

THE IMPACT OF INGESTION OF BREADS OF VARYING COMPOSITION ON  
BIOMARKERS OF GLUCOSE METABOLISM IN OVERWEIGHT AND OBESE ADULTS

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## **ABSTRACT**

### **THE IMPACT OF INGESTION OF BREADS OF VARYING COMPOSITION ON BIOMARKERS OF GLUCOSE METABOLISM IN OVERWEIGHT AND OBESE ADULTS**

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This thesis examined the impact of ingestion of breads of varying composition on biomarkers of glucose metabolism in overweight and obese adults. In study 1, the metabolic responses to acute ingestion of 50 g available carbohydrate of 4 breads (sourdough, whole-wheat, whole-wheat barley and white) were examined after first and second meals. Ingestion of sourdough bread significantly lowered blood glucose and GLP-1 responses in the two meal periods while ingestion of whole-wheat breads did not change the metabolic responses compared to white bread. In study 2, metabolic responses to acute ingestion of whole-grain (11-grain, sprouted-grain and 12-grain), sourdough and white breads, were examined in a 2-part study. In part 1, ingestion of 50 g available carbohydrate of sprouted-grain bread significantly lowered the blood glucose response compared to sourdough and white breads. The GLP-1 response for sourdough bread was significantly lower than all the other breads. In part 2, ingestion of a fixed portion (107 g) of sprouted-grain bread significantly lowered the blood glucose and serum insulin responses compared to sourdough bread. In both study parts, the GLP-1 response for sprouted-grain bread was significantly greater than white bread. In study 3, the impact of 6 wk ingestion of 11-grain or white bread, on biomarkers of glucose metabolism was examined in metabolically challenged obese men and postmenopausal women, and healthy subjects. In the former group, 6 wk ingestion of 11-grain bread reduced the glycemic response, compared to white bread. The

insulinogenic index increased post-11-grain bread, compared to post-white bread, in the control group and there was a trend ( $P = 0.06$ ) in the metabolically challenged group. Taken together, these results suggest that sourdough bread ingestion may improve postprandial glycemia while whole-wheat bread ingestion does not. Among whole-grain breads, ingestion of sprouted-grain bread lowered postprandial glycemia, while acute ingestion of 11-grain bread has no impact on glucose metabolism; this suggests that metabolic response to whole-grain breads vary among different recipes. Furthermore, chronic ingestion of 11-grain bread may improve glycemia in metabolically challenged obese population and increase insulin sensitivity in healthy population.

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## LIST OF ABBREVIATIONS

<i>Abbreviation</i>	<i>Definition</i>
AUC	Area under the curve
CHO	Carbohydrate
CVD	Cardiovascular disease
C <sub>max</sub>	Peak serum concentration
dAUC	Detrimental AUC
DF	Dietary fiber
DPP-4	Dipeptidyl peptidase-4
GI	Glycemic index
GIP	Glucose dependent insulinotropic polypeptide
GIPR	GIP receptor
GLP-1	Glucagon-like peptide-1
GLPR	GLP-1 receptor
IAUC	Incremental AUC
IGI	Insulinogenic index
IGT	Impaired glucose tolerance
HGI	High glycemia/insulinemic
HOMA-IR	Homeostasis model for insulin resistance
NEFA	Non-esterified fatty acids
NGI	Normal glycemia/insulinemic
OGTT	Oral glucose tolerance test
SCFA	Short chain fatty acids
T2D	Type 2 Diabetes
T <sub>max</sub>	Time until maximum concentration

# **CHAPTER ONE**

## **INTRODUCTION**

## 1.1 Introduction

Diabetes, specifically Type 2 Diabetes (T2D), is a significant health problem in North America (1,2). In Canada alone, there are 60,000 new cases of T2D per year. In Ontario, a recent study reported a 69% increase in the prevalence of T2D from 1995 to 2005 (3). The Canadian public bears the financial burden for the immediate and long-term care for diabetes which, in 1998 was estimated \$1.6 billion (1). Prevalence of obesity, a strong risk factor for developing T2D, is growing as well. In 2001, Mokhdad et al. (4) surveyed approximately 200,000 Americans and reported a 61% increase (from 7.7 to 19.8%) in the prevalence of obesity. Due to this alarming increase in both obesity and T2D, and subsequent burden to the health care system, a great deal of attention is being directed to the identification of key environmental and lifestyle factors that contribute to the prevention and management of these conditions.

The most promising approach in prevention of T2D and obesity is modification of risk factors through diet and physical activity (5-7). Nutritional research has focused on the role of dietary macronutrient quantity and quality in the progression of obesity and T2D and demonstrated that carbohydrate (CHO) is the major dietary factor influencing glycemic control (6,8,9). Therefore, there is an urgent need for broader strategies that influence the nutritive value of CHO foods without changing their popular appeal.

Traditionally, the impact of ingestion of CHO on biomarkers related to T2D has been examined in the fasted state. North Americans spend at least 18 h a day in a postprandial state. Postprandial responses to CHO ingestion are associated with changes in various biomarkers related to chronic diseases such as T2D, suggesting that postprandial state plays a key role in development of T2D (10,11). In recent years, the value of investigating T2D risk in the postprandial state has been recognized and an increasing number of studies have examined the

metabolic responses to CHO ingestion in this state. The primary endpoints in postprandial CHO research have been glucose and insulin and results have shown that they both can be altered by the amount and quality of the CHO consumed (5,12,13). There are many biomarkers in addition to blood glucose and insulin that can be altered by CHO ingestion including cholesterol, lipoproteins, cytokines and incretin hormones. (14,15). It is necessary and informative to consider a wider spectrum of biomarkers that relate to T2D risk as research progresses in the area of postprandial CHO metabolism. Incretin hormones, which will be a focus of this thesis, are gut hormones released in response to meal ingestion and play a major role in the regulation of postprandial insulin and glucose homeostasis (16-18).

## **1.2 Incretin Hormones**

The communication between the intestine and endocrine pancreas was confirmed when it was shown that insulin secretion is greater following an oral dose of glucose compared to an intravenous administration of the same dose of glucose (19,20). This phenomenon has been termed the ‘incretin effect’, and is estimated to account for approximately 50% of the total insulin secreted after oral glucose administration (18,21). By definition, incretins are hormones that are secreted from the gastrointestinal tract into the circulation in response to nutrient ingestion that enhance glucose-stimulated insulin secretion. To date, only glucose dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) fulfill the definition of an incretin hormone and together they account for the incretin effect in humans (22). GIP and GLP-1 share many common actions in the pancreas but have distinct actions beyond those of the pancreas. The following section provides an overview of GIP and GLP-1 secretion, regulation, biological actions, degradation and function in T2D.

### 1.2.1 GIP

In 1971, Brown isolated and deduced the amino acid structure of a peptide isolated from intestinal mucosa. Exogenous administration of the peptide inhibited gastric acid secretion in dogs, so he called it gastric inhibitory polypeptide (GIP). Brown and colleagues subsequently found that GIP had insulinotropic properties and suggested to rename it glucose-dependent insulinotropic polypeptide, retaining the acronym GIP (23). Therefore, the first incretin hormone described was GIP.

GIP is a 42 amino acid peptide belonging to the glucagon-secretin family of peptides. The members of this family show pronounced sequence homology, particularly in the N-terminal part of the molecule (24). GIP is processed from a precursor of 153 amino acids (**Fig 1**), but specific function for the other fragments of the precursor have not been identified (25). GIP is produced and released predominantly from k-cells which exhibit the highest density in the proximal small intestine, but recent studies have indicated that GIP-containing cells are found in the entire small intestine (25-27). GIP is secreted in response to nutrient ingestion, especially glucose and fat (25,28,29). Oral fat ingestion alone without any carbohydrate being present, induces GIP secretion, but this is not sufficient to stimulate insulin secretion at fasting glucose concentrations, indicating that the effects of GIP on insulin release do not occur if plasma levels of glucose are also not concurrently increasing; GIP-mediated insulin secretion is glucose dependent (29,30).

In humans, fasting systemic venous plasma GIP levels are approximately 9 to 11 pM and peak plasma concentrations of 50 to 120 pM are achieved after eating, depending on the health status of the subject and the amount and quality of the food consumed (16,25,28). The half life of bioactive GIP is about 2 min owing to the rapid degradation by proteolytic enzyme dipeptidyl

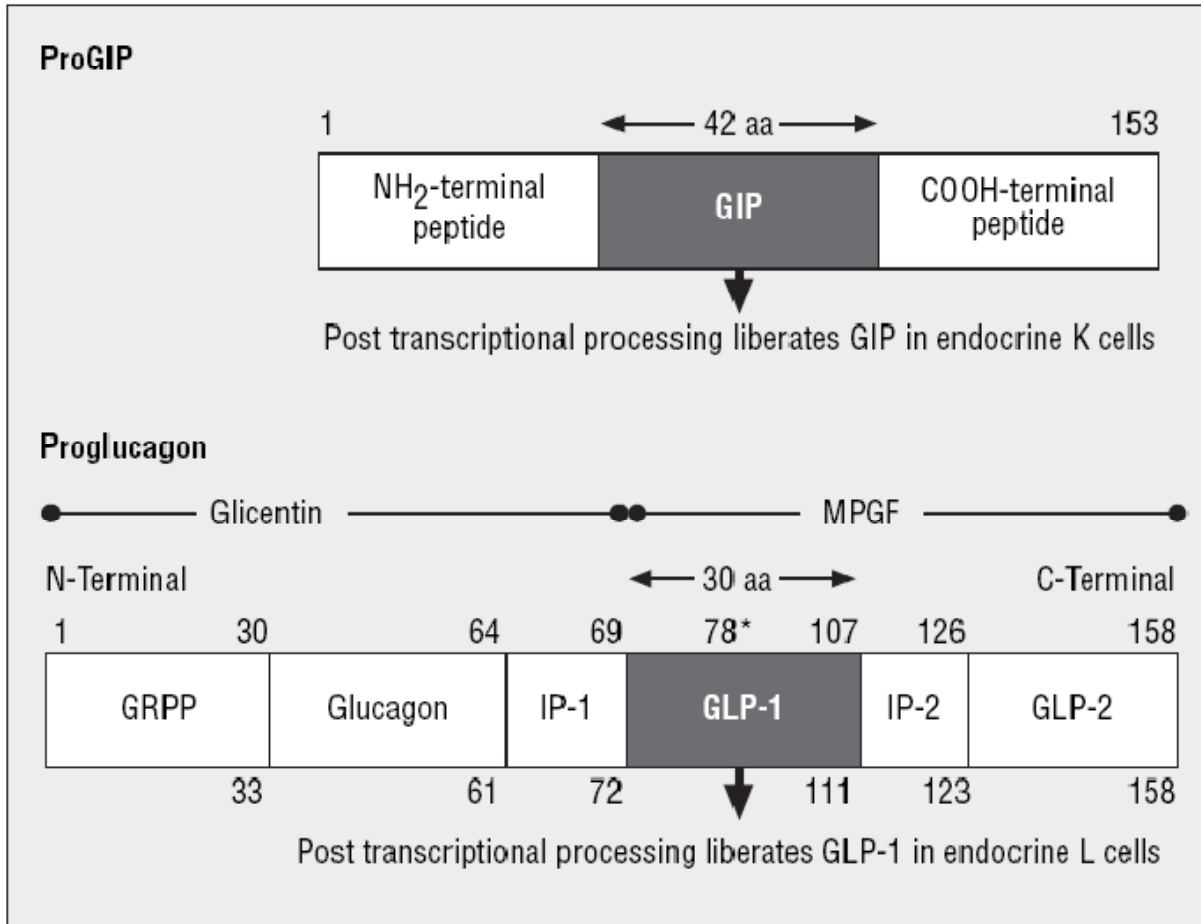
peptidase-4 (DPP-4), which is bound to lymphocytes and endothelial cells of blood vessels of gut and liver as well as being present in soluble form in the circulation. This enzyme cleaves a dipeptide from the N-terminus of oligo-peptides or proteins that contain an alanine or proline residue in position 2, thereby modifying or inhibiting their activity (28,30). The full length GIP (1-42) converts to GIP (3-42) which is then inactivated by DPP-4 and excreted by the kidney (26,28,30,31). The elimination rates of GIP (1-42) and GIP (3-42) are similar in those with and without T2D (32).

GIP receptor (GIPR) has been identified in the stomach, adipose, bone, brain, pancreas as well as adrenal cortex (26,28,33). The actions of GIP are less well studied than those of GLP-1. Pancreatic actions of GIP include stimulating glucose-induced insulin secretion and  $\beta$ -cell expansion (22,26,28,34). Interaction of GIP with GIPR on the pancreatic beta-cells causes an increase of cAMP levels, which in turn increases the intracellular calcium concentration and enhances the exocytosis of insulin-containing granules (25,26,35). GIP has also been shown to promote beta-cell proliferation and reduce beta-cell apoptosis (30).

In addition to affecting the pancreas, GIP alters fat metabolism in adipocytes. The anabolic effects of GIP in adipose tissue include stimulation of fatty acid synthesis and re-esterification, enhancement of insulin-stimulated incorporation of fatty acids into triglycerides, up-regulation of lipoprotein lipase synthesis, and reduction of glucagon-stimulated lipolysis (36). Vilsboll et al. (37) reported increased fasting GIP concentration and an increased early phase GIP response in obese, healthy subjects compared to lean, healthy subjects. Marks (31) hypothesized that GIP might function as an obesity-promoting hormone and Miyawaki (38) showed that GIP knock-out mice were resistant to obesity and insulin resistance induced by high

fat diet, compared to normal mice. These observations suggest that high GIP levels may predispose to the development of obesity.

Studies employing the immunoneutralization of endogenous GIP activity indicated that an intestinal hormone other than GIP contributes substantially to the incretin effect. A major contribution to the incretin effect from the lower gastrointestinal tract was shown in studies of patients after varying degree of resection of small intestine, providing further evidence indicative of the presence of an additional incretin hormone released from the distal small intestine (16,17,22).



**Figure 1.1 Structure of proGIP and proglucagon encoding GIP and GLP-1 (25).**

### 1.2.2 GLP-1

The discovery of a second incretin hormone, glucagon-like peptide-1 (GLP-1), followed the cloning and sequencing of mammalian proglucagon genes. In 1983, Bell et al. identified the sequence of two additional glucagon related peptides from the hamster proglucagon gene that were approximately 50% homologous to glucagon, and were accordingly named glucagon-like peptide-1 and glucagon-like peptide-2 (17).

Glucagon is the main product of post transcriptional processing of proglucagon in the endocrine pancreas. GLP-1 is produced together with GLP-2 and glicentin (enteroglucagon) as the main products in the enteroendocrine L cells (**Fig 1**). Despite close structural homology, GLP-2 does not share the same biological action as GLP-1, but rather acts as a regulator of growth in the intestinal tract (25). GLP-1 is mainly expressed in mucosal L-cells which are found in highest density in the distal intestine. Both K-cells and L-cells are open type endocrine cells, meaning that they can be influenced by direct contact with nutrients found in intestinal lumen (39). Recent studies revealed a high degree of co-localization of GLP-1 and GIP-secreting cells, suggesting simultaneous secretion of both incretin hormones (27).

Ingestion of a meal rich in CHO and fat is the primary stimulus for GLP-1 secretion but individual nutrients including glucose and other sugars, fatty acids, essential amino acids and dietary fiber (DF) can also stimulate its secretion (29). GLP-1 is released rapidly into the circulation after food ingestion and its secretion occurs in a biphasic pattern, with an early phase beginning within 5 to 15 min and a prolonged second phase following within 30 to 60 min. Thus, the early phase and the prolonged second phase of GLP-1 secretion may be due to both direct nutrient contact with the L cells and K/L cells in upper small intestine and by neural, and other gut-derived (and even non-gut-derived) endocrine factors activating L-cells in the distal intestine

(17,22,25,29). Several studies proposed vagal cholinergic muscarinic regulation of GLP-1 secretion (17,29,40). A duodeno-ileal endocrine loop has also been proposed as another mechanism to explain the rapid GLP-1 response to meal ingestion. In this loop GIP, acetylcholine, and gastrin releasing peptide act as mediators: the afferent vagus nerve is activated by GIP, which subsequently stimulates GLP-1 secretion through the efferent vagus nerve and enteric neurons that release acetylcholine and gastrin-releasing peptide (41).

Typical fasting levels of bioactive GLP-1, measured from peripheral veins, are in the range of 5 to 10 pM and increase by 2- to 3-fold after meal ingestion, depending on the size and composition of meal (18,22,28). The first two N-terminal amino acids (His, Ala) of native GLP-1 are rapidly cleaved by DPP-4, and the resulting GLP-1 (9-36) fragment is not insulinotropic (42). GLP-1 is also degraded *in vitro* by neutral endopeptidase 24.11 (NEP-24.11), which is a membrane-bound zinc metallopeptidase. High levels of this enzyme are found in the kidney and GLP-1, and its metabolites are rapidly cleared through the kidneys, suggesting that NEP-24.11 is involved in renal clearance of GLP-1 (16,28,29). GIP is also degraded by NEP 24.11 *in vitro*, but at a much slower rate. It was suggested that the larger size of GIP (42 amino acids compared to 30 for GLP-1) may be one factor determining its suitability as a substrate for NEP 24.11, since the enzyme has a preference for smaller peptides (26,28).

The distribution of GLP-1 receptor (GLP1R) seems to be more widespread than GIPR. GLP-1R is expressed in the brain, lung, pancreatic islets, stomach, hypothalamus, heart, intestine and kidney. The physiological role of GLP-1 in some of the organs in which GLP1R is expressed is uncertain (16,18,25). GLP-1 induces insulin secretion after food intake by binding to GLP1R on pancreatic beta-cells, which is positively coupled to increase intracellular cAMP and  $Ca^{2+}$  levels in beta-cells and stimulation of exocytosis of insulin (18,35). Insulinotropic effect of

GLP-1 is glucose-dependent. In addition, GLP-1 stimulates beta-cell proliferation and inhibits beta-cell apoptosis. GLP-1 also stimulates the transcription of the genes coding for beta-cells involved in the process of glucose sensing and insulin synthesis and secretion (16,18). GLP-1 has been shown to inhibit glucagon secretion and this contributes to the glucose-lowering effect of GLP-1 (43).

The presence of GLP1R in the central nervous system is indicative of a role for GLP-1 in central nervous functions. Several studies showed that food intake and appetite are reduced by GLP-1 administration (36,44-46). In healthy humans, Flint et al. reported a 21% reduction in food intake as well as increase in satiety and fullness during GLP-1 administration (46).

GLP-1 has been shown to delay gastric emptying. Delayed gastric emptying and consequently delayed CHO absorption may be beneficial to glucose tolerance, especially in early T2D where gastric emptying is accelerated (43,46). Pancreatic and extra-pancreatic GLP-1 functions make it a suitable therapeutic agent for management of T2D.

### **1.2.3. Incretin Function in Diabetes**

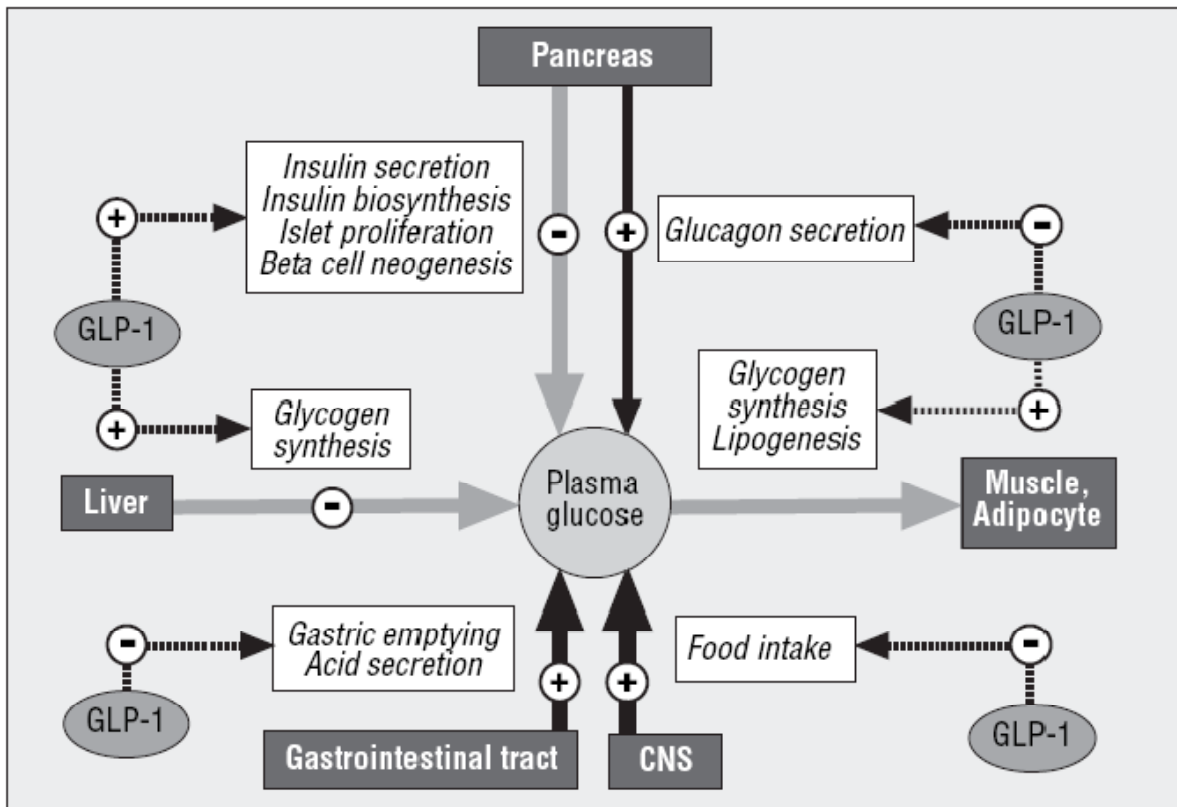
There are many publications on the secretion of the incretin hormones in T2D and the majority of them reported that GLP-1 concentrations were significantly decreased while GIP concentrations were approximately normal. They also found that the insulinotropic effect of GLP-1 was preserved while the insulinotropic effect of GIP was blunted or absent, suggesting that T2D patients are resistant to the biological effects of GIP (37,43,47-49). It has been shown that type 2 diabetic patients have normal early-phase but defective late-phase insulin secretion in response to GIP, whereas their insulin response to GLP-1 is high (49). The mechanisms underlying the diminished GIP responsiveness in diabetes are not fully understood but may involve genetic variants of the beta-cell receptor for GIP, reduced expression of GIP receptor or chronic

desensitization of the receptor (18). Possible explanation for diminished GLP-1 secretion may include altered gastric emptying rate, which might influence the absorption rate in the proximal intestine, resulting in less foods reaching the distal intestine, where the GLP-1 producing L-cells are more numerous (50). An alternative aetiology for diminished GLP-1 action in T2D may be hyperglycemia-induced down regulation of GLP-1 receptor expression in pancreatic islets (51). Although both GLP-1 and GIP act as incretin hormones in normal subjects, only GLP-1 can be used to treat T2D.

The glucose-lowering effect of GLP-1 and number of additional GLP-1 actions, make it a very good candidate for treatment of type 2 diabetic patients. T2D is associated with an impaired insulin secretion, particularly loss of first phase insulin secretion (52-54). It is evident that GLP-1 stimulates glucose dependent insulin secretion and restores first phase-insulin secretion (25,35,55,56). Additionally, abnormal gastric emptying may occur in diabetes. A reduced entry of nutrients into the circulation based on the slow gastric emptying is a major mechanism under glucose-lowering effect of GLP-1 in the postprandial state (43). Furthermore, effects of GLP-1 on differentiation, growth and regeneration of pancreatic beta-cells suggests that GLP-1 or its derivatives could be used to preserve or increase the reduced beta-cell mass in newly diagnosed patients with type 1 diabetes (50,57).

The observation that native GLP-1 is cleaved rapidly by DPP-4 at the position 2 alanine, led to the development of degradation resistant GLP-1R agonists, such as Exendine-4, for the treatment of T2D. Exendine-4 is a 39 amino acid peptide which contains a glycine at position 2 is not a substrate for DPP-4 and exhibits a longer half life compared to GLP-1 (22,50,58). Other synthetic agents that have been developed were DPP-4 inhibitors. Typically, DPP-4 inhibitors result in a 2-fold increase in GLP-1 levels. DPP-4 is an enzyme with many substrate including

neuropeptides, cytokines, other gastrointestinal hormones, and chemokines; as such, concerns have been raised regarding possible effects of DPP-4 inhibition on other systems and physiologic functions; for example, DPP-4 is expressed on lymphocytes, raising a potential concern about adverse effects of DPP-4 inhibitors on immune function. However, clinical trials to date indicate that DPP-4 inhibitors are well tolerated (51). GLP-1R agonists and DPP-4 inhibitors have not demonstrated major adverse effect in preclinical studies or phase 3 clinical programs; however the long-term consequences of their administration remain unknown (59). Further results are expected to complete the efficacy/safety profile of these agents, and to define their role in the therapeutic strategy of T2D.



**Figure 1.2. Biological and Physiological Actions of GLP-1 (25)**

### **1.3 Dietary Carbohydrate**

Carbohydrates are a diverse macronutrient group with varying structure and biological functions comprising a considerable portion of the daily dietary intake of Canadians. According to the Canadian Community Health Survey, approximately 50% of energy in the Canadian diet is comprised of CHO (60). The specific nature of the dietary CHO has great impact on postprandial glucose excursion and is the primary stimulus for postprandial insulin release (5,14,61).

Bread makes up a considerable part of Canadian intake of starch and fiber. It constitutes the main part of one or more of the daily meals and is considered an indispensable element in the food of all age groups (60). Many studies used bread as a challenge in postprandial CHO research and showed that several factors can influence postprandial glycemia by affecting gastric emptying rate and/or the rate of digestion and absorption of starch in the small intestine (62-66). Some of these factors are related to the botanical composition of the raw material, while others are linked to the processing of the dough and baking conditions. Recent studies applied various methods and strategies to design the optimum bread, which improves glycemia, to help prevent and manage T2D, as discussed in detail below.

#### **1.3.1 Strategies to Optimize the Metabolic Responses to Carbohydrate Foods**

Differences in postprandial glucose response to various CHO-rich foods have been demonstrated in healthy and diabetic subjects (62,67). The glycemic index (GI) is a physiological classification for CHO-rich foods, with implication in health and disease (61,68). The GI is defined as the incremental area under (IAUC) of the blood glucose curve to a 50 g available CHO portion of a given food expressed as related to the response to 50 g available CHO of a reference food (generally glucose or white bread) taken by the same subject (61,68-70). From a

physiological perspective, the GI serves as an indicator of the rate at which a given food is digested and absorbed. The principle is that the slower the rate of CHO absorption, the lower the rise in glucose level and the lower the GI value (61,68). Recent studies have shown that GI is positively associated with the risk of developing T2D and cardiovascular disease (CVD) (61,71,72). While the GI only considers postprandial glucose excursion, the insulinemic index is a measure of the postprandial insulin response to a given food item and is likely to be of considerable importance and is not always reflected by GI. For example, Juntunen et al. (73) found in healthy, postmenopausal women, that despite a lack of difference in postprandial glucose response after ingestion of rye breads as compared to white wheat breads, the postprandial insulin response was significantly lower with the rye breads, suggesting that less insulin was needed to maintain a similar postprandial glucose excursion following rye bread ingestion versus a white wheat bread. This finding highlights the importance of extending dietary studies beyond blood glucose measures to include other potential biomarkers for T2D.

