POSTPRANDIAL METABOLIC RESPONSES TO MACRONUTRIENT IN HEALTHY, HYPERINSULINEMIC AND TYPE 2 DIABETIC SUBJECTS

by

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A thesis submitted in conformity with requirements for the degree of Doctor of Philosophy, Graduate Department of Nutritional Sciences, Faculty of Medicine, University of Toronto

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TYPE 2 DIABETIC SUBJECTS

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Abstract

The literature comparing macronutrient metabolism in healthy and diabetic subjects is abundant; however, little data exists on how non-diabetic subjects with insulin resistance handle macronutrient. We did two studies to investigate the postprandial responses to macronutrient in healthy, hyperinsulinemic and type 2 diabetic (T2DM) subjects.

In the first study, twenty-five healthy, non-diabetic subjects [9 with fasting serum insulin (FSI) < 40 pmol/L; 8 with 40 ≤ FSI < 70 pmol/L; and 8 with FSI ≥ 70 pmol/L] were fed eleven test meals (50g oral glucose with 0-30g doses of canola oil or whey protein) after an overnight fast. There were no significant FSI × fat (p=0.19) or FSI × protein (p=0.08) interaction effects on glucose response, suggesting that the effects of fat or protein on glycemia were independent of FSI of the subjects. In addition, the changes in relative glucose response per gram of fat (r = -0.05, p = 0.82) or protein (r = -0.08, p = 0.70) were not related to FSI of the subjects.
In the second study, Healthy (FSI < 40pmol/L), Hyperinsulinemic (FSI ≥ 40pmol/L), and T2DM were fed five foods with 50g available carbohydrate. Among the subject-groups, the Glycemic Index (GI) values were not significantly different for each food, and the mean (±SEM) GI values of all foods were not significantly different (p>0.05). However, the mean (±SEM) Insulinemic Index of the foods was higher in T2DM (100±7, n=10) than those of Healthy (78±5, n=9) and Hyperinsulinemic subjects (70±5, n=12) (p=0.05). The Insulinemic Index was inversely associated with insulin sensitivity (r=-0.66, p<0.0001), positively related to fasting- and postprandial-glucose (both r=0.68, p<0.0001) and hepatic insulin extraction (r=0.62, p=0.0002).

The oral-glucose data were pooled from the two studies to investigate whether there was any relationship between GLP-1 and insulin sensitivity, β-cell function and hepatic insulin extraction. No significant correlation was observed (p>0.05).

The results suggest that the glucose-lowering effect of fat and protein is not affected by insulin sensitivity. GI is independent of the metabolic status of the subjects; however, unlike GI, Insulinemic Index is influenced by the metabolic status of the subjects, and thus may have limited clinical utility.

Abstract Word Count: 349
To My Baby, My Little Love
Taras (Dundun, 敦敦)

献给妈妈的最爱
敦敦
ACKNOWLEDGEMENTS

As I draw near the completion of the Ph.D program, Qu Yuan’s poem (ca. 340 BCE – 278 BCE) keeps resounding in my ears “the road ahead is long and rugged; I see no ending; yet high and low I’ll search with my will unbending (路曼曼其修远兮, 吾将上下而求索)”. I dare not compare the Ph.D journey to Qu Yuan’s lofty pursuit of truth, beauty and ideal political ambition, nor by no mean am I using the ancient poet to elevate the status of successfully completing a Ph.D. At this moment of embarking on a new academic journey, I could find no better way to describe the current state of my mind. Looking back at the road travelled, I am in deep gratitude to so many people.

I would like to thank the many participants involved in the two clinical studies, the nurses from the Risk Factor Modification Center at St.Michael’s Hospital, the staff and fellow students of Dr.Wolever’s Lab, the staff at St.Michael’s Core Laboratory and Banting and Best Diabetes Core Laboratory. Special thanks also go to my three research assistants, Michelle Liu, Jonathan Leung, and Cindy Huang. They not only helped me run the studies but also offered joyful conversation and friendship.

I also would like to thank Dr. Deborah O’Connor and Dr. Lindsay Robinson (University of Guelph) whom gave insightful and critical appraisal of my thesis. My committee members: Dr. Harvey Anderson, Dr. Anthony Hanley and Dr. Qinghua Wang who gave me so many constructive suggestions and such good guidance on numerous occasions.

My greatest gratitude goes to my supervisor Dr.Wolever, who has constantly offered me support and guidance. His scientific ingenuity and passion for research I greatly admire.
Family and friends have supported me in various ways. My mother-in-law and sister-in-law helped to take care of my baby while I was in Toronto for various meetings. My father-in-law encouraged me in my academic pursuit. The delightful friendship with Derong, Suchang, Xuefei and Yixin give me much-needed escape from the perils of graduate student life.

It is not possible to complete this thesis without the unconditional love of my parents who are so proud of me. They have always been there for me even though I lived across the ocean from them. My parents made a long hard trip from China to U.S to take care of me and my new-born so I would be able to concentrate on my thesis.

I would not be what I am today without the devoted support of my beloved husband, Ihor, a gentleman and scholar, without whom, there is nothing…

This thesis is dedicated to my baby boy Taras (Dundun), who I carried throughout the laborious process of thesis writing, and has been the joy, hope and light of my life since his birth.
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<td>Analysis of covariance</td>
<td>ANCOVA</td>
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<td>Analysis of variance</td>
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<td>Area under the curve</td>
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<td>Blood pressure</td>
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<td>BMI</td>
<td>Body Mass Index</td>
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<td>Carbohydrate</td>
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<td>Cholecystokinin</td>
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<td>Coefficient of variation</td>
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<td>C-reactive protein</td>
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<td>Fasting serum insulin</td>
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PUBLICATIONS AND PRESENTATIONS ARISING FROM DISSERTATION

Peer reviewed publications


Presentations

28th International Symposium on Diabetes and Nutrition of the Diabetes and Nutrition Study Group (DNSG) of the EASD, July 1-4, 2010, Oslo, Norway. “Are the glycemic and insulinemic index values of carbohydrate foods the same in normal, hyperinsulinemic and type 2 diabetic patients?”

The 69th Scientific Session of American Diabetes Association. New Orleans, LA, USA. June 5-9, 2009. “Attenuated GLP-1 response after oral glucose is associated with reduced β-cell function in humans” and “The postprandial insulin-raising effect of protein is not due to increased insulin secretion but due to reduced hepatic insulin extraction”.

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CHAPTER 1

INTRODUCTION
1. Introduction

High postprandial blood glucose concentrations, even within the non-diabetic range [i.e. impaired glucose tolerant (IGT) subjects with 2-hour postprandial glucose between 7.8 and 11.0 mmol/l in the 75g oral glucose tolerance test (1)], are associated with increased risk of cardiovascular disease (2), diabetes (3) and cancer (4). In order to maintain the blood glucose concentration within a narrow physiological range, an intricate system of neural, hormonal and direct nutrient responses are initiated after a meal, which are all mediated in varying degrees by macronutrient (carbohydrate, fat and protein) (Figure 1.1); however, in individuals with obesity, diabetes and insulin resistance, the physiological mechanisms elicited by the macronutrient may become abnormal, thereby disrupting substrate use and glucose homeostasis (Figure 1.2). Many studies have compared the postprandial metabolic responses elicited by macronutrient in healthy and diabetic subjects; however, less attention has been paid to non-diabetic subjects with insulin resistance/ hyperinsulinemia.

Studies comparing the acute effects elicited by macronutrient in healthy and diabetic subjects have demonstrated that in the latter, a series of metabolic responses to macronutrient is adversely affected. For example, the glucose-lowering effects of fat and protein were attenuated or absent in diabetic subjects (5, 6) and insulin secretory response following carbohydrate ingestion or a mixture of carbohydrate and protein hydrolysate was substantially impaired in type 2 diabetes compared to the control subjects (6). Furthermore, the secretion and action of gut hormones such as cholecystokinin (CCK), peptide YY (PYY), glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) may also be attenuated as well in diabetic subjects. For instance, the meal-induced increase in CCK was lower in type 2 diabetic subjects than in the control (7) and the circulating PYY response to a meal was blunted in people
with type 2 diabetes compared to BMI-matched control subjects (8). These various abnormalities of metabolic response to macronutrient in diabetic subjects suggest that the same may occur in non-diabetic subjects with insulin resistance/hyperinsulinemia. Therefore, it is important to understand the impact of insulin resistance/hyperinsulinemia on macronutrient metabolism.

