherent in the plasma extraction procedure.

Serum paracetamol

Serum paracetamol and paracetamol concentrations in the test breads were measured by enzyme immunoassay (Emit Acetaminophen Assay, Behring Diagnostics Inc, Cupertino, Canada). The interassay CV's were 8.8, 9.0 and 9.9 % in the concentrations of 72 (n=18), 220 (n=18) and 799 µmol/L (n=18), respectively.

4.3.3 Statistical analysis

Statistical analysis in the *Studies I-III* was done by comparing the data of the experimental rye products and pasta with the refined wheat bread with the exception that, as a consequence of different portion sizes in *Study I*, wholemeal rye bread was compared to rye crispbread. In addition, in *Study III* the rye breads were compared to each other. The comparisons were made at each time point as well as among the AUCs which were calculated from the initial postprandial rise above the fasting level (321). Statistical significance of the differences was assessed using the non-parametric Friedman's test followed by the Wilcoxon's test for pairwise comparisons. To control the overall level of significance, the Bonferroni adjustment was used.

In *Study IV* the percentage changes in nutrient intakes from the run-in period to the rye bread period and from the run-in period to the wheat bread period were compared by the Wilcoxon's test since the values were not normally distributed. Because the percentage change in the intake of protein from the run-in period to the rye bread period was greater, and the corresponding value in the intake of total fat smaller than those from the run-in period to the wheat bread period, the differences in these protein and fat variables were used as covariates in the covariance analysis. Normal distribution and

homogeneity of variance were checked, and logarithmic transformations were made for all the variables included in covariance analysis if needed. To obtain normally distributed variables for the proportional changes in AIR, the basic values were at first logarithmically transformed, and then the proportional change variables were calculated. The proportional changes in S_I , S_G and AIR, as well as the changes in body weight, and fasting plasma glucose and insulin concentrations at the beginning and at the end of the bread periods were analyzed by covariance analysis of repeated measures (322).

The comparisons in the single measurement points during the FSIGT and in the frequency of exercise among the run-in, rye bread and wheat bread periods were made by using the Friedman's test. The nonparametric Mann-Whitney U test was used to compare the changes in fasting glucose and insulin, S_I , S_G and AIR during the rye and wheat bread periods according to glucose tolerance, use of thyroid hormone, estrogen replacement therapy, and body weight.

In all analyses, P values < 0.05 were considered to be statistically significant. The results are expressed as mean \pm SD or SEM. Data were analyzed with SPSS for Windows 7.5 program in *Study I* and 8.0 program in *Studies II-IV* (SPSS, Chicago, IL, USA) (323, 324).

5. RESULTS

5.1 Rye bread and postprandial glucose metabolism (*Studies I-III*)

5.1.1 Glucose, insulin and C-peptide

The fasting glucose values or responses to rye breads and pasta during the first two hours postprandially did not differ as compared to the response to the refined wheat bread (Figure 4) nor was there any difference in the corresponding glucose AUCs. The refined wheat bread had a steeper return below the fasting level (2-3 hours postprandially in *Studies II* and *III*) as compared to the rye breads and pasta which remained near the baseline (Figure 4). In *Study I* the glucose response to the wholemeal rye bread was higher than that detected for the rye crispbread at 150 and 180 minutes. In general, responses in plasma glucose and insulin were higher in the postmenopausal women in *Study III* than seen in the younger adults in *Studies I-II*. Similar differences were also found in GIP and GLP-1 responses (see 5.1.2).

There were no differences between the fasting insulin values obtained for the experimental products and the refined wheat bread with the exception of the high-fiber rye bread in *Study III* (Figure 5). The postprandial responses to rye breads and pasta were consistently reduced at several measuring points (Figure 5), as were the insulin AUCs after the wholekernel rye breads in *Studies I-II*, endosperm rye and the traditional rye breads in *Study III*, and pasta in *Study II* (Figure 6). Also the AUC after the high-fiber rye bread in *Study III* tended to be reduced as compared to that obtained after the refined wheat bread ($P=0.06$). Additionally, the 90 minute glucose and insulin AUCs were calculated in *Study II* showing similar glucose and insulin areal responses over 180 minutes, except in the case of the β -glucan rye bread which exhibited a smaller insulin AUC as compared to the refined wheat bread ($P=0.006$).

The fasting C-peptide values did not differ significantly among the test products in *Study III*, but both the postprandial C-peptide responses (Figure 7) and the C-peptide AUCs (Figure 8) were reduced after experimental rye breads in a similar manner to the insulin responses as compared to those of the refined wheat bread.