The glycemic response to different bread types varies widely. The GI of breads varies from 27 (barley bread with 75% whole grain) to 95 (French baguette) (64). This extreme variability reflects very different rates of starch digestion. Various components of breads have been studied in relation to their ability to modulate the rate of starch digestion and consequently postprandial glycemia. Among these components, dietary fibre (DF) has received the most research attention due to its ability to affect the rate and extent of starch degradation (74). DFs are mainly derived from plant material, including cell wall material. Their common characteristic is that they escape digestion in the small intestine and reach large intestine where they undergo fermentation (75). Traditionally, DFs have been classified on the basis of their solubility in the water. Thus, they have been defined into soluble and insoluble fibers. Insoluble DFs are non-

viscous with negligible effect on gastric emptying rate (76). Research has shown that they reduce the transit time within the small intestine, increase the stool weight and act as a bulking agent (76,77). Weickert et al. (78) showed that ingestion of fiber-enriched bread (white bread enriched with 31.2 g insoluble fiber/day) over a 72 h period, improved whole-body insulin sensitivity, compared to control bread (white bread) in overweight and obese women. More studies are needed to clarify the mechanism linking cereal fiber (insoluble fiber) intake and reduced risk of T2D.

Soluble fibre has been shown to reduce the rate of starch digestion and alter the rate of glucose absorption. The viscosity-altering behaviour of soluble DFs within the small intestine should account for some of their nutritional benefits (79). Several types of soluble fiber have been studied: oat fiber, barley fiber rich in  $\beta$ -glucan, arabinoxylans, psyllium and guar. Among the tested soluble fibers, there is an emerging interest in barley as functional food ingredient. Barley grain has a high concentration of the soluble fiber,  $\beta$ -glucan (linear chains of  $\beta$ -glucose residues joined through both  $\beta$ -(1-3) and  $\beta$ -(1-4) linkages) (80). It has been shown that inclusion of barley in CHO-rich foods can modulate the postprandial glucose and insulin responses (81-83). Casiraghi et al. (84) evaluated the acute glucose and insulin responses to two different products obtained from flour enriched with  $\beta$ -glucan (8.5%) in comparison with similar products prepared with whole-wheat flour in healthy subjects. Barley products (cookie and crackers) were prepared by replacing 60% wheat flour with the barley flour. Similar whole-wheat products were made by incorporating 60% wheat bran to wheat flour. The results showed that products prepared from barley flour enriched with  $\beta$ -glucan exhibited favourable responses on glucose and insulin responses (84).

$\beta$ -glucan is believed to be the active component responsible for the observed reduction of blood glucose and insulin response after ingestion of a meal containing barley. Wood et al. (80) suggested that the lowered postprandial glucose response after a single meal containing  $\beta$ -glucan is related to the impairment intestinal transit and absorption due to meal viscosity. Viscous fibers like  $\beta$ -glucan rapidly absorb water within the gastrointestinal tract, which increases the viscosity of the food(s) consumed and limit the absorption of glucose (79,82,85). In order to have a positive effect, the viscose fiber must not be destructed, since there is a risk that their rheological properties will be modified in the intestine. For instance due to its linear structure, barley  $\beta$ -glucan is very sensitive to depolymerisation during the baking process (80).

Another strategy to lower the metabolic responses to bread and other CHO-rich foods is increasing the levels of organic acids by prolonged fermentation time or direct addition of organic acids. Studies have demonstrated that the organic acids formed during fermentation of bread (86,87) may improve the glucose tolerance to starch. In a study by Maioli et al. (88) the postprandial glycemic and insulinemic responses were evaluated in subjects with impaired glucose tolerance (IGT) who had a meal containing sourdough bread leavened with lactobacilli, in comparison to a reference meal containing bread leavened with baker's yeast. In IGT subjects, sourdough bread induced a significantly lower glucose and insulin responses at 30 min and a smaller glucose and insulin area under the curve compared to the reference bread. Östman et al. (89) showed that addition of acetic acid (given as vinegar) to bread meals lowered the blood glucose response in healthy volunteers. Similarly Liljeberg et al. (90) examined the effect of acetic acid (given as vinegar) in a white bread on the postprandial glucose and insulin responses in healthy subjects. Presence of acetic acid in the white bread lowered the acute glucose and

insulin responses as well as the gastric emptying rate (based on lowered paracetamol concentrations) (90).

The mechanism underlying the favourable effect of organic acids on glucose tolerance has not been completely explained. Suggestions have included that organic acids interfere with starch digestion and that the glucose lowering effect of organic acids cause an inhibition of digestive amylases (86). However, *in vitro* measurement of the rate of starch hydrolysis using a simulated gastrointestinal model did not reveal any amylase inhibition in the case of acetic acid containing bread (91). It is also possible that the mechanism at gastrointestinal level differs for different acids. In some studies acetic acid (given as vinegar) and in others, lactic acid were added to the bread. (It should be noted that sourdough bread contains lactic acid produced during the fermentation.) In the case of acetic acid added to bread meals, Liljeberg et al. (90) reported lowered glycemia and reduced rate of gastric emptying. In other studies, lowered glycemia after consumption of bread with added sodium propionate by healthy subjects was attributed to lowered rate of gastric emptying as determined by using ultrasonography (63) or paracetamol (87). However, in a study by Östman et al. (91) addition of lactic acid to bread improved glycemia but did not affect the rate of gastric emptying. This presents the possibility that acetic acid mediates its favourable effect on glycemia by reducing the rate of gastric emptying whereas lactic acid lowers the rate of starch digestion. More studies are needed to explain the mechanism by which organic acids improve glycemia.

Other important factors influencing the postprandial glycemia following ingestion of CHO-rich food are botanical origin and amylose/amylopectin ratio. Starch is composed of amylose and amylopectin. Due to its linear structure, amylose is hydrolyzed more slowly than amylopectin, whose branched structure is more accessible to  $\alpha$ -amylase (92,93). In a study by

Behall et al. (94) consumption of breads containing 50-70% amylose-cornstarch resulted in lower glucose and insulin responses compared to breads made with standard cornstarch containing 30% amylose and 70% amylopectin.

Other factors are particle size, the extent of starch gelatinization and the integrity of the plant cell wall (93). Boiled intact cereal grains such as rye, oats, barley and wheat cause lower glucose and insulin responses. However, when the cereal grains are ground into flours before boiling, the postprandial glucose and insulin increase significantly (93). **Table 1** summarises the various methods to reduce the GI of bread.

**Table 1.1. Different means to reduce the glycemic index of bread (64).**

Modifications realised	Effect obtained
On the raw materials Amylose:amylopectin ratio Starch encapsulation by protein or fibre Incorporation of intact cereal kernels	Limitation of the starch accessibility to $\alpha$ -amylase within the food matrix
On the raw materials Adding soluble fibre ( $\beta$ -glucans, arabinoxylans) Adding organic acids	Modification of digestive physiology Increased viscosity of the digestive medium Slowing of gastric emptying rate
On the technological process Use of leaven (organic acids) instead of regular yeast Short kneading and/or long fermentation time	More compact structure and slowing of gastric emptying rate

### 1.3.2 Whole-Grain Consumption and Type 2 Diabetes Risk

Current recommendations in Canada suggest that at least half of daily grain intake should be in the form of whole-grains (95). The 1994-1996 Continuing Survey of Food Intake in Individuals showed that USA adults consumed an average of 6.9 servings of grain products per day, but only one serving per day was whole-grain. Only 8% of adults consumed three or more servings of whole-grain foods per day (96).

By definition, whole-grain foods are composed of the bran, germ, and endosperm in the same proportions as the cereal grain in which it was obtained while refined-grains lose a

substantial amount of DF and contain a relatively high amount of starch, because most of the bran and germ is removed in the refining process. The majority of the fiber and nutrients in the whole-grain are retained in the bran and germ, while the endosperm is particularly rich in starch (97,98).

The rationale behind the current dietary recommendations stems from a consensus of literature supporting the protective role of whole-grains against the development of T2D and CVD. Fung et al. (99) examined prospectively the association between whole- and refined-grain intake and the risk of T2D in a large cohort of healthy men. The results showed that the risk of T2D in men in the highest quintile of whole-grain intake (quintile median 3.2 serving/d) was reduced 42% during follow up after 12 y. The Nurses' Health Study, which followed over 75,000 women over a 10-y period, found a significant, inverse relationship between incidence of coronary heart disease (CHD) and intake of whole-grains, with subjects in the highest quintile of intake (median intake 2.70 serving/d) having a 25% reduced risk of CHD (100). In a cross-sectional analysis of participants in the Baltimore Longitudinal Study of Aging (101), the association between dietary intake of whole-grain foods and chronic disease risk factors was examined. Dietary intakes were assessed with 7-d dietary food records and quantified in g/d. The results showed that whole-grain consumption was inversely associated with 2-h blood glucose, total cholesterol and LDL cholesterol. Compared with subjects in lowest quintile (median intake of 0.68 g/d), the subjects in highest quintile (median intake 45.8 g/d) had lower BMI, weight and smaller waist circumference (101).

Additional studies examined the influence of whole-grain intake on insulin resistance and showed that consumption of whole-grains may reduce insulin resistance and improve insulin sensitivity. For example the consumption of whole-grain was inversely correlated with insulin

resistance in the Insulin Resistance Atherosclerosis Study (102) and in cross-sectional examinations of Framingham Offspring Cohort (103). Steffen et al. (96) tested the hypothesis that whole-grain intake is associated with greater insulin sensitivity and lower BMI in a cohort of adolescents. Two 127-item food frequency questionnaires were administered at the mean ages of 13 and 15 years to 285 adolescents who underwent two, euglycemic insulin clamps. Adolescents, who consumed more than 1½ serving of whole-grain foods per day, were leaner and more insulin sensitive than those who consumed less than ½ serving per day (96). The effect of whole-grain intake on insulin sensitivity has been studied in adults in a crossover feeding study among hyperinsulinemic, overweight men and women (104). The results demonstrated that insulin sensitivity increased during consumption of a diet rich in whole-grain foods for 6 weeks but not while subjects were consuming a refined grain diet for 6 weeks. **Table 2** summarizes the studies examining the effect of consumption of whole-grain cereals on the risk of T2D.

The mechanism by which whole-grain foods induce their protective effect against chronic diseases, such as T2D and CVD, is not fully understood. The protective effect of whole-grain may depend on presence or interaction of several biologically active components including DF, vitamin E, magnesium, folate and other nutrients and non-nutrients (97,98,100,105,106). Magnesium, a rich constituent of the grain germ, is associated with low insulin concentrations (77,103). In epidemiologic studies vitamin E, folate, and fiber in whole-grains have independently associated with a reduced risk of CVD (77,100,107). However, diets rich in whole-grain foods have been associated with reduced risk of CVD and T2D, independent of the effects of the selected nutrients found in whole-grains (108). Another proposed mechanism to explain the protective benefits of whole-grains is an increased production of short chain fatty acids (SCFA) from the high content of indigestible carbohydrates, as well as the high content of

antioxidant, lignans and other phytochemicals (107-109). More studies are needed to explain the mechanism through which whole-grain consumption confers protection against chronic diseases such as T2D.

**Table 1.2. Summary of the reported effects between consumption of whole-grain cereals and the risk of diabetes.**

Study	Design/ Duration of Study	Effects
McKeown et al. (103)	Cross-sectional	Reduction in diabetes risk
Liese et al. (102)	Cross-sectional	Increase of the insulin sensitivity
Esmailzadeh et al. (110)	Cross-sectional	Reduction in metabolic syndrome risk
de Munter (111)	Prospective/ 12-18y	Reduction in diabetes risk
Jacops et al. (105)	Prospective/ 17y	Reduced risk of inflammatory mortality
Fung et al. (99)	Prospective/ 12 y	Reduction in diabetes risk
Rave et al. (112)	Randomized crossover/ 4 wk treatment	Improved metabolic risk factors for T2D
Pereira et al. (104)	Randomized crossover/ 6 wk treatment	Improvement of the $\beta$ -cell function, increased insulin secretion
Andersson et al. (113)	Randomized crossover/ 6 wk treatment	No significant metabolic changes

### **1.3.3. Incretins' Response to Ingestion of Carbohydrates**

It has become evident that the gastrointestinal tract plays a key role in the interaction between the contents of a meal and subsequent metabolic and hormonal responses. In the past decades the gastrointestinal tract has been found to release peptide hormones into circulation in response to ingestion of a meal. These hormones, particularly GIP and GLP-1, have important biological actions including stimulation of glucose-induced insulin secretion (19,25,114). Thus, it is very important to understand how different nutrients influence the secretion of gut hormones. Elliott et al. (115) examined the acute effects of different macronutrients on the secretion of GLP-1 and GIP in healthy subjects. The subjects consumed three equicaloric (375 kcal) test meals of carbohydrate, fat and protein. Small increases in plasma GLP-1 were found after all meals. Levels reached a maximum 30 min after the carbohydrate and 150 min after the fat load. Ingestion of both carbohydrate and fat induced substantial rises in GIP secretion, but the protein meal had no effect on GIP secretion (115). Another study evaluated the postprandial incretin concentrations in response to ingestion of a small and a large meal (260 kcal and 520 kcal) in type 1 and type 2 diabetic patients as well as in two groups (lean and obese) of healthy subjects (37). The incretin responses were significantly higher in all groups after the large meal, compared with the small meal, with correspondingly higher C-peptide responses. The author concluded that it might be possible to modulate incretin and insulin secretion in diabetic patients as well as obese healthy individuals by giving them a large meal, compared to a small meal (37).

Among macronutrients, CHO has a substantial effect on postprandial glucose excursion and insulin release. It also stimulates the secretion of incretin hormones which play an important role in the regulation of glucose metabolism and energy storage (5).

Because CHOs vary in their rate of digestion and thus in glucose release and absorption, it is conceivable that the ability to stimulate incretin hormone secretion differs among the various types of CHOs. The capacity of CHO to induce the secretion of incretin hormones secretion could be one of the factors affecting the postprandial glucose response and therefore the metabolic quality of CHOs.

Some studies investigated the effect of ingestion of different types of CHO on secretion of incretin hormones and other biomarkers related to T2D. As previously mentioned, Juntunen et al. (116) evaluated the effect of rye fiber in rye breads on glucose metabolism in healthy postmenopausal women. The results showed that postprandial insulin, GIP, and C-peptide responses to the rye breads were significantly lower than the response to the control while glucose and GLP-1 responses to the rye breads were not significantly different from those to the control. *In vitro* starch hydrolysis was slower in all rye breads than in the control, and the structure of continuous matrix and starch granules differed between the rye and control breads (116). In another study (73) healthy subjects ingested two types of rye bread (one had a high content of whole kernels and the other bread had a high content of oat  $\beta$ -glucan), dark durum wheat pasta (positive control) and white wheat bread (reference food). The glucose responses and the rate of gastric emptying after consumption of the 2 rye breads and pasta did not differ from those after consumption of white wheat bread. However, insulin, GIP, and GLP-1 responses, were lower after the consumption of rye breads and pasta than after consumption of white wheat bread (73). The data from these studies by Juntunen et al. suggest that the structural and compositional properties of fiber play a more important role in the regulation of insulin response than does the amount of fiber consumed. It also suggests that these effects on the insulin response may be mediated through GIP and GLP-1.

These findings suggest that different types of CHO differ considerably in their ability to stimulate the secretion of incretin hormones. More research is needed to clarify how different types of CHO influence incretin secretion.

#### **1.4. Effect of Carbohydrates on the Postprandial Response to a Subsequent Meal**

Examination of the postprandial effects of dietary CHO on biomarkers related to T2D can extend to a subsequent meal. Since meals are consumed one after another throughout the day, it is logical that previous meals can affect the physiological responses to subsequent meals. The finding that a low GI meal improves the glycemic tolerance to the following meal was reported first by Jenkins et al. (117). The phenomenon can be seen from breakfast to lunch (118) but also from dinner to breakfast (119).

Östman et al. (120) examined the effect of a low GI breakfast containing barley bread with organic acids on the glucose tolerance at the subsequent lunch meal. They found that a breakfast of a lactic acid bread improved second meal glucose tolerance associated with a high GI lunch meal 4 h later in healthy subjects. In another study, the effects of cereal-based test meals that varied in GI features and content of indigestible carbohydrates on glucose tolerance following standardized meals in healthy subjects were investigated (121). In series 1, the test meals were consumed at breakfast, and postprandial blood glucose IAUCs were calculated after the test breakfast, standardized lunch, and standardized dinner. In series 2, the subjects consumed test evening meals and IAUCs were calculated after a subsequent standardized breakfast. Barley or rye kernel breakfasts lowered the blood glucose IAUC (0–120 min) at breakfast, at a subsequent lunch, and also the cumulative IAUCs (breakfast+ lunch+ dinner) when compared with white-wheat bread. Blood glucose IAUC after lunch was positively correlated with breakfast blood glucose IAUC. Breath hydrogen excretion (a reflection of colonic

fermentation) was negatively correlated with blood glucose IAUCs after lunch and dinner. A barley kernel evening meal resulted in lower glucose IAUCs and higher hydrogen excretion after a subsequent breakfast compared with white-wheat bread. They concluded that glucose tolerance at subsequent meals can be notably improved during the course of a whole day or overnight by choosing specific low-GI, whole-grain cereal products, and the benefits appear to be mediated through colonic fermentation (121).

Several physiologic mechanisms may be involved in the extended metabolic effects of a diet characterized by low GI foods. It has been proposed that the prolonged digestive phase after ingestion of a low GI meal will suppress the release of fatty acids for a longer time which inhibits insulin resistance and improve the insulin economy in the postprandial phase (117,119). Another proposed mechanism is the increased colonic fermentation of the indigestible CHO content of the low GI first meal. It appears that fermentable CHO, independent of its effect on the food's GI, has the potential to regulate the postprandial non-esterified fatty acids (NEFA) and lower the gastric emptying. The mechanism of the second meal effect remains to be elucidated (118).

## 1.5 Summary

People with T2D are at increased risk for CVD and other comorbidities. This risk can be lowered by improving glycemic control through life style modifications such as changes in diet and physical activities (2,4,5). It has been shown that dietary CHO plays a major role in glucose excursion and stimulating insulin secretion (5). In recent years, there has been considerable discussion regarding the effect of modifying dietary CHO on blood glucose, insulin and management of T2D.

Differences in postprandial glucose response to various CHO-rich foods have been demonstrated in healthy and diabetic subjects (62,67). A number of factors can influence postprandial glycemia by affecting gastric emptying rate and/or rate of digestion and absorption of starch. Some of these factors include viscous fibers, organic acids, inclusion of whole kernels, particle size, structure of the starch and fiber and processing of the CHO food (13,64,67,73,84,122). For instance, it has been shown that increasing the level of organic acids in bread, by direct addition or prolonged fermentation time would lower the glycemic response not only after the first meal, but also following a subsequent meal (86,87,120). Addition of viscous fiber, such as  $\beta$ -glucan in barley, has been shown to improve the glucose and insulin responses in healthy subjects by increasing the viscosity of food and limiting the glucose absorption (79,80,84).

Recently, a great deal of attention has been given to the association between whole-grain consumption and reduced risk of T2D. Epidemiologic (98,108) and clinical studies (104) showed an inverse association between whole-grain consumption and T2D risk. The amount of whole-grain servings that need to be consumed and the period of time required in order to get a positive effect is not yet determined. The mechanism underlying

the beneficial effect of whole-grain consumption is not fully understood and needs further research.

It has become increasingly evident that the gastrointestinal tract plays a major role in the interplay between the meal composition and the subsequent metabolic and hormonal responses. Recently, it has been shown that the gastrointestinal tract releases peptide hormones in response to meal ingestion. Some of these hormones, such as incretin hormones, are closely involved with glucose metabolism. Incretin hormones, GIP and GLP-1, have several important metabolic actions, including stimulating the glucose-induced insulin secretion, expansion of pancreatic  $\beta$ -cells and energy storage (17,21,26,27,29,31). However, the mechanisms governing gastrointestinal peptide secretion remain unclear. Dietary CHO has been shown to be an important stimulus for the secretion of incretins (29). It has been suggested that different types of CHO differ in their ability to stimulate secretion of incretins. For example, Watchers-Hagedoorn et al. (123) showed that slowly available CHO induced late and prolonged GIP and GLP-1 release. Juntunen et al. (73) demonstrated that rye breads lower insulin, GIP and GLP-1 responses without any impact on glucose response. It is possible that changes in glucose and insulin responses to various CHO foods are mediated through the changes in postprandial GIP and GLP-1 responses. Knowledge about how different types of CHO modulate incretin responses could contribute in developing CHO foods that are beneficial in prevention and management of T2D.

**CHAPTER TWO**

**PURPOSE OF RESEARCH**

## 2.1 Purpose of Research

The overall purpose of the current research was to examine the impact of ingestion of breads varying in CHO composition on biomarkers related to T2D in obese individuals. Specifically, the focus of the thesis was to gain a better understanding of how different breads affect the secretion of incretin hormones and how altered incretin responses would impact the insulin and glucose responses. To do this, two studies with the acute ingestion of breads were performed as well as one in which breads were ingested for 6 weeks. The objectives of the studies are outlined as follows:

- The primary objective of study 1 was to determine the acute postprandial effects of laboratory prepared breads varying in composition (white wheat, white sourdough, whole-wheat, and whole-wheat barley) on blood glucose, insulin, GIP, GLP-1 and rate of gastric emptying in overweight and obese males. The secondary objective of this study was to determine the acute postprandial effect of ingestion of the above test breads on the postprandial response to a second meal on these biomarkers in overweight and obese males.
- The objective of study 2 was to determine the postprandial impact of various commercial whole-grain breads (sprouted-grain, 11-grain and 12-grain) and a laboratory prepared sourdough bread, compared to commercial white bread, on blood glucose, insulin, GIP and GLP-1 in overweight and obese men.
- The objective of study 3 was to determine the effect of 6 wk of dietary substitution, in which habitually consumed bread products were replaced with either a whole-grain, 11-grain bread or a refined white bread, on blood glucose, insulin, glucagon, GIP and GLP-1 in both normal glycemic/insulinemic (NGI) and hyperglycemic/hyperinsulinemic (HGI) men and postmenopausal women.

## **2.2 Research Hypothesis**

The general hypothesis addresses in this thesis was that ingestion of sourdough, whole-wheat, sprouted-grain and whole-grain breads would lower the metabolic responses compared to white bread in overweight and obese subjects. Specific hypothesis are outlined as follows:

- In study 1, it was hypothesized that ingestion of sourdough, whole-wheat and whole-wheat barley breads would improve the metabolic responses, compared to white bread following the first and second meals.
- In study 2, it was hypothesized that ingestion of sourdough and whole-grain (sprouted-grain, 11-grain and 12-grain) breads would improve the metabolic responses, compared to white bread.
- In study 3, it was hypothesized that 6 wk ingestion of whole-grain (11-grain) bread would improve the metabolic responses, compared to white bread in the HGI group compared to the NGI group.

**CHAPTER 3**

**THE ACUTE IMPACT OF INGESTION OF BREAD**

**OF VARYING COMPOSITION ON BLOOD**

**GLUCOSE, INSULIN AND INCRETINS**

**FOLLOWING FIRST AND SECOND MEALS**

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## **ABSTRACT**

**Background:** The botanical origin, structural characteristics and baking conditions influence the metabolic responses to carbohydrate (CHO) containing foods. We hypothesized that ingestion of whole-wheat or sourdough breads would have an optimal effect on biomarkers of glucose homeostasis than that observed with consumption of white bread after first and second meals.

**Subjects and Methods:** Ten volunteers consumed 50 g available CHO (randomized crossover design) of each of the 4 breads (white, whole-wheat, sourdough and whole-wheat barley) followed 3 h later by the ingestion of a standard second meal. Venous blood samples were collected for 3 h following bread ingestion and a further 2 h after the second meal for determination of glucose, insulin, paracetamol (indirect marker of gastric emptying rate), GIP and GLP-1 concentrations.

**Results:** Glucose and GLP-1 responses to sourdough bread were significantly ( $P < 0.05$ ) lower than whole-wheat and whole-wheat barley breads. Glucose area under the curve (AUC) for sourdough bread was lower than those for whole-wheat ( $P < 0.005$ ) and whole-wheat barley ( $P < 0.03$ ) breads for the entire study. Furthermore, GIP AUC after sourdough bread ingestion was lower compared to white ( $P < 0.004$ ) and whole-wheat barley ( $P < 0.002$ ) breads following the second meal. There were no significant differences in insulin and paracetamol concentrations among the test breads.

**Conclusions:** The ingestion of whole-wheat breads did not result in postprandial metabolic responses that were lower than those of white bread. However, the consumption of sourdough bread resulted in lower glucose and GLP-1 responses compared to those of whole-wheat breads during the two meal period.

**Keywords:** carbohydrate, glucose homeostasis, insulin sensitivity, GIP, GLP-1, whole-wheat, barley.

## INTRODUCTION

North Americans spend at least 18 h a day in a postprandial state and postprandial responses to either carbohydrate (CHO) or fat ingestion are associated with changes in various biomarkers for metabolic disorders such as type 2 diabetes (T2D) and cardiovascular diseases (CVD) (124,125). Although CHOs are a major component of the diet, minimizing the postprandial disturbances in blood glucose and insulin is thought to be of health benefit.

Breads are a major component of the wide range of CHO-containing foods in the North American diet (126,127). The botanical origin and milling of the grains, processing of the dough and baking conditions can all influence the metabolic responses to the breads by influencing factors such as the rates of gastric emptying, hydrolysis of the starches and their absorption and hence the metabolic responses (65,66,128,129). Strategies to optimize blood glucose and insulin responses to bread consumption include replacement of wheat flour with flour types richer in DF such as barley flour. Some studies have suggested that beta-glucan, the viscous fiber in barley, is capable of decreasing glucose and insulin responses by increasing the viscosity of the meal bolus, decreasing the rate of gastric emptying and consequently reducing the rate of intestinal absorption of CHO and glucose delivery to the blood. Another strategy to lower the metabolic response to bread is sourdough fermentation or the direct addition of organic acids. Organic acids are thought to prolong the rate of gastric emptying and/or decrease the rate of starch digestion but the mechanisms are not established (86,87,90,120).

In recent years, investigations have suggested that the incretin hormones glucose-dependent insulintropic polypeptide (GIP) and glucagon-like peptide-1 (GLP)-1 are intimately involved in the postprandial regulation of CHOs. These gut-derived hormones

have been shown to be responsible for approximately half of the postprandial insulin release and are thought to be important in pancreatic beta cell integrity. The major stimulus for incretin secretion is the presence of CHO in the gut (16,114,130). However, our understanding of the impact of different types of CHOs on the incretin response is in its infancy.

There is accumulating data suggesting that a low glycemic index (GI) meal not only lowers the acute glycemia but also improves glycemia and insulinemia after the subsequent (second) meal, a phenomenon called the second meal effect (119,131-133). The physiologic mechanism of how the glycemia of the first meal influences the second meal is not well-established. However, one theory is that the prolonged absorptive phase after the first meal will suppress short chain fatty acid release, thus improve insulin sensitivity at the time of the next meal (119).