Insulin resistance is a pathologic state in which the target cells fail to respond or have reduced sensitivity to metabolic actions of normal levels of circulating insulin (9). It is well known that beside β-cell failure, insulin resistance is the major pathophysiological event contributing to the development of type 2 diabetes mellitus (10-12). Further, insulin resistance is tightly associated with major public health problems such as obesity, hypertension, coronary artery disease, dyslipidemia and a cluster of metabolic and cardiovascular abnormalities that define the metabolic syndrome (13, 14). Therefore, it is of great importance to understand the impact of hyperinsulinemia/insulin resistance on macronutrient metabolism, specifically whether the degree of insulin sensitivity of the subjects influences the effects of macronutrient (carbohydrate, fat and protein) on acute postprandial glucose and insulin responses.

The blood glucose raising potential of carbohydrates in foods is numerically classified as Glycemic Index (GI), which is calculated as the incremental area under the blood glucose response curve (AUC) for each food expressed as a percentage of the area after taking the same amount of carbohydrate as glucose (15,16). Many foods have been tested for their GI values; however, this is usually done either in healthy or diabetic subjects (17). Though GI values of foods did not differ significantly among a group of heterogeneous subjects with diabetes (18) and were similar in healthy and diabetic subjects (19-21), the GI of foods has not been determined in non-diabetic, insulin resistant/hyperinsulinemic subjects, a population highly susceptible to
diabetes. It is not known whether higher fasting insulin concentration and higher postprandial insulin response of the subjects affect the Glycemic Index, thus its validity and clinical utility in the prevention and management of diabetes.

Chronic hyperinsulinemia plays a significant role in the pathogenesis of insulin resistance and associated chronic diseases (22-27); therefore, the extent of insulin response elicited by food is as important as that of the glucose response. Some investigators have expressed concern that the GI does not adequately address concurrent insulin response (i.e. lack of close association between GI and Insulinemic Index), therefore, they have began to report values for Insulinemic Index (28-30). Insulinemic Index is calculated similarly to GI so as to measure the extent to which the available carbohydrate in food raises plasma insulin (31). The plasma insulin response elicited by a meal is not only glucose-dependent but also tightly connected with the normal function of gastrointestinal hormones (i.e. GLP-1, GIP, and CCK), the activity of the enteroinsulin axis, β-cell function and hepatic insulin extraction. All of these may be abnormal in individuals with obesity, diabetes and insulin resistance/hyperinsulinemia.

Adding fat and/or protein to carbohydrate food reduces blood glucose response compared to carbohydrate alone (32-34); The exact mechanisms associated with the hypoglycemic effect of fat and protein are not clear, though they are suggested to be through similar mechanisms such as delaying gastric emptying (35) and/or enhancing insulin secretion through augmented GIP and GLP-1 secretion (36); however, a study in mice found that glucose-induced incretin hormone release was differently modulated by fat and protein (37). In the same study, it was also found that insulin response to glucose was 3-fold augmented by protein, and was associated with enhanced oral glucose tolerance, whereas, the addition of fat resulted in only a 1.5 fold increase in insulin with no accelerated glucose disposal (37). This is consistent with the result of a human
study that protein reduced glucose response 2 to 3 times more than fat, and there was no
significant fat \times protein interaction (38). A recent study in rats found that upper small intestinal
lipids activate a gut-brain-liver neural axis to inhibit liver glucose production and decrease
plasma glucose level (39); however, it is not known whether protein also exerts similar effects.
Taken together, both animal and human studies suggest that fat and protein may modulate
postprandial glucose response through different mechanisms.

In summary, there is evidence that the acute effects of macronutrient (carbohydrate, fat
and protein) on glucose and insulin responses are adversely affected in patients with diabetes
compared to healthy subjects; however, it is not known whether the same occurs in non-diabetic
subjects with insulin resistance/hyperinsulinemia. In addition, the underlying mechanisms
associated with the hypoglycemic effects of fat and protein are unclear. In terms of the clinical
utility of GI and Insulinemic Index, it remains to be determined whether insulin
resistance/hyperinsulinemia modifies GI and Insulinemic Index, and whether GI and Insulinemic
Index are valid measures of the biological effects of carbohydrate foods in all conditions
regardless of subject’s glucose tolerance status or degrees of insulin sensitivity.
Figure 1.1 The mechanisms regulating postprandial glycaemia in healthy subjects. Many substances in foods can affect postprandial glycaemic response. The major ones are carbohydrate, fat and protein. Blood glucose rises after meal ingestion. In order to maintain glucose homeostasis, the human body orchestrates both the peripheral and central nervous system (CNS) to lower postprandial glucose levels. Three major pathways have been identified: first, glucose stimulates pancreatic β-cell secretion of insulin. Insulin regulates glucose concentration by acting upon the peripheral organs (i.e. insulin inhibits free fatty acids release from adipose tissue, inhibits liver glucose production, and stimulates both skeletal muscle and adipose tissue uptake of glucose). The second pathway is through gastrointestinal hormones such as GLP-1, GIP, CCK and PYY, which work either by delaying gastric emptying, inhibiting food intake, or through incretin mediated glucose-dependent insulin secretion. The third pathway is that upper intestine lipids [long chain fatty acid (LCFA) is the main metabolite of dietary fat digestion, and LCFA-CoA is the metabolite of LCFA] activate intestine-brain-liver neural axis to increase liver insulin sensitivity and reduce hepatic glucose production (39). This pathway, though well demonstrated in animal models, is extremely difficult to show in humans. Nevertheless, through the concerted efforts of these different pathways, the glucose level goes down.
Figure 1.2 The mechanisms regulating postprandial glycemia are disrupted in diabetes, obesity and insulin resistance. For example, the subjects may have altered release pattern of gut hormones or there is a reduced effect of gut hormones on gastric emptying, food intake and insulin secretion, or they may have acquired deficits in nutrient sensing (i.e. in diabetes and obesity, nutrient-sensing mechanisms in both the gut and the brain are disrupted, leading to a disregulation of glucose levels), or insulin loses its ability to carry out the functions such as inhibition of free fatty acids release from adipose and suppression of hepatic glucose production. Because of these disruptions, the blood glucose remains elevated.
CHAPTER 2

LITERATURE REVIEW
2. 1 Effects of Macronutrient on Postprandial Glucose and Insulin Responses

2.1.1 Dietary Carbohydrate

Dietary carbohydrate is the main dietary component affecting postprandial blood glucose and insulin responses (40). In addition, carbohydrate foods provide energy, water-soluble vitamins and minerals, and fiber. It is recommended that diets provide 45-65% of calories from carbohydrate, with a minimum intake of 130g carbohydrate/day for adults (41).

The important dietary carbohydrates consist of monosaccharide (i.e. glucose, fructose [a component of sucrose and also presents as a monosaccharide in fruits] and galactose [a component of lactose or milk sugar]), disaccharides (pairs of monosaccharide linked together, such as maltose, sucrose and lactose), oligosaccharides (chains of 3 to 10 glucose or fructose polymers), and polysaccharides (i.e. starch and dietary fiber, which consists of long chains of glucose or other monosaccharide molecules).

Dietary carbohydrates influence glucose and insulin responses through at least 4 major mechanisms: 1) the type of monosaccharide absorbed; 2) the amount of carbohydrate ingested; 3) the rate of carbohydrate digestion and absorption; and 4) the colonic fermentation (42). It is recognized that both the type and amount of carbohydrate in a food influence the postprandial glucose and insulin responses (43).