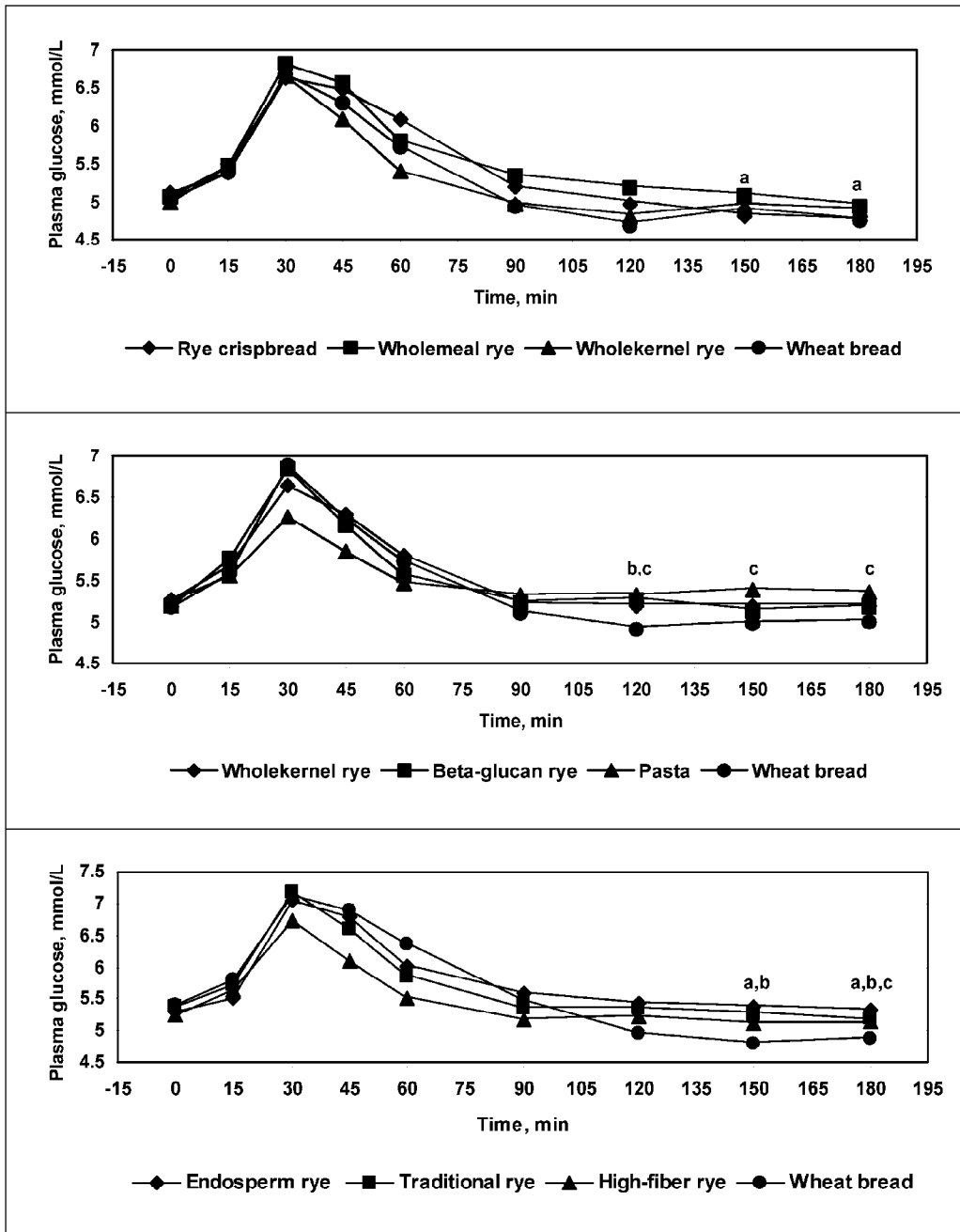


Figure 4. Mean fasting values and postprandial glucose responses to rye and wheat products over 180 minutes. In Study I (upper) the response to wholemeal rye bread (a) was significantly different from that to rye crispbread. In Study II (middle) the responses to β -glucan rye bread (b) and pasta (c) were significantly different from that to wheat bread. In Study III (lower) the responses to endosperm rye (a), traditional rye (b), and high-fiber breads (c) were significantly different from that to wheat bread. $P < 0.05$, Wilcoxon

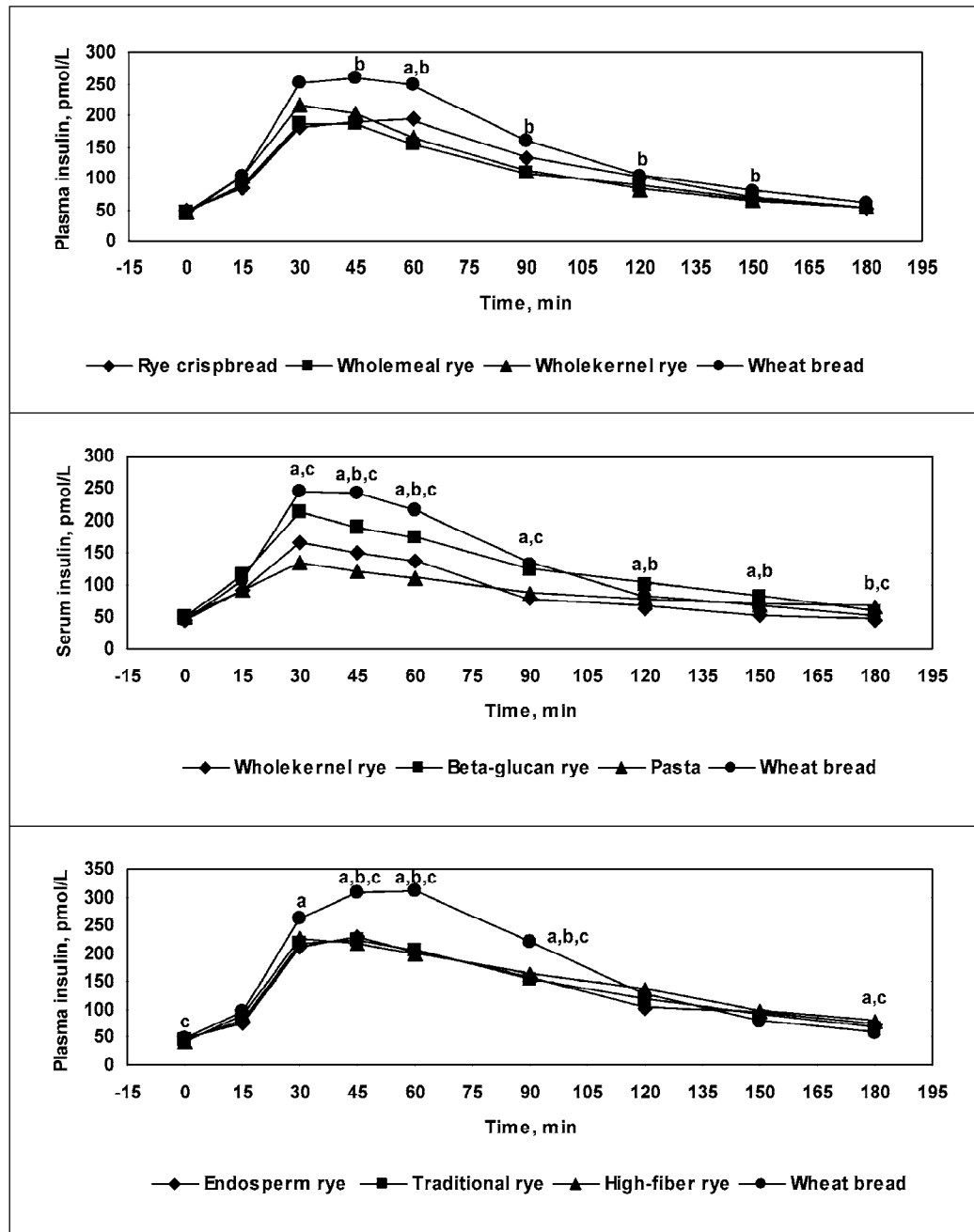


Figure 5. Mean fasting values and postprandial insulin responses to rye and wheat products over 180 minutes. In Study I (upper) the responses to wholemeal (a) and wholekernel rye breads (b) were significantly different from those to rye crispbread and wheat bread, respectively. In Study II (middle) the responses to wholekernel rye bread (a), β -glucan rye bread (b) and pasta (c) were significantly different from that to wheat bread. In Study III (lower) the responses to endosperm (a), traditional (b), and high-fiber rye breads (c) were significantly different from that to wheat bread. $P < 0.05$, Wilcoxon