The primary objective of the present study was to determine the acute postprandial effect of 50 g of available CHO in the form of breads made with different grain flours or leavening processes on the rate of gastric emptying and biomarkers of glucose homeostasis. A secondary objective was to examine the impact of these breads on the above biomarkers following consumption of a standard second meal. We hypothesized that white bread would be associated with the most extreme metabolic responses while more favorable responses would occur following ingestion of sourdough and whole-wheat breads.

## **SUBJECTS AND METHODS**

### **Subjects**

Eleven overweight or obese males were recruited from the Guelph, Ontario vicinity through advertisement in local newspapers. The subjects were all non-smokers

and reported no history of gastrointestinal disease, gluten allergy, dyslipidemia, diabetes or any other chronic diseases. They were not taking any medications (with the exception of two subjects who were taking hypertension medication) or natural health products. Potential subjects were screened for diabetes or glucose intolerance based on blood glucose at the end of a 2-h oral glucose tolerance test. The protocol of the study was approved by the University of Guelph Human Ethics Committee and written consent was obtained from all subjects.

### **Study protocol**

The subjects were asked to maintain their usual diet and general lifestyle throughout the study, and to abstain from strenuous physical activity and ingestion of alcohol, caffeine substances and analgesic drugs that contain paracetamol for 48 h prior to testing. Dietary records were kept for the three days preceding each of the study days. The night before each study day, the subjects were provided with the same commercially prepared frozen meal for consistency.

Each subject consumed four different test breads on separate occasions, at least one week apart, in a single blind, randomized, crossover design. On each occasion, the subject reported following a 12 h, overnight fast. A venous catheter was inserted into the forearm by a trained technician, and kept patent for the duration of the experiment with a slow saline infusion. After a fasting blood sample was taken, subjects consumed a serving of bread together with 250 mL of water within 10 min. Three h following bread consumption, subjects consumed a standard, commercially prepared lunch (6-inch Subway® sandwich on white bread with 300 mL of orange juice) within 15 min and were studied for an additional 2 h. The serving of the test breads provided 50 g available CHO and 1 g paracetamol, required portions of 98, 138, 109 and 127 g for white, whole-wheat,

sourdough and whole-wheat barley bread, respectively. Blood samples were taken at 0 (fasting), 15, 30, 60, 90, 120 and 180 min after the consumption of the bread as well as at 15, 30, 60, 90 and 120 min after consumption of the second meal.

### **Study test breads**

Four different test breads (white bread, whole-wheat bread, sourdough bread and whole-wheat barley bread) were developed and produced specifically for this study, were prepared by the primary investigator conducting the study. Paracetamol (13-14 g) was added to the flour in order to estimate gastric emptying rate. The recipes are summarized in **Table 3.1**. The same investigator prepared all breads in a commercial kitchen as follows: after a floor time of 10 min at room temperature, the dough was divided into 480 g pieces and proofed for 60 min at 30°C (relative humidity: 70%), except for the sourdough which was proofed at room temperature for 3 h. Baking was performed at 180°C for 30 min and then bread was allowed to cool at room temperature for about 4 h. The crust was removed and 3-4 slices of bread were wrapped in aluminum foil and stored at -20°C until used. The breads were heated in a microwave for 20 s before consumption.

A portion (100 g) of each study test bread was analyzed by two different laboratories (Industrial Laboratories of Canada (ILC) in Tilsonburg, ON and Agriculture and Agri-Food Canada (AAFC) in Guelph, ON) and the results were averaged (**Table 3.2**).

**Table 3.1. Study Test Bread Recipes**

	White	Whole-wheat	Sourdough	Whole-wheat barley
White flour (g)	1255		1056	
Whole-wheat flour (g)		900		922
Whole-grain flour (g)		365		
Barley flour (g)				366
Gluten (g)		39		67
Sourdough starter <sup>1</sup> (g)			633	
Yeast (g)	27	26	15	30
Sucrose (g)	50	51	57	53
Salt (g)	25	26	28	27
Oil (g)	50	51	57	53
Paracetamol (g)	13	13	13	14
Water (g)	761	912	470	949

<sup>1</sup>Made from white flour, water and dry yeasts.

**Table 3.2. Nutrient composition of study test bread servings used to provide 50 g of available CHO<sup>1</sup>**

	White	Whole-Wheat	Sourdough	Whole-Wheat Barley
Portion size (g)	98	138	109	127
Available carbohydrate (g) <sup>2</sup>	50	50	50	50
Fat (g)	4.2	6.1	4.7	6.1
Protein (g)	9.7	16.0	9.8	15.1
Total fiber (g)	1.5	6.3	1.0	5.5
Energy content (kcal)	282.6	356.1	285.5	337.3

<sup>1</sup> Test breads were analyzed by two different laboratories and the results were averaged.

<sup>2</sup> Available CHO was calculated using this formula: total CHO - total dietary fiber.

### **Biochemical and dietary analysis**

A single blood sample consisted of 14 mL and was partitioned as follows: Approximately 2 mL of blood for glucose analysis was put into tubes containing 72 USP units sodium heparin. Four mL blood for serum insulin and paracetamol analysis was collected into tubes without any anticoagulants. After centrifugation (1341× g; 10 min) at 4°C; serum was stored at –20°C for later analysis. For GIP total and GLP-1 total analysis, 6 mL of blood was collected into ice-chilled tubes containing 10.8 mg potassium EDTA and 1824 KIU aprotinin. Ten µl DPP-4 inhibitor per mL of blood was added to the samples in less than 30 s. After centrifugation (1000× g; 15 min) at 4°C, plasma was stored at –80°C until assayed.

All analyses were conducted in duplicate. Blood glucose was determined using a semiautomatic glucose and lactate analyzer (STAT 2300; YSI, Yellow Springs, OH,

USA). Serum insulin was analyzed by radioimmunoassay (Linco Research Inc., St. Charles, USA). The calculated inter-assay coefficient of variation (CV) for serum insulin was 2.59%. Serum paracetamol concentration was determined using a paracetamol assay kit (Cambridge Life Science, UK). The inter-assay CV for serum paracetamol was 3.7%. The plasma GIP total concentrations were determined by GIP total ELISA kit (Linco Research Inc., St. Charles, USA). The inter-assay CV for plasma GIP total was 6.6%. Plasma GLP-1 total concentrations was measured by GLP-1 total RIA kit (Linco Research Inc., St. Charles, USA) after extraction with 70% ethanol. The inter-assay CV for plasma GLP-1 total was 7.2%. For all of the assays, all samples from each subject were analyzed together to eliminate the effect of inter-batch variation.

Energy and nutrient intakes were calculated by using the ESHA Food Processor program (version 9.5, Salem, OR, USA). The data were analyzed for energy, macronutrients, cholesterol and dietary fiber. Intake data was averaged across the three days.

### **Calculations and statistical analysis**

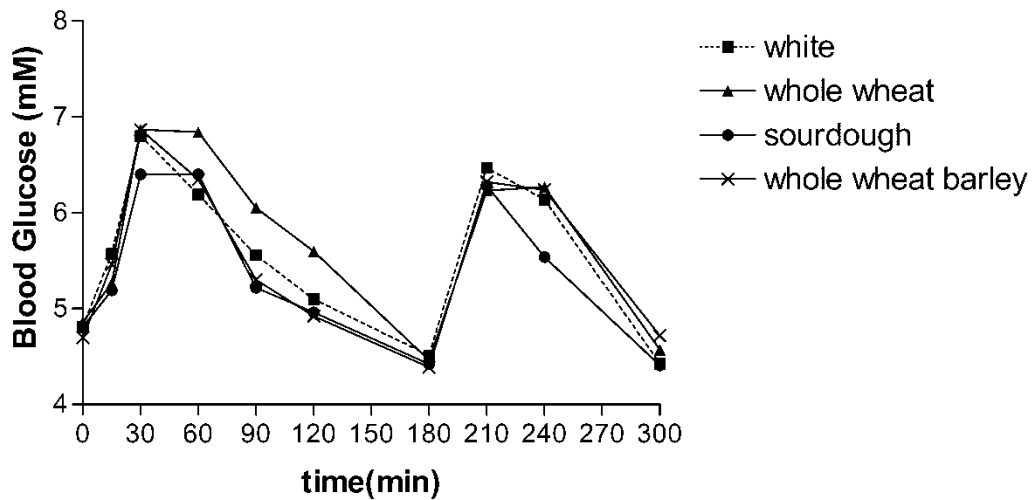
Area under the curve (AUC) was determined for blood glucose, serum insulin and paracetamol, plasma GIP and GLP-1 (GraphPad Prism, version 3.0, San Diego). Data were analyzed for the time periods 0-300 min (defined as entire study), 0-180 min (defined as the first meal) and 180- 300 min (defined as the second meal). Fasting (0 min) concentrations were used as baseline in second meal AUC calculations. Insulin sensitivity index (ISI) was calculated using the method described by Matsuda and DeFronzo (134). For the characterization of paracetamol absorption, the ratio of peak serum concentration ( $C_{max}$ ,  $\mu\text{mol/l}$ ) to time until peak concentration ( $T_{max}$ , min) was calculated (135).

All statistical analyses were performed using the Statistical Analysis System (SAS) (SAS Institute Inc., version 9.1 Cary, NC). Significance ( $P < 0.05$ ) was tested by two-factor repeated measure analysis of variance (ANOVA) using a mixed model (treatment: fixed effect and subject: random effect) followed by the Tukey's test for multiple comparisons. Results are given as mean  $\pm$  SEM.

## RESULTS

A total of 11 subjects were recruited for the study. One subject discontinued because of illness. Based on this, the statistical analysis was performed on a total of 10 subjects ( $59.0 \pm 2.41$  years old, BMI=  $30.8 \pm 0.95$  kg/m<sup>2</sup>, mean  $\pm$  SE).

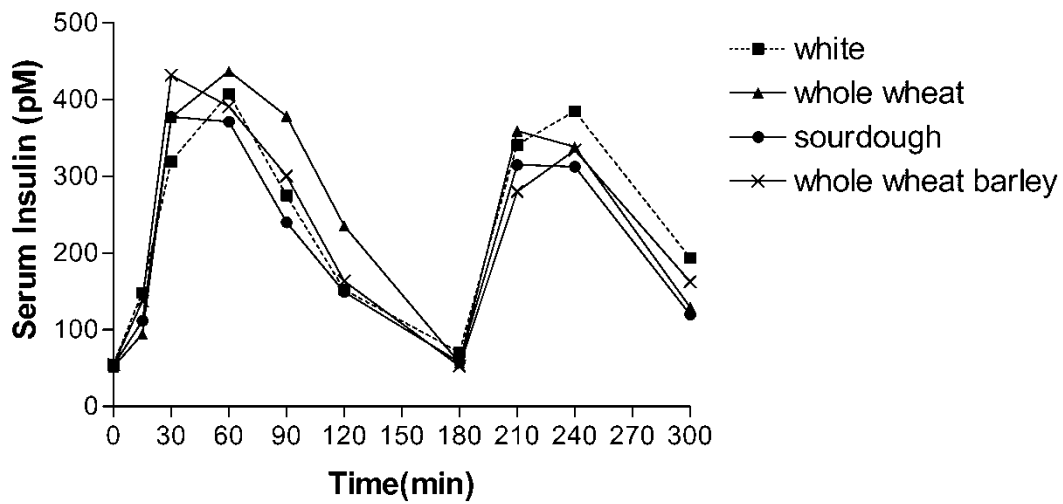
**Blood Glucose.** The mean blood glucose response to the test breads following consumption of the first and second meals is shown in **Fig. 3.1**. Fasting blood glucose concentrations did not significantly differ among the test breads. There were significant differences in overall glucose responses to the test breads. Glucose response to sourdough was significantly lower than both white ( $P < 0.05$ ) and whole-wheat ( $P < 0.007$ ) breads, glucose response to whole-wheat barley was significantly lower ( $P < 0.05$ ) than whole-wheat for the entire study. Glucose AUC for the white bread was lower ( $P < 0.02$ ) than whole-wheat bread for the entire study. Similar to the overall glucose response, glucose AUC for sourdough was lower than both whole-wheat ( $P < 0.005$ ) and whole-wheat barley ( $P < 0.03$ ) breads. Additionally, whole-wheat barley tended ( $P = 0.09$ ) to have lower glucose AUC compared to whole-wheat for the entire study. During the first meal period, glucose AUC for sourdough was significantly lower ( $P < 0.01$ ) than that for whole-wheat and during the second meal period, glucose AUC for sourdough was lower than both whole-wheat ( $P < 0.03$ ) and whole-wheat barley ( $P < 0.005$ ) breads (**Table 3.3**).



**Figure 3.1. Fasting and postprandial glucose responses to the test breads after the first and second meals.** Test bread was ingested at 0 min followed by ingestion of a standardized lunch meal at 180 min. Data are means and standard errors are not included for the clarity of the figure,  $n = 10$ .

**Serum Insulin and Insulin Sensitivity.** Fasting serum insulin concentrations were not significantly different among the test breads. There were no significant differences in overall insulin responses to the test breads (**Fig. 3.2**). Similarly, no significant difference was found in insulin AUC among the test breads (Table 3).

Although there were no significant differences in ISI among the test breads (data not shown) ISI after the ingestion of sourdough bread was 16% and 19% higher than after white and whole-wheat bread consumption in the first meal, respectively. Furthermore, ISI for sourdough was 21% higher than both white and whole-wheat breads following the second meal.



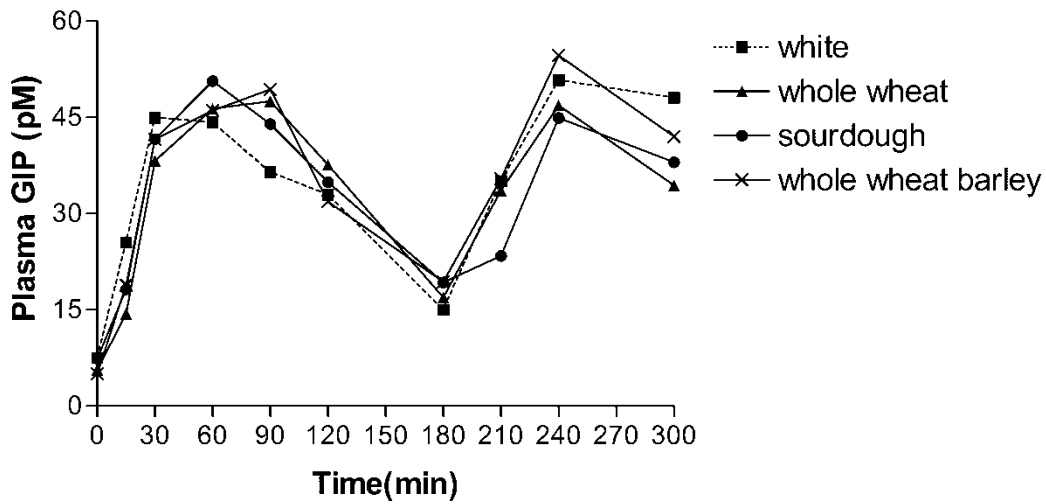
**Figure 3.2. Fasting and postprandial serum insulin responses to the test breads after the first and second meals.** Test bread was ingested at 0 min followed by ingestion of a standardized lunch meal at 180 min. Data are means and standard errors are not included for the clarity of the figure,  $n = 10$ .

**Table 3.3. Area under the curve of blood glucose and serum insulin responses to the test breads for entire study, first and second meals, ( $n = 10$ )**

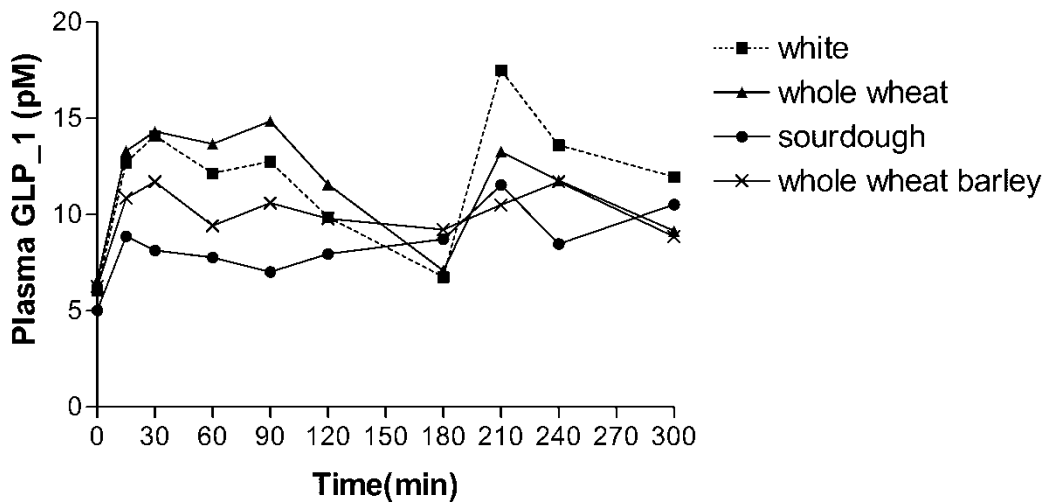
Time/Parameter	White	Whole-Wheat	Sourdough	Whole-Wheat Barley
<b><i>Entire study (0-300 min)</i></b>				
Glucose (mmol/L. 5 h)	222.1 <sup>a,c,d</sup> ± 40.7	272.7 <sup>b</sup> ± 52.4	161.8 <sup>a,c</sup> ± 39.3	250.5 <sup>b,d</sup> ± 37.3
Insulin (nmol/L. 5 h)	66.9 <sup>a</sup> ± 25.3	66.3 <sup>a</sup> ± 25.2	63.3 <sup>a</sup> ± 24.3	57.4 <sup>a</sup> ± 21.7
<b><i>First meal (0-180 min)</i></b>				
Glucose (mmol/L. 3 h)	124.0 <sup>a,b</sup> ± 28.5	167.7 <sup>a</sup> ± 32.9	96.3 <sup>b</sup> ± 31.3	131.7 <sup>a,b</sup> ± 21.0
Insulin (nmol/L.3 h)	39.6 <sup>a</sup> ± 15.0	39.2 <sup>a</sup> ± 15.0	37.5 <sup>a</sup> ± 14.2	33.9 <sup>a</sup> ± 12.8
<b><i>Second meal(180-300 min)</i></b>				
Glucose (mmol/L. 2 h)	98.1 <sup>a,b</sup> ± 12.2	105.0 <sup>a</sup> ± 19.5	65.5 <sup>b</sup> ± 8.1	118.8 <sup>a</sup> ± 16.3
Insulin (nmol/L.2 h)	27.3 <sup>a</sup> ± 10.3	27.1 <sup>a</sup> ± 10.2	25.8 <sup>a</sup> ± 9.8	23.3 <sup>a</sup> ± 8.9

Mean values within a row with unlike superscript letters were significantly different ( $P < 0.05$ ).

**Plasma GIP and GLP-1.** Fasting GIP and GLP-1 concentrations did not significantly differ among the test breads. No overall treatment effect was found in GIP responses to the test breads. While there were no significant differences for GIP AUC among the test breads following the first meal, GIP AUC after sourdough bread ingestion was significantly lower compared with both white ( $P < 0.004$ ) and whole-wheat barley ( $P < 0.002$ ) breads after the second meal (**Table 3.4**). Furthermore, GIP AUC after whole-wheat was significantly lower than that for whole-wheat barley ( $P < 0.05$ ). GIP AUC after white bread ingestion tended to be higher than after whole-wheat bread ( $P = 0.09$ ). Postprandial GLP-1 concentrations following ingestion of the test breads didn't follow a pattern similar to that of GIP concentrations. There were significant differences in overall GLP-1 responses to the test breads (**Fig. 3.4**) with sourdough bread being lower than both white ( $P < 0.0001$ ) and whole-wheat ( $P < 0.0001$ ) breads. GLP-1 response to the sourdough tended to be lower ( $P = 0.06$ ) than whole-wheat barley bread. In addition, GLP-1 concentration was significantly higher after white bread than whole-wheat barley bread ( $P < 0.02$ ). GLP-1 response to whole-wheat was higher than whole-wheat barley bread ( $P < 0.03$ ). Despite the significant treatment effect in overall GLP-1 response, no significant difference was found in GLP-1 AUC among the test breads (Table 3.4).

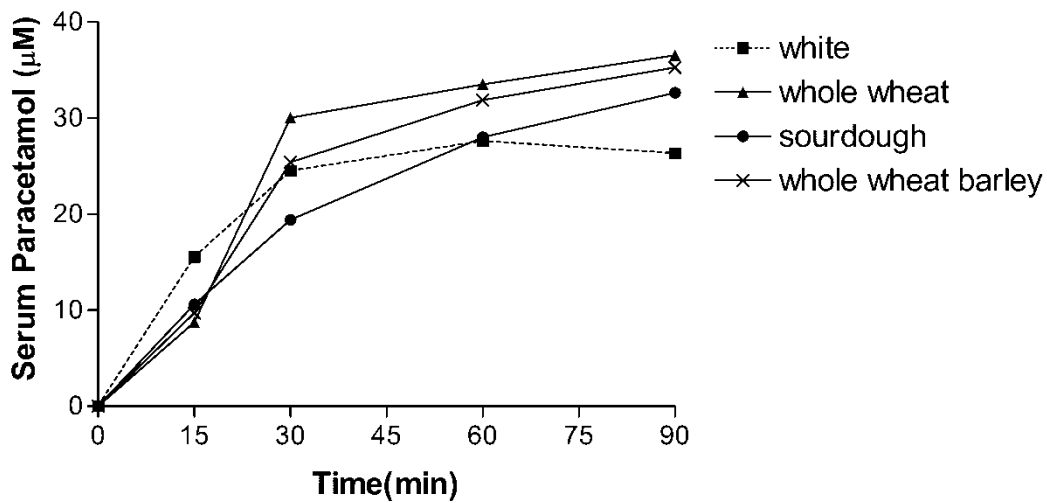


**Figure 3.3. Fasting and postprandial GIP responses to test breads after the first and second meals.** Test bread was ingested at 0 min followed by ingestion of a standardized lunch meal at 180 min. Data are means and standard errors are not included for the clarity of the figure,  $n = 10$ .



**Figure 3.4. Fasting and postprandial GLP-1 responses to the test breads after the first and second meals.** Test bread was ingested at 0 min followed by ingestion of a standardized lunch meal at 180 min. Data are means and standard errors are not included for the clarity of the figure,  $n = 10$ .

**Serum Paracetamol.** While there was no significant difference in paracetamol concentration among the breads, paracetamol concentration after sourdough at time point 30 min tended to be lower than whole-wheat bread ( $P = 0.07$ ) (**Fig. 3.5**). Furthermore, no difference was found in paracetamol AUC and  $C_{max}:T_{max}$  among the breads (data not shown), suggesting that gastric emptying was not different among the treatments.



**Figure 3.5. Fasting and postprandial paracetamol responses to test breads over 90 min after the first meal.** Test bread was ingested at 0 min. Data are means and standard errors are not included for the clarity of the figure,  $n = 10$ .

**Table 3.4. Area under the curve of plasma GIP and GLP-1 responses to the test breads for the entire study, the first and second meals, ( $n=10$ )**

Time/Parameter	White	Whole-Wheat	Sourdough	Whole-Wheat Barley
<b><i>Entire study (0-300 min)</i></b>				
GIP (nmol/L. 5 h)	8.8 <sup>a</sup> ± 0.8	8.9 <sup>a</sup> ± 0.8	8.2 <sup>a</sup> ± 0.8	9.7 <sup>a</sup> ± 0.1
GLP-1 (nmol/L. 5 h)	1.7 <sup>a</sup> ± 0.4	1.4 <sup>a</sup> ± 0.5	1.1 <sup>a</sup> ± 0.3	1.5 <sup>a</sup> ± 0.4
<b><i>First meal (0-180 min)</i></b>				
GIP (nmol/L. 3 h)	4.4 <sup>a</sup> ± 0.5	5.1 <sup>a</sup> ± 0.4	4.9 <sup>a</sup> ± 0.6	5.2 <sup>a</sup> ± 0.7
GLP-1(nmol/L. 3 h)	0.8 <sup>a</sup> ± 0.3	0.9 <sup>a</sup> ± 0.3	0.5 <sup>a</sup> ± 0.1	0.9 <sup>a</sup> ± 0.3
<b><i>Second meal (180-300 min)</i></b>				
GIP(nmol/L. 2 h)	3.6 <sup>a,c</sup> ± 0.2	3.2 <sup>b,c</sup> ± 0.4	2.8 <sup>b</sup> ± 0.3	3.5 <sup>a</sup> ± 0.4
GLP-1 (nmol/L. 2 h)	1.1 <sup>a</sup> ± 0.2	0.5 <sup>a</sup> ± 0.1	0.5 <sup>a</sup> ± 0.1	0.5 <sup>a</sup> ± 0.1

Mean values within a row with unlike superscript letters were significantly different ( $P < 0.05$ ).

## DISCUSSION

We examined the postprandial and second meal effects of ingestion of various breads in middle-aged, sedentary, overweight and obese men as this population represents a group that are at increased risk for T2D. The breads were designed to cover a broad range of properties that have been suggested to influence assimilation and biomarkers of health. The nature of the breads ingested influenced the metabolic responses to not only their own assimilation but also to the processing of a standard second meal 3 h later. However, the results from the present study do not support our hypothesis that white bread would induce the highest postprandial responses in blood glucose, insulin and incretins following both the first and second meals. In addition, for most measures, ingestion of sourdough bread induced the least disturbance in glucose homeostasis. Surprisingly, neither whole-wheat nor whole-wheat barley breads were associated with ‘superior’ postprandial responses. In fact, whole-wheat bread generally induced the highest postprandial glucose responses in the subjects.

The overall impact of sourdough bread ingestion on blood glucose responses was internally consistent with other parameters measured in the current study. The overall glucose response to the sourdough bread was significantly lower than white bread ( $P < 0.05$ ). It is important to note that, sourdough and white breads shared the same recipe and baking process, except for using yeast culture (sourdough starter) and longer proof time for sourdough. We measured pH of both breads and sourdough had lower pH compared to the white bread (5.07 vs. 5.77, respectively) which is indicative of the increased level of organic acids in sourdough bread. Glucose AUC for sourdough bread was significantly lower than for whole-wheat ( $P < 0.005$ ) for each meal and for whole-wheat barley ( $P < 0.03$ ) bread over all and for the second meal. In light of sourdough generally resulting in

more modest blood glucose disturbance together with no differences between breads for serum insulin, suggests that sourdough may increase insulin sensitivity. While the ISI was 15-20% higher for sourdough, the differences were not significant. These findings agree with those of a previous study in which lowered glycaemia was noted with a sourdough bread containing lactic acid compared to a whole-wheat bread (87). Improved glycaemia following a bread meal supplemented with vinegar (acetic acid) has also been reported (89). It has been suggested that the presence of organic acids could reduce the gastric emptying rate (87,90,91); however, the paracetamol data in the present study do not support this possibility nor could it account for the impact of sourdough on the second meal. No significant differences in postprandial paracetamol concentrations were noted, (the AUCs and T<sub>max</sub>:C<sub>max</sub> were similar), indicating that the rate of gastric emptying was not different among the test breads. This implies that the presence of organic acids in the sourdough bread interfered with the rate of digestion. Our results agree with those of an earlier study by Liljeberg and Bjorck (87), who showed no significant differences between blood paracetamol responses to bread with added lactic acid and those to whole meal bread. They also reported that lactic acid either produced during sourdough fermentation or added directly to the bread reduced the rate of in vitro starch hydrolysis, suggesting that lactic acid interferes with the digestive process. It is possible that lactic acid in sourdough bread causes interaction between the gluten and starch, resulting in reduced starch bioavailability (91). More work is needed to clarify the mechanism by which organic acids present in sourdough bread improve glycaemia.