1) The type of monosaccharide absorbed

The major monosaccharides absorbed are glucose, galactose and fructose. However, glucose is the only monosaccharide that appears in blood to any large extent (5-7 mmol/L) whereas neither fructose (44) nor galactose (45) raises plasma glucose or insulin appreciably.
The differences in glucose and insulin raising potential of the monosaccharides (glucose, fructose and galactose) resulted from their uniquely different metabolism. Take fructose for example. Unlike glucose, fructose stimulates only modest insulin secretion and does not require the presence of insulin to enter cells (46). In addition, fructose is rapidly utilized by the liver and it bypasses the early, rate-limiting steps of glucose metabolism and is rapidly converted to fructose-1-phosphate, which is subsequently converted to lactate, glucose, and glycogen (47). Studies in both healthy and diabetic subjects demonstrated that fructose produces smaller postprandial increases in plasma glucose and insulin responses than other common carbohydrates (48-51); however, adding fructose (or sucrose) in large amounts to the diet is not recommended because it adversely affects serum lipids and raises serum triglycerides (52, 53). These undesirable effects may be due to lower insulin excursion after fructose, which results in less activation of adipose tissue lipoprotein lipase and impaired triglyceride clearance (51).

2) The amount of carbohydrate ingested

Plasma glucose and insulin concentrations increase as the amount of carbohydrate consumed increases. In healthy subjects, the AUC of plasma insulin increases linearly as the dose of carbohydrate ingested increases from 0 to 100 g; however, the shape of the dose-response curve for blood glucose is non-linear, with the curve flattening off as the dose of carbohydrate increases, particularly over 50g (44, 54). The shape of dose-response curve for glucose is very similar for healthy (54) and diabetic subjects (55).

3) The rate of carbohydrate digestion and absorption
The glycemic impact of carbohydrate foods is thought to be determined by the rate at which they are digested and absorbed (15, 56). There are three lines of evidence to support this: first, the rate of liberation of the carbohydrate products of digestion in vitro over 3-5 hrs is closely associated with postprandial blood glucose response in vivo (57); second, a human ileostomy model showed that carbohydrate malabsorption was insufficient to account for the reduced glycemic response (58); third, consuming carbohydrate slowly mimics the metabolic responses elicited by slowly digested carbohydrate foods (59, 60).

Numerous factors influence the digestion and absorption of carbohydrates in the small intestine: type of starch (amylose vs. amylopectin) (61), style of preparation (i.e. cooking method and time, amount of heat and moisture used, degree of processing)(62-64), the physical form of foods (i.e. juice vs. whole fruit, mashed potato vs. whole potato)(43), amount of fiber, coingestion of fat and proteins (65, 66), presence of antinutrients such as α-amylase inhibitors and phytic acid (67, 68), and transit time (rapidly digested starch vs. slowly digested starch )(69).

The rate and extent of starch digestion in vitro is commonly measured using the Englyst method (69). Using controlled enzymic hydrolysis with pancreatin and amyloglucosidase, the Englyst procedure measures rapidly digestible starch, slowly digestible starch and resistant starch in carbohydrate foods. The released glucose is measured by colorimetry, using a glucose oxidase kit (69). This technique also gives a value for rapidly available glucose, which includes rapidly digestible starch, free glucose and the glucose moiety of sucrose (70). Using this technique, Englyst et al measured the rapidly available glucose of 39 carbohydrate foods, and found that the reported GI values were positively associated with the measured rapidly available glucose \( r=0.76, p<0.001 \) (70). This finding further confirms the concept that GI allows foods to be ranked on the basis of the rate of digestion and absorption of the available carbohydrates.
4) Colonic fermentation

Carbohydrates resistant to digestion and those that escape absorption in the small intestine enter the colon where they are fermented with the production of hydrogen, methane and short chain fatty acids. The short chain fatty acids consist primarily of acetate, propionate and butyrate (71, 72). The colonic fermentation of the undigested carbohydrates can be assessed by measuring breath hydrogen and methane (73), or through the more quantitative method by measuring acetate kinetics with $^{13}$C-labelled acetate (73, 74).

The short chain fatty acids influence systemic glucose and lipid metabolism via direct or indirect mechanisms. For example, though acetate has no direct effect on glucose metabolism (75), it can reduce blood glucose in the long term by reducing serum free fatty acids concentrations (76, 77); however, colonic propionate is a gluconeogenic substrate that raises blood glucose (78) but inhibits the utilization of acetate for cholesterol synthesis (78).

2.1.1.1 Glycemic Index

The concept of Glycemic Index (GI) was founded on the notion that slowly digested carbohydrates may reduce the metabolic risk factors associated with diabetes and coronary heart diseases (79). The GI is a measure of carbohydrate quality. It ranks carbohydrate foods by measuring the extent to which available carbohydrate in foods raises blood glucose relative to that of oral glucose (15). The alternative measure of glycemic response to carbohydrate-containing foods is glycemic load (GL), the product of dietary GI and the amount of available dietary carbohydrate, which combines both the qualitative and quantitative measures of carbohydrate and reflects the overall glycemic impact of the diet (80).
Since its conception decades ago, the GI has elicited much controversy and its clinical relevance has been questioned and vigorously debated (34, 81-84). One of the major criticisms of GI is that it does not apply in mixed meals because of the confounding effects of fat and protein on glycemic response (34, 85). Studies of GI in mixed meals yield conflicting results - some find the observed glycemic response to mixed meals were predicted by the GI and carbohydrate contents of the foods (86, 87) whereas others do not (85, 88, 89). Venn et al examined the studies on mixed meals, and concluded that summing the GI values of individual components of a meal does not reliably predict the observed GI of the meal as a whole (90). Furthermore, they argued that the association of low GI foods with a low glycemic response alone does not necessarily justify a health claim (90). The rationale being that low GI foods are usually naturally occurring and minimally processed carbohydrate containing cereals, vegetables and fruit, and these foods have nutritional qualities (i.e. phytochemicals, antioxidants) other than their immediate impact on postprandial glycemia as a basis to recommend their consumption (90). Out of concern for these limitations, the 2007 FAO/WHO Scientific Update on Carbohydrates in Human Nutrition cautioned that food selection should not be based on GI alone and suggested that “GI is perhaps most appropriately used to guide food choices when considering similar carbohydrate-containing foods, for example bread with a low GI may be preferable to a higher GI bread, with a resultant lower glycemic load (GL)”(91).

Despite the controversies over the clinical utility of GI, evidence is accumulating that low-GI or low-GL foods may play a role in the prevention and management of chronic diseases such as diabetes, coronary heart disease, and certain types of cancer. The most recent 2011 American Diabetes Association position statement states that “for individuals with diabetes, use of GI and GL may provide a modest additional benefit for glycemic control over that observed
when total carbohydrate is considered alone”(92). The following paragraphs illustrate the role of low-GI or low-GL diet in the prevention and management of chronic diseases such as diabetes, coronary heart disease, and certain types of cancer.

**GI and diabetes mellitus**

Several randomized clinical trials have reported that low GI diets were effective in controlling glycemia in diabetic subjects (93-99), but others have not confirmed this effect (100-102); therefore, to clarify the issue of the effect of low-GI diets in the management of diabetes, Brand-Miller et al conducted a meta-analysis of 14 randomized controlled trials to compare low-GI diets with conventional or high-GI diets in the management of type 1 and type 2 diabetes. The meta-analysis showed that glycated proteins were reduced by 7.4% and hemoglobin A1C (HbA1c) by 0.43% more on the low-GI diet than on the high-GI diet. This result is clinically significant and is on par with that achieved by pharmacological agents (103). Recently, a meta-analysis of 37 prospective cohort studies found that diets with a high GI or GL independently increased the risk of type 2 diabetes (GI RR=1.40, 95% CI:1.23, 1.59; GL RR=1.27, 95% CI:1.12, 1.45)(104).

In the most recent Cochrane review (11 randomized control trials involving 402 participants), the authors compared low- and high-GI diets for people with either type 1 or type 2 diabetes mellitus. They found a significant reduction in HbA1c (weighted mean difference of -0.5%, 95% CI: -0.9, -0.1, P=0.02); moreover, the episodes of hypoglycemia were significantly fewer in low- compared to high-GI diet (105). Recently, an updated version of a Cochrane review including 12 randomized control trials and 612 participants found that low-GI diets lowered % HbA1c levels by 0.4% (95% CI: -0.7, -0.20, P=0.001) compared with the comparison
diets (high-GI diet, carbohydrate exchange diet, and high-cereal fiber diet)(106). The reduction of 0.4–0.5% HbA1C is clinically significant and is comparable to decreases achieved through medications for newly diagnosed type 2 diabetes (107, 108).