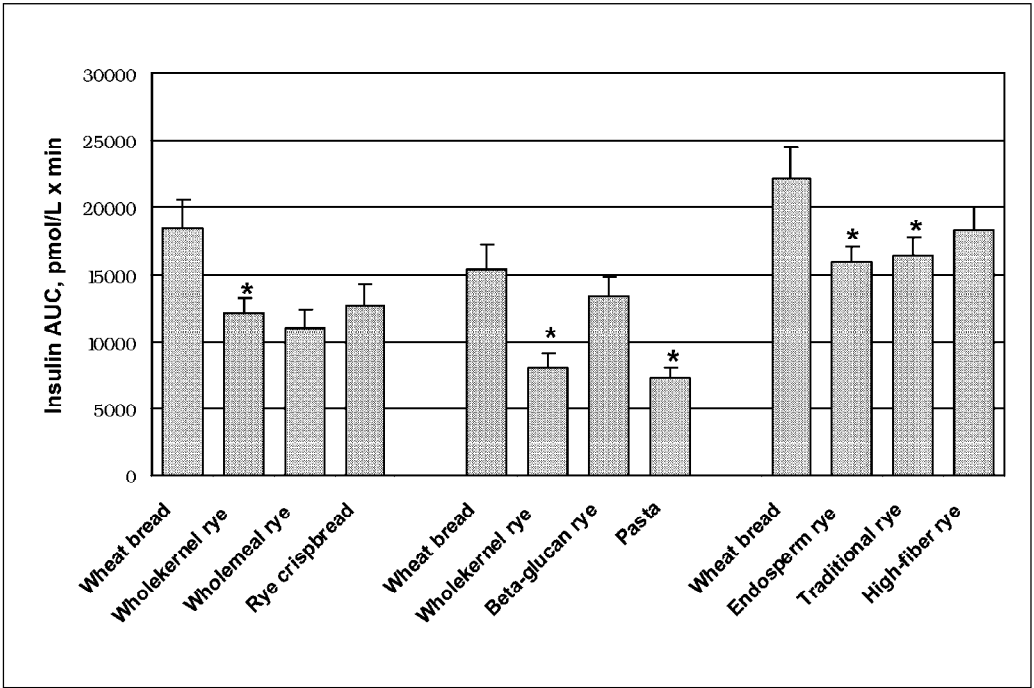


Figure 6. Insulin areas under the curve (AUC) after the ingestion of rye and wheat products in Studies I-III. Bars represent means and SEM.
* significantly different as compared to wheat bread. $P < 0.05$, Wilcoxon

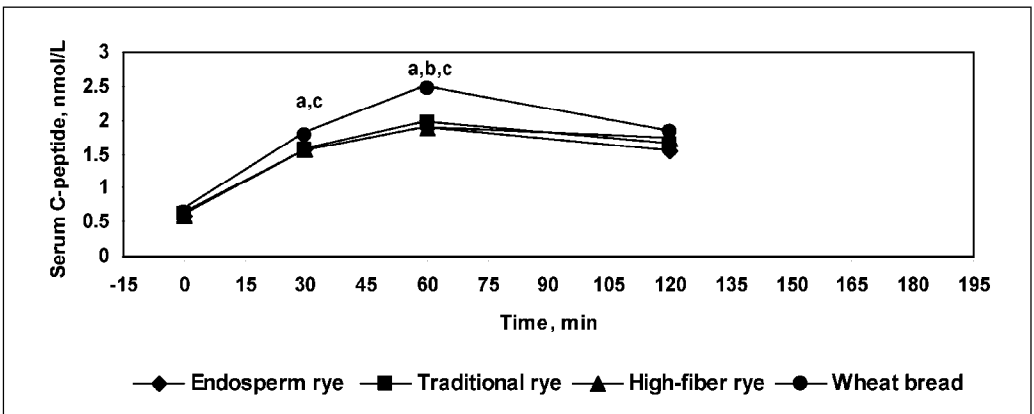


Figure 7. Mean fasting values and postprandial C-peptide responses to rye and wheat products over 180 minutes. In Study III the responses to endosperm (a), traditional (b), and high-fiber rye breads (c) were significantly different from that to wheat bread. $P < 0.05$, Wilcoxon

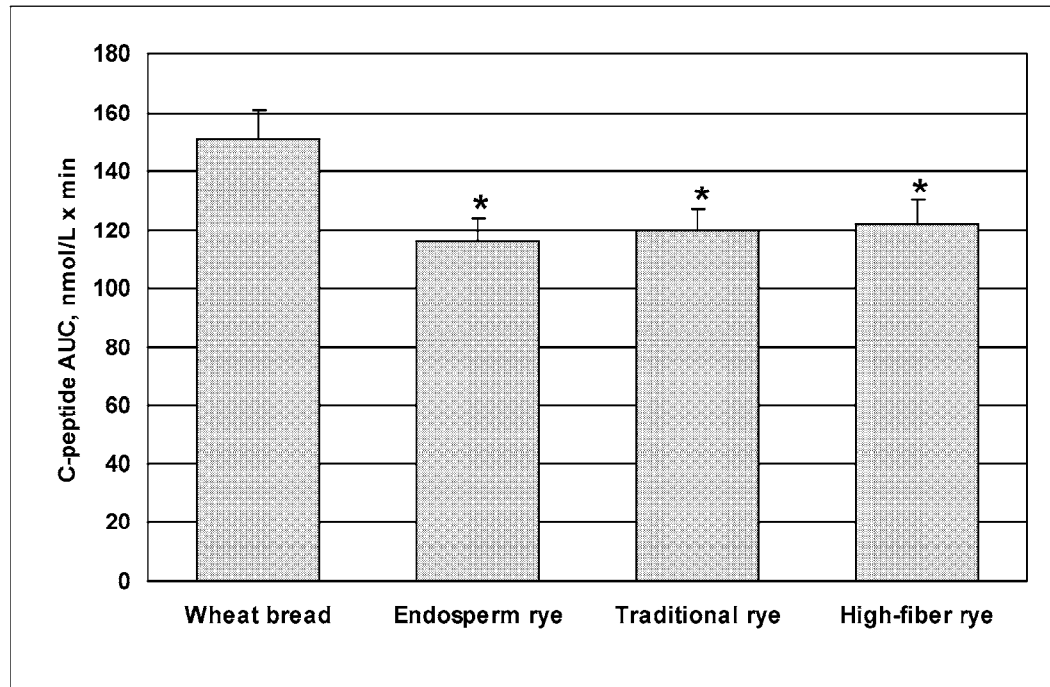


Figure 8. C-peptide areas under the curve (AUC) after the ingestion of rye and wheat products in Study III. Bars represent means and SEM.

* significantly different as compared to wheat bread. $P < 0.05$, Wilcoxon

5.1.2 GIP and GLP-1

There were no differences in the fasting values of GIP among the bread groups in *Studies II-III*. Plasma GIP responses mirrored the insulin responses (Figures 9 and 10) since the responses at several postprandial measurement points after the experimental products were reduced (Figure 9) and GIP AUCs to all experimental rye breads and pasta were smaller (Figure 10) than those to refined wheat bread. In addition, the GIP response at 30 and 60 min was lower ($P=0.030$ and $P=0.048$, respectively; Figure 9), and the GIP AUC to traditional rye bread was smaller than the equivalent values for endosperm rye bread (Figure 10). The response to high-fiber rye bread was reduced as compared to that of endosperm rye bread at 30, 45 and 60 min ($P=0.006$, $P=0.012$ and $P=0.042$, respectively; Figure 9).

GLP-1 AUCs did not differ among the test products in *Studies II-III*, but the fasting value of β -glucan rye bread and postprandial responses to experimental products at several time points in *Study II*, as well as the responses to high-fiber rye bread at 150 and 180 min in *Study III* were different as compared to the refined wheat bread (Figure 11).