The observed metabolic effects were carried forward to the next meal as the glucose AUC in the second meal period of the sourdough trial was reduced significantly compared to the whole-wheat ( $P < 0.03$ ) and whole-wheat barley ( $P < 0.005$ ) trials.

Östman et al. (120) found that the addition of lactic acid to barley bread reduced the glycemic and insulinemic responses to a second meal (high GI lunch) by 25% in healthy subjects. They speculated that this was due to the prolonged digestive phase after the first meal suppressing the plasma levels of short chain fatty acids and that this improved insulin sensitivity at the time of the next meal. The incretin results may also offer an explanation, as discussed below.

Recently there has been a great deal of attention on the responses of the incretins, GIP and GLP-1, to ingestion of carbohydrates and their impact on postprandial metabolism. Little is known about the effect of different types of CHO on secretion of incretins. In the current study, ingestion of sourdough bread resulted in a lower overall GLP-1 response compared with any of the other breads. In addition, GIP AUC in the second meal was lower by 33% and 36% following ingestion of sourdough bread compared to white ( $P < 0.04$ ) and whole-wheat barley ( $P < 0.002$ ) breads, respectively. Thus the nature of the bread influenced both glucose and incretin responses in our sample of overweight and obese men. Up to 50% of insulin secretion in response to feeding has been attributed to the incretins (16,114); however, in the present study the nature of the breads was associated with marked differences in the incretin and blood glucose responses but not that for insulin. This implies that the relationship between the incretins-insulin-glucose is not straightforward. While GLP-1 has also been proposed to enhance insulin sensitivity (56), in the present investigation, sourdough had the lowest GLP-1 responses and yet appeared to be associated with more optimal blood glucose management.

Similarly, Juntunen et al. (136) found that ingestion of white bread, in comparison to rye bread (with or without the addition of beta-glucan), did not affect blood glucose responses or rates of gastric emptying. However, insulin data in that study were

remarkably different as were the GIP and GLP-1 responses. In addition, Bakhøj et al. (137) showed that ingestion of Einkorn honey-salt leavened and whole-grain breads reduced postprandial GIP, but not that of GLP-1, compared to the conventional yeast bread and proposed that this was due to an increased level of organic acids (based on reduction of the pH in the dough). These studies as well as the present investigation demonstrated that the nature of bread results in altered blood glucose and/or insulin responses as well as those of the incretins. However, the mechanisms and the physiological significance remain to be elucidated.

Dietary recommendations have emphasized the replacement of white bread with whole-wheat bread or dark bread to improve glycemic control in individuals at risk for T2D. However, in our study, there was no indication of white bread having a greater glycemic or insulinemic response compared to our two whole-wheat breads; in fact, whole-wheat bread generally resulted in higher glycemic responses compared to white bread. This finding may be attributed to the small particle size of the ultra fine ground whole-wheat flour used in our study. Furthermore, it has been reported that only soluble fiber has the ability to lower postprandial glucose and insulin responses whereas insoluble fiber has negligible effects on glycemic control. Soluble fiber increases the viscosity and decreases gastrointestinal motility and subsequently reduces the rate of delivery of glucose to the blood (13,79,85). It is possible that the total fiber content of our whole-wheat bread (without barley) consisted predominantly of insoluble fiber (we did not measure the amount of soluble and insoluble fiber). The overall glucose response to the whole-wheat barley was significantly lower than whole-wheat bread ( $P < 0.05$ ). In the current study, whole-wheat and whole-wheat barley breads shared similar recipe and baking process (Table 2). Lack of ultra fine ground whole-grain flour and presence of

barley flour containing beta glucan, a soluble fiber, in whole-wheat barley bread might be responsible for the lowered overall glucose response compared to whole-wheat bread.

The most surprising finding of this study was the lack of what could be interpreted as positive health effects of the whole-wheat breads. While this might be due to the small number of subjects, the data are internally consistent. For example, sourdough bread had the lowest responses in virtually every measure. As noted above, the nature of the flour for the whole-wheat bread selected may be one reason for this finding. In addition, to achieve the goal of having the subjects ingest the same amount of available CHO, the whole-wheat barley and whole-wheat challenges, resulted in the subjects ingesting approximately 29% and 40% more mass (in comparison to white bread), respectively. Similarly, the two whole-wheat bread challenges resulted in consumption of approximately 20% and 25% more energy (in comparison to white bread), respectively. While these factors did not appear to influence gastric emptying, they may have influenced hormonal responses and/or aspects of intestinal function that were not evaluated in the current study. It may be that any putative health benefits are only apparent after long term dietary interventions. Further work is thus necessary to elucidate if these factors impact the metabolic responses by altering the digestion and absorption of the bread consumed.

## **CONCLUSION**

The ingestion of 50 g of available CHO in the form of different breads by middle-aged overweight or obese men influenced not only the postprandial response to this meal but also those of a standardized second meal. Contrary to our hypothesis, the ingestion of white bread did not result in postprandial glycaemic or insulinemic responses that were greater than those of whole-wheat breads. In addition, the consumption of sourdough

bread resulted in lower overall glucose and GLP-1 responses compared to those of whole-wheat and whole-wheat barley breads.

### **Acknowledgements**

We wish to thank Hayhoe Mills Ltd. and ADM Milling Company for providing the flour for the breads and Food Development Group (Dr. Phillip Lee Wing) for assisting with developing the bread recipes. Special thanks to Premila Sathasivam and Mehrnoosh Kashani for their excellent technical assistance. The financial support from Food Research Program of the Ontario Ministry of Agriculture and Food is greatly appreciated. This work was also supported by an industrial NSERC scholarship for A Mofidi sponsored by Stone-Mill Bakehouse.

**CHAPTER 4**

**THE ACUTE IMPACT OF INGESTION OF**

**SOURDOUGH AND COMMERCIAL WHOLE-**

**GRAIN BREADS ON BLOOD GLUCOSE, INSULIN**

**AND INCRETINS**

## ABSTRACT

**Background:** Consumption of whole-grain and sourdough breads is associated with improved glucose homeostasis.

**Objective:** To examine the impact of various commercial whole-grain breads and a sourdough bread, as compared to white bread, on biomarkers of glucose homeostasis.

**Design:** In a two-part randomized crossover study, 23 overweight and obese males ingested test breads (11-grain, sprouted-grain, 12-grain, sourdough and white) on different occasions, matched for available CHO (50 g) in part 1 and bread mass (107 g) in part 2. In both parts, blood samples collected at various time intervals over 3 h were analyzed for blood glucose, serum insulin and plasma GIP and GLP-1. Parts 1 and 2 followed the same protocol.

**Results:** In part 1 (matched for available CHO), blood glucose response for sprouted-grain was lower than 11-grain ( $P < 0.009$ ), sourdough ( $P < 0.001$ ) and white ( $P < 0.006$ ) breads. Insulin AUC for sourdough and white were lower ( $P < 0.05$ ) than 11-grain and sprouted-grain breads. GIP response did not differ among the breads while GLP-1 response to sourdough was lower ( $P < 0.05$ ) than all other breads. In part 2 (matched for bread volume), glucose and insulin AUC for sourdough was greater ( $P < 0.05$ ) than for 11-grain, sprouted-grain and 12-grain breads. GIP AUC for 12-grain was lower ( $P < 0.03$ ) than white bread while sprouted-grain had the greatest ( $P < 0.05$ ) GLP-1 response among the breads.

**Conclusion:** Sprouted-grain bread improved glycemia by lowering glucose response and increasing GLP-1 response. Twelve-grain bread lowered glucose response while 11-grain bread did not have any favourable impact on glucose metabolism.

**Key Words:** carbohydrate, glucose homeostasis, insulin, GIP, GLP-1, whole-grain, sourdough, sprouted-grain, dietary fiber.

## INTRODUCTION

There is substantial interest in the role of dietary carbohydrate (CHO) in preventing and managing T2D (138). In North America, bread is the predominant CHO-containing food and consumption of white bread is 5 times that of whole-wheat, rye and other dark breads (139). Replacing white bread with whole-grain breads is often recommended to improve glycemic control (140). Epidemiologic (99,101,102,110,141) and clinical (104,112) studies have reported strong inverse associations between whole-grain consumption and the risk of T2D and cardiovascular disease. It has been suggested that the fiber content of whole-grain foods improves glucose/insulin metabolism by reducing the rate of CHO breakdown and absorption (78,107,142).

The incretin hormones, GIP and GLP-1 are intimately involved in postprandial regulation of glucose homeostasis. It is estimated that approximately half of the postprandial insulin release in response to CHO ingestion is caused by these gut-derived hormones (16,34,114,143). Thus the magnitude of the incretin response is vital to both the acute insulinemic and glycemic responses to CHO ingestion. However, our understanding of the impact of different types of CHO on the incretin response is in its infancy.

Previously, we showed that ingestion of whole-wheat and whole-wheat barley breads did not result in lower metabolic responses compared with white bread (144). Furthermore, sourdough white bread resulted in lower glucose and GLP-1 responses for two subsequent meal periods (144). In our previous work, ultra-finely ground whole-wheat flour was used rather than whole-grain flour. In addition, in order to equalize the amount of available CHO (50 g) across treatments, the bread volume consumed varied from 98 to 138 g resulting in higher energy, fat, protein and fiber intake for the whole-

wheat bread treatments. Further study is needed to examine if bread volume influences the metabolic responses to bread.

The sprouting treatment of cereal grains is reported to decrease starch content and increase the content and availability of nutrients including vitamins, minerals and antioxidants (145). One clinical study reported improved glycemia following consumption of pre-germinated brown rice, compared to white rice, in healthy and T2D subjects (146). To our knowledge, the metabolic effect of breads baked with sprouted wheat flour has not been studied.

The present investigation tested the hypothesis that consumption of laboratory prepared sourdough bread and commercial whole-grain and sprouted-grain breads would result in lower metabolic responses compared with commercial white bread. This hypothesis was tested using 2 approaches including normalizing consumption of breads according to available CHO (part 1) and bread volume (part 2).

## **SUBJECTS AND METHODS**

The study protocol was approved by the University of Guelph Human Research Ethics Board and each subject provided written informed consent. Subjects were recruited from the Guelph, Ontario area through advertisement in local newspapers. Subjects were non-smokers and had no history of gastrointestinal disease, gluten allergy, dyslipidemia or diabetes. Subjects did not take medications (with the exception of antidepressants and/or antihypertensives) or natural health products. Potential subjects were screened for glucose intolerance and diabetes at a pre-study visit using a standard 2-h oral glucose tolerance test (OGTT) (Truтол<sup>®</sup> Custom Laboratories Inc., Baltimore, MD). Subjects were excluded if they had impaired fasting plasma glucose ( $>6.1$  mmol/L), impaired glucose tolerance ( $>7.8$  mmol/L at 2 h), or impaired fasting insulin ( $>90$  pmol/L).

## **General Protocol**

Parts 1 and 2 of the investigation followed the same protocol with the exception of the amount of bread consumed. A single-blind, randomized crossover design was used and the 5 study days were at least 1 week apart. Throughout the study, subjects were instructed to maintain their usual diet and lifestyle but were required to avoid alcohol, caffeine substances and strenuous physical activity 48 h prior to each study day and to report to the laboratory after an overnight (12 h) fast. Dietary records were kept for three days prior to each study day and in the evening before each study day, subjects were instructed to consume a standardized meal, consisting of vegetable lasagne (President's Choice Blue Menu Reduced Fat Vegetable Lasagne<sup>®</sup>) and a cereal bar (Kellogg's Nutri-grain Cereal Bar<sup>®</sup>).

On each study day a venous catheter was inserted into the forearm by a trained technician, and kept patent for the duration of the experiment with a slow saline infusion. After collection of a fasting blood sample (time point -15 min), subjects consumed a serving of test bread with 250 mL of water within 15 min. Subsequently, blood samples were collected at 15, 30, 45, 60, 90, 120, 150, and 180 min.

### **Part 1: Acute postprandial effect of ingestion of breads matched for available carbohydrate.**

Twelve overweight or obese males (body mass index (BMI): 25-35 kg/m<sup>2</sup>) were recruited in part 1. The test breads were prepared to provide 50 g of available CHO which required portions of 151, 157, 107, 122 and 110 g for 11-grain (whole-grain, with sourdough culture, Stone-mill Bakehouse Ltd.<sup>®</sup>, Scarborough, ON, Canada), sprouted-grain (whole-grain, with sourdough culture, Stone-mill Bakehouse Ltd.<sup>®</sup>, Scarborough, ON, Canada), sourdough white (as described previously (144) and baked at the Guelph

Food Technology Centre at the University of Guelph), 12-grain (whole-grain, Dempsters<sup>®</sup>, Canada Bread Ltd., Brampton, ON, Canada), and white bread (Wonder Bread, Weston Bakeries Ltd.<sup>®</sup>, Toronto, ON, Canada), respectively. Breads were sliced, de-crusts and stored at -20°C until consumption. Before consumption, the bread slices were thawed in a microwave for 15 s and weighed. The nutrient composition of the test breads consumed in part 1 is summarized in **Table 4.1**.

**Part 2: Acute postprandial effect of ingestion of breads matched for volume.**

Eleven overweight or obese males were recruited in part 2. The test breads were prepared to provide a consistent portion of 107 g of the same test breads as in part 1. This resulted in intakes of 35, 34, 50, 43 and 48 g of available CHO for 11-grain, sprouted-grain, sourdough white, 12-grain and white bread, respectively (**Table 4.2**).

**Table 4.1. Nutrient composition of the test breads delivering 50 g available CHO (Part 1)<sup>1</sup>**

	11-grain	Sprouted-grain	Sourdough	12-grain	White
Total Bread (g)	151.0	157.2	107.3	122.2	110.3
Available CHO (g) <sup>2</sup>	50.0	50.0	50.0	50.0	50.0
Energy (kcal)	320.2	336.4	277.9	317.8	273.7
Starch (g)	44.9	46.3	45.4	42.5	43.6
Total Sugars (g)	5.1	3.6	4.5	7.4	6.4
Soluble Fiber (g)	0.9	0.6	0.3	1.1	0.3
Insoluble Fiber (g)	11.9	11.4	4.9	9.9	4.6
Dietary Fiber (g)	12.8	12.1	5.2	11.0	4.9
Protein (g)	16.9	22.3	9.0	12.6	9.8
Fat (g)	3.1	2.9	4.3	5.2	3.6

<sup>1</sup> Test breads were analyzed by Laboratories of Canada Incorporated (ILC) in Tilsonburg, ON

<sup>2</sup> Available CHO was calculated using this formula: starch + total sugar

**Table 4.2. Nutrient composition of the test breads delivering a consistent portion size (Part 2) <sup>1</sup>**

	11-grain	Sprouted-grain	Sourdough	12-grain	White
Total bread (g)	107.3	107.3	107.3	107.3	107.3
Available CHO (g) <sup>2</sup>	35.5	34.0	50.0	43.8	48.6
Energy (kcal)	227.4	229.6	277.9	278.9	266.0
Starch (g)	31.2	31.6	45.4	37.3	42.3
Total Sugars (g)	4.3	2.4	4.5	6.5	6.2
Soluble Fiber (g)	0.6	0.4	0.3	0.9	0.3
Insoluble Fiber (g)	8.4	7.8	4.9	8.6	4.5
Dietary Fiber (g)	9.1	8.2	5.2	9.6	4.8
Protein (g)	12.0	15.2	9.0	11.0	9.5
Fat (g)	2.2	2.0	4.2	4.6	3.5

<sup>1</sup> Test breads were analyzed by Laboratories of Canada Incorporated (ILC) in Tilsonburg, ON

<sup>2</sup> Available CHO was calculated using this formula: starch + total sugar

### **Blood collection, biochemical and dietary analysis**

For analysis of blood glucose, blood samples were collected at all time points into vacutainers containing 72 USP units sodium heparin, immediately put on ice, and subsequently analyzed using a semiautomatic glucose analyzer (YSI 2300, Yellow Springs, OH, USA). For analysis of serum insulin, blood samples were collected at all time points into vacutainers without anticoagulants and centrifuged (1341 x g for 10 min at 4°C). Serum supernatant was aliquoted and frozen at -20°C until analysis using a solid phase <sup>125</sup>I radioimmunoassay (Coat-A-Count<sup>®</sup>, Diagnostic Products Corporation, CA, USA) with an intra- and inter-assay variability of 5.2% and 7.3%, respectively.

For analysis of the incretin hormones, blood samples were collected at all time points into ice-chilled tubes containing 10.8 mg K<sub>2</sub>EDTA, 1824 KIU aprotinin and 10 µL/mL blood dipeptidyl peptidase-4 inhibitor. Following centrifugation (1000 x g for 15 min at 4°C), plasma was separated and stored at -80°C until analysis. Plasma GIP total concentrations were measured using a Human GIP (Total) ELISA kit (Linco Research Inc., St Charles, USA) with 100% cross reactivity to human intact GIP, GIP (1-42), and the N-terminally truncated metabolite, GIP (3-42). Intra- and inter-assay variability for GIP were 6.5% and 3.4%, respectively. Plasma GLP-1 total concentrations were measured by GLP-1 total RIA kit (Linco Research Inc., St Charles, USA) after extraction with 70% ethanol. The antibody used in this kit binds specifically with C-terminal portion of GLP-1, both amidated and non-amidated forms. Intra- and inter-assay variations for GLP-1 were 4.0% and 9.9%, respectively.

Food record data were analyzed for energy, macronutrients, cholesterol and dietary fiber by using ESHA Food Processor program (version 9.5, Salem, OR, USA) and averaged across each 3-day food record.

## Calculations and statistical analysis

Incremental area under the curve (AUC) was determined for blood glucose, serum insulin, plasma GIP, and GLP-1 (GraphPad Prism, version 3.02, San Diego, CA, USA). Insulin Sensitivity Index (ISI) was calculated using the method described by Matsuda and DeFronzo (134).

All statistical analyses were performed using the Statistical Analysis System (SAS Institute Inc., version 9.1 Cary, NC, USA). Univariate analysis was used to examine the normal distribution of each variable and logarithmic transformations were applied to data that was not normally distributed (specific variables are identified in data tables). Significance ( $P < 0.05$ ) was tested by two-factor repeated measure analysis of variance (ANOVA) using a mixed model (treatment: fixed effect and subject: random effect) followed by the Tukey's test for multiple comparisons. Results are given as mean  $\pm$  SEM.

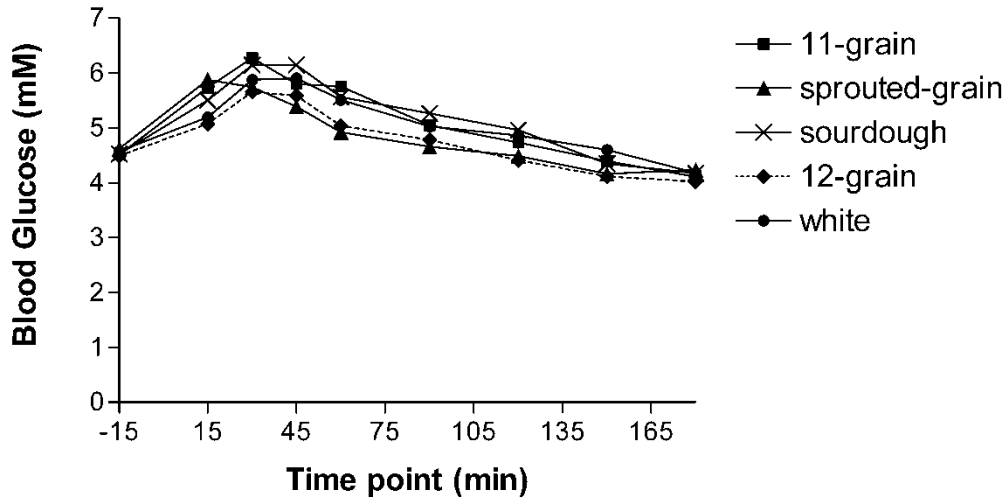
## RESULTS

### Part 1: Acute postprandial effect of ingestion of breads matched for available CHO.

**Subjects.** Twelve subjects (age:  $54.9 \pm 2.0$  y, BMI:  $29.1 \pm 1.1$  kg/m<sup>2</sup>, fasting blood glucose:  $4.5 \pm 0.1$  mmol/L, fasting serum insulin:  $50.8 \pm 4.8$  pmol/L) completed part 1 of the study.

**Blood Glucose.** Significant overall treatment effects were found in glucose responses to the breads (**Fig. 4.1**). Sprouted-grain bread was significantly lower than 11-grain ( $P < 0.009$ ), sourdough ( $P < 0.001$ ) and white ( $P < 0.006$ ) breads. Furthermore, 12-grain bread was significantly lower than 11-grain ( $P < 0.04$ ) and sourdough ( $P < 0.003$ ) breads. Similarly, glucose AUC for sprouted-grain bread was significantly lower than 11-grain ( $P < 0.007$ ), sourdough ( $P < 0.004$ ) and white ( $P < 0.05$ ) breads (**Table 4.3**). Furthermore,

glucose AUC for 12-grain bread was significantly lower than 11-grain ( $P < 0.01$ ) and sourdough ( $P < 0.009$ ) breads (Table 4.3).

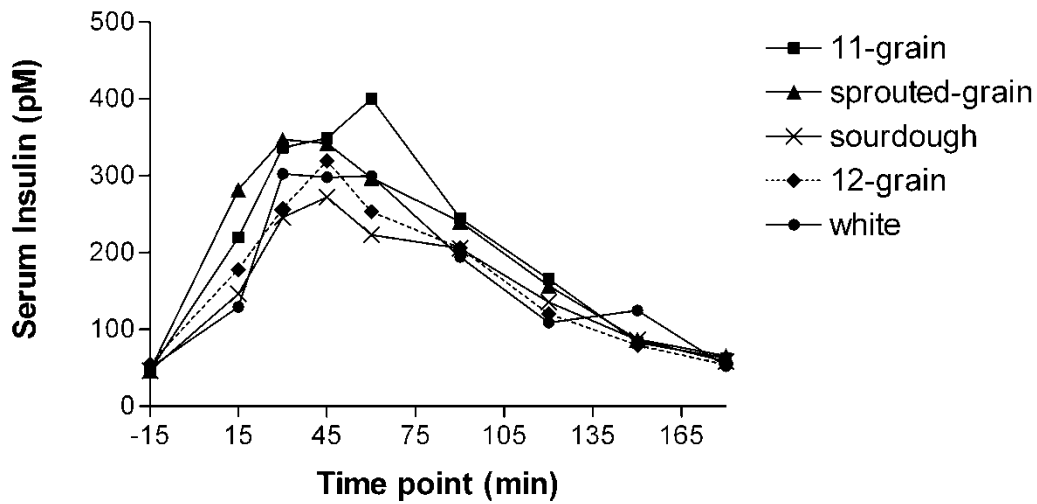


**Figure 4.1. Fasting and postprandial glucose responses to the ingestion of 50 g available carbohydrate of the test breads.** Test bread was ingested at 0 min. Data are means. Standard errors are not included for clarity,  $n = 12$ .

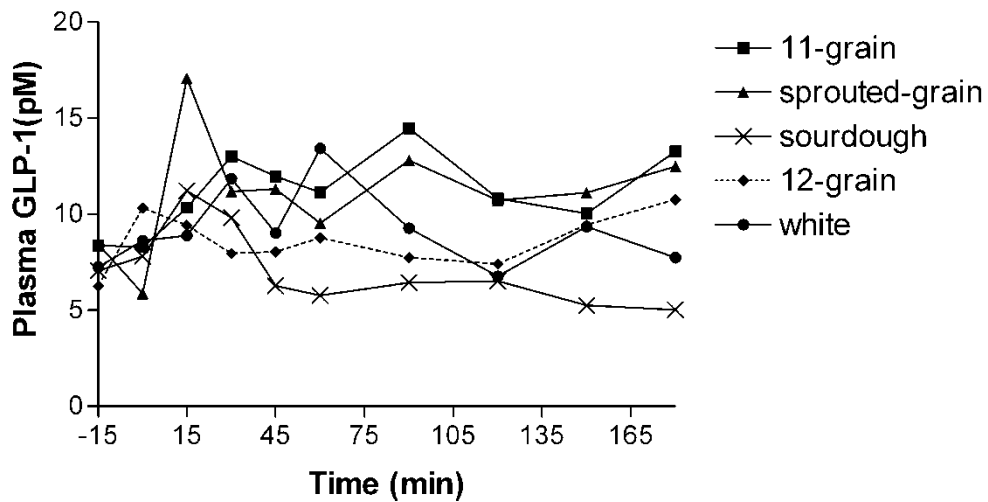
**Serum Insulin and Insulin Sensitivity.** Significant overall treatment effects were found in insulin responses to the breads with 11-grain bread being higher than sourdough ( $P < 0.005$ ) and white ( $P < 0.03$ ) breads (**Fig. 4.2**). Furthermore, insulin AUC for 11-grain and sprouted-grain breads were significantly ( $P < 0.05$ ) greater than sourdough and white breads (Table 3). ISI was not significantly different among the breads (data not shown).

**Plasma GIP and GLP-1.** Despite the difference in insulin responses, there was no significant overall treatment effect in GIP responses to the breads (data not shown). Similarly, bread treatment did not significantly affect GIP AUC (Table 4.3).

The significant differences in overall GLP-1 response to the breads did not correspond with those for insulin. The GLP-1 response to sourdough bread was lower than 11-grain ( $P < 0.0001$ ), sprouted-grain ( $P < 0.0001$ ) and white ( $P < 0.02$ ) breads. Additionally, the GLP-1 response 11-grain bread was greater than 12-grain ( $P < 0.03$ ) and white ( $P < 0.03$ ) breads, while the GLP-1 response to sprouted-grain bread was greater than 12-grain ( $P < 0.009$ ) and white ( $P < 0.05$ ) breads (**Fig. 4.3**). Despite these differences, bread treatment did not significantly affect GLP-1 AUC (Table 4.3).



**Figure 4.2. Fasting and postprandial insulin response to the ingestion of 50 g available carbohydrate of the test breads.** Test bread was ingested at 0 min. Data are means. Standard errors are not included for clarity,  $n = 12$ .



**Figure 4.3. Fasting and postprandial GLP-1 responses to the ingestion of 50 g available carbohydrate of the test breads.** Test bread was ingested at 0 min. Data are means. Standard errors are not included for clarity,  $n = 12$ .