Two plausible mechanisms linking the development of type 2 diabetes with high-GI diet have been proposed (109). First, high-GI foods produce higher blood glucose concentrations and a greater demand for insulin. Chronic hyperinsulinemia may eventually result in pancreatic β-cell failure as the result of overstimulation, consequently, impaired glucose tolerance and frank diabetes. Second, high-GI diet may result in insulin resistance because it directly leads to postprandial hyperglycemia, counter-regulatory hormone secretion and increased late postprandial serum free fatty acids.

**GI and coronary heart disease**

A low-GI diet also has implications in the prevention of coronary heart disease. Clinical, metabolic and epidemiological studies have unanimously demonstrated that low-GI foods may favorably affect metabolic predictors of coronary heart disease. In a recent 6-month randomized parallel trial comparing the effect of low-GI diet vs. a high-cereal fiber diet on type 2 diabetes, low-GI diet increased high-density lipoprotein (HDL) cholesterol by 1.7mg/dl, and dietary GI was negatively related to HDL concentration (r=-0.19, p=0.009)(110). In a metabolic study, higher GI scores were associated with higher fasting triglycerides and lower HDL-cholesterol levels (111). In epidemiological studies, the British Adults Study demonstrated a significant negative relation between serum HDL-cholesterol concentration and the GI of the diet for both men and women (112), and the Nurse’s Health Study showed a positive relation between GI and fasting serum triglycerides (113).
In a recent systematic analysis of prospective cohort studies and randomized trials investigating dietary exposures in relation to coronary heart disease, the authors implemented Bradford Hill criteria to derive a causation score and identified strong evidence that there is a causal association between coronary heart disease and the consumption of high-GI diet (114).

**GI and cancer**

The insulin/colon cancer hypothesis suggests that diets with a high glycemic response and consequent hyperinsulinemia may play a role in colorectal carcinogenesis (115). The biologically plausible mechanism being that the diets with high-GI and/or high-GL may influence cancer risk via hyperinsulinemia and insulin-like growth factor axis, the major determinants of proliferation and apoptosis (116). However, epidemiological studies investigating the association between dietary GI and GL and the risk of cancers of digestive tract have yielded inconsistent findings (117). In addition, a recent meta-analysis did not find any association between dietary GI/GL and colorectal or pancreatic cancer risk (118).

For the role of GI/GL in the risks of breast cancer, the findings are conflicting as well. A recent prospective cohort study of Swedish women (n=61433, mean follow-up of 17.4 years) showed a significant positive relationship between both high-GI and high-GL diets and the risk of developing breast cancer (119); however, in the Shanghai women’s health study, a population-based cohort study (n=74942, mean follow-up of 7.35 years), only diets with high-GL were found associated with breast cancer risk in premenopausal women (<50 yr old). GI was not found to be associated with breast cancer risk, which was ascribed to the narrow range and centered distribution of the observed GI values (120). Even the meta-analyses show inconsistent results: two meta-analyses did not find any significant association between GI/GL and the risk of
breast cancer (121, 122), whereas one meta-analysis of 37 prospective cohort studies found that diets with a high GI independently increased the risk of breast cancer, the rate ratio (RR) for the comparison of the highest with the lowest quartiles of GI is 1.08 (95% CI: 1.02, 1.16) (104).

The lack of significant association between GI/GL and cancer risks doesn’t warrant the rejection of the insulin/colon cancer hypothesis; in fact, the heterogeneity in epidemiological studies investigating the association between GI/GL and cancer risks may be due to the lack of specifically selected and validated dietary questionnaires to assess GI or GL, and thus may lead to under- or over-estimation of GI or GL values. Indeed, a recent study has revealed several methodological challenges in assigning GI values to food items using existing GI tables and dietary questionnaires that were not designed specifically for the study purpose (123).

2.1.1.2 Insulinemic Index

Because of the implication of raised plasma insulin in the pathogenesis of chronic diseases (22-27) and the concern that GI fails to consider the insulin response (124), some investigators have begun to report values for Insulinemic Index (28-30). Insulinemic Index is calculated in similar way as GI [the area under the blood insulin response curve for test food expressed as a percentage of the area after taking the same amount (50g) of carbohydrate as glucose] to measure the extent to which the available carbohydrate in foods raises plasma insulin (31). In general, there is a good correlation between the GI of the foods and their Insulinemic Index (r=0.69, p<0.001) (125), with the exceptions of items such as milk (126) and protein-rich foods (30), which elicited insulin responses that were disproportionately higher than their glycemic responses. Although statistically significant, the GI only explains about 25-50% of the variation in Insulinemic Index, which may be because protein or fat in a carbohydrate-rich meal
evokes additional or synergistic insulin secretion to contribute to the reduction in glycemia (127-130). Nevertheless, this lack of close association between GI and Insulinemic Index is one of the major criticisms of the GI (34) and the rationale for advocating using food Insulinemic Index to classify foods (131).

A recent study in lean young healthy subjects found that the observed insulin responses to mixed meals were strongly correlated with insulin demand predicted by the Insulinemic Index of component foods (131). Hence, the authors suggested using the Insulinemic Index of composite meals to estimate pre-prandial insulin doses for patient with type 1 diabetes (131). However, it is recognized the worsening defects of insulin secretion in diabetic subjects may prevent the detection of Insulinemic Index differences among foods (131). The bottom line is that for Insulinemic Index to be a valid property of a food and be applicable in a broader population, its values should be similar in different subjects regardless of their glucose tolerance status or degree of insulin sensitivity.

2.1.2 Dietary Fat

Numerous studies have demonstrated that adding fat to carbohydrate reduces the blood glucose response (129, 132, 133); however, adding a large amount of fat to diet is not advised due to its detrimental effects on lipid metabolism. For example, adding a large amount of sunflower oil (40g) to pasta initially delays postprandial glycemia, but 4 hours later, it leads to high levels of triglycerides, non-esterified fatty acids and insulin resistance (134).

It is proposed that adding dietary fat to carbohydrate attenuates postprandial glycemic response either through reduced rate of gastric emptying, resulting in a delay in carbohydrate absorption (129, 135, 136) or the incretin hormone effect, mainly GLP-1 and GIP mediated
glucose-dependent insulin secretion (35, 137). Recently, a new pathway of the lipid-mediated regulation of glucose homeostasis has been established. It was found that in rodents, upper intestinal lipids activate a gut-brain-liver neural axis to inhibit liver glucose production and decrease plasma glucose concentration (39).

The effects of fat on acute glycemic responses may differ in solid vs. liquid meals. For example, adding 0, 5, 10, 20 and 40g solid fat (canola margarine) to white bread reduced glycemic response in a dose-dependent, but non-linear manner (138). By contrast, adding 0, 5, 10 or 30g corn oil to oral glucose reduced glycemic responses in a linear manner (38).

Besides the physical form of fat, the fat source also differentially influences postprandial glucose responses. Plasma glucose after butter-containing meals was higher than after meals containing olive or corn oil (139). This may be due to differences in the ability of different types of fat to stimulate insulin (140) and GLP-1 secretion (140). For example, monounsaturated fatty acids stimulate much higher insulin secretion than saturated fatty acids (140), and fats rich in monounsaturated fatty acids (i.e. olive oil) induced higher GLP-1 concentration than butter (141). Another reason could be due to the differences in the proportion of fatty acids absorbed via the portal vein as opposed to chylomicrons which, in turn, may influence hepatic glucose output (134) and/or hepatic insulin extraction (142).

2.1.3 Dietary Protein

It is generally thought that adding protein to carbohydrate reduces glycemic responses by stimulating insulin secretion (127) and/or delaying gastric emptying (143). However, the reported effects of protein on postprandial glucose and insulin responses are inconsistent, and the magnitude of the response varies tremendously (127, 144-146). These variations do not seem to
be explained by subject groups since a variety of effects are seen in both healthy and diabetic subjects (147). Review of the literature suggests that the most likely explanations are the differences in digestion kinetics and the source of protein.