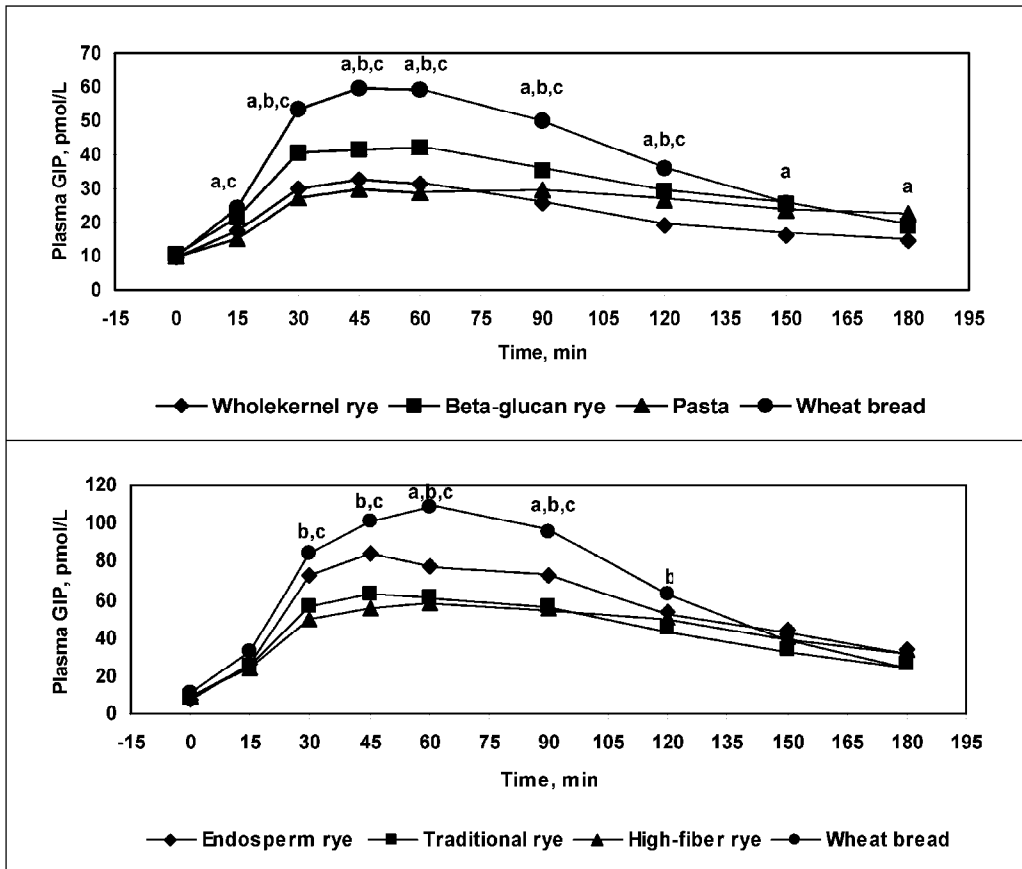


Figure 9. Mean fasting values and postprandial glucose-dependent insulinotropic polypeptide (GIP) responses to rye and wheat products over 180 minutes. In Study II (upper) the responses to wholekernel rye bread (a), β -glucan rye bread (b) and pasta (c) were significantly different from that to wheat bread. In Study III (lower) the responses to endosperm (a), traditional (b), and high-fiber rye breads (c) were significantly different from that to wheat bread. $P < 0.05$, Wilcoxon

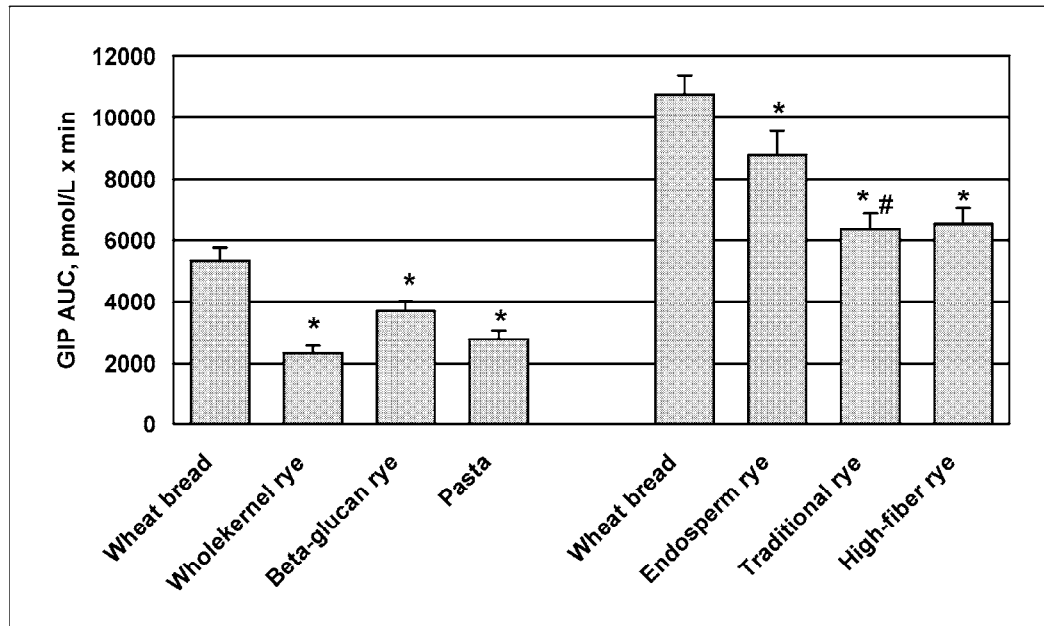


Figure 10. Glucose-dependent insulinotropic polypeptide (GIP) areas under the curve (AUC) after the ingestion of rye and wheat products in Studies II-III. Bars represent means and SEM.

* significantly different as compared to wheat bread, and # as compared to endosperm rye bread, respectively. $P < 0.05$, Wilcoxon