**Table 4.3. Area under the curve for blood glucose, serum insulin, plasma GIP and GLP-1 after ingestion of 50 g available CHO of the test breads for 180 min (Part 1)<sup>1,2</sup>**

	11-grain	Sprouted-grain	Sourdough	12-grain	White
Glucose (mM/l*180min)	114.4 <sup>a</sup> ± 7.4	39.5 <sup>b</sup> ± 31.2	119.4 <sup>a</sup> ± 30.5	47.3 <sup>bc</sup> ± 21.0	91.5 <sup>ac</sup> ± 31.2
Insulin (nM/l*180min)	31.6 <sup>a</sup> ± 6	30.4 <sup>a</sup> ± 5	21.4 <sup>b</sup> ± 3.3	25.9 <sup>ab</sup> ± 6.8	24.1 <sup>b</sup> ± 4.5
GIP (nM/l*180min)	3.2 ± 0.4	3.7 ± 0.3	3.6 ± 0.4	3.6 ± 0.4	3.3 ± 0.2
GLP-1 <sup>3</sup> (pM/l*180 min)	0.6 ± 0.2	0.6 ± 0.3	0.0 ± 0.1	0.4 ± 0.2	0.4 ± 0.31

<sup>1</sup> All values are mean (± SEM); (*n* =12) except for GLP-1(*n* =11) because of technical problems.

<sup>2</sup> Mean values within a row with unlike superscript letters were significantly different (*P* < 0.05).

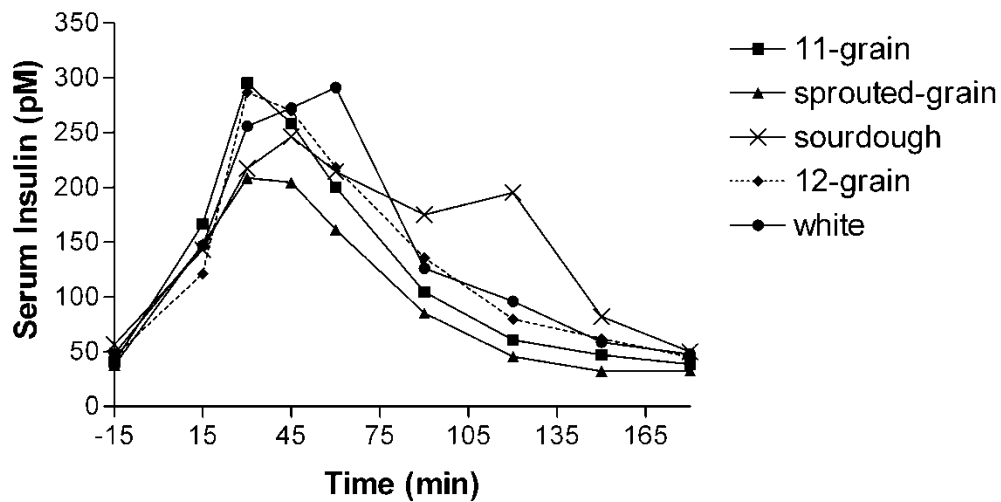
<sup>3</sup> Data was log transformed prior to statistical analysis and is presented as the geometric mean ± SEM

## **Part 2: Acute postprandial effect of ingestion of breads matched for volume.**

**Subjects.** Eleven subjects (age:  $53.9 \pm 1.7$  y, BMI:  $28.6 \pm 0.7$  kg/m<sup>2</sup>, fasting glucose:  $4.6 \pm 0.1$  mmol/L, fasting insulin:  $40.6 \pm 5.7$  pmol/L) completed part 2 of the study.

**Blood Glucose.** There were no significant overall treatment effects in glucose responses to the breads (data not shown). Glucose AUC for sourdough bread was significantly greater than 11-grain ( $P < 0.002$ ), sprouted-grain ( $P < 0.01$ ), 12-grain ( $P < 0.001$ ) and white ( $P < 0.04$ ) breads (Table 4.4).

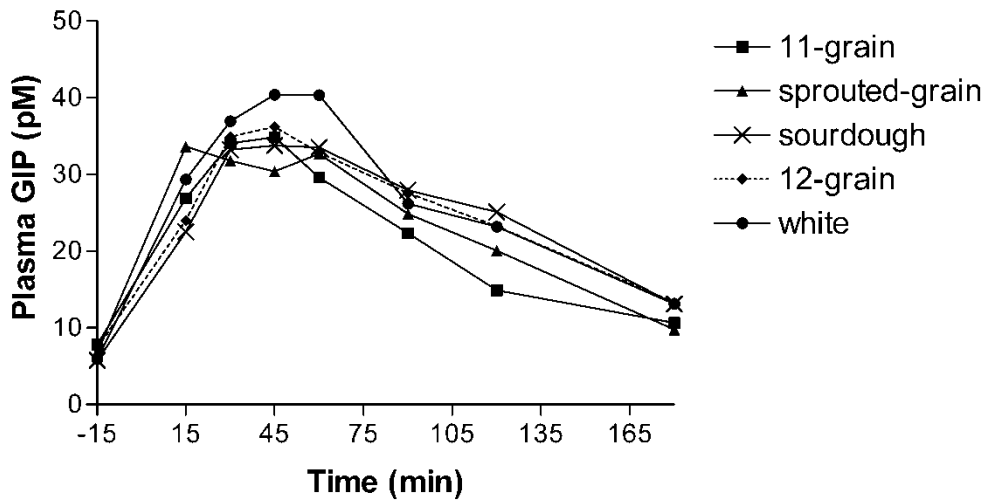
**Serum Insulin and Insulin Sensitivity.** Significant overall treatment effects were found in insulin responses to the breads with sprouted-grain being lower than 12-grain ( $P < 0.03$ ) bread and 12-grain bread being lower than sourdough ( $P < 0.001$ ) and white ( $P < 0.001$ ) breads (Fig. 4.4). Insulin AUC for 11-grain ( $P < 0.03$ ), sprouted-grain ( $P < 0.05$ ) and 12-grain ( $P < 0.0007$ ) breads were significantly lower than sourdough bread. In addition, insulin AUC for 12-grain was lower than white bread ( $P < 0.03$ ) (Table 4.4). ISI was not significantly different among the breads (data not shown).



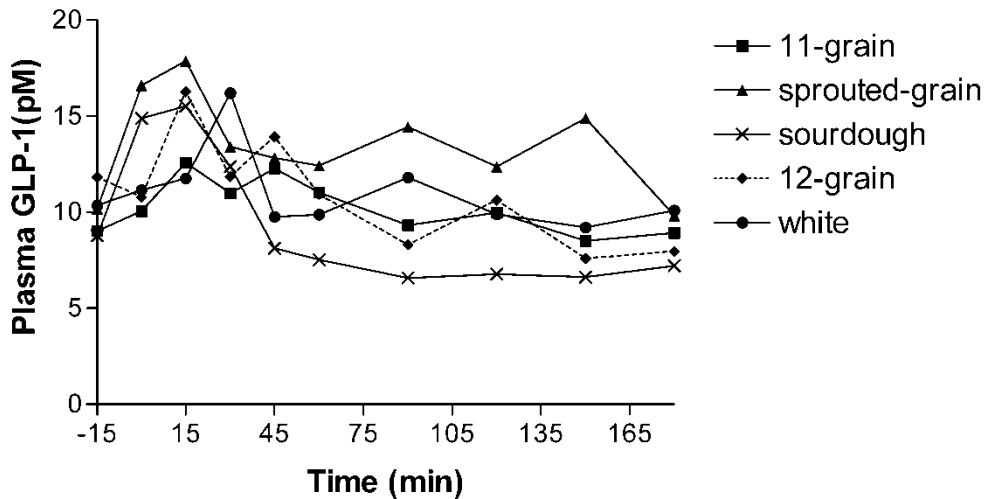
**Figure 4.4. Fasting and postprandial insulin responses to the ingestion of a consistent amount of the test breads.** Test bread was ingested at 0 min. Data are means. Standard errors are not included for clarity,  $n = 11$ .

**Plasma GIP and GLP-1.** As in part 1, incretin responses did not correspond with the postprandial insulin response. Overall GIP response to 11-grain was lower than sourdough bread ( $P < 0.008$ ) (**Fig. 4.5**). GIP AUC for 11-grain bread was significantly lower than sourdough ( $P < 0.03$ ) and white ( $P < 0.001$ ) breads (Table 4.4). Despite the modest difference in the available CHO consumed, GIP AUC for 12-grain was lower than white bread ( $P < 0.03$ ) (Table 4.4).

Similarly, GLP-1 response did not relate to the amount of available CHO consumed as the overall GLP-1 response to sprouted-grain bread was significantly greater than 11-grain ( $P < 0.008$ ), sourdough ( $P < 0.001$ ), 12-grain ( $P < 0.04$ ) and white ( $P < 0.04$ ) breads (**Fig. 4.6**). GLP-1 AUC for sprouted-grain was significantly greater than sourdough ( $P < 0.05$ ) and 12-grain ( $P < 0.01$ ) breads (Table 4.4).



**Figure 4.5. Fasting and postprandial GIP responses to the ingestion of a consistent amount of the test breads.** Test bread was ingested at 0 min. Data are means. Standard errors are not included for clarity,  $n = 11$ .



**Figure 4.6. Fasting and postprandial GLP-1 responses to the ingestion of a consistent amount of the test breads.** Test bread was ingested at 0 min. Data are means. Standard errors are not included for clarity,  $n = 10$ .

**Table 4.4. Area under the curve for blood glucose, serum insulin, plasma GIP and GLP-1 responses after ingestion of set amount of the test breads for 180 min (Part 2) <sup>1,2</sup>**

	11-grain	Sprouted-grain	Sourdough	12-grain	White
Glucose (mM/l*180min)	56.9 <sup>a</sup> ± 22.9	31.7 <sup>a</sup> ± 27.0	130.5 <sup>b</sup> ± 34.5	75.3 <sup>a</sup> ± 21.4	83.8 <sup>a</sup> ± 26.4
Insulin (nM/l*180min)	16.20 <sup>ac</sup> ± 2.1	12.7 <sup>ac</sup> ± 1.9	21.5 <sup>b</sup> ± 2.7	16.8 <sup>a</sup> ± 2.4	18.1 <sup>bc</sup> ± 3.4
GIP (nM/l*180min)	2.70 <sup>a</sup> ± 0.3	3.1 <sup>ab</sup> ± 0.3	3.5 <sup>bc</sup> ± 0.3	3.3 <sup>ac</sup> ± 0.4	4.0 <sup>b</sup> ± 0.7
GLP-1 <sup>3</sup> (pM/l*180 min)	0.5 <sup>ab</sup> ± 0.2	0.8 <sup>a</sup> ± 0.3	-0.1 <sup>b</sup> ± 0.1	-0.2 <sup>b</sup> ± 0.5	0.1 <sup>ab</sup> ± 0.3

<sup>1</sup> All values are mean (± SEM); (*n* = 11) except for GLP-1 (*n* = 10) because of technical problems.

<sup>2</sup> Mean values within a row with unlike superscript letters were significantly different (*P* < 0.05).

<sup>3</sup> Data was log transformed prior to statistical analysis and is presented as the geometric mean ± SEM.

## DISCUSSION

The purpose of the current study was to determine the acute effects of breads of variable CHO composition on postprandial glucose, insulin and incretin responses in sedentary, overweight/obese males as this population represents a group that are at increased risk for T2D. We hypothesised that the sprouted-grain, whole-grain and sourdough breads would lower the postprandial metabolic responses, in comparison to white bread, in both parts 1 and 2 of the study.

When 50 g of available CHO was ingested (part 1), the glucose response (overall and AUC) to sprouted-grain bread was significantly less than 11-grain, sourdough and white breads. Additionally, the glucose response (overall and AUC) for 12-grain bread was significantly lower than sourdough and 11-grain breads. The favourable glucose responses to the sprouted-grain and 12-grain breads support our hypothesis. Greater fiber content in sprouted-grain and 12-grain breads (Table 1) may explain the lowered glycemia following their ingestion compared to white and sourdough breads. Dietary fiber is reported to attenuate glycemic response through its physical action in the gut which lowers the rate of CHO digestion and absorption (84,85,107,142). However, the glucose lowering effect of cereal fiber has been attributed only to soluble fiber (79,84,107) and in the present study, the fiber content of the sprouted-grain and 12-grain breads was predominantly insoluble fiber, suggesting that soluble fiber may not be the only component responsible for improving glycemia. Other nutrients and components in the sprouted-grain and 12-grain breads may have positive effects. It has been suggested that the sprouting treatment of cereal grains increases the content and availability of vitamins, minerals and antioxidants (145) and whole-grains are known to contain higher amounts of vitamins, minerals, phytoestrogens and antioxidants. The presence of micronutrients such as magnesium, vitamin E,

antioxidants, phenolic compounds and phytoestrogens may act synergistically to lower glycemia (99,100,102,105,147).

The lack of difference in postprandial glucose response between the 11-grain and white bread was unexpected. It should be noted that there are several factors influencing the metabolic responses to breads including the flour particle size, kneading protocol, leavening process and baking procedure (65,66,128,129,148), but we are currently unable to identify which specific factor may have accounted for the findings in the present study. While it is possible that any positive effect of the 11-grain bread would be apparent only after a long term intervention, our findings clearly highlight that whole-grain breads are not the same. Eleven-grain bread had sourdough culture and did not improve glycemia suggests that one cannot generalize across whole-grain products and the metabolic responses to whole-grains are different for each recipe.

The insulin results did not support our hypothesis. When matched for available CHO, insulin AUCs for 11-grain and sprouted-grain breads were greater than sourdough and white breads (Table 4.3). This is consistent with the glucose data for sprouted-grain and sourdough breads, but does not explain the glucose result for 11-grain bread, suggesting that glycemia does not always predict insulinemia. In the present study, acute ingestion of 50 g available CHO from whole-grain and sprouted-grain breads did not improve insulinemia or insulin sensitivity (as assessed by calculation of ISI) compared to white bread. Limited literature is available on acute intervention and the results from epidemiologic (99,110,141) and chronic interventional studies (104,112) suggest that any positive effect of whole-grain food intake on insulinemia and insulin sensitivity is only apparent after a chronic intervention. These findings may explain the lack of positive effect of acute ingestion of whole-grain breads on insulinemia and insulin sensitivity in our study.

It should be noted that the magnitude of the glucose and insulin (Table 4.3 and 4.4) responses to the sourdough bread were similar in parts 1 and 2 of the study, respectively, and that these data are consistent with those reported previously from our laboratory (144). While we previously showed that sourdough bread resulted in a more favourable postprandial response compared with whole-wheat bread, the breads were all prepared in the laboratory (144). In the present study the comparison was with whole-grain (not whole-wheat) breads and the breads were commercially prepared. In the former investigation (144), the breads were all administered to control for available CHO and thus subjects ingested different volumes of breads. In part 2 of the current study, matching the treatments for volume of bread consumed resulted in a large difference in available CHO content among the breads. The lower glucose and insulin AUCs for the whole-grain breads compared to those of sourdough bread can be attributed to the lower available CHO and greater DF content of the whole-grain treatments.

Incretins are potent insulin-releasing hormones that play an important role in glucose homeostasis. Previously we observed that sourdough bread resulted in lower GLP-1 response (144). In part 1 of the present study, GIP responses to the ingestion of 50 g available CHO of the breads did not differ significantly among the test breads. However, in part 2, ingestion of equal volumes of the test breads resulted in significantly lower GIP AUC for 11-grain and 12-grain breads compared to white breads, a result that may be attributed to the lower available CHO content of these breads. However, the GIP response to sprouted-grain bread, with the lowest available CHO content, was not lower than those to white bread. In both parts 1 and 2, the insulin responses did not appear to follow that of the incretins. These findings suggest that postprandial responses for different whole-grain breads are complex and cannot be explained only by the available CHO content.

Consistent with our previous study (144), in part 1 of the present study, overall GLP-1 response to sourdough bread was significantly lower than 11-grain, sprouted-grain and white breads. Consistently, insulin response to sourdough bread in part 1 was significantly lower than 11-grain and sprouted-grain breads. Bakhøj et al. (137) reported lowered postprandial GIP responses to the ingestion of Einkorn honey-salt leavened and whole-grain breads compared to the conventional yeast bread and proposed that this was due to an increased level of organic acids (based on reduction of the pH in the dough). Dietary fiber has also been shown to increase GLP-1 secretion in rats (149) and dogs (150). A study by Massimino et al. (150) found that highly fermentable dietary fibers were more potent stimulators of GLP-1 secretion compared to low fermentable fibers. Given that the fermentable insoluble fibre content was greatest in the sprouted-grain and lowest in the sourdough bread, it is reasonable to speculate that the GLP-1 response observed in the present study may in part be influenced by insoluble fiber content of the breads.

Overall, the results of the current investigation suggest that glucose metabolism is complex and multi-factorial. The simple model of glucose stimulates insulin secretion and incretins regulate postprandial insulin release does not always apply. Additionally, GIP and GLP-1 do not respond in a similar manner with respect to the CHO ingested. In our previous (144) and present studies we showed that the nature of the bread consumed has an impact on glucose, insulin and incretin responses, but the mechanism is complex and requires further study.

To our knowledge this is the first study to compare postprandial responses to ingestion of various breads delivering an identical amount of available CHO (thus different volumes) with the postprandial effect of ingestion of a fixed portion size (thus same volume, but different amounts of available CHO) of the same breads in overweight and obese men. It appears that bread volume

and fiber content may play a role but are not the dominant factors in determining the metabolic responses to the breads as in part 2 of the study, 11-grain, sprouted-grain and 12-grain breads, with similar volume and fiber content, induced different results in almost every measure. These results suggest that the nature of the ingredients is an important factor influencing the metabolic responses to the breads. Lack of difference between 11-grain and white breads was unexpected but it may be that any positive impact of 11-grain on glucose metabolism only occurs after a chronic dietary intervention.

## **CONCLUSION**

The ingestion of 50 g available CHO and a fixed portion of different breads by overweight and obese males resulted in altered postprandial glucose, insulin and incretin responses. Glycemic control was specific to the type of whole-grain bread consumed. Sprouted-grain bread improved glycemia by lowering glucose response and increasing GLP-1 response. Twelve-grain bread resulted in lowered glucose response while 11-grain bread did not have any favourable impact on glucose metabolism.

## **Acknowledgements**

We wish to thank Stone-mill Bakehouse for generously donating the 11-grain and sprouted-grain breads and Hayhoe Mills Ltd. for providing the flour for the sourdough bread. Special thanks to Premila Sathasivam and Mehrnoosh Kashani for their excellent technical assistance. Financial support from the Food Research Program of the Ontario Ministry of Agriculture, Food and Rural Affairs is greatly appreciated. This work was also supported by an industrial NSERC scholarship to A Mofidi sponsored by Stone-Mill Bakehouse, Ontario, Canada.

**CHAPTER 5**

**THE IMPACT OF CHRONIC INGESTION OF  
WHOLE-GRAIN BREAD ON BLOOD GLUCOSE,  
INSULIN, GLUCAGON AND INCRETINS IN  
SUBJECTS AT RISK FOR TYPE 2 DIABETES  
AND HEALTHY CONTROLS**

## **ABSTRACT**

**Background:** Chronic ingestion of whole-grain bread may improve glucose metabolism and reduce the risk of developing T2D.

**Objective:** To examine the impact of chronic consumption of whole-grain (11-grain) bread, as compared to white bread, on biomarkers of glucose metabolism in population at increased risk of developing type 2 diabetes compared to healthy controls.

**Design:** Twenty eight men and postmenopausal women, classified as obese hyperglycemic/hyperinsulinemic (HGI; n=14) or non-obese normal glycemic/insulinemic (NGI; n=14), were randomly assigned to two 6-wk treatment periods in which either 11-grain bread (11G) or white bread (WB) was substituted for their habitually consumed bread products. Pre and post each treatment period, an oral glucose tolerance test was administered and blood samples collected at various time intervals for 3 h were analyzed to measure blood glucose, serum insulin and plasma glucagon, GIP and GLP-1.

**Results:** No treatment effect was found on fasting blood glucose, serum insulin, plasma glucagon, GIP and GLP-1 in either the HGI or NGI group. Glucose AUC was significantly ( $P < 0.05$ ) lower post-11G as compared to post-WB in the HGI group. Insulinogenic index was significantly ( $P < 0.05$ ) higher post-11G as compared to post-WB in the NGI group and there was a similar trend ( $p=0.06$ ) for the HGI group. There was no significant difference in AUC values for insulin, glucagon, GIP and GLP-1 between the bread treatments in either the HGI or NGI group.

**Conclusion:** Six wk ingestion of 11G lowered postprandial blood glucose in the HGI group, increased insulinogenic index in the NGI group but had no impact on fasting and postprandial insulin, glucagon, GIP and GLP-1 in either the HGI or NGI group.

**Keywords:** Whole-grain, carbohydrate, GIP, GLP-1, dietary fiber, risk factors, insulin resistance.

## INTRODUCTION

The incidence of T2D and its associated co-morbidities are dramatically increasing worldwide (54,140). Obesity is often associated with insulin resistance which results in hyperglycemia and hyper-insulinemia (151). Men and women with obesity are particularly at increased risk of developing T2D (138,152). Thus dietary interventions capable of alleviating insulin resistance are especially important for the prevention of T2D in these high-risk individuals (140).

There is substantial interest in the role of CHO in preventing and managing T2D (112,142). CHO have a great impact on postprandial glucose excursion and insulin secretion (153). In Canada grain products account for more than 40% of intake of CHO (126). Although current dietary guidelines recommend the intake of 3 or more daily servings from whole-grain foods, the actual intake is far from the recommended level (154). Whole-grain foods are composed of the bran, germ, and endosperm and are rich source of DF, vitamins and minerals. In contrast, in refined-grain products removal of the bran and germ produces a product relatively rich in starch and lower in fibre (99,102,110).

Prospective studies have shown an inverse association between whole-grain intake and T2D in men and women (99,101,102,110) and some clinical studies (104,155) demonstrated that whole-grain consumption improved insulin resistance. The observed health effect of whole-grain consumption might be due to the presence and/or interaction of bioactive components such as fibre, magnesium, zinc, vitamin E and other nutrients and non-nutrients (99,105,108,110). However, the mechanisms linking whole-grain consumption and reduced risk of T2D is not completely understood.

The postprandial regulation of glucose is partly due to the actions of incretins, GIP and GLP-1 (16,114). These gut-derived hormones can be responsible for 50% of postprandial insulin release. They also promote insulin gene expression and beta cell proliferation and GLP-1 also inhibits glucagon secretion (16,114,156). The postprandial response of incretins varies depending on the size and composition of the meal (157). However, studies examining the impact of different types of CHOs on the incretin response are needed.

Previously we found that ingestion of sourdough bread lowered glycemia compared to white bread and was associated with altered incretin responses (144). In the same study, whole-wheat breads did not result in lowered glycemia compared to white bread. We subsequently found that ingestion of sprouted-grain and 12-grain breads lowered glycemia compared to 11-grain bread (158). Surprisingly, no significant difference was found in the metabolic responses between 11-grain and white breads (158). It is possible that any positive effect of 11-grain bread would appear only after a chronic intervention. On this basis, in the present study we tested the hypothesis that the consumption of a commercial whole-grain (11-grain) bread for 6 weeks, as compared to a commercial white bread, would improve glucose metabolism and lower T2D biomarkers response in obese subjects with elevated glucose and/or insulin levels as compared to control, healthy subjects.

## **SUBJECTS AND METHODS**

### *Subjects*

The subjects were recruited by advertisement in local newspapers in Guelph, Ontario. Non-smoking men and postmenopausal women (defined as the absence of menses for  $\geq 1$  year), between the ages of 43-70 years, were considered for inclusion into the study. Potential subjects were screened according to the following exclusion criteria: reported the presence of any serious

medical condition (i.e. cardiovascular disease, diabetes mellitus, hepatic disease, renal failure, autoimmune conditions or cancer), consuming  $\geq 2$  alcoholic beverages/d, taking any lipid-lowering medication, daily low-dose aspirin or hormone replacement therapy, reported any major body weight changes (defined as a weight change of  $> 5\%$  of usual body weight within the last 6 months) and gluten allergy.

Individuals who met the above criteria were invited to the lab to have their weight, height and waist circumference measured and also for administration of a standard 75 g oral glucose tolerance test (Trutol<sup>®</sup>, Nerl Diagnostics, East Providence, RI; OGTT) to determine their glycemic and insulinemic status. Venous blood was sampled in the fasted state and 2 h after the OGTT for determination of blood glucose and serum insulin concentrations. The subjects were classified into the hyperglycemic/insulinemic (HGI) group if they were obese (BMI  $> 30 \text{ kg/m}^2$  or waist circumference  $\geq 102 \text{ cm}$  for men and  $\geq 88 \text{ cm}$  for women) and met one or more of the following criteria: fasting plasma glucose  $> 6.1 \text{ mmol/L}$ , 2-h post OGTT plasma glucose  $> 7.8 \text{ mmol/L}$ , fasting serum insulin  $> 90 \text{ pmol/L}$ . The subjects were classified into normoglycemic /normoinsulinemic (NGI) group if they were non-obese (BMI  $< 25 \text{ kg/m}^2$  or waist circumference  $< 102 \text{ cm}$  for men and  $< 88 \text{ cm}$  for women) and normoglycemic (fasting plasma glucose  $< 6.1$ , 2-h post OGTT plasma glucose  $< 7.8 \text{ mmol/L}$ ) and euinsulinemic (fasting serum insulin  $< 90 \text{ pmol/L}$ ).

In total, 28 men and postmenopausal women were enrolled in the study (HGI  $n=14$ ; NGI  $n=14$ ). The study protocol was approved by the University of Guelph Research Ethics Board and each subject read and signed consent forms before enrollment in the study.

### *Study Protocol*

The intervention study used a randomized crossover design. The subjects' habitually consumed bread products were substituted with commercially available white bread (WB; Wonder bread, Weston Bakeries Limited<sup>®</sup>, Toronto, ON, Canada) and 11-grain, whole-grain bread (11G; Stone-mill Bakehouse<sup>®</sup>, Toronto, ON, Canada) during two 6 wk treatment periods, separated by a 4 wk washout period. The first cohort of subjects (n=14) completed the study between May to November 2007 (summer cohort), while the second cohort (n=14) completed the study between January to July 2008 (winter cohort).

Subjects completed an OGTT test at baseline and after each treatment period, in the morning after a 12 h overnight fast. A catheter was inserted into a subcutaneous forearm vein by a medically trained technician and kept patent for the duration of the experiment using a slow normal saline (0.9%) infusion. After collecting a blood sample (time point 0) in the fasted state, a standard 75 g OGTT was administered. Blood samples were collected at 15, 30, 45, 60, 120, and 180 min after the start of the OGTT for the measurement of blood glucose, serum insulin, plasma glucagon, GIP and GLP-1 concentrations. Body weight, waist circumference and body composition (as measured by bioelectrical impedance analysis, BIA; BodyStat 1500<sup>®</sup>) were measured on all study days before taking the fasting blood sample. Duplicate blood pressure measurements (Dinamap<sup>™</sup> Plus Vital Signs Monitor, Johnson & Johnson Medical Inc., Tampa, FL) were taken 60 min after OGTT administration.

Subjects were required to abstain from alcohol, caffeine and exercise 48 h prior to each study day. In addition, the subjects were asked to discontinue the use of dietary and herbal supplements, with the exception of a multivitamin, for the duration of the study. A standardized

dinner was supplied to the subjects to be consumed the evening before each study day for consistency.