Dietary proteins are heterogeneous with regards to their rate of digestion and absorption (148), which affects insulin response to varying degrees. It has been demonstrated in both healthy (149) and type 2 diabetic subjects (150) that ingestion of whey protein (a fast protein, which is digested and absorbed quickly from the gut) with a medium calorie, high protein mixed meal resulted in greater postprandial amino acid concentrations and in a 13-40% greater β-cell response (evaluated as the rise in insulin, C-peptide, and proinsulin) than ingestion of casein (a slow protein). Gannon et al found that in type 2 diabetic patients, ingestion of 50g of cottage cheese resulted in significantly higher plasma insulin concentration compared to an equivalent amount of egg white (127), which was attributed to the relatively poor digestibility of egg white relative to cottage cheese (151).

Numerous studies have shown that different sources of protein resulted in different glucose and insulin responses (127, 150, 152, 153). The discrepancies may be either due to different amino acid composition of the proteins (153), different effect on gastric emptying (152) or differential effects of varying protein sources on incretin hormone secretion (154). For example, Van et al reported that in healthy subjects, a carbohydrate drink containing a mixture of free leucine, phenylalanine, and arginine produced a much larger insulinotropic effect than pea, whey hydrolysate, or intact casein, and the differences were attributed to the different amino acid composition of the proteins (153). Lang et al found that in healthy, normal-weight men, a mixed meal containing 50g soy protein elicited an earlier metabolic response (insulin, glucagon, and glucose) than an equivalent amount of casein, with gelatin presenting intermediate results (152).
It was suggested that the differences may be explained by modifications in the rate of gastric emptying (152) since it is well known that casein precipitates in the stomach and significantly reduces the rate of gastric emptying (155). For the varying effects of different types of protein on incretin hormone secretion, Nilsson et al showed that in healthy subjects, whey protein was a much stronger GIP secretagogue than other food proteins such as cod, gluten, and cheese (156). There is also evidence that whey protein induces higher GLP-1 response than casein (150).

2.2 Insulin Resistance and Compensatory Hyperinsulinemia

Insulin resistance refers to a reduced sensitivity to insulin-stimulated glucose uptake (9). It results in inability of insulin to provide normal glucose and lipid homeostasis, and is manifested as impaired suppression of hepatic glucose production (157), impaired glucose uptake by skeletal muscle (158), and disinhibited lipolysis in adipose tissue (159). In order to maintain glucose homeostasis, hyperinsulinemia occurs as an obligatory compensation for insulin resistance and is the hallmark of insulin resistance (160). Chronic hyperinsulinemia can also down-regulate insulin action, indeed, when hyperinsulinemia was induced experimentally over a 48-72 hr period at normal-glycemic conditions, the healthy subjects became insulin resistant (161).

Insulin resistance plays an important etiological role in the development of type 2 diabetes. In an individual with normal blood glucose concentrations, insulin resistance is usually associated with a compensatory increase in plasma insulin levels (hyperinsulinemia) (162). As long as pancreatic β-cells are able to augment their secretion of insulin sufficiently to compensate for insulin resistance, glucose tolerance remains normal (163). However, over time,
pancreatic β-cell begins to fail and can no longer compensate for insulin resistance, impaired glucose tolerance occurs, and overt type 2 diabetes develops (10-12).

Currently, there is disagreement over whether insulin resistance and hyperinsulinemia are the cosegregated metabolic traits and whether they play a different role in the pathogenesis of clinical phenotypes associated with the two abnormalities. Ferrannini et al measured insulin resistance (by euglycemic-hyperinsulinemic clamp) and insulin response to oral glucose tolerance test (OGTT) in a cohort of 1308 non-diabetic European subjects. They found that only 60% of the most insulin-resistant individuals (bottom quartile of insulin sensitivity) had the highest insulin response (top quartile of insulin response) (164). Henceforth, they suggested that insulin resistance and hyperinsulinemia may be two dis-associated entities and carry different pathogenic potential. However, Kim & Reaven argued that Ferrannini et al’s analysis did not provide any information on the percentage of individuals in quartiles of insulin response by quartiles of insulin sensitivity (165). Taking this issue into consideration, they found that insulin resistance and hyperinsulinemia are well correlated ($r=0.76$, $p<0.001$) and rarely exist in isolation from one another in a nondiabetic population; in addition, there were minimal differences in CVD risk factors between individuals with different insulin responses but within the same insulin sensitivity quartile (165). Therefore, Kim and Reaven suggested that from a pathophysiological point of view, it is better to view insulin resistance and compensatory hyperinsulinemia as one entity in nondiabetic individuals rather than using statistical method to discern which abnormality is caused by insulin resistance and which is caused by hyperinsulinemia (165).
2.2.1 Methods to measure insulin sensitivity/resistance

A number of direct, indirect and surrogate measurements of insulin sensitivity have been developed (Table 2.1). The direct measures of insulin sensitivity include insulin suppression test (166) and the “gold-standard” hyperinsulinemic-euglycemic glucose clamp, in which insulin is infused at a constant rate and glucose is held at basal levels by glucose infusion (167). The indirect measurement of insulin sensitivity includes the minimal model analysis of frequently sampled intravenous glucose tolerance test (168). These methods allow the measure of maximum insulin action and minimize the effect of non-insulin-mediated glucose uptake. However, the expense and technical difficulties associated with these methods render them rather impractical for routine screening. Due to that reason, a number of surrogate indices of insulin sensitivity/resistance based on fasting glucose and insulin such as homeostasis model assessment (HOMA) (169) and quantitative insulin-sensitivity check index (QUICKI) (170) have been developed. Though both methods have been widely used, their sensitivity and specificity in detecting insulin resistance has been questioned. A study compared the insulin sensitivity assessment indices (including HOMA and QUICKI) with euglycemic-hyperinsulinemic clamp data after a dietary and exercise intervention in older adults, and found that these indices vary substantially from the clamp method in their ability to assess insulin sensitivity (171). One reason could be that surrogate indices based on fasting glucose and insulin concentrations reflect primarily hepatic insulin sensitivity/resistance, and do not represent whole-body insulin sensitivity (172). In response to this, a number of oral-glucose-tolerance-test (OGTT) based indices of insulin sensitivity have been developed to evaluate whole-body insulin sensitivity. The most frequently used ones are: 1) Matsuda’s insulin sensitivity index calculated as $10,000/(\text{fasting glucose} \times \text{fasting insulin} \times \text{mean glucose} \times \text{mean insulin})^{0.5}$, which takes into
account both hepatic and muscle insulin sensitivity, and is highly correlated ($r = 0.73, p < 0.0001$) with the rate of whole-body glucose disposal during the euglycemic-hyperinsulinemic clamp (173); 2) Mari’s oral glucose insulin sensitivity based on a physiological glucose-insulin model, which is in good agreement with hyperinsulinemic-euglycemic glucose clamp ($r=0.77, p<0.0001$) (174). To differentiate the relative contribution of liver and muscle to whole-body insulin sensitivity, insulin resistance at the site of liver (hepatic insulin resistance) was calculated as the product of total AUC of glucose and insulin during the first 30 minutes ($\text{glucose}_{0-30}[\text{AUC}] \times \text{insulin}_{0-30}[\text{AUC}]$) (172) and muscle insulin sensitivity was calculated as the rate of decay of plasma glucose concentration from its peak value to its nadir divided by the mean insulin concentration (172). Both indices of hepatic insulin resistance and muscle insulin sensitivity derived from OGTT have been validated using hyperinsulinemic-euglycemic clamp (172).