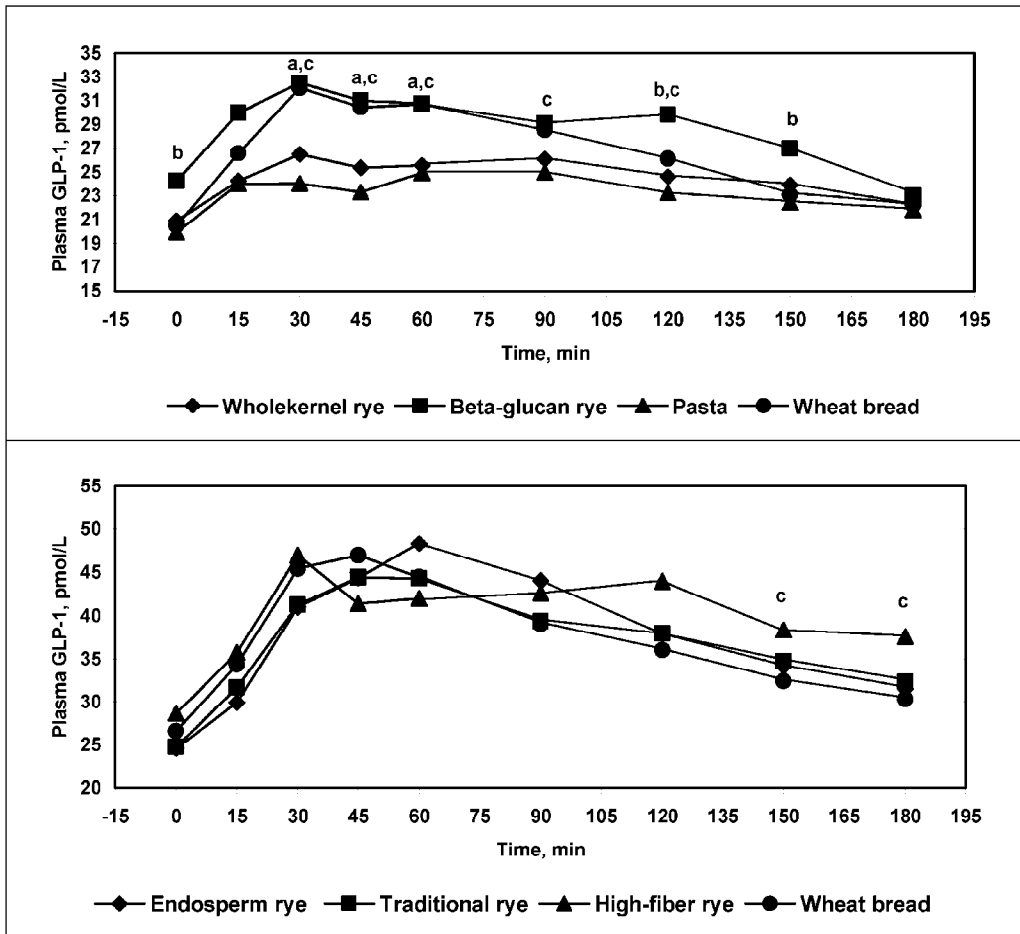


Figure 11. Mean fasting values and postprandial glucagon-like peptide-1 (GLP-1) responses to rye and wheat products over 180 minutes. In Study II (upper) the responses to wholekernel rye bread (a), β -glucan rye bread (b) and pasta (c) were significantly different from that to wheat bread. In Study III (lower) the response to high-fiber rye bread (c) was significantly different from that to wheat bread. $P < 0.05$, Wilcoxon

5.1.3 Gastric emptying

Serum paracetamol responses as a measure of gastric emptying in *Study II* did not differ between the experimental rye breads and the refined wheat bread. Only wholekernel rye bread had a lower response than that of the refined wheat bread at 120 min ($53.5 \pm 10.2 \mu\text{mol/L}$ for wholekernel rye bread and $62.3 \pm 3.2 \mu\text{mol/L}$ for refined wheat bread; $P = 0.004$, Wilcoxon).

5.1.4 Food and dietary factors

The microscopy of the test breads revealed differences in the macrostructure of rye and wheat breads. The continuous matrix and the starch granules of rye and wheat breads after baking were different. In wheat bread starch was inside the granules and was swollen and gelatinized, with the starch granules trapped within gluten network which formed the continuous matrix. In rye bread, amylose starch had leached out and surrounded the granules, and changed to RS. The continuous matrix was formed by closely packed starch granules.

The rate of *in vitro* starch hydrolysis was expressed as HI values which differed significantly among the test products in *Study II* ($P=0.017$) and *Study III* ($P=0.029$) (Table 6). When the total amount of available carbohydrate was determined after subtraction of free sugars in *Study I* the HIs among the breads were significantly different ($P=0.003$) (Table 6) whereas the determination of the available carbohydrate as total sugars (starch and free sugars in bread) gave similar HIs.

Table 6. The hydrolysis indices, portion sizes and eating times of test products in postprandial *Studies I-III*.

	Hydrolysis index ¹	Portion size, g	Eating time ²
Study I			
White wheat ³	100 ± 0	121.1	8 min 33 s
Wholekernel rye ³	78 ± 8	148.4	9 min 2 s
Wholemeal rye ³	87 ± 7	98.2	9 min 55 s
Rye crispbread ³	101 ± 3	79.4	11 min 19 s
Study II			
White wheat	100 ± 0	112.4	7 min 3 s
Wholekernel rye	72 ± 3	135.0	8 min 51 s
β-glucan rye	97 ± 3	169.0	8 min 23 s
Pasta	56 ± 2	179.1	6 min 43 s
Study III			
White wheat	100 ± 0	105.5	7 min 46 s
Endosperm rye	82 ± 3	111.9	8 min 24 s
Traditional rye	76 ± 2	142.7	9 min 26 s
High-fiber rye	71 ± 4	199.4	12 min 42 s

¹ mean ± SD, n=6 in *Study I* and n=3 in *Studies II-III*

² means of 20 subjects in *Studies I-II* and of 19 subjects in *Study III*

³ total amount of available carbohydrate was determined after subtraction of free sugars

The rye bread containing oat β -glucan concentrate in *Study II* was offered as fresh-baked in order to avoid the effect of freezing on β -glucan. However, due to the endogenous β -glucanase activity in the rye flour, the proportion of high-molecular weight β -glucans decreased and that of the low-molecular weight β -glucans increased in the rye bread as compared to the β -glucan concentrate. Nonetheless the β -glucan rye bread reduced the postprandial insulin responses (Figure 5) and the 90 minute insulin AUC was smaller as compared to that after the refined wheat bread. Since the insulin responses of all rye breads were consistently reduced in *Study III* (Figure 5), the total content or quality of rye fiber was concluded not to have any effect on postprandial responses in the amounts of total rye fiber content of 6.1-29.0 g/portion and of soluble and insoluble fiber contents of 3.0-4.8 g and 3.1-24.1 g/portion, respectively. Also the breads containing whole kernels in *Studies I-II* showed reduced insulin responses (Figure 5).

There were significant differences in the times taken to eat the test products ($P=0.0001$ in *Study I*, $P=0.0001$ in *Study II*, and $P=0.0001$ in *Study III*, respectively; Table 6). The differences seemed to be related to the portion size (Table 6) and to the edibility of the bread, since the rye crispbread and high-fiber rye bread took the longest to eat. In addition, the adjustment of the bread portions according to the content of available carbohydrates lead to some variation in the nutrient contents of the bread portions (Table 4).

5.2 Glucose metabolism after 8-week consumption of rye bread (*Study IV*)

The proportional change of AIR from the run-in period to the end of the rye bread period was greater than that from the run-in period to the end of the wheat bread period (9.9 ± 24.2 vs. 2.8 ± 36.3 %, respectively; $P=0.047$; Figure 12), and the result was independent of the difference in energy intakes from protein and total fat, and the order of the bread periods. Other measured values (S_I , S_G , and fasting glucose and insulin concentrations) did not change significantly during the study, and there were no consistent differences at the single measurement points either in glucose or insulin during the FSIGT.