#### *Dietary intervention and assessment*

Canada's Food Guide (95) recommends that males and females between the ages of 18-50 y consume 8 or 6-7 servings of grain products per day, respectively. Based on this, treatment breads comprised approximately 65% of the daily recommended grain servings during each intervention period. In order to standardize the amount of bread given to the subjects, a serving of bread was considered to be 35 g, as indicated in Eating Well with Canada's Food Guide (95). This equated to 4 and 5 slices of WB for women and men, respectively, and 5 and 6 slices of 11G for women and men, respectively. In order to substitute habitually consumed bread products with the treatment breads, subjects were required to eliminate all non-treatment breads (e.g. bagels, pitas, dinner rolls, English muffins, buns, etc.) from their diet during the treatment periods. The subjects were instructed to maintain their habitual dietary and activity levels throughout the study. Every 2 weeks the subjects made laboratory visits to collect the study bread and have their weight measured. The study breads were provided pre-sliced in 14-day supplies packaged in re-sealable bags and pre-portioned into daily packages. The nutrient composition of the test breads is summarized in **Table 5.1**.

Compliance with the dietary intervention was monitored by keeping a study diary where the time and manner in which the test bread was consumed were recorded. Subjects were advised to record physical activity and medication use changes as well as illness in their study diary. Subjects kept 3-day food records prior to each study day and 4-day food records (3 week days and 1 weekend day) during the second and fourth week of the treatment period. Subjects were given detailed instruction on keeping accurate record of food intake.

All food records were analyzed for energy, macronutrients and fiber using ESHA Food Processor Version 8.1 (ESHA Research, Salem, OR).

**Table 5.1. Nutritional composition of daily portion of study breads<sup>1</sup>**

	11G		WB	
	men <i>7 slices</i>	women <i>6 slices</i>	men <i>5 slices</i>	women <i>4 slices</i>
Weight (g)	188	150	175	150
Energy (kcal)	398	318	434	372
Total carbohydrate (g)	69.4	55.5	79.8	68.4
Available carbohydrate (g)	62.1	49.7	79.3	68.0
Starch (g)	54.6	43.7	69.1	59.3
Sugars (g)	5.3	4.2	10.2	8.7
Total fibre (g)	15.9	12.8	7.9	6.8
Soluble fibre (g)	1.1	0.9	0.5	0.5
Insoluble fibre (g)	14.8	11.9	7.4	6.3
Protein (g)	21.0	16.8	15.6	13.4
Fat (g)	3.9	3.2	5.8	5.0

<sup>1</sup> Analysis for all the study breads was completed by Industrial Laboratories of Canada Incorporated (ILC) in Tilsonburg, ON.

#### *Blood Collection and Biochemical Analysis*

Blood samples intended for blood glucose analysis were collected at all time points into sodium heparinized tubes, immediately put on ice, and analyzed using a semiautomatic glucose analyzer (YSI 2300, Yellow Springs, OH, USA). For measurement of serum insulin, blood samples were collected at all time points into vacutainers without anticoagulants and centrifuged (1341 xg for 10 min at 4°C). Serum supernatant was aliquoted and stored at -20°C for later analysis of insulin using a solid phase <sup>125</sup>I radioimmunoassay (Coat-A-Count®, Diagnostic Products Corporation, Los Angeles, CA, USA) with an intra- and inter-assay variability of 3.1% and 7.2%, respectively.

For measurement of plasma glucagon, blood samples were collected in K<sub>2</sub> EDTA vacutainer tubes with 90 µL of aprotinin (Sigma-Aldrich, St. Louis, MO, USA) added. Samples were centrifuged (1000 xg for 15 min at 4°C) to obtain plasma and stored at -80°C until analysis.

Plasma glucagon was determined using RIA kit (Linco Research, St. Charles, MO, USA). Intra- and inter-assay variations were 4.0 % and 7.3% respectively.

For analysis of the incretin hormones, blood samples were collected at all time points into ice-chilled tubes containing 10.8 mg K<sub>2</sub>EDTA, 1824 KIU aprotinin and 10 µL/mL blood dipeptidyl peptidase-4 inhibitor. Following centrifugation (1000 x g for 15 min at 4°C), plasma was separated and stored at -80°C until analysis. Plasma GIP total concentrations were measured using a Human GIP (Total) ELISA kit (Linco Research Inc., St Charles, USA). Intra- and inter-assay variations for GIP were 6.5% and 3.4%, respectively. Plasma GLP-1 total concentrations were measured by GLP-1 total RIA kit (Linco Research Inc., St Charles, USA) after extraction with 70% ethanol. Intra- and inter-assay variations for GLP-1 were 4.0% and 9.9 %, respectively.

#### *Data and Statistical Analysis*

The homeostasis assessment of insulin resistance (HOMA-IR) was calculated as fasting whole blood glucose (mmol/L) x fasting serum insulin (pmol/L)/22.5 (159). To evaluate the early phase insulin secretion, the insulinogenic index (IGI) was calculated as the ratio of the change in serum insulin concentration (pmol/L) during the first 30 min after an OGTT to change in blood glucose concentration (mmol/L) during the first 30 min after the OGTT (insulin at 30 min – fasting insulin)/ (glucose at 30 min – fasting glucose) (160). To evaluate metabolism, glucagon/insulin ratio (G/I) was calculated as the ratio of plasma glucagon (pmol/L) to serum insulin (pmol/L) (161). Incremental area under the curve (AUC) was determined for blood glucose, serum insulin, plasma GIP and GLP-1. As glucagon concentration is suppressed in response to an OGTT, decremental area under the curve (dAUC) was calculated. GraphPad Prism (version 3.02, San Diego, CA, USA) was used to calculate AUCs.

Univariate analysis was utilized to examine the normal distribution of each variable and logarithmic transformations were applied to data that was not normally distributed (specific variables are identified in data tables).

Baseline and post bread treatment data comparisons between the HGI and NGI groups were determined using Student's *t*-test. The effect of bread treatment on all study endpoints was determined for the HGI and NGI groups using analysis of covariance (ANCOVA), including study day 1 values and cohort group (summer and winter) as covariates, followed by Tukey's Studentized Range Test for multiple comparisons. ANCOVA was used to compare the post-WB with the post-11G concentration at each time point, using the study day 1 values and cohort group (summer or winter) as covariates. Student's *t*-test was used to determine if dietary composition differed prior to or during each of the bread treatments.

All data are presented as mean  $\pm$  SEM and were analyzed using SAS software version 9.1 (SAS Institute, Inc., Cary, NC). *P* values  $\leq 0.05$  were considered statistically significant.

## **RESULTS**

### *Subject characteristics at study entry*

A total of 28 subjects were recruited into the study (NGI: n=14; HGI: n=14). One female subject in the HGI group dropped out after completion of the first treatment period due to the time commitment involved. Data for the completed bread treatment for this subject was used in the statistical analysis. Subject characteristics at entry into the study are presented in **Table 5.2**. At baseline, the HGI group had significantly higher body weight ( $P < 0.001$ ), BMI ( $P < 0.001$ ), waist circumference ( $P < 0.001$ ), percentage body fat ( $P < 0.01$ ), fasting whole blood glucose ( $P < 0.001$ ), fasting serum insulin ( $P < 0.001$ ), HOMA-IR ( $P < 0.05$ ), and G/I ratio ( $P < 0.0001$ ), as compared to the NGI group. Furthermore, in the HGI group baseline AUC for glucose ( $P <$

0.001), insulin ( $P < 0.001$ ) and glucagon ( $P < 0.001$ ) were significantly greater than those in the NGI group.

### *Diet*

There were no significant differences in energy or macronutrient intake prior to initiation of the 11G and WB treatments in either the NGI or HGI group. Compliance with the test breads was determined to be 95%. Fiber intake during the 11G bread treatment was significantly ( $P < 0.01$ ) greater than that during the WB treatment in both the NGI and HGI groups. Furthermore, in the NGI group intake of monounsaturated fat during the 11G bread treatment was significantly ( $P < 0.05$ ) higher as compared to the WB (**Table 5.3**).

**Table 5.2. Subject baseline characteristics at study entry<sup>1</sup>**

Measurements	NGI Group (n=14)			HGI Group (n=14)			<i>P</i> <sup>2</sup>
Age (y)	53	±	6	57	±	7	0.10
Sex (n; Male/Female)	10/4			10/4			
Body weight (kg)	79.4	±	13.7	107.4	±	19.1	0.001
BMI (kg/m <sup>2</sup> )	26.5	±	2.9	35.7	±	5.7	0.001
Waist circumference (cm)	93	±	8	116	±	11	0.001
Body Fat (%)	27.8	±	7.6	36.0	±	8	0.01
Systolic blood pressure (mm Hg)	116.9	±	15.2	126.3	±	11.4	0.08
Diastolic blood pressure (mm Hg)	66.0	±	7.9	68.8	±	9.4	0.44
Fasting whole blood glucose (mmol/L)	4.5	±	0.1	5.1	±	0.1	0.001
Fasting serum insulin (pmol/L) <sup>3</sup>	31.9	±	2.1	98.2	±	6.0	0.001
Fasting plasma glucagon (pmol/L) <sup>3</sup>	58.8	±	4.0	74.2	±	5.7	0.005
Fasting plasma GIP(pmol/L)	9.5	±	4.4	8.9	±	1.0	0.7
Fasting plasma GLP-1(pmol/L) <sup>3</sup>	12.4	±	0.9	13.4	±	1.5	0.4
Baseline AUC glucose (mmol/L*180min)	106.7	±	14.2	264.0	±	29.4	0.001
Baseline AUC insulin (nmol/L*180min)	30.5	±	3.1	60.1	±	7.1	0.001
Baseline dAUC glucagon (pmol/L*180min)	67.0	±	8.8	134.5	±	9.1	0.001
Baseline AUC GIP (nmol/L*180min)	5.2	±	0.4	4.1	±	0.5	0.9
Baseline AUC GLP-1 (nmol/L*180min)	1.3	±	0.2	0.7	±	0.2	0.08
HOMA-IR <sup>4</sup>	0.9	±	0.2	3.1	±	0.7	0.05
Baseline G/I ratio <sup>3,5</sup>	0.6	±	0.01	0.2	±	0.05	0.0001

<sup>1</sup> All values are presented as mean ± SD.

<sup>2</sup> Student's unpaired *t* test for difference between NGI and HGI at study entry.

<sup>3</sup> Data was log transformed prior to statistical analysis and is presented as the geometric mean ± SD.

<sup>4</sup> Homeostasis assessment of insulin resistance (fasting plasma glucose\*fasting serum insulin/22.5).

<sup>5</sup> Glucagon(pmol/L)/insulin (pmol/L).

**Table 5.3. Dietary intake pre and during 11G and WB treatments<sup>1</sup>**

	11-Grain				White Bread				<i>P</i> <sup>5</sup>
	pre-11G <sup>3</sup>		During <sup>4</sup> 11G		pre-WB <sup>3</sup>		During <sup>4</sup> WB		
<b>NGI<sup>2</sup> Group (n)</b>	14		14		14		14		
Energy (kcal)	2350	± 104	2323	± 114.6	2291	± 172	2385	± 121.8	0.71
Protein (g)	86	± 3.8	93	± 5.2	86	± 6.4	88	± 4.4	0.45
Total Carbohydrates (g)	314	± 23	292	± 22.3	304	± 24	324	± 23.0	0.33
Total Fibre (g)	25	± 1.9	31	± 2.0	24	± 2.4	22	± 1.9	0.01
Saturated fat (g)	28	± 1.7	29	± 2.4	27	± 3.0	27	± 1.6	0.45
Monounsaturated Fat (g)	18	± 1.3	20	± 1.6	19	± 2.8	17	± 1.0	0.05
Polyunsaturated Fat (g)	11	± 1.9	12	± 1.0	12	± 2.7	10	± 1.1	0.20
Cholesterol (mg)	240	± 26	229	± 28.6	232	± 30	228	± 15.6	0.33
<b>HGI Group (n)</b>	13		13		14		14		
Energy (kcal)	2385	± 145	2422	± 149.2	2186	± 104	2540	± 120.9	0.54
Protein (g)	102	± 7.8	104	± 8.7	89	± 6.3	108	± 5.8	0.71
Total Carbohydrates (g)	298	± 22	284	± 20.0	270	± 18	307	± 18.7	0.42
Total Fibre (g)	27	± 3.4	30	± 2.1	20	± 2.1	20	± 1.6	0.01
Saturated fat (g)	31	± 2.6	29	± 2.9	29	± 2.6	30	± 1.7	0.84
Monounsaturated Fat (g)	22	± 2.9	26	± 2.7	19	± 2.9	27	± 2.5	0.76
Polyunsaturated Fat (g)	11	± 1.5	13	± 1.2	9	± 1.0	13	± 1.2	0.98
Cholesterol (mg)	269	± 35	327	± 34.6	288	± 29	373	± 31.7	0.98

<sup>1</sup> Data presented as mean ± SEM.

<sup>2</sup> Abbreviations: 11G 11-grain bread; WB white bread; NGI normal glycemic/insulinemic group; HGI hyperglycemic and/or insulinemic group; kcal kilocalorie.

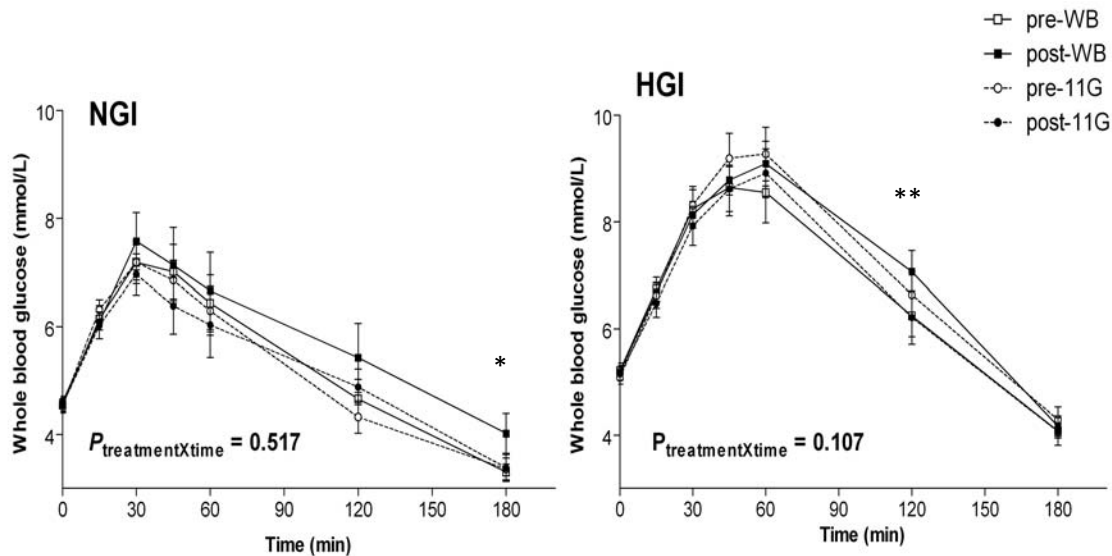
<sup>3</sup> No significant difference in nutrient composition pre-WB vs. pre-11G.

<sup>4</sup> Average of all food records collected during a specified treatment period, with the exception of the 4-day food records collected pre-intervention.

<sup>5</sup> *P* for difference during WB vs. during 11G interventions using Student's *t*-test.

## Blood Glucose

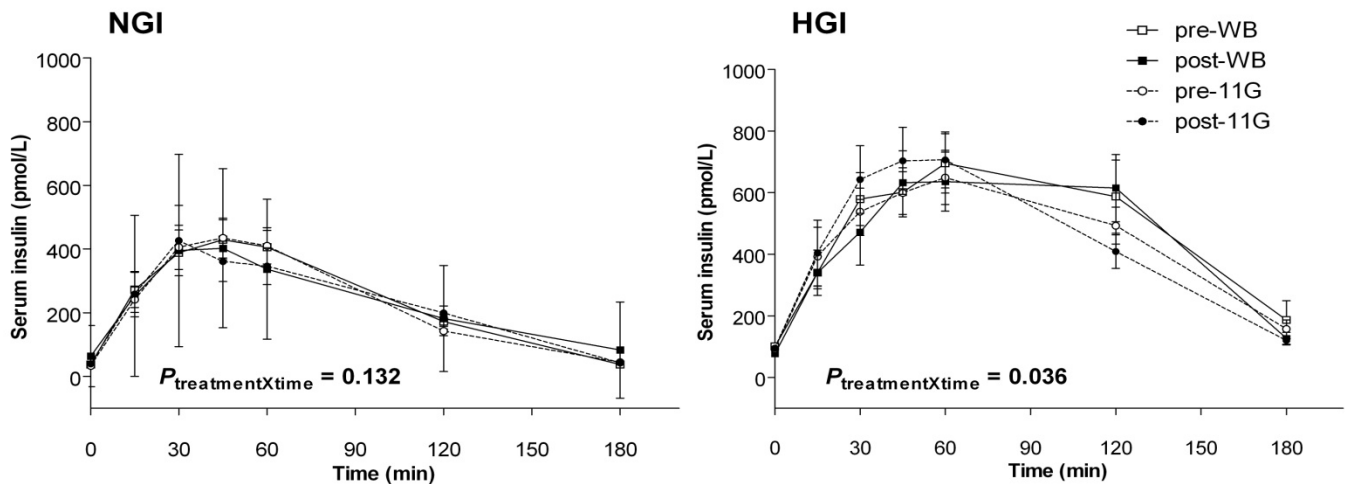
The fasting values for glucose did not significantly differ at the start and end of each bread treatment in either the HGI or NGI group. Postprandial glucose response pre- and post-bread treatments in both HGI and NGI groups are shown in **Fig. 5.1**. Glucose concentration at time point 120 min was significantly ( $P < 0.004$ ) lower post-11G as compared with post-WB in the HGI group. Glucose concentration at time point 180 min post-11G was significantly ( $P < 0.05$ ) lower compared to post-WB in the NGI group. Additionally, glucose AUC was significantly ( $P < 0.05$ ) lower post-11G as compared to post-WB in the HGI group (**Table 5.4**). There was no significant difference in glucose AUC post-bread treatments between the HGI and NGI groups.



**Figure 5.1. Postprandial whole blood glucose response to the ingestion of 75g glucose (OGTT) in NGI and HGI groups before and after 11-grain and white bread treatments.** Data is presented as means  $\pm$  SEM. \* post-WB vs. post-11G;  $P < 0.05$ , \*\* post-WB vs. post-11G;  $P < 0.004$ .

### Serum Insulin, IGI and HOMA-IR

The fasting values for insulin did not significantly differ at the start and end of each bread treatment in either the HGI or NGI group. No treatment within time effect was found in either the HGI or NGI groups (**Fig. 5.2**). In the NGI group IGI was significantly ( $P < 0.05$ ) higher post-11G as compared to post-WB and in the HGI group IGI tended to be higher ( $P = 0.06$ ) post-11G as compared to post-WB (**Table 5.5**). There were no significant differences in HOMA-IR and insulin AUC between the bread treatments in either the HGI or NGI groups (Table 5.4 and 5.5). There was no significant difference in insulin AUC post bread treatment between the HGI and NGI groups.

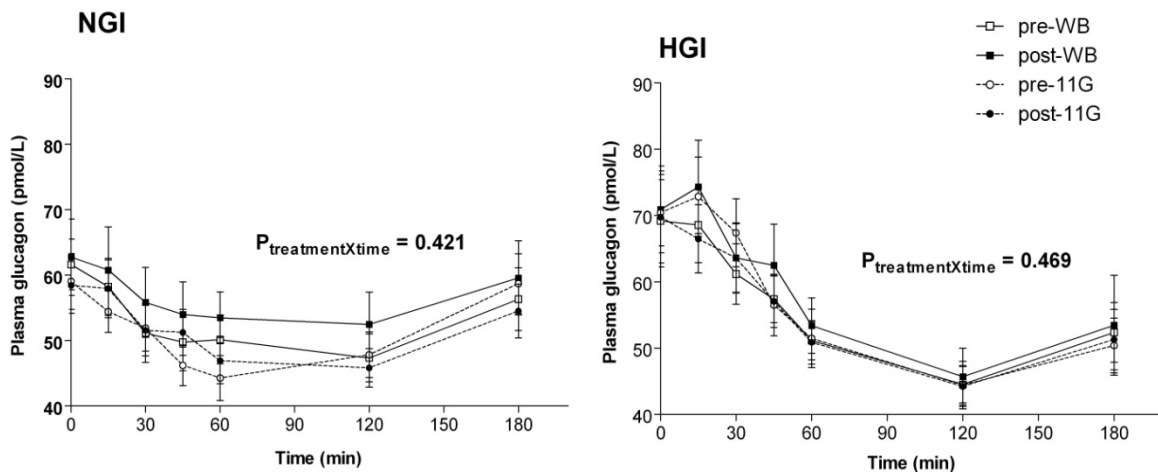


**Figure 5.2. Postprandial serum insulin response to the ingestion of 75g glucose (OGTT) in NGI and HGI groups before and after 11G and WB treatments.** Data is presented as means  $\pm$  SEM. No treatment within time effect was found in either the HGI or NGI group.

### 5. Plasma Glucagon and G/I ratio

The fasting values for glucagon did not significantly differ at the start and end of each bread treatment in either the HGI or NGI group. No treatment within time effect was found in either the HGI or NGI group (**Fig. 5.3**). No significant difference was found in the glucagon dAUC post-11G as compared to post-WB in either the HGI or NGI groups (Table 5.5). In the HGI group the glucagon dAUC post-11G bread was significantly ( $P < 0.04$ ) greater than those in the NGI group.

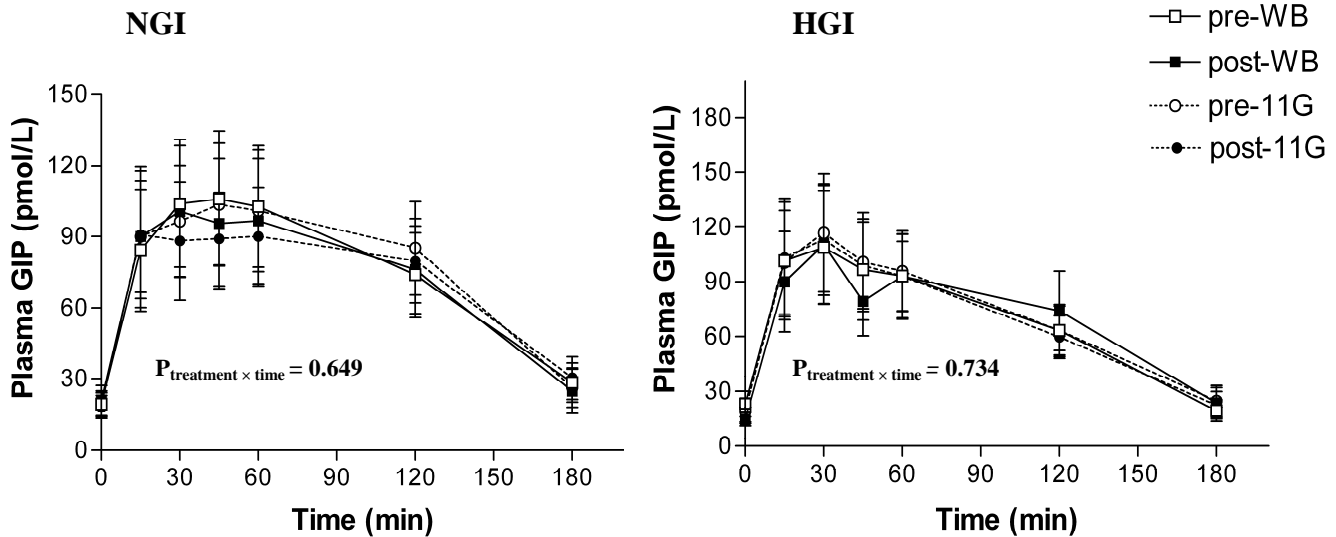
Fasting values for G/I ratio did not differ at the start or end of each bread treatments within either group. Significant difference was noted in fasting G/I ratio post-11G ( $P < 0.001$ ) and post-WB ( $P < 0.001$ ) between the HGI and NGI groups.



**Figure 5.3. Postprandial plasma glucagon response to the ingestion of 75g glucose (OGTT) in NGI and HGI groups before and after 11G and WB treatments.** Data is presented as means  $\pm$  SEM. No treatment within time effect was found in either the HGI or NGI group.

## Plasma GIP

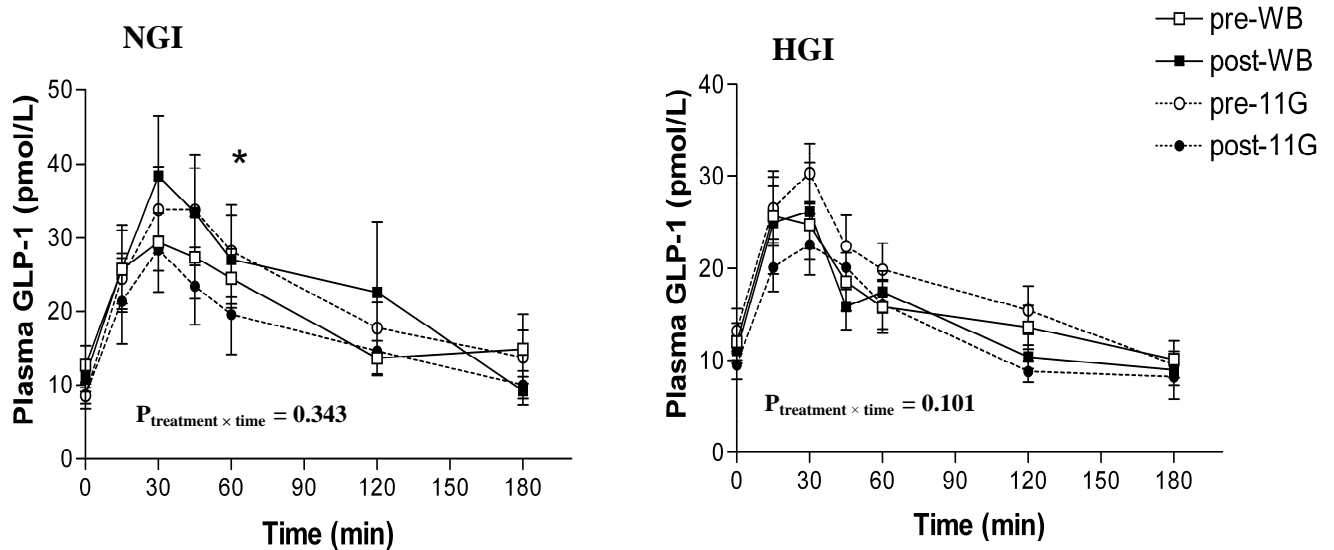
The fasting values for GIP did not significantly differ at the start and end of each bread treatment in either the HGI or NGI group. Postprandial GIP responses pre- and post- bread treatments in both the HGI and NGI groups are shown in **Fig. 5.4**. No treatment within time effect was found in either the HGI or NGI group. GIP AUC did not significantly differ between the bread treatments in either the HGI or NGI group (Table 5.5). There was no significant difference in GIP AUC post bread treatments between the HGI and NGI groups.



**Figure 5.4. Postprandial plasma GIP response to the ingestion of 75g glucose (OGTT) in NGI and HGI groups before and after 11G and WB treatments.** Data is presented as means  $\pm$  SEM. No treatment within time effect was found in either the HGI or NGI group.