Fasting insulin is often used as a surrogate measure of insulin resistance based on the finding that among non-diabetic subjects, high fasting insulin level is moderately well correlated with the euglycemic-hyperinsulinemic clamp (175) and minimal model techniques (176). However, since fasting insulin levels are determined not only by the degree of insulin sensitivity but also by pancreatic $\beta$-cell secretory function and hepatic insulin clearance, it is recognized that fasting insulin is not an exact surrogate measure of insulin resistance. Nevertheless, it is acknowledged that fasting insulin can be used as a simple, economical, and routine screening tool for subjects in large clinical or population-based epidemiological studies (177).
Table 2.1 Methods to measure insulin sensitivity/resistance

<table>
<thead>
<tr>
<th>Methods</th>
<th>Primary Organ</th>
<th>Formula</th>
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<tbody>
<tr>
<td><strong>Direct measure</strong></td>
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<tr>
<td>Hyperinsulinemic-euglycemic glucose clamp</td>
<td>Whole-body</td>
<td>For detailed procedure, see ref (167)</td>
</tr>
<tr>
<td>Insulin suppression test</td>
<td>Whole-body</td>
<td>For detailed procedure, see ref (166)</td>
</tr>
<tr>
<td><strong>Indirect measure</strong></td>
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<tr>
<td>Minimal model analysis of FSIVGTT</td>
<td>Peripheral &amp; Liver</td>
<td>For detailed procedure, see ref (168)</td>
</tr>
<tr>
<td>OGTT-derived indices</td>
<td></td>
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<tr>
<td>Matsuda’s insulin sensitivity index</td>
<td>Liver &amp; Muscle</td>
<td>$10,000/(\text{fasting glucose} \times \text{fasting insulin} \times \text{mean glucose} \times \text{mean insulin} )^{0.5}$ (173)</td>
</tr>
<tr>
<td>Mari’s oral glucose insulin sensitivity</td>
<td>Liver &amp; Muscle</td>
<td>For this calculation, go to: <a href="http://webmet.pd.cnr.it/ogis/index.php">http://webmet.pd.cnr.it/ogis/index.php</a> (174)</td>
</tr>
<tr>
<td>Hepatic insulin resistance</td>
<td>Liver</td>
<td>$\text{glucose}<em>{0-30}[\text{AUC}] \times \text{insulin}</em>{0-30}[\text{AUC}]$ (172)</td>
</tr>
<tr>
<td>Muscle insulin sensitivity</td>
<td>Muscle</td>
<td>$\frac{dG}{dt} ÷ \text{mean plasma insulin concentration}$ (172)</td>
</tr>
<tr>
<td>Fasting glucose/insulin based indices</td>
<td></td>
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<tr>
<td>Homeostasis model assessment (169)</td>
<td>Liver</td>
<td>For this calculation, go to: <a href="http://www.dtu.ox.ac.uk/homacalculator/index.php">www.dtu.ox.ac.uk/homacalculator/index.php</a></td>
</tr>
<tr>
<td>Quantitative insulin-sensitivity check index</td>
<td>Liver</td>
<td>$\frac{1}{\log(I_{0\mu U/mL}) + \log(G_{0mg/dL})}$ (170)</td>
</tr>
</tbody>
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FSIVGTT: Frequently sampled intravenous glucose tolerance test; G:glucose; I:insulin.
dG/dt: the rate of decay of plasma glucose concentration from its peak value to its nadir.
2.3 Insulin Resistance/Hyperinsulinemia and the Risk of Chronic Diseases

Recent years have witnessed rapidly increasing trends in insulin resistance and compensatory hyperinsulinemia. Li et al found that the prevalence of hyperinsulinemia increased by 35.1% overall (38.3% among men and 32.1% among women) in the past decade among non-diabetic U.S adults (age ≥ 20 years) based on the Third National Health and Nutrition Examination Survey (NHANES III, 1988-1994) and NHANES 1999-2002 data (178). Recently, the same research group also found that hyperinsulinemia was independently associated with pre-diabetes. In addition, the prevalence of pre-diabetes (impaired fasting glucose and/or IGT) was four times higher among adolescents (12-19y) with hyperinsulinemia, based on the most recent NHANES 2005-2006 data (179).

Insulin resistance/hyperinsulinemia is associated with increased risk of a cluster of chronic diseases. In 1988, Reaven suggested that insulin resistance, characterized by compensatory hyperinsulinemia, is the central element, and in large measure the cause of a cluster of related abnormalities including glucose intolerance, dyslipidemia and hypertension (180), which lead to a variety of clinical syndromes. For example, insulin resistance/hyperinsulinemia has been associated with increased risk of type 2 diabetes (180, 181), coronary heart disease (26, 182, 183), essential hypertension (184), congestive heart failure (185), polycystic ovarian syndrome (186), nonalcoholic fatty liver disease (187), Alzheimer’s disease (188) and cognitive decline (189) and cancers of certain sites such as prostate (190), colon and rectum (191) and breast (192).
2.3.1 Mechanisms of insulin resistance induced clinical syndromes

The mechanisms through which insulin resistance/hyperinsulinemia increased the risks of the aforementioned abnormalities and clinical syndromes are complicated and multifaceted. It is proposed that the series of events that lead to the abnormalities and clinical syndromes are in most instances, if not all, a result of differential tissue insulin sensitivity (14). The most important organs involved are probably skeletal muscle, adipose tissue, kidney and liver. There are a few lines of evidence to support this hypothesis: kidney retains normal insulin sensitivity in the presence of muscle and adipose tissue insulin resistance (193), and the elevated plasma insulin concentration enhances renal sodium reabsorption, thus contributing to the development of hypertension (194). The second example is the etiology for nonalcoholic fatty liver. It was suggested that peripheral insulin resistance (muscle and adipose tissue) led to increased lipolysis and delivery of free fatty acid to the liver, which remains insulin sensitive to stimulate hepatic triglyceride, thereby predisposing to the development of fatty liver (187). The third example is the pathogenesis of breast cancer. Insulin has been shown to reduce sex hormone binding globulin (SHBG) production in a hepatoma cell line (195), and in humans, insulin levels are inversely related to SHBG and SHBG-bound estradiol; therefore, more circulating estradiol becomes available with hyperinsulinemia (192), and estradiol is generally regarded as the most important hormonal promoter of developing breast cancer (196). The fourth example is polycystic ovary syndrome. High insulin acts through its own receptor (rather than the insulin-like growth factor-1 receptor) on insulin sensitive ovary and adrenal to augment androgen production (186). It is known that hyperandrogenemia is the hallmark of the polycystic ovary syndrome (197).
Though the differential tissue insulin sensitivity hypothesis seems to explain the majority of the insulin resistance induced clinical syndromes, certain diseases may not fall into this category. Take Alzheimer’s disease for example. It was suggested that brain hyperinsulinemia (due to cerebral insulin resistance) may be associated with reduced amyloid-β clearance and eventual accumulation of amyloid-β peptide, a pathological hallmark of Alzheimer’s disease (198). This is because both amyloid-β peptide and insulin compete for the insulin-degrading enzyme for its own degradation; however, the affinity for the binding of insulin-degrading enzyme to insulin is much greater than that for the amyloid-β peptide, as the result of which, less amyloid-β peptides were cleared (199). It was even suggested that Alzheimer’s disease could be qualified as “an insulin resistant brain state” due to the observation that activation of the insulin receptor was impaired in brain autopsy sample of Alzheimer’s disease patients (200).

2.4 Hepatic Insulin Extraction in the Regulation of Peripheral Insulin Concentration

Peripheral insulin concentration after meal ingestion is determined not only by pancreatic insulin secretion, but also hepatic insulin clearance (Figure 2.1) (201, 202); therefore, the liver plays a critical role in determining peripheral insulin levels. The liver is the primary site of degradation of circulating insulin (203). In non-diabetic subjects, approximately 50–70% of the insulin secreted into the portal system is removed by the liver, measured either by hepatic vein catheterization techniques (204, 205) or by calculating the ratio of AUC of peripheral C-peptide concentration to that of the insulin (206).

Reduced hepatic insulin clearance is usually observed after oral glucose or meal ingestion, thereby increasing the systemic availability of insulin to aid glucose disposal (207-209); however, the regulation of hepatic insulin clearance is not a static process, but rather
influenced by both physiological and pathophysiological states of individuals (Figure 2.1). For example, increased hepatic insulin clearance had been reported in elderly men and women (210-212) and patients with essential hypertension (213), whereas decreased hepatic insulin clearance was observed in obese subjects (214, 215) and in patients with liver cirrhosis (216); in diabetic patients, the results are conflicting as both normal (217, 218), decreased (219, 220), or even increased (221) hepatic insulin clearance had been reported. The reasons for such divergent findings are not clear. It is possible that the variations in liver fat content (222, 223) may contribute to this. It is known that increased liver fat content is associated with impaired hepatic insulin clearance (224).