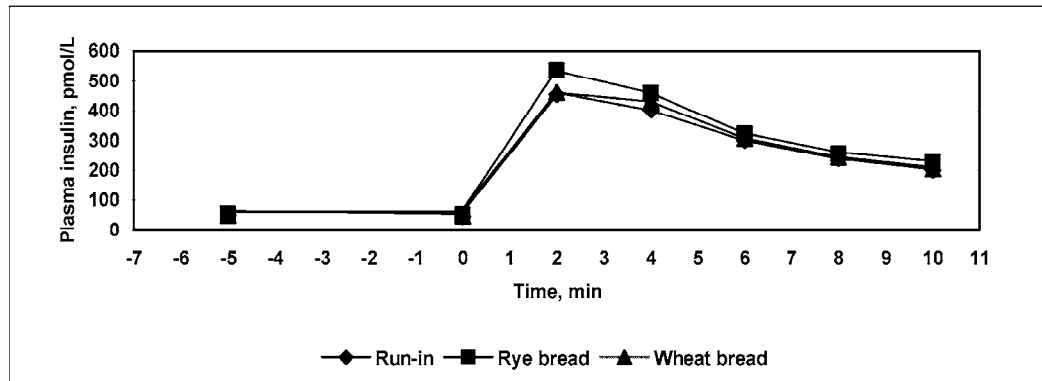


Figure 12 . Mean fasting values and acute insulin responses (AIR, 0-10 min) at the run-in and at the end of rye (RB) and wheat bread (WB) periods during the frequently sampled intravenous glucose tolerance tests in Study IV. The proportional change of AIR from the run-in period to the end of the RB period was significantly greater than that from the run-in period to the end of WB period. $P=0.047$, repeated measures analysis of covariance

6. DISCUSSION

6.1 Glucose metabolism

No differences were found in the present studies in the postprandial glucose responses to rye products as compared to refined wheat bread nor were there any changes of glucose responses in FSIGT after the consumption of rye or wheat breads for eight weeks. However, some qualitative differences in the postprandial glucose responses were evident based on the shapes of glucose curves for different breads. Insulin responses to rye breads were decreased in parallel with the C-peptide responses, suggesting that the postprandially lowered insulin response resulted from diminished insulin secretion and not from increased liver extraction of secreted insulin.

Our result that rye bread produces a similar glucose response to that of refined wheat breads is in agreement with one earlier postprandial study on conventional rye breads in healthy subjects (212), though in two other studies the consumption of rye breads containing whole kernels did decrease glucose responses (163, 222). In healthy adults, circulating glucose is maintained within narrow limits, and carbohydrate or glucose loads result in increased secretion of insulin and other hormones (325). This quickly normalizes the blood glucose concentration by enhancing the tissue uptake of glucose. Another mechanism for regulating plasma glucose is the hepatic suppression of glycogenolysis and gluconeogenesis caused by binding of insulin to its receptors in the liver (121). Decreased postprandial insulin secretion after rye bread presumably results in reduced insulin binding to hepatocytes when compared to the situation with refined wheat bread. Consequently, partial endogenic glucose production continues at a higher rate after ingestion of rye bread and compensates for the slower release of glucose from rye bread as indicated by the present *in vitro* hydrolysis studies. Thirdly, the increase in postprandial plasma glucose results in a counter-regulatory metabolic response to glucose, and consequently in a restoration of the plasma glucose back to baseline (143). The rapid rise of plasma glucose, as shown after the refined wheat bread, results in efficient secretion of insulin and a decrease in plasma glucose below the fasting level. Subsequent release of FFAs by counter-regulatory hormones may over time predispose to insulin resistance (130). In the case of rye bread, the slower starch digestion delays the rise of plasma glucose and reduces the need for insulin. Consequently, the later fluctuations of plasma glucose are inhibited, as shown by glucose levels in the latter part of the 3-hour postprandial period.

The incretin hormones are an integral component of physiological mechanisms in the gastrointestinal tract which influence glucose metabolism by modifying insulin

secretion. In the present studies, GIP was reduced in parallel with insulin, whereas no consistent changes in GLP-1 or in gastric emptying were found. One earlier study has reported the effects of rye bread on the incretin response. In contrast to our result, the GIP response in type 2 diabetics was unaffected after consumption of wholemeal rye bread (215). No other data in humans exists on the effects of rye bread on GLP-1 and on gastric emptying. The altered incretin hormone response has been proposed to serve as an indicator of regional changes in the absorptive function of the small intestine (326). Thus, the depression of GIP response would indicate a reduced rate of carbohydrate absorption in the duodenum and jejunum. This *in vivo* finding is in line with the reduced rate of *in vitro* starch hydrolysis of rye breads and pasta found in our pilot studies and in some earlier *in vitro* studies with rye breads (163, 212). Our findings on gastric emptying after different breads indicate that the passage of the rye bread from the stomach to the small intestine is similar to that of wheat bread, and therefore cannot explain the differences between these breads in their insulin and incretin responses.

We used FSIGT to demonstrate for the first time that AIR can be enhanced by daily consumption of rye bread. Apparently, rye bread can influence pancreatic insulin secretion independently of the insulin potentiating effect of the enteroinsular axis, as indicated by intravenous glucose administration in the present study. AIR is known to be critical in the effective regulation of the postprandial rise in plasma glucose and also in the maintenance of normal insulin secretion (132). The enhanced AIR may, therefore, be related also to the reduced demand of postprandial insulin observed in the present studies. It is possible that regular consumption of rye bread could over time affect the main pathogenetic mechanisms of type 2 diabetes, i.e. prevent or delay β cell exhaustion and insulin resistance. Further support for this suggestion is obtained from the findings that low AIR (132, 327) and high fasting and postprandial insulin concentrations (328, 329) predict the development of type 2 diabetes, and that there is an inverse association between whole grain consumption and fasting insulin concentrations (227, 228). In epidemiological studies, high intakes of whole grain cereal products (6, 8, 186), cereal fiber (6, 7, 9) and consumption of a low-GI diet (7, 9) have been associated with a smaller risk for the development of type 2 diabetes. However, the data on the health effects of consumption of rye bread in intervention studies is scanty (234, 235), and the present study found no differences in fasting glucose and insulin, insulin sensitivity nor in glucose effectiveness between rye and wheat bread consumption. Therefore, more well-controlled intervention studies, especially in risk groups, are needed.

6.2 Relevance of the results to glycemic index

In our postprandial studies in healthy adults, there were no differences in glucose responses indicating non-differing GI values of different rye breads and refined wheat breads. These results are in clear contrast to findings from many previous studies reported by Foster-Powell and her coworkers in the International Table of GI and GL Values (10). However, the International Table of GI does reveal major variations in the GI values of different foodstuffs even between the products of the same food group. For example, the variations in values for wholemeal rye breads from different studies range from 58-94, for spaghetti they are 38-97 and for boiled potatoes 77-144.