## Plasma GLP-1

The fasting values for GLP-1 did not significantly differ at the start and end of each bread treatment in either the HGI or NGI group. Postprandial GLP-1 response pre- and post- bread treatments in both the HGI and NGI groups are shown in **Fig. 5.5**. A treatment within time effect ( $P < 0.03$ ) was found only in the NGI group at time point 60 min between bread treatments with post-WB GLP-1 concentration being greater than post-11G. GLP-1 AUC did not significantly differ between the bread treatments in either the HGI or NGI group (Table 5.4). There was no significant difference in GLP-1 AUC post-bread treatment between the HGI and NGI groups.



**Figure 5.5. Postprandial plasma GLP-1 response to the ingestion of 75g glucose (OGTT) in NGI and HGI groups before and after 11G and WB treatments.** Data is presented as means  $\pm$  SEM. \* post-WB vs. post-11G;  $P < 0.03$

**Table 5.4. Clinical characteristics pre and post 11G and WB treatments in the NGI and HGI groups<sup>1</sup>**

	11-Grain						White Bread						<i>P</i> <sup>2</sup>
	pre			post			pre			post			
<b>NGI Group (n)</b>	14			14			14			14			
Body weight (kg)	80.1	±	3.6	79.6	±	3.6	79.4	±	3.6	79.3	±	3.7	0.56
BMI (kg/m <sup>2</sup> )	26.8	±	0.8	26.6	±	0.8	26.5	±	0.8	26.5	±	0.8	0.75
Waist circumference (cm)	94	±	2.0	93	±	2.1	95	±	2.0	94	±	2.0	0.29
Body fat (%)	28.7	±	2.2	27.8	±	2.3	27.0	±	2.3	26.5	±	2.3	0.06
Systolic blood pressure (mm Hg)	116.0	±	4.1	117.9	±	3.4	117.5	±	4.3	121.6	±	5.1	0.30
Diastolic blood pressure (mm Hg)	65.1	±	1.9	67.1	±	1.9	67.4	±	2.5	68.3	±	2.7	0.97
Fasting whole blood glucose (mmol/L)	4.5	±	0.1	4.6	±	0.1	4.5	±	0.1	4.7	±	0.1	0.45
Fasting serum insulin (pmol/L) <sup>3</sup>	35.1	±	2.9	35.8	±	5.0	38.4	±	4.1	69.9	±	30.9	0.06
Fasting plasma glucagon (pmol/L) <sup>3</sup>	59.0	±	4.3	58.5	±	4.3	61.6	±	3.8	62.8	±	5.8	0.52
Fasting plasma GIP (pmol/L)	10.1	±	1.3	10.5	±	1.9	9.6	±	1.2	10.1	±	1.7	0.43
Fasting plasma GLP-1 (pmol/L) <sup>3</sup>	10.0	±	1.9	8.6	±	1.6	13.0	±	2.5	15.5	±	5.3	0.33
HOMA-IR <sup>3,4</sup>	1.0	±	0.1	1.0	±	0.1	1.1	±	0.1	2.2	±	1.1	0.07
Fasting G/I ratio <sup>3,5</sup>	0.56	±	0.06	0.79	±	0.31	0.55	±	0.06	0.57	±	0.15	0.12
<b>HGI Group (n)</b>	13			13			14			14			
Body weight (kg)	106.6	±	5.9	106.0	±	5.7	106.9	±	5.0	106.4	±	5.0	0.95
BMI (kg/m <sup>2</sup> )	35.6	±	1.7	35.2	±	1.7	35.5	±	1.5	35.4	±	1.5	0.25
Waist circumference (cm)	116	±	3.2	116	±	3.2	116	±	3.2	116	±	2.9	0.90
Body fat (%)	36.4	±	2.1	37.2	±	2.2	35.7	±	2.1	36.6	±	2.3	0.15
Systolic blood pressure (mm Hg)	126.2	±	3.7	124.8	±	4.1	127.7	±	4.0	129.3	±	4.6	0.17
Diastolic blood pressure (mm Hg)	68.8	±	2.5	68.3	±	2.5	68.7	±	2.6	72.7	±	4.6	0.25
Fasting whole blood glucose (mmol/L)	5.0	±	0.1	5.2	±	0.1	5.2	±	0.2	5.1	±	0.1	0.30
Fasting serum insulin (pmol/L) <sup>3</sup>	91.5	±	7.6	101.7	±	9.5	92.0	±	6.3	82.5	±	7.6	0.16
Fasting plasma glucagon (pmol/L) <sup>3</sup>	70.4	±	5.0	69.8	±	6.9	69.2	±	7.0	70.9	±	6.5	0.53
Fasting GIP (pmol/L)	8.9	±	1.4	7.6	±	1.3	11.5	±	2.1	7.6	±	1.0	0.51
Fasting plasma GLP-1 (pmol/L) <sup>3</sup>	13.5	±	2.5	9.5	±	1.5	12.0	±	2.0	11.1	±	1.4	0.54
HOMA-IR <sup>3,4</sup>	2.8	±	0.2	3.2	±	0.3	3.0	±	0.2	2.7	±	0.2	0.12
Fasting G/I ratio <sup>3,5</sup>	0.23	±	0.01	0.22	±	0.01	0.21	±	0.02	0.27	±	0.02	0.11

<sup>1</sup> Data presented as mean ± SEM.

<sup>2</sup> *P* for difference in post-WB vs post-11G using ANCOVA with cohort (summer or winter) and pre-treatment values as covariates.

<sup>3</sup> Data was log transformed prior to statistical analysis and is presented as the geometric mean ± SEM.

<sup>4</sup> Homeostasis assessment of insulin resistance (fasting plasma glucose\*fasting serum insulin/22.5).

<sup>5</sup> Glucagon(pmol/L)/insulin (pmol/L)

**Table 5.5. Incremental area under the curve of glucose, insulin, glucagon, GIP and GLP-1 responses pre and post 11G and WB treatments in the NGI and HGI groups<sup>1</sup>**

	11-Grain						White Bread						<i>P</i> <sup>2</sup>
	pre			post			pre			post			
<b>NGI Group (n)</b>	14			14			14			14			
<i>Glucose</i>	118	±	30	126	±	37	142	±	38	215	±	76	0.24
AUC (mmol/L*180 min)													
<i>Insulin</i>	35.6	±	4.5	35.1	±	4.9	36.5	±	3.9	30.8	±	4.5	0.24
AUC <sup>3</sup> (nmol/L*180 min)													
IGI <sup>3,4</sup>	160	±	34	165	±	47	225	±	76	145	±	42	0.04
<i>Glucagon</i>	1.5	±	0.4	1.5	±	3.4	1.8	±	429	1.3	±	0.4	0.63
dAUC (nmol/L*180min)													
<i>GIP</i>	5.1	±	0.6	4.9	±	0.7	4.8	±	0.5	4.4	±	0.6	0.82
AUC (nmol/L*180min)													
<i>GLP-1</i>	2.4	±	0.6	1.6	±	0.3	1.2	±	0.3	1.1	±	0.7	0.53
AUC (nmol/L*180min)													
<b>HGI Group (n)</b>	13			13			14			14			
<i>Glucose</i>	359	±	41	285	±	44	273	±	47	351	±	45	0.02
AUC (mmol/L*180 min)													
<i>Insulin</i>	65.6	±	7.4	64.3	±	6.3	72.2	±	12.1	72.7	±	11.6	0.35
AUC <sup>3</sup> (nmol/L*180 min)													
IGI <sup>3,4</sup>	145	±	25	222	±	55	152	±	24	128	±	27	0.06
<i>Glucagon</i>	3.1	±	0.4	3.1	±	0.8	3.0	±	0.7	2.8	±	0.4	0.88
dAUC (nmol/L*180min)													
<i>GIP</i>	4.8	±	0.7	4.5	±	0.6	4.2	±	0.5	4.8	±	0.5	0.91
AUC (nmol/L*180min)													
<i>GLP-1</i>	0.8	±	0.4	0.7	±	0.3	0.6	±	0.3	0.7	±	0.2	0.66
AUC (nmol/L*180min)													

<sup>1</sup> Data presented as mean ± SEM.

<sup>2</sup> *P* for difference in post-WB vs post-11G using ANCOVA with cohort (summer or winter) and pre-treatment values as covariates.

<sup>3</sup> Data was log transformed prior to statistical analysis and is presented as the geometric mean ± SEM.

<sup>4</sup> IGI insulinogenic index (insulin at 30 min – fasting insulin)/ (glucose at 30 min – fasting glucose).

## **DISCUSSION**

The purpose of the present study was to determine the impact of chronic ingestion of 11G, as compared to WB, on glucose metabolism in obese subjects with elevated glucose/insulin, as well as in healthy, control subjects. At study entry (Table 5.2), the HGI group had significantly greater BMI, body fat % and waist circumference compared to the NGI group. Furthermore, baseline fasting and AUC values for most measures (glucose, insulin, glucagon) were significantly greater in the HGI group compared to the NGI group, suggesting that the subject groups were very different in fasting state and also after ingestion of CHO. We hypothesized that chronic ingestion of 11G would result in improved glucose metabolism in the HGI, compared to the NGI group.

The results showed that glucose AUC post-11G was significantly lower than that of post-WB in the HGI group. Additionally, glucose concentrations were significantly lower at time point 120 and 180 min post-11G in the HGI and NGI groups, respectively. This finding is in agreement with studies showing that the association of whole-grain intake with reduced risk of T2D was observed to be stronger in an overweight adult population (96,101,104,105). Greater fiber and lower available CHO intake in 11G treatment may explain the lower glycaemia post-11G compared to post-WB (Table 3). Furthermore, it has been suggested that in addition to dietary fiber, other bioactive components such as vitamins, minerals and antioxidants in whole-grain may act synergistically to lower the risk of chronic diseases such as T2D (99,108,113).

Previously (158), we showed that when bread consumption was based on volume of the breads (107 g), acute ingestion of 11G did not lower postprandial glucose or insulin responses compared to WB. In the same study, when bread consumption was based on the amount of

available CHO (50 g), there was no difference in glucose response between 11G and WB while 11G produced greater insulin response compared to WB. In the current study we matched daily bread intake on the basis of total weight rather than on the amount of available CHO in the breads. Further, we felt that this method was more representative of the dietary habits of consumers given that consumers are unlikely to ingest a larger total quantity of whole-grain breads, as compared to white bread, as a means to compensate for available CHO intake. The results showed that despite the lower glucose AUC post-11G in the HGI group, no difference was found in HOMA-IR and insulin AUC between the bread treatments in either the HGI or NGI group. Our insulin result is consistent with the study with similar design (113) which demonstrated that substitution of whole-grains for refined-grain products in the habitual daily diet of healthy overweight adults for 6 wk did not affect insulin response and insulin sensitivity. Conversely, Pereira et al. (104) reported improved insulin sensitivity and reduced fasting insulin after 6 wk of whole-grain consumption, compared with refined-grains, in hyperinsulinemic overweight and obese men. One potential explanation between our results and those of Pereira et al. (104), may relate to the quantity of whole-grains provided. With the exception of bread products, our subjects maintained their habitual intake of other grain products whereas Pereira et al. (104) provided all cereal and grain products (e.g. pasta, rice, bread, breakfast cereal, muffins, cookies etc.) in the form of whole-grains.

We found that 6 weeks of 11G consumption, compared to WB, resulted in improved IGI ( $P < 0.04$ ), a marker of the first phase insulin response, in the NGI group (Table 5). Additionally, a strong trend ( $P = 0.06$ ) towards improvement in IGI post-11G compared to post-WB was observed in the HGI group. Laaksonen et al. (155) showed that supplementing the diet with whole-grain rye bread and pasta over a period of 12 wk (as opposed to 6 wk in our

study) led to a significant improvement in the IGI in 72 subjects (as opposed to 14 subjects in the HGI group in our study) with metabolic syndrome. In the present study, despite the significantly higher insulin baseline fasting and AUC values in the HGI as compared to the NGI group, the trend towards improved IGI post-11G did not reach significance ( $P < 0.06$ ). It is possible that with longer intervention, a more extreme diet modification and/or larger sample size we might have seen improvement in IGI and insulin sensitivity in the HGI group. It should be also noted that the main assessment was conducted with ingestion of 75 g of simple CHO and not with the test bread. The subjects may have adaptations to the daily bread that was consumed that are not detected with our protocol.

Previously (158), we showed that when bread consumption was based on volume of the test breads (107 g), acute ingestion of 11G resulted in lower ( $P < 0.001$ ) GIP response compared to WB, with no impact on GLP-1 response. Lowered GIP response to 11G has been attributed to lower available CHO content in 11G as compared to WB (158). In the present study, despite the lower available CHO intake with the test bread in the 11G treatment compared to WB treatment, 6 wk ingestion of 11G did not alter GIP and GLP-1 responses compared to WB. As noted above, this is based on the response to 75 g of simple CHO and not the test breads. According to previous studies in animals (149,150), highly fermentable insoluble DF promotes secretion of GLP-1, an effect suggested to be mediated by bacterial colonic fermentation and formation of short chain fatty acids. In the present study, we did not measure colonic fermentation but the insoluble fiber intake (Table 1) was greater in 11G treatment compared to WB treatment for 6 wk and it did not affect GLP-1 response. Therefore, lack of difference in GIP and GLP-1 response between the bread treatments remained unexplained.

Glucagon response did not significantly differ between the bread treatments in either the HGI or NGI group. Given that we did not observe a significant bread treatment effect on insulin, GIP and GLP-1 responses, it is perhaps not surprising that ingestion of 11G did not alter glucagon and G/I ratio compared to WB. This may suggest that merely changing the type of bread ingested, without changing the background diet of the subjects, is not enough of a stimulus to alter the T2D biomarkers evaluated in the present study.

Fisher et al. (162) examined the association between the protective effect of whole-grain consumption and the genetic variation of *TCF7L2* (gene involved in the process of insulin secretion in the pancreatic  $\beta$ -cells). The results provided evidence that the favorable effect of whole-grain intake on diabetes risk is modified by *TCF7L2* rs 7903146. This genetic aspect may explain the lack of consistency among the studies looking at the impact of whole-grain intake on insulin demand and insulin sensitivity.

A strength of the present study is that using a randomized crossover design allowed each subject to serve as his/her own control and eliminated any intra-subject variability. Additionally, comparing a healthy group to a metabolically impaired group allowed us to clarify the differential effects of whole-grain bread on individuals with varying metabolic status. The non-blinded provision of the bread treatments may be considered a limitation of the study; however, this was unavoidable given the visual difference between whole-grain and white breads. Duration of the intervention and the sample size are additional limitations of the study. As noted above, a limitation is that our assessment was conducted within an OGTT. The subject may have experienced daily adjustment to the insulin, glucagon and incretin secretion that we could not detect.

## **CONCLUSION**

Six weeks of 11G consumption improved IGI in the NIG group and lowered glycemia in the HIG group as well as having a strong trend for improved IGI in the HIG group. There was no change in fasting and postprandial insulin, glucagon, GIP and GLP-1 responses post bread treatment. We suspect that changing one component of the diet without changing the background diet for 6 weeks was not sufficient to impact the glucose metabolism in obese people with elevated glucose/insulin. It is possible that a longer intervention and consumption of greater quantity of 11G may improve glucose and insulin homeostasis, but further research is needed to confirm this.

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**CHAPTER SIX**

**GENERAL DISCUSSION**

## 6.1 General Discussion

The overall aim of this thesis was to determine the impact of ingestion of different types of breads varying in CHO composition (sourdough, whole-wheat, whole-wheat barley, whole-grain and white breads) on biomarkers related to T2D in obese and overweight adults. It was hypothesized that the sourdough, whole-wheat and whole-grain breads would improve the glucose homeostasis compared to the white bread. In order to test the hypothesis, 3 studies were performed with middle-aged sedentary males. These studies were designed to address not only basic nutritional science questions but also to investigate issues of applied nutrition.

In study 1, sourdough bread reduced glucose response compared to whole-wheat bread by 60% and white bread by 30% in the first meal period. This was associated with modification in incretin responses, but gastric emptying rate was not affected. In this study sourdough and white bread shared a similar recipe; they only differed in the use of sourdough culture and long fermentation time for sourdough bread, suggesting an effect of organic acids formed during the fermentation. However, the mechanism by which organic acids exert their positive effects on glycemia is not clearly understood. One theory is that the presence of organic acids may interfere with starch digestion (86). Another possibility is that organic acids influence the rate of gastric emptying (87). However, in study 1, ingestion of sourdough bread lowered the glucose response without changing the rate of gastric emptying. This observation suggests that lowered glycemia after ingestion of sourdough bread could not be attributed to reduced rate of gastric emptying and instead a mechanism related to slowing digestive phase was likely involved. Östman et al. (91) examined the impact of lactic acid on the rate of *in vitro* starch hydrolysis and showed that the presence of lactic acid in bread reinforced the interactions between starch and protein (gluten) which should reduce starch bioavailability (enzyme

accessibility) and consequently reduce the rate of starch hydrolysis. This study (91) provided strong evidence that organic acid in sourdough bread exerts its effect on glycemia by reducing the rate of starch digestion.

A complementary explanation for the glucose lowering effect of sourdough bread might be the presence of oligosaccharides in the sourdough bread. Oligosaccharides are CHO which have 3-10 monosaccharide linked together. They are found naturally in small amounts in many plants including chicory roots, artichokes, onions, asparagus and legumes. Oligosaccharides have attracted attention recently because the small intestine cannot break them down and 90% of those consumed reach the colon and undergo fermentation (163,164). Thus they have actions similar to DF. In preliminary work conducted with Dr Steve Cui at Agriculture and Agri-food Canada, oligosaccharides in laboratory-prepared sourdough and white breads were measured using size exclusion chromatography. The preliminary results (**Table 6.1**) showed that sourdough bread contained a far greater amount of oligosaccharides than white bread. It is possible that prolonged fermentation in sourdough bread preparation resulted in the breakdown of CHO and production of oligosaccharides. Similarly, Rouzaud and Anaya (165) detected higher amount of oligosaccharides in yeasted sourdoughs compared to unfermented doughs. This is an important guide for the food industry because it suggests that rather than adding DF or resistant starch to a bread product, it is possible to increase the fiber/indigestible CHO content by processing per se. It is not known whether the greater amount of oligosaccharides in sourdough bread was partly responsible for the glucose lowering effect of this bread. More work is needed to measure the level of oligosaccharides in various breads and to examine the impact of the presence of oligosaccharides in bread products on glucose metabolism.

In study 2, acute ingestion of 11-grain bread containing sourdough culture did not improve glycemia in overweight and obese subjects. It has been suggested that changes in CHO fraction due to the prolonged fermentation (8-24h) can be affected by external parameters such as dough consistency, fermentation temperature, flour extraction rate and type of starter. These factors affect the biochemical activity of the fermenting flora (165). This may explain the lack of positive effect of the sourdough culture used in baking of 11-grain bread in study 2.

**Table 6.1. Oligosaccharide profile in sourdough and white breads.**

	Sourdough bread	White bread
Maltotriose (ug/mL)	2.06	0.89
Maltotetraose (ug/mL)	4.53	0.64
Maltohexaose (ug/mL)	15.70	0.0
Maltoheptaose (ug/mL)	10.16	0.0
Total (ug/mL)	32.45	1.53

It has been shown that inclusion of barley in CHO-rich foods lowers glucose and insulin responses in healthy subjects (79,166). However in Study 1, ingestion of whole-wheat barley bread did not result in lower glucose and insulin responses compared to the white bread. It has been suggested that  $\beta$ -glucan (soluble fiber) found in barley is responsible for the glucose lowering effect.  $\beta$ -glucan content of whole-wheat barley bread was not measured in the present study but it is possible that the amount of  $\beta$ -glucan was not sufficient to influence the glucose metabolism. Liljeberg et al. (128) showed that 50 and 80% substitution of the barley flour in barley-based breads lowered the GI (compared to reference bread) by 30 and 40 units, respectively. In the present study, the substitution of barley flour in whole-wheat barley bread

was 27%. Adding more barley flour to the whole-wheat barley bread recipe was attempted but the dough did not rise and the final bread was very dense and unappetizing. Twenty seven % substitution of the barley flour resulted in a final bread with similar appearance and texture as other test breads (sourdough, whole-wheat and white).

Another explanation for the lack of difference between the whole-wheat barley and white breads is that  $\beta$ -glucan may have been destroyed during the baking process. It has been reported that due to its linear structure,  $\beta$ -glucan is very sensitive to depolymerisation during the technological process (baking) which subsequently reduces its viscosity and putative functionality *in vivo* (80). The present study provides more evidence that the glucose lowering effect of barley may depend on the quantity added to the bread. Furthermore, it clearly demonstrates that merely adding an ingredient that has potentially favourable metabolic impact does not ensure the effect and the processing of the food may have a major impact on its function. This is an important finding as often food products are marketed to the consumer based on a specific ingredient (e.g. 'contains barley and beta glucan')

In study 1, the observed metabolic effects in the first meal period were carried forward to the next meal as the sourdough bread had the capacity to lower the glucose AUC at the second meal, compared to the whole-wheat breads. Several mechanisms have been suggested to explain the second meal effect. One theory is that when the CHO absorption is prolonged in the first meal, there is less of a tendency for the blood glucose to undershoot below the basal level. As a result, free-fatty acids are not released to compensate for the low blood glucose and the suppression of free-fatty acids release could improve insulin economy and sensitivity, hence improve glycemia at the time of subsequent meal (167,168). Another proposed mechanism for the second meal effect is the colonic fermentation of indigestible CHO (resistant starch, DF and

oligosaccharides) resulting in formation of SCFA, mainly acetic, propionic and butyric acids (121). Brighenti et al. (118) showed that soluble/fermentable CHOs have the potential to improve the postprandial response to a second meal by decreasing NEFA concentrations. However, the results of the above studies suggest that SCFAs of colonic origin may shift the metabolism towards glucose oxidation rather than fat oxidation. The results of the current study do not support the role of indigestible CHO in second meal effect since the result of study 1 indicated greater DF content of whole-wheat breads did not improve the glycemic response to the second meal. This might be due to the short time (3 h) between the first and second meal in our study. The indigestible CHO content of the first meal is more likely to explain a second meal effect from evening meal to breakfast. It is not known whether oligosaccharides reach colon and undergo fermentation faster than the other indigestible CHO like resistant starch or DF. More studies are needed to examine the effect of oligosaccharide content of foods on second meal effect.

Fiber intake has been shown to improve glucose and insulin metabolism in patients with T2D (7,107). Dietary guidelines suggest intake of whole-wheat and other dark breads as one way to increase the fiber intake and improve glycemia (153). However, in study 1, ingestion of whole-wheat breads did not result in lower glucose and insulin responses compared to white bread. This lack of impact agrees with those studies reporting similar glycemic responses to white and whole-wheat breads (64,68), suggesting similar digestive process despite the greater fiber content in whole-wheat bread. The high glycemic response to whole-wheat bread might be due to the small particle size of the ultra fine-ground whole-wheat flour used in my study. Whole-wheat flour corresponds to white flour with the added bran (insoluble fiber) fraction (64). It has been reported that only soluble fiber influence the glucose metabolism by increasing

the viscosity of digesta and forming a physical barrier, leading to reduced rate of glucose absorption (77,79). Only total DF was measured, but it is very likely that the total DF content of the whole-wheat bread consisted predominantly of insoluble fiber. This is another example of the complexity of evaluating the benefits of various food items and consumers must be able to discriminate not only the amount of fiber available in a product but also the type of fiber.

Another explanation for the lack of a positive effect of whole-wheat bread on glycemia is that adding bran to the white flour cannot restore the intact physical structure and interaction between starch and fiber; instead it could disrupt the gluten network and favour the digestive process (64). Instead of making flour by adding bran to white flour, it is recommended (64) to directly use whole-grain flour with the totality of wheat fractions (bran, germ, and endosperm). In study 1, whole-wheat flour was used because traditional dietary research showed that whole-wheat bread consumption was generally high compared to that of whole-grain breads and thus the results could be applied to a commercial product (141). Recently, consumers have become more interested in whole-grain consumption. Nielson consumer survey data suggests that from 2004 to 2006 there has been a steady rise in the relative proportion of whole-grain products purchased within the cereal and bread categories (169). Whole-grain flour contains particles with a more intact structure that are favourable to slow starch digestion, and therefore lower GI (99,111). This is in agreement with the results of part 1 of study 2, in which the ingestion of sprouted-grain bread lowered the glucose response in compared to sourdough and white breads. However, ingestion of 11-grain bread with high fiber content did not alter glycemia compared to white bread. This finding suggests that metabolic responses to various whole-grain breads are different for each recipe and therefore one cannot generalize about the metabolic responses to ingesting whole-grain products. It should be noted that there are many factors influencing the

metabolic effect of bread products other than the type of flour including kneading protocol, fermentation time, baking temperature and storage condition. Once again the conclusion is that the specific ingredients and their processing can dramatically influence the postprandial physiological responses and there is no simple advice that one can give the consumer.

The lack of positive effect of whole-wheat bread on CHO management should not be interpreted as a negative health effect. Whole-wheat consumption has many health benefits. For instance, the Institute of Medicine, USA, recommends that children and adults consume 14 grams of fiber for every 1,000 calories of food they eat each day (111). Whole-wheat bread consumption would increase the daily DF intake and help to improve the gastrointestinal health and prevent developing constipation and diverticulitis (170,171). Whole-wheat intake also increases the intake of minerals such as manganese and magnesium (111).

The practice of sprouting of cereal grains has become popular in the western world. Sprouted grains are thought of as having exceptional nutritive value. Sprouting of grains causes increased activity of hydrolytic enzymes, an increase in total protein, a decrease in starch and anti-nutrients, a slight increase in crude fiber, and slightly higher amounts of B-group vitamins and certain minerals (172). Most of the increases in nutrients are not true increases in amount but rather they are increases in concentration and reflect the decrease in dry matter, mainly in the form of CHO during sprouting. As total CHO decreases, the percentage of other nutrients increases. The magnitude of the nutritional improvement is, however, influenced by the type of cereal, seed quality and sprouting conditions (145,172). In study 2, no conventional flour was used in baking the sprouted-grain bread. The fully developed wheat sprouts were processed to dough like consistency, and then sourdough culture and dry yeast were added to the dough (G Boeringer, personal communication). It is possible that the increased vitamins and minerals

worked synergistically with the reduced CHO amount and lowered the glucose response after the ingestion of sprouted-grain bread in study 2. Another possibility is that the partial hydrolysis of CHO during sprouting resulted in an increased level of oligosaccharides and was partly responsible for the glucose lowering effect of sprouted-grain bread. More research is needed to explain the mechanism by which ingestion of sprouted- grain bread impacts glucose metabolism.

Surprisingly in study 2, acute ingestion of 11-grain bread did not improve glycemia or insulinemia compared to the white bread. Limited literature is available on acute intervention of such foods. The results from epidemiologic (99,110,141) and chronic interventional studies (104,112) suggest that any positive effect of whole-grain food consumption on insulinemia and insulin sensitivity is only apparent after a chronic intervention. In study 3, 6 wk ingestion of 11-grain bread lowered glucose response compared to white bread in the HGI group which was attributed to the lowered glucose response post-11-grain to the greater DF intake in 11-grain bread treatment (15.9 g /d) compared to white bread treatment (7.9 g /d). The latter result suggests that greater DF intake in the form of whole-grain products is beneficial for glycemic control in metabolically challenged people compared to individuals with normal glucose and insulin responses. These results are in agreement with those of other studies (96,101,105) reporting the positive effect of whole-grain consumption in overweight or obese individuals, suggesting that the inverse association of whole-grain consumption and reduced risk of T2D is greater in overweight and obese population. In study 3, the IGI was significantly increased in the NGI group and there was a strong trend ( $P = 0.06$ ) for this in the HGI group. It is also possible that daily consumption of either a greater dose of whole-grain products and/or longer

intervention period are required to improve glucose and insulin responses in both HGI and NGI groups.