Hepatic insulin clearance is decreased in obesity (214, 215), particularly in abdominal obesity (225), which contributes to hyperinsulinemia in obesity. The cause for the reduction in insulin clearance in obesity is not clear. It was suggested that pathological alterations in liver metabolism may play a role (215). Evidence exist that elevated free fatty acids levels, which commonly occur in obesity, impact on hepatic insulin extraction. For example, the in vitro studies showed that free fatty acids impair insulin binding and degradation in isolated rat hepatocytes (226-228). Furthermore, an in vivo study in dogs found that free fatty acids impair hepatic insulin extraction (229). The study in non-diabetic humans found that increased liver fat was associated with impaired liver insulin clearance (230). Fortunately, the diminishment of hepatic insulin clearance in obesity seems to be reversible. In obese children and adolescents, hepatic insulin clearance increased after weight loss, resulting in near normalization of insulin levels (231).

Pharmacological agents also impact on hepatic insulin extraction, although their effects vary tremendously. Take antidiabetic drugs for example, glyburide augments hepatic insulin
extraction whereas glipizide had no effect (232, 233); metformin enhances hepatic insulin extraction in non-diabetic, first degree relatives of African Americans with type 2 diabetes (234); and thiazolidinediones increase hepatic insulin clearance after oral glucose challenge in subjects with impaired glucose tolerance and type 2 diabetics (235).

Hormones (sex or growth hormones) also affect hepatic insulin extraction. Monti et al showed that the girls with Turner's syndrome [a chromosomal aberration caused by complete or partial X chromosome monosomy in a phenotypic female (236)] have higher insulin clearance than their healthy counterparts, and after growth hormone therapy, insulin clearance was normalized, possibly to counteract hepatic insulin resistance (237). Krakover et al (238) investigated the effects of female sex hormones on insulin binding and receptor-mediated insulin degradation in hepatocytes from ovariectomized rats. They found that estradiol and progesterone increased insulin binding and degradation, whereas testosterone decreased degradation. Thus, they suggested that abnormalities in sex hormone levels could contribute to altered insulin metabolism and peripheral hyperinsulinemia in androgenised women with abdominal obesity (238).

There are a few methods to measure hepatic insulin extraction. The direct measurement of hepatic insulin extraction involves hepatic vein catheterization techniques, with catheters placed in artery and hepatic veins (204). The invasive nature of this technique prompted the development of indirect, non-invasive approaches based on mathematical models to infer hepatic insulin extraction from plasma measurements of C-peptide and insulin. Polonsky et al proposed using the ratio of AUC of C-peptide to that of the insulin to reflect hepatic insulin extraction (239). The rationale being that C-peptide is secreted from the pancreatic β-cells in equimolar concentration with insulin, but not extracted by the liver (239). Toffolo et al assessed hepatic
insulin extraction during an insulin-modified intravenous glucose tolerance test (240). Hepatic insulin extraction was determined by calculating insulin secretion rate (ISR) using plasma C-peptide concentrations and the C-peptide minimal model and by calculating post-hepatic insulin delivery rate using plasma insulin concentrations and the insulin minimal model (240). The rationale for this method is that the simultaneous modeling of insulin secretion rate from C-peptide concentration and insulin delivery rate in plasma after its passage through the liver from insulin concentration provides an estimate of hepatic extraction profile and index (240).
Figure 2.1  **Factors affecting peripheral insulin concentration.** Peripheral insulin concentration is determined by both pancreatic β-cell insulin secretion and hepatic insulin extraction. Hepatic insulin extraction is influenced by multiple factors such as the physiological and pathophysiological status of the individuals and use of pharmacological agents and hormones. Various factors such as glucose, amino acids, free fatty acids, acetylcholine and incretin hormones stimulate pancreatic insulin secretion.
2.5 Effects of Macronutrient on Postprandial Responses in Hyperinsulinemic Subjects

2.5.1 Dietary Carbohydrate

Glycemic Index (GI) and Insulinemic Index are quantitative measures of the blood glucose and insulin raising potential of available carbohydrate in foods. The GI values of foods are similar in healthy control and diabetic subjects (both type 1 and type 2 diabetes) (21, 241, 242). However, the GI values of foods have never been determined in non-diabetic, insulin resistant/hyperinsulinemic subjects; thus we do not know whether they are similar in healthy control, hyperinsulinemic, and diabetic subjects. It is important to clarify this issue because for GI to have clinical utility, their values must be similar in different subjects regardless of glucose tolerance status or degree of insulin sensitivity.

There are few data comparing the relative glucose and insulin responses to carbohydrate test meals in different groups of subjects. A multicentre trial examining the relative glucose and insulin response to a prototype standardized mixed meal (50g available CHO, 11g fat and 12g protein) found that although the relative glucose responses were similar in lean normal, obese normal, subject with impaired glucose tolerance (IGT) and subjects with type 2 diabetes, the relative insulin responses were significantly different among obese normal, IGT and type 2 diabetic subjects (243). In another study comparing the acute plasma glucose and insulin responses elicited by breakfast cereals with either high or low fiber content in 77 non-diabetic subjects divided a priori into 35 with fasting plasma insulin (FPI) < 41pmol/L and 42 with FPI ≥ 41pmol/L, it was found that the glycemic impact of a high fiber cereal, relative to that of a low fiber cereal, did not differ in hyperinsulinemic subjects compared to healthy subjects. However, relative insulin responses were inversely related to fasting insulin concentration (244). These studies suggest that the relative insulin responses elicited by different carbohydrate meals differ
in hyperinsulinemic subjects compared to healthy subjects; however, these studies used meals containing a mixture of foods and macronutrient. Thus no firm conclusion can be drawn. Therefore, to see if different carbohydrates elicit similar relative glucose and insulin responses in hyperinsulinemic and healthy subjects, it is important to study pure carbohydrates (i.e. sugars) and carbohydrate foods fed alone and to control the amount of available carbohydrate in the different test meals.

2.5.2 Dietary Fat

There is evidence that fat has no effect on postprandial glucose and insulin responses in people with diabetes (5, 245). Simpson et al studied the glycemic and hormonal responses to macronutrient in healthy subjects, patients with type 1 diabetes and patients with type 2 diabetes. They found that fat markedly reduced the glycemic response to oral carbohydrate in non-diabetics. However, in both type 1 and type 2 diabetes, the presence of fat had no significant effect on the glycemic response (245). Since delaying gastric emptying is the presumed mechanism for the reduced glycemic response in non-diabetic subjects, the authors suggest that the absence of a fat effect in diabetic subjects may be due to the presence of mild gastric stasis due to unrecognized autonomic neuropathy (245). Gannon et al found that adding 50g fat (butter) to 50g carbohydrate reduced glycemic response in healthy, young subjects (246); however, when the same study was done in older people with type 2 diabetes, there was essentially no effect on blood glucose by ingestion of butter with carbohydrate meal (247). The attenuated effect of fat on glucose-lowering is not a “monopoly” of diabetes; a pilot study comparing the effects of fat and protein on glycemic response in healthy control and hyperinsulinemic subjects showed that adding fat to carbohydrate has a smaller effect in
reducing the glycemic response in hyperinsulinemic subjects than healthy subjects (38); however, this result needs to be confirmed in larger studies.

The mechanism for the attenuated hypoglycemic effect of fat in diabetic subjects is not clear. In rats with insulin resistance induced by high-fat diets, intraduodenal lipid infusion failed to suppress glucose production, suggesting that lipid-induced gut-brain-liver neuronal network in the regulation of glucose homeostasis may be impaired following high-fat diets (39). Therefore, it is possible that the upper intestine of insulin resistant and diabetic subjects may have acquired defect(s) in lipid sensing (through an intestine-brain-liver neurocircuitry), thus hindering glucose homeostasis regulation, or they may have altered release pattern of insulin and gut hormones, or there is a reduced effect of gut hormones on gastric emptying and insulin secretion. The exact mechanisms still warrant investigation.

2.5.3 Dietary Protein

The stimulating effect of protein on plasma insulin concentration was found in both healthy (248) and type 2 diabetic subjects (249). However, compared to the healthy subjects, the insulinotropic effect of protein was attenuated in people with type 2 diabetes (6). A possible explanation may be the impaired insulinotropic effect of GIP (250), and/or reduced postprandial secretion of GLP-1 in type 2 diabetes (251). The insulinotropic capacity of protein has seldom been investigated in non-diabetic subjects with insulin resistance/hyperinsulinemia.