There are numerous factors which may contribute to the observed variation in GI values. The glucose tolerance status of the subjects is probably one of the most important factors affecting GI. The present studies were carried out in healthy subjects in whom the glucose concentration is regulated within a narrow range, as discussed in section 6.1. Therefore our finding of unaffected glucose response to different breads would be expected, even differing from many other studies reporting GI values using subjects with IGT or diabetes. In general, the more impaired the glucose metabolism, the larger the variation in the glucose response (166). IGT may also explain the decreased glucose response to whole kernel breads reported earlier (163, 222). A large variation in GI values may also be expected if the compositional and structural characteristics of the study products deviate greatly from conventional products. Only edible and palatable products should be studied for their beneficial effects on glucose metabolism. Our experimental rye bread containing 60 % whole kernels was regarded as unpalatable and difficult to eat. Even though it has beneficial glucose responses, is debatable whether the experimental rye bread containing as much as 80 % whole kernels could be incorporated into the daily diet (163).

In our study, venous blood was used for glucose analysis, and no consistent differences in the glucose responses between rye and wheat breads were observed. However, some earlier rye bread studies with healthy subjects have used capillary blood for glucose analysis (163, 212, 222). The rise in capillary blood after carbohydrate ingestion has been shown to be greater and less variable than the equivalent values in venous blood (330, 331), and therefore capillary samples have been recommended for the determination of GI values (15). A recent interlaboratory study also showed that within-subject variation from two centers using venous blood was twice that from five centers using capillary blood (332). However, in the present study larger volumes of blood were needed to determine glucose and hormone concentrations than could be obtained from capillary samples. In addition, the capillary blood sample may be diluted

by the interstitial fluid and the venous blood sampling through cannula is ethically more justified than pricking the finger several times during each study visit.

Differences in the length of postprandial study periods and in the methods of calculating response areas for the determination of GI values may contribute to differences in GIs. Two hours have been found to be adequate in healthy individuals for measuring the initial blood glucose rise (333). Even though the glucose response was measured over three hours, the AUC calculation was based on only the values above the baseline level, and usually included only two hours. In addition, AUC was calculated according to the recommended protocol by ignoring the area below the fasting level (15, 321). The 2-hour study period and the method of area calculation based on the incremental response have been used also by others studying the effects of rye bread on postprandial glucose response in healthy subjects (163, 212, 222).

The small number of the subjects used for determining the glucose response may contribute to the large variability in the reported GI values. The number of study subjects has typically varied from 6 to 12 in the studies reported in the International Table of GI and GL Values (10), while in the present studies the number of subjects was 19-20.

According to the present studies, GI values cannot be calculated in subjects having normal glucose tolerance even in a well-controlled postprandial setting due to the strict physiological control of the plasma glucose level. Furthermore, our results show that insulin is more sensitive than glucose in differentiating the impact of foods and diet factors on glucose metabolism in healthy subjects. Therefore an insulin index, determined in a similar manner as GI, could be used when ranking individual foods in respect of their effects on glucosc metabolism in healthy individuals.

6.3 Effects of food and dietary factors on postprandial glucose and insulin

The novel finding of the reduced postprandial insulin response to rye breads in the present studies can be explained by the macrostructure of the breads. Differences in starch gelatinization and continuous matrix between rye and wheat breads cause differences in bread hardness and structure. The structural firmness and tightness of rye bread may impair starch hydrolysis, as indicated also by the slower *in vitro* starch hydrolysis of rye bread as compared to wheat bread, whereas the porosity and softness of wheat bread makes it readily digestible. Furthermore, these structural characteristics of rye bread are inherent to rye as an ingredient and cannot be achieved during baking

wheat bread in a similar way as rye baking is prepared (Juntunen et al, unpublished results, 2002).

The present studies confirmed previous findings on the importance of the preserved food structure (163, 231, 264) and particle size (263) on postprandial glucose and insulin responses. After controlling for the structure effect, i.e. all breads were baked by milled flours, it was also shown that the content or quality of dietary fiber in rye bread, per se, have no impact on postprandial glucose and insulin responses. The presence of fiber may rather act as a marker for the structural intactness of rye kernels or contribute to those characteristics of the bread macrostructure that are associated with the reduced postprandial insulin response in the present studies. However, the contribution of soluble fiber to postprandial responses cannot be excluded, as indicated by earlier studies (13, 220) and by the present finding with β -glucan rye bread. The 90 minute insulin AUC of the latter bread, calculated to evaluate the initial rise in blood glucose and to exclude the tail effect of carbohydrate ingestion, showed that soluble β -glucan concentrate may cause a lower postprandial insulin response even though the molecular size of β -glucan and therefore probably its viscosity became reduced during the baking. The impact of the firm macrostructure of rye bread, in addition to the β -glucan, cannot also be excluded. The reduced viscosity was also found in the rye bread as compared to the raw material extracts of rye (205), emphasizing together with the altered β -glucan composition found in the present study, the importance of the processing conditions for the preservation of viscosity in soluble fiber.

Dietary fiber complex contains numerous bioactive substances but their effects on glucose metabolism were not investigated in these present studies. The current evidence of beneficial effects of such compounds is based on *in vitro* studies (294, 300, 301), and on animal studies (302, 303), as well as on epidemiological evidence (7, 9, 334) and on the effects of these compounds from sources other than rye in humans (177, 294, 295). The results show that these compounds may have beneficial effects on glucose metabolism both at the level of gastrointestinal tract and pancreatic β cells and they can affect insulin sensitivity and oxidative stress. Therefore the impact of various bioactive substances in the rye bread matrix on glucose metabolism needs to be characterized in clinical trials in humans.

The content of fat and protein were not standardized in test bread portions even though they can modify glucose and insulin responses to carbohydrate foods by slowing gastric emptying (335), or stimulating insulin secretion (336). However, the range of protein and fat in amounts as used in our postprandial studies and found in most foods (330) or meals (331), have not shown to cause any detectable effects on postprandial

glucose and insulin, at least in normal subjects. On the contrary, the content and source of carbohydrates together have been shown to explain 85-94 % of the glucose and insulin responses (330). If the amount of carbohydrate is kept constant, as was the target in the present studies, it would be anticipated that postprandial responses reflect the actual impact of carbohydrate type on glucose and insulin responses.

The extension of eating time from 10 to 30 minutes (337) or the consumption of small meals (325) such that the variation in the portion sizes was in the same range as in our studies, have not affected glucose and insulin responses. The differences in portion sizes raise the question of whether the portions should be determined as edible portions, which people usually consume during one meal. This approach, however, excludes the possibility to compare the results to earlier findings, and would require an adoption of a different study design.

6.4 Methodological considerations

After the determination of glucose and insulin responses among healthy younger adult population in *Studies I and II*, we concentrated on studying a homogenous group of elderly women in *Studies III and IV*. Deterioration of glucose tolerance is a typical feature in elderly individuals, and therefore they also have an elevated risk for type II diabetes (329). The data of elderly women in *Study IV* was also analyzed separately for those subjects with and without IGT but the analysis showed no differences between groups, and therefore results were combined and presented for whole group. In general, the results of this thesis can be applied to the healthy adult population with normal glucose tolerance.