It has been reported that typical fasting levels of bioactive GLP-1 and GIP are in the range of 5 to 10 pM and 9 to 11 pM, respectively, and that both increase quickly after meal ingestion (18,22,28). Furthermore, GLP-1 secretion occurs in a biphasic pattern, with an early phase beginning within 5 to 15 min and a prolonged second phase following within 30 to 60 min (17,22,25,29). The GIP and GLP-1 results from the current research are in agreement with the literature. In study 1, the biphasic pattern of GLP-1 secretion was demonstrated (Chapter 3, Fig 4) with an early phase around 15 min and the second phase following around 90 min. In study 2 and 3 this pattern was not clearly shown. It has been proposed that the early phase of GLP-1 secretion may be due to neural, and other gut-derived (and even non-gut-derived) endocrine factors activating L-cells in the distal intestine and the prolonged second phase may be due to direct nutrient contact with the L cells and K/L cells in upper small intestine (17,22,25,29).

It is far from clear whether a successful dietary intervention should increase or decrease the incretin response. It must be appreciated that glucose management is complex and that a single change in one component cannot easily be interpreted without information regarding other elements. Vilsboll et al. (37) reported increased fasting GIP concentration in obese, healthy subjects compared to lean, healthy subjects. Miyawaki (38) showed that GIP knock-out mice were resistant to obesity and insulin resistance induced by high fat diet, compared to normal mice. These observations suggest that high GIP levels may predispose individuals to the development of obesity. In study 3, there was no significant difference in fasting GIP levels

between the NGI and HGI groups. More research is needed to clarify the impact of GIP secretion and function on the development of obesity.

Dietary CHO is the major stimulus for postprandial incretin secretion but little is known how different types of CHO alter the secretion of incretins (46,114) and of course the foods are not merely CHO but a combination of CHO in various forms, fat and protein. In study 1, the GLP-1 response to sourdough bread was lower than to whole-wheat and white breads for the first and the second meals. Additionally, in part 1 of study 2, ingestion of sourdough bread resulted in the lowest GLP-1 response among the test breads. It is possible that the increased levels of organic acids in sourdough bread lowered the GLP-1 response compared to other test breads but the mechanism is not understood. The lowered GLP-1 response to sourdough bread compared to whole-wheat and whole-grain breads might be due to the lower DF content of sourdough bread. DF has been shown to increase GLP-1 secretion in animals (149,150). It has been demonstrated that soluble/fermentable DF was more potent stimulator of GLP-1 secretion compared to non-soluble DF (150). This is in agreement with the results of study 2, in which sprouted-grain and 11-grain breads with highest soluble fiber content had the greatest GLP-1 response among the test breads. However, the results of study 3 do not support the role of DF in postprandial GLP-1 secretion. The greater DF intake in 11-grain bread treatment for 6 weeks did not alter the GLP-1 response compared to white bread treatment. More studies are needed to clarify the mechanism by which organic acids and DF influence postprandial GLP-1 secretion.

It has been suggested that increased secretion of GLP-1 in response to ingestion of CHO foods stimulates postprandial insulin secretion (25,50,114). Furthermore, GLP-1 has a role in expansion of pancreatic  $\beta$ -cells and inhibition of  $\beta$ -cell apoptosis and as a result has the potential to be used as therapeutic agent in management of T2D (25,47,50,57). Additionally,

GLP-1 has been shown to reduce the rate of gastric emptying (43,46). In study 1, ingestion of sourdough bread reduced glucose and GLP-1 responses, with no impact on insulin response, in overweight or obese subjects. It is not known whether the lowered GLP-1 response to sourdough bread would have an unfavourable impact on insulin secretion,  $\beta$ -cells expansion and gastric emptying in the extended period of time. It is speculated that a bread product containing a greater amount of indigestible CHO, resulting in a lowered glucose response and insulin demand and increased GLP-1 response would be useful in preventing and managing T2D. In study 2, ingestion of sprouted-grain bread reduced glucose response and increased GLP-1 response compared to other breads. Future research should examine the impact of chronic ingestion of sprouted-grain bread on biomarkers of glucose metabolism in a population at risk of T2D and healthy controls.

The results for the GLP-1 response in study 1 and part 1 of study 2 suggest that the difference in volume of bread (vs. available CHO) consumed may influence the GLP-1 response to the test breads. In study 1, sourdough bread had a lower volume and GLP-1 response, compared to whole-wheat and whole-wheat barley breads. In part 1 of study 2, 11-grain and sprouted-grain breads had the greatest volumes, and the greatest GLP-1 response. However, in part 2 of study 2, equal volumes of the test breads were ingested and sprouted-grain bread still induced the greatest GLP-1 response among the breads. This observation suggests that the volume of the bread may be one factor but that its influence can be modulated by other aspects of the food in postprandial secretion of GLP-1.

Ingestion of the test breads altered the postprandial glucose, insulin and incretin responses but no clear association was found between insulin and glucose/incretins. Furthermore, GIP and GLP-1 responded differently to different breads. In study 1 and part 1 of

study 2, ingestion of sourdough bread lowered the glucose and GLP-1 responses compared to white and whole-wheat breads without any impact on GIP and insulin responses. In part 2 of study 2 ingestion of 11-grain bread lowered the GIP response compared to white bread while glucose, insulin and GLP-1 responses to this bread remained unchanged. Additionally, in study 3 the glucose response was lowered post-11-grain bread compared to post-white bread in the HGI group but no significant difference was noted in insulin, glucagon and incretin responses between the 11-grain and white bread treatments in either the HGI or NGI group. These findings suggest that metabolic responses to breads are varied across different recipes and they are not reflected by merely GI, or in other words, GI is not the sole parameter reflecting nutritional quality of bread products. In addition, while it is commonly stated that the incretins account for 50% of postprandial insulin secretion (18,21,27), the data from this research demonstrate that this is not a simple relationship.

Despite of the novel findings of the current research, there were some limitations. In study 1, the test breads were not analyzed for soluble and insoluble fiber. Furthermore, the preliminary work on oligosaccharides was conducted only on sourdough and white breads and no data is available for whole-wheat breads. A major limitation in study 2 was that no detailed information on recipe and baking process for the commercial breads was available to assist in examining possible reasons for the different responses to the whole-grain breads. On the other hand, examining the commercial breads provided information about the metabolic impact of breads that are already available to the people and is more applied. In study 3, the lack of impact of chronic ingestion of 11-grain bread on glucose metabolism might be due to the insufficient bread dose or intervention time. It is also possible that changing one component of

a diet will not provide sufficient stimulus and changing the background diet is required to improve the glucose metabolism.

## **6.2 Summary and Future Direction**

In summary, the current research demonstrated that ingestion of different types of bread varying in composition altered the glucose, insulin, GIP and GLP-1 responses in overweight and obese adults. While whole-wheat breads did not provide improvement in the postprandial responses, sourdough and sprouted-grain breads lowered glycemia and may have a beneficial effect in prevention and management of T2D. Acute ingestion of whole-wheat, whole-wheat barley, 12-grain and 11-grain breads did not result in improved glucose metabolism; however, 6 wk ingestion of 11-grain bread lowered the glucose response in subjects at increased risk of developing T2D compared to the healthy controls.

In my research, in many cases the glucose data was not consistent with the changes in the insulin data and in turn the insulin response did not follow incretin response; this suggests that glucose metabolism is very dynamic and multi-factorial. This research provides evidence that the simple model of dietary CHO stimulates incretin secretion and subsequently insulin secretion and incretins regulate postprandial insulin release does not always apply. Furthermore, GIP and GLP-1 responses to the CHO consumed do not follow a similar pattern. The mechanism of impact of bread on glucose metabolism is complex and needs further research.

The present study also indicated that the bread mass consumed may play a role but it is not the sole or major determinant factor in metabolic impact of bread. Furthermore, the DF content and compositional properties of bread do not explain the metabolic impacts of a bread meal. Thus, there must be other factors related to the nature of the ingredients, structural

properties, kneading and proofing protocol and baking process influencing the metabolic response to bread. One of the factors influencing starch digestion and consequently glucose response to bread ingestion might be the presence of oligosaccharides in the bread products. For instance, prolonged fermentation time and sprouting of the grain may result in partial hydrolysis of the starch and increased the level of oligosaccharides. These factors should be studied further to produce CHO foods that could be beneficial in preventing and managing T2D.

The current research demonstrated that ingestion of various whole-grain breads resulted in different metabolic responses in overweight and obese men. The consumer would like a simple message such as ingestion of any whole-grain bread has favourable impact on glycemic control. However, the results of the present research do not support this and showed that whole-grain breads are not the same in terms of inducing metabolic responses. Future research should examine how different factors related to the ingredients; recipe and baking process impact the metabolic effects of whole-grain breads.

The results of this research provide a guide for the food industry and can be applied to develop healthier bread products which can be useful in prevention of T2D in population at increased risk of developing T2D. For instance, using sourdough culture instead of dry yeast and prolonging the fermentation time resulted in increased level of organic acids and may improve glycemic control. In addition, using whole-grain flour (with totality of wheat fractions) and sprouted-grain flour instead of whole-wheat flour (white flour with added bran fraction) may favour slow rate of CHO digestion and absorption and improve glycemic control. The future research should examine different ways of processing bread in order to limit starch degradation and glucose absorption and reduce the metabolic response following bread ingestion.

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# APPENDICES

## **Appendix 1. Certification of ethics approval**

RESEARCH ETHICS BOARD

Certification of Ethical Acceptability of Research

Involving Human Participants

APPROVAL PERIOD: [August 31, 2004](#) to [August 31, 2008](#)

REB NUMBER: 04AU003

TYPE OF REVIEW: Delegated Type 1

RESPONSIBLE FACULTY: TERENCE GRAHAM

DEPARTMENT: Human Health & Nutritional Sciences

SPONSOR: OMAFRA

TITLE OF PROJECT: IMPACT OF BREADS ON BIOMARKERS FOR TYPE 2

DIABETES AND CARDIOVASCULAR DISEASE

The members of the University of Guelph Research Ethics Board have examined the protocol which describes the participation of the human subjects in the above-named research project and considers the procedures, as described by the applicant, to conform to the University's ethical standards and the Tri-Council Policy Statement.

The REB requires that you adhere to the protocol as last reviewed and approved by the REB. The REB must approve any modifications before they can be implemented. If you wish to modify your research project, please complete the Change Request Form. If there is a change in your source of funding, or a previously unfunded project receives funding, you must report this as a change to the protocol.

Adverse or unexpected events must be reported to the REB as soon as possible with an indication of how these events affect, in the view of the Responsible Faculty, the safety of the participants, and the continuation of the protocol.

If research participants are in the care of a health facility, at a school, or other institution or community organization, it is the responsibility of the Principal Investigator to ensure that the ethical guidelines and approvals of those facilities or institutions are obtained and filed with the REB prior to the initiation of any research protocols.

The Tri-council Policy Statement requires that ongoing research be monitored by, at a minimum, a final report and, if the approval period is longer than one year, annual

reports. Continued approval is contingent on timely submission of reports.

Membership of the Research Ethics Board: F. Caldwell, Student Health Services ; J. Dickey, HHNS , M. Dwyer, Legal Representative; M. Fairburn, Ethics and External, B. Ferguson, Economics, C. Harvey-Smith, N.D. and External ; J. Minogue , EHS ; L Trick, Psychology ; P. Salmon, SETS ; J. Tindale, FRAN , T. Turner; Sociology & Anthropology.

Approved: Date: \_\_\_\_\_

per

Chair, Research Ethics Board

**SUBJECT SCREENING QUESTIONNAIRE**

Your responses to this questionnaire will be kept confidential and you are asked to complete it for your own health and safety. Please answer each question with as much detail as possible. If you feel at all uncomfortable answering any of these questions please feel free to leave them blank.

**I. Personal Data (Please Print):**

Name: \_\_\_\_\_ Age: \_\_\_\_\_

DOB (dd/mm/yy): \_\_\_\_\_

**Mailing Address:**

Street: \_\_\_\_\_ City: \_\_\_\_\_  
Province: \_\_\_\_\_ Postal Code: \_\_\_\_\_  
Phone: (    ) \_\_\_\_\_  
Email: \_\_\_\_\_

Best way to communicate: \_\_\_\_\_

**II. Subject Information:**

**Exercise Patterns**

How often do you exercise in one week? \_\_\_\_\_

If you are active, please list what type of activities you participate in, and the duration each activity:

Activity

Duration

- a)
- b)
- c)
- d)

**III. Medical History**

Please circle "Yes" or "No" to the following questions and/or fill in the blanks.

1. When did you last see your family doctor? \_\_\_\_\_
2. When was your last physical examine administered by your family doctor? \_\_\_\_\_
3. Do you currently smoke? YES NO  
    a. If no, have you ever smoked? YES NO  
    b. How long ago did you quit? \_\_\_\_\_
4. Do you have any allergies to food? YES NO  
    If yes, please list: \_\_\_\_\_  
\_\_\_\_\_
5. Do you have any allergies to any environmental factors? YES NO  
    If yes, please list: \_\_\_\_\_  
\_\_\_\_\_
6. Do you have any allergies to any medications? YES NO  
    If yes, please list: \_\_\_\_\_  
\_\_\_\_\_
7. Do you currently take any prescription medication? YES NO  
    If yes, please list: \_\_\_\_\_  
\_\_\_\_\_
8. Do you currently take any over-the-counter medications (e.g. aspirin)? YES NO  
    If yes, please list: \_\_\_\_\_  
\_\_\_\_\_
9. Is there any major medical condition with which you have been diagnosed and are under the care of a physician, i.e. diabetes, high blood pressure, gallbladder-related illness? YES NO  
    If yes, please list: \_\_\_\_\_  
\_\_\_\_\_
10. Have you EVER been diagnosed with impaired fasting glucose, impaired glucose tolerance, or diabetes? YES NO  
    If yes, please list: \_\_\_\_\_

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11. Have you had any stomach problems such as ulcers? YES NO  
If yes, please list: \_\_\_\_\_

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12. When you experience a cut do you take a long time to stop bleeding? YES NO

13. Do you develop bruises easily? YES NO

14. Have you ever been told that you have a heart problem? YES NO  
If yes, please list: \_\_\_\_\_

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15. Have you ever been told that you have a breathing problem? YES NO  
If yes, please list: \_\_\_\_\_

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16. Have you ever experienced a seizure? YES NO  
If yes, do you experience these regularly? YES NO

17. Have you ever been told that you have kidney problems? YES NO  
If yes, please list: \_\_\_\_\_

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18. Are there any other mental or physical health issues we should be aware of? YES NO

If yes, please list: \_\_\_\_\_

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**IV. Body Weight**

19. Has your body weight changed a lot in the past 6 months? YES NO  
If yes, please explain \_\_\_\_\_

20. Are you in the process of gaining or losing weight? YES NO  
If yes, would you be comfortable with maintaining your body weight for the duration of this study?  
YES NO

**V. Diet-Related Questions**

21. Do you currently use any alternative therapies [e.g. high garlic intake, see a Naturopath (ND)]?  
YES NO

If yes, please list type and how long you have used these products/therapies:

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22. Do you currently use any dietary supplements? YES NO

If yes, please list: \_\_\_\_\_

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23. Are you on a special diet? YES NO

If yes, please list: \_\_\_\_\_

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24. Are you a vegetarian? YES NO

If yes, do you eat dairy products? YES NO

If yes, do you eat eggs? YES NO

If yes, do you eat fish? YES NO

25. Do you consume alcohol? YES NO

If yes, how many drinks per week? \_\_\_\_\_

(1 drink = 12oz beer, 4oz wine, 1oz hard liquor)

26. Do you consume beverages with caffeine (e.g. coffee, coke) YES NO

If yes, how many times a week? \_\_\_\_\_

**VI. Study-Related Questions:**

27. Are you participating in any other research studies currently? YES NO

28. What days of the week would you be able to come in for study days?

MON TUES WED THURS FRI

Note we are trying our best to schedule the study visits during the week. If it is the case that you can only do weekends, we will try our best to accommodate that. Please discuss this with the study coordinator.

29. Why do you want to participate in this study?

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Subject's signature

Date

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Investigator's signature

Date

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**Appendix 3: Sample of Subject Consent Form used in study 1**

-Printed on University of Guelph letterhead-

**INFORMED CONSENT FORM**

**"Impact of breads on biomarkers for type 2 diabetes and cardiovascular disease"**

You are asked to participate in a research study being conducted by Dr. Terry Graham, a Professor from the Department of Human Biology and Nutritional Sciences at the University of Guelph. Dr. Graham is collaborating with Professors Arend Bonen, Alison Duncan, and Lindsay Robinson (Department of Human Biology and Nutritional Sciences), Rickey Yada (Department of Food Science), and James Rush (University of Waterloo) to conduct this study. This research project has been reviewed for its scientific merit and is being funded by a research grant from the Ontario Ministry of Agriculture and Food through the Food Program.

If you have any concerns about the research study please feel free to contact:

Trish Parsons (MSc. Student) at 824-4120 ext. 58081 or email her at [pparsons@uoguelph.ca](mailto:pparsons@uoguelph.ca)

Anita Mofidi (Ph.D. student) at 824-4120 ext. 58081 or email her at [amofidin@uoguelph.ca](mailto:amofidin@uoguelph.ca)

If you require any further information that Trish or Anita cannot provide please feel free to contact:

Dr. Terry Graham (Professor and Chair) at 824-4120 ext 56168 or email him at [terrygra@uoguelph.ca](mailto:terrygra@uoguelph.ca)

Dr. Lindsay Robinson (Assistant Professor) at 824-4120 ext 52297 or email her at [lrobinso@uoguelph.ca](mailto:lrobinso@uoguelph.ca)

Dr. Alison Duncan (Assistant Professor) at 824-4120 ext 53416 or email her at [amduncan@uoguelph.ca](mailto:amduncan@uoguelph.ca)

The purpose of the study is to determine whether ingestion of different types of breads result in changes in various biomarkers (risk factors) for diabetes and cardiovascular disease.

**Outline of Study:**

1. Preliminary Session
2. Preliminary Testing – OGTT
3. Experimental Trial – Four study days in total

If you volunteer to participate in this study, we would ask you to do the following things:

### Preliminary Sessions:

You (the subject) will be required to attend a pre-trial session to complete a Subject Screening Questionnaire, to have your weight and height measured, and to have your body composition assessed by bioelectrical impedance. This is a common, non-invasive method that involves sending a low-level, safe electrical current through the body. The current passes freely through the fluids contained in muscle tissue, but encounters resistance when it passes through fat tissue. This allows us to determine your percentage body fat. You will also be asked to read the **Study Information Sheet** and **Informed Consent Form** that identify the potential risks associated with participation in the study. You will also be asked to attend one preliminary testing sessions where you will undergo an oral glucose tolerance test (OGTT). This test involves coming to the laboratory after an overnight (12 hour) fast and ingestion of a glucose beverage. Blood samples will be taken at 0 and 120 minutes following the consumption of the glucose beverage. This information will be used to assess your metabolic response to the carbohydrate ingested.

### Experimental Trials:

#### A. Study Requirements:

On four occasions, separated by at least a one week period, you will have to perform the following experimental protocol:

- ✓ Abstain from alcohol, caffeine, and exercise for 48 hours prior to each study day
- ✓ Abstain from all supplements unless told otherwise by investigators for the entire study
- ✓ Abstain from using any products that contain ASA (aspirin) and/or ibuprofen for the entire study
- ✓ You will need to keep detailed **dietary records** for **3 days** prior to each study day.
- ✓ The night before each study day you will eat the **dinner meal provided** for you and then you will not eat anything **after 8pm**
- ✓ You will report to the laboratory after an **overnight (12 hour) fast**.
- ✓ Each trial will be administered in a randomized, manner, meaning that the order of the test breakfast meals will not be the same for all subjects.

#### B. Study Days:

During the four separate study days you will complete the following protocol:

- a. Report to the Animal Science and Nutrition Building (ANNU) Room **304** (Human Testing Laboratory) after an overnight (12 hour) fast
- b. You will meet with an investigator who will check that your 3-day diet record is completed correctly
- c. You will have a venous catheter inserted into a vein by a fully-trained technician, to allow for blood collection throughout the day
- d. A blood sample will be taken from the catheter for the baseline measurement (1 sample)

- e. You will ingest a carbohydrate-rich breakfast meal of bread and water
- f. Blood samples will be taken from the catheter at 15, 30, 60, 90, 120 and 180min after bread consumption (6 samples)
- g. After three hours a high-glycemic LUNCH will be consumed. This lunch will consist of a 6" submarine sandwich from Subway restaurants and 300ml of Orange Juice
- h. Blood samples will be taken from the catheter at 30, 60 and 120 minutes following the lunch meal (3 samples in total)
- i. 2 hours after the Lunch meal, the venous catheter will be removed and new 3-day diet record sheets will be given to you
- j. You will confirm the next date for your next study day

#### Blood sampling:

A medically trained and approved technician will place a small catheter in a vein in your forearm. The Teflon catheter is inserted with the assistance of a small needle and subsequently the needle is removed. The discomfort of this procedure is transient and is very similar to having an injection by a needle; once the needle is removed there should be no sensation from the catheter. The insertion of a venous catheter is a very common medical practice and it involves few risks. However, there may be internal bleeding from the vein if adequate pressure is not maintained on the insertion point upon removal of the catheter. This may cause bruising and some minor discomfort. On rare occasions, injury of the internal wall of the vein may occur causing the formation of a small blood clot, which will normally dissolve soon after the experiment. There is a remote chance of infection in the area of the venipuncture, although this is very rare.

In any one trial we will take a total of 10 blood samples, which amounts to a total blood loss of approximately 180 mL. This amount is less than the blood loss that occurs during blood donation (approximately 330 mL). It is not enough of a blood loss to affect your physical performance and function in any way. After a blood sample is taken, the catheter is flushed with a sterile saline solution. This is a salt solution similar to your own plasma and it will help to prevent blood from clotting in the catheter. The blood samples and the associated data will not be used for any purposes other than those outlined in the study. You will have complete access to your data as well as the summary data that represents all of the subjects in the study.

#### C. Pain medication

If at all possible any use of ASA (aspirin) should be avoided for the entire time that you (the subject) are participating in this investigation. If a pain medication is needed during the time you are participating in this study (i.e. for a headache, etc) an acetaminophen (Tylenol) product is suggested.

#### Time Commitment:

This study involves a large time commitment and each subject should give serious consideration to all that is involved before making a decision as to whether or not they want to participate. You (the subject) will be expected to come to the laboratory a total of six times, ideally over a 6-7 week period. The six visits will include one initial session of 1-2 hours, one preliminary sessions (ideally separated by one week) of 2-3 hours (oral glucose tolerance test) and four experimental trials (ideally once per week for four weeks) of 6-7 hours each. During this time, you will remain in the laboratory resting or sitting quietly. You may read, watch movies (laboratory is equipped with TV/VCR), or bring a laptop to work on, but you will not be allowed to leave the laboratory during this time. You will be required to come early in the morning (typically 8 am) and you will be at the laboratory until about 3 pm each time. To compensate you for your time and effort we will give you an honourarium of \$320 upon completion of the study. Your decision to be a subject in this study is voluntary and you are free to withdraw from the study at any time, including during an experimental trial, without consequences of any kind (should this occur, you will receive a portion of the honourarium based on the amount of time you participated in the study). You have the option to withdraw your data from the study. The investigators may withdraw you from the study if circumstances arise that warrant doing so, for example when you cannot attend the necessary sessions or wish not to participate in some of the procedures in the study.

The benefits of the study do not accrue directly to you as an individual. The study is designed to determine how different types of carbohydrates that are common in the diet impact on metabolic responses and biomarkers (risk factors) for diabetes and cardiovascular disease and this in turn may have implications for designing nutritional strategies for optimal health.

Every effort will be made to ensure confidentiality of any identifying materials obtained during the study. Samples and data are coded immediately and are stored in password protected computer files in this manner. Thus your individual data is not identifiable with your name. Any results published or presented will always be done using group data; no individual data will be presented.

You may withdraw your consent at any time and discontinue participation without penalty. You will receive remuneration (an honourarium) in the amount of \$320 for your participation in the entire study (6 visits to the laboratory in total). However, if you choose to withdraw from the study at any time, you will receive remuneration for the amount of time that you participated in the study. For example, you will receive \$20 for participation in the initial pre-test sessions and \$75 for each of the four experimental trials. You are not waiving any legal claims, rights or remedies because of your participation in this research study.

This study has been reviewed and received ethics clearance through the University of Guelph Research Ethics Board. If you have questions regarding your rights as a research participant, please contact:

Research Ethics Officer

University of Guelph

437 University Centre

Guelph, ON N1G 2W1

Telephone: (519) 824-4120 ext 56606

Email: [sauld@uoguelph.ca](mailto:sauld@uoguelph.ca)

FAX: (519) 821-5236

I have read the information provided for the study “Impact of breads on biomarkers for type 2 diabetes and cardiovascular disease” as described herein. My questions have been answered to my satisfaction, and I agree to participate in this study. I have been given a copy of this form.

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(Name of participant *(please print)*)

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(Signature of participant)

Date

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(Name of witness *(please print)*)

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(Signature of witness *(please print)*)

Date

Appendix 4: Calculations for Determination of Daily Bread Dose

	<b>MEN</b>	<b>WOMEN</b>
<b>Recommended food guide servings/day for Grain Products</b>	8 servings	6-7 servings
<b>Total Grams of Bread Required per day</b>	8 servings x 35g per food guide serving x 65% grain product servings  = 182 g of bread per day	6-7 servings x 35g per food guide serving x 65% of grain product servings  = 137 – 160 g/d (average 149g/d)
<b>Quantity of White Bread per day</b>  <b>(37.5g/slice)<sup>1</sup></b>	=182 g per day / 37.5g per slice  = 4.8 slices per day <b>(round up to 5 slices per day)</b>	=149 g per day / 37.5g per slice  = 3.98 slices per day <b>(round up to 4 slices per day)</b>
<b>Quantity of 11-Grain Bread per day</b>  <b>(25g/slice)<sup>2</sup></b>	=182 g per day / 25 g per slice  = 7.3 slices per day  <b>(round down to 7 slices per day)</b>	=149 g per day / 25 g per slice  = 6 slices per day  <b>(6 slices per day)</b>

<sup>1</sup> As indicated by manufacturer (Stone-mill Bakehouse).

<sup>2</sup> As indicated by manufacturer (Weston Bakeries Limited)

**Appendix 5:** Sample of Study Diary used in study 3

## STUDY DIARY

Date	Time	No. of slices of bread	How I ate the bread	Exercise	Health Notes ** please note illness; medication changes; new prescriptions; etc**
1					
2					
3					
4					

**Appendix 1:** Sample of Food Record Sheet used in study 3

<b>Meals</b>	<b>Time</b>	<b>Description or food, drink, condiments</b>	<b>Portion</b>	<b>How was it prepared</b>
<b>B*reakfast</b>				
<b>am snack</b>				
<b>Lunch</b>				
<b>Supper</b>				
<b>Evening snack</b>				