Bread is a good vehicle to modify the carbohydrates in the diet, especially the intake of fiber, because it provides 24-28 % of the dietary carbohydrates and it is the main source of dietary fiber in the Finnish diet (55 % of the total fiber in men and 44 % in women, respectively) (181). Even though there was a major increase in the dietary fiber intake compared to the control wheat period as well as from conventional diet to rye bread period, it was surprisingly well tolerated. Only one of the 20 women participating in the 2 x 8 weeks intervention study reported severe gastrointestinal symptoms at the beginning of the rye bread period.

Even though both serum and plasma insulin samples were used for the determination of postprandial insulin responses in the present studies, this should not have any effect on the observed levels of insulin between the studies, since the radioimmunoassay method used at the Department of Clinical Nutrition gives similar results from plasma

and serum samples (Maritta Siloaho, unpublished results, 2002). The specificity of insulin measurement by radioimmunoassay has been shown to be influenced by the crossreactivity with proinsulin and proinsulin split products (338). Since the subjects in all studies were healthy, this may, however, have only a minor effect on total measured insulin concentrations.

The correlation in the assessment of S_I between the FSIGT and the glucose clamp is strong (339, 340). Even though glucose clamp is regarded as the gold standard method, the FSIGT requires less specialized equipment, is less labor intensive and is a cheaper alternative in non-diabetic subjects. In diabetic patients, however, the MINMOD program is not very applicable to the estimation of S_I as a result of partially or totally suppressed insulin response to glucose. However, that was not the problem in the present study since the subjects were healthy with respect to glucose metabolism. MINMOD analysis of FSIGT has been shown to be reproducible when manageable confounding factors have been controlled (45, 341), as in the present study.

The AutoDELFIA C-peptide assay was used for the determination of insulin secretion in the present study. The overall performance of that assay is very satisfactory as evaluated on the basis of imprecision and accuracy data of the external quality control hormone analysis scheme carried out by Labquality (Helsinki, Finland).

Both the GLP-1 and GIP assays used in the present studies for the determination of the secretion rate of these incretins measured the “total hormone” which is the sum of the biologically active intact hormone, and the inactive metabolites (85, 320). Since the degradation of GLP-1 is rapid (half-life of 1-1.5 min, and clearance rate exceeding cardiac output), the secretion of the L cells cannot always be measured as the increased plasma concentrations of the intact peptide whereas increased concentrations of metabolites are evident (92). For GIP, the dipeptidyl-peptidase IV degradation is not so extensive as that of GLP-1, and therefore almost all of the GIP that leaves the gut is intact (342). The assay used in the present studies has been shown to be extremely reliable, sensitive and very specific (79). In particular, it does not cross-react with the so-called 8-kDa GIP, whose chemical nature and relation to GIP secretion is uncertain.

Many confounding factors in the incretin response, such as age (343), glucose tolerance (342) and feeding status (344, 345), were controlled in the present studies by selecting homogenous study groups and by providing detailed instructions concerning the preparation for the studies. However, the lack of randomization of the eating order in the case of β -glucan bread in *Study II* may have contributed to the significantly higher fasting value of GLP-1 in that bread as compared to control wheat bread.

Paracetamol absorption kinetics after the oral ingestion of the drug was used in the present study as an indirect marker of gastric emptying (346) since that technique is cheap, easy to perform, does not require any special equipment, and it has been shown to correlate well to scintigraphy, the gold standard for measuring gastric emptying. The use of paracetamol as a marker requires an intact small bowel to achieve normal absorption, and therefore it was suited to our subjects who did not have gastrointestinal disorders. The use of paracetamol has been criticized because of the potential interaction with food components and this can impact on gastric release of nutrients (347), though this claim has not been confirmed after the ingestion of white bread (348).

7. SUMMARY AND CONCLUSIONS

This doctoral thesis is based on four original publications investigating the impact of rye bread on glucose metabolism in healthy subjects. The data have been collected from three postprandial studies in which healthy subjects were given a single meal of different breads in a random order (*Studies I-III*), and from one intervention study (*Study IV*) in which the subjects consumed high-fiber rye bread or white wheat bread daily for eight weeks in a cross-over design. The results can be summarized as follows:

1. Both conventional commercially available and experimental rye breads significantly decreased the postprandial insulin and C-peptide responses but had no effect on the glucose response as compared to the refined wheat bread. This indicates that after rye bread consumption less insulin is needed and secreted to regulate the postprandial glucose response than is the case after refined wheat bread.
2. With respect to the physiological factors affecting glucose metabolism, the reduced GIP response indicated changes in the absorptive function of the upper small intestine. This finding was also supported by the reduced rate of starch digestion of rye breads *in vitro*. There were, however, no significant differences between the rye and wheat breads with respect to GLP-1 responses or gastric emptying.
3. The firm bread matrix of rye bread as well as preserved food structure in the form of whole kernels were associated with a decreased postprandial insulin response, but the response was unaffected by the quantity and quality of rye fiber.
4. The daily consumption of the high-fiber rye bread over 8 weeks resulted in an enhanced acute insulin response as compared to that of the refined wheat bread. However, other indicators of the glucose metabolism, i.e. indexes of insulin sensitivity and glucose effectiveness or fasting glucose and insulin, did not change significantly.

The postprandial studies in this thesis focused on the types and quality of carbohydrates in rye bread associating with the glucose response and GI. However, the results showed that in healthy subjects, the insulin response is a better indicator of glucose metabolism than the plasma glucose response. The unaffected postprandial glucose response in healthy individuals is not an unexpected finding. It is most probably explained by the continuous regulation of plasma glucose by hormone secretions and by

the modification of endogenic glucose production after binding of insulin to its receptors in liver. According to the present results, GI cannot be used among the non-diabetic population in ranking of different types of carbohydrates with respect of their postprandial glycemc response, at least in the case of products like rye and wheat breads. However, the blood insulin response might be used for this purpose and it should be tested also for foods other than bread.

In the studies of this thesis the ingestion of rye bread reduced the need for postprandial insulin and improved the first-phase insulin secretion, both factors associated with the effective regulation of glucose metabolism and reduced risk of type 2 diabetes. The intervention study showed that rye bread is easily incorporated into the daily diet in amounts (approximately 25 % of daily intake of energy or 50 % of the carbohydrate containing foods) capable of eliciting beneficial effects on glucose and lipid metabolism in intervention studies comparing the effects of low- vs. high-GI diets (193). Rye bread can also be consumed as part of the breakfast which has been shown to be crucial in controlling of insulin sensitivity for the rest of the day (174). Therefore, consumption of rye bread, a traditionally eaten staple food in Finland, is likely to be beneficial for glucose metabolism among the general population. If these effects are confirmed in individuals at risk of developing impaired glucose metabolism, rye bread could also be used in the prevention and in the long-term treatment of glucose intolerance and diabetes.

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