A recent study found that adding soy protein to glucose reduced glycemic response in both healthy and hyperinsulinemic subjects, but 5g protein tended to have lesser effect on reducing glycemic response in hyperinsulinemic than it had on healthy subjects (38), suggesting that in hyperinsulinemic subjects, the insulinotropic effect of protein may be attenuated.
Miller et al studied the effect of protein (lean beef steak) in lean healthy Caucasian subjects, and found a significant inverse relationship between insulin sensitivity and the extent of the decline in plasma glucose (33). These results must be interpreted with caution because both had wide scatter of data around the line of best fit (33, 38).

2.6 Mechanisms by Which Fat and Protein Modulate Postprandial Glycemia

It is generally thought that adding fat and protein to carbohydrate reduces glycemic response through similar mechanisms such as delaying gastric emptying (35) and/or enhancing insulin secretion through augmented GIP and GLP-1 secretion (36). However, there is emerging evidence that fat and protein may modulate glucose homeostasis through different mechanisms. A recent study in rats found that upper intestine lipids activate gut-brain-liver neuronal axis to down-regulate hepatic glucose production and reduce plasma glucose concentration (39); however, it is not known if protein has similar effect. In a mouse model, it was found that glucose-induced incretin hormone release was differently modulated by fat (oleic acid) and protein (whey protein) (37). Whey protein resulted in more GLP-1 secretion than oleic acid and significantly increased GIP; however, oleic acid had no effect on GIP secretion. In addition, whey protein deactivated the dipeptidyl peptidase-4 (the enzyme that inactivates GLP-1) activity, whereas oleic acid doesn’t have such an effect (37). The same study also found that insulin response to oral glucose was 3-fold augmented by protein, and was associated with enhanced oral glucose tolerance, whereas, the addition of fat resulted in only 1.5 fold increase in insulin and there was no accelerated glucose disposal (37). This is consistent with the human study that protein reduced glucose response 2-3 times more than fat, and there was no significant fat × protein interaction (38). Both the animal and human studies suggest that the effects of fat and
protein on postprandial metabolic responses are independent of each other, and the mechanisms by which protein reduces glycemic responses may differ from those for fat.

2.7 The Effects of Habitual Diet on Postprandial Responses

Besides the macronutrient, the habitual dietary intake might also influence postprandial hormonal and metabolic responses. There is evidence that in humans, a high fat diet potentiates the effect of oral glucose on GIP secretion (252) and influences gastrointestinal transit (253, 254), suggesting that the acute glucose-lowering effects and other metabolic effects of fat may be influenced by habitual fat intake.

Animal studies found that habitual dietary fiber intake up-regulates proglucagon messenger RNA, increases GLP-1 concentration and reduces glucose level (255, 256). However, in humans, the addition of the soluble fibre psyllium to a pasta meal did not alter gastric emptying or GLP-1 concentrations (257). A low dose of 1.7g psyllium was used in this study, which might explain the lack of effects (257).

A pilot study from our lab found that higher habitual dietary fiber intake amplifies the effects of protein in lowering glycemic response (38). The mechanism that could explain such effect remains unclear though it was suggested that fiber intake may be associated with increased GLP-1 secretion (38). This hypothesis is consistent with the results of animal studies that higher fiber diet up-regulates GLP-1 secretion, possible via short chain fatty acids, the products of the fermentation of fiber in the colon (255). Recently, this effect was confirmed in humans. A study in hyperinsulinemic subjects found that wheat fiber intake increased plasma butyrate and GLP-1 concentrations, although it takes 9-12 month for these changes to occur (258).
CHAPTER 3

RATIONALE, HYPOTHESES, AND OBJECTIVES
3.1 Rationale

To maintain glucose homeostasis, the normal functioning of the gastrointestinal tract (liver, gut and pancreas), β-cell function, central nervous system, and entero-insulin axis is essential. However, in people with diabetes, obesity, and insulin resistance, these physiological systems may become abnormal, and the mechanisms by which macronutrient (carbohydrate, fat and protein) modulate postprandial blood glucose and insulin responses may be altered as well. Previous studies suggest that the effects of macronutrient on glucose and insulin responses differ in healthy control and hyperinsulinemic subjects, and this discrepancy may be due to differences in gut hormone secretion, pancreatic β-cell insulin secretion and/or hepatic insulin extraction. Clarification of the mechanisms is important in lieu of the fact that insulin resistance/hyperinsulinemia is at the early stage of the pathogenic pathway towards type 2 diabetes, and the complex interactions between macronutrient and hyperinsulinemia at the cellular level remain unclear. In addition, by testing GI and Insulinemic Index in “pure” carbohydrate and carbohydrate foods, this thesis may clarify the controversial issues surrounding the clinical utility of GI and Insulinemic Index.

3.2 Overall Objective

The overall objective of this thesis is to determine whether macronutrient (carbohydrate, fat and protein) elicit different effects on acute postprandial metabolic responses in healthy control, hyperinsulinemic and type 2 diabetic subjects, and to explore potential mechanisms that may explain any differences observed.
3.3 Specific Hypotheses

1. Adding fat and protein to carbohydrate results in less attenuation of glucose response in non-diabetic, hyperinsulinemic subjects than in the healthy control subjects.

2. The effects of macronutrient on postprandial responses are affected by habitual intakes of fat and dietary fibre.

3. The GI values of carbohydrate foods depend on differences in their relative rates of digestion and absorption which do not differ in healthy control, hyperinsulinemic and diabetic subjects; therefore, the GI values of carbohydrate foods will not differ in these subject groups. The GI value of sucrose depends on the hepatic handling of fructose which might differ in these subject groups.

4. The Insulinemic Index of carbohydrate foods depends not only on their relative glycemic impact but also on β-cell function, hepatic insulin extraction and incretin hormone effects, which differ in healthy control, hyperinsulinemic and type 2 diabetic subjects. Hence Insulinemic Index of carbohydrate foods will differ in these subject groups.

5. Insulin sensitivity, β-cell function and hepatic insulin extraction are interconnected. GLP-1 as an incretin hormone may play a role in these parameters. Therefore, the endogenous plasma GLP-1 response to oral glucose is associated with insulin sensitivity, β-cell function and hepatic insulin extraction, a triad important for glucose homeostasis.

In order to address the research questions above, two studies were carried out. The aim of the first study was to determine the postprandial metabolic effects of adding fat (canola oil) and protein (whey protein) to carbohydrate (50g oral glucose drink) in healthy control and hyperinsulinemic subjects (Chapter 4); The aim of the second study was to determine the effects of different sources of carbohydrate foods on postprandial glucose and insulin responses in
healthy control, hyperinsulinemic and type 2 diabetic subjects (Chapter 5). Since in both studies, the oral glucose drink was repeated 3 times for each subject, data from the oral glucose drink was pooled together to analyze if there were any associations between plasma active GLP-1 concentration and the indirect measures of insulin sensitivity, β-cell function and hepatic insulin extraction (Chapter 6).
CHAPTER 4

THE HYPOGLYCEMIC EFFECT OF FAT AND PROTEIN IS NOT ATTENUATED BY

INSULIN RESISTANCE

A partial content of this chapter is published in American Journal of Clinical Nutrition (354)
4.1 Abstract

Background: The glucose-lowering effects of fat and protein are attenuated or absent in diabetic patients, suggesting the same may occur in insulin resistant subjects without diabetes.

Objective: To determine whether the postprandial metabolic responses elicited by fat and protein were influenced by insulin sensitivity of the subjects and to see whether fat and protein modulate glucose response through different mechanisms.

Design: Overnight fasted, healthy, non-diabetic subjects aged 18-45 y, 9 with fasting serum insulin (FSI) < 40pmol/L; 8 with 40 ≤ FSI < 70pmol/L; and 8 with FSI ≥ 70 pmol/L took 50g oral glucose with 0-30g doses of canola oil and whey protein on 11 separate mornings. The relative glycemic response (RGR) was expressed as incremental area under the curve (AUC) after each test meal divided by the mean AUC of the 3 glucose controls in each subject.

Results: Protein significantly reduced glucose (p<0.0001), hepatic insulin extraction (p<0.0001), and increased insulin (p<0.0001) and GLP-1 (p=0.004); however, protein had no significant effect on C-peptide (p=0.69) and insulin secretion rate (p=0.13). Fat (30g) only significantly increased GLP-1 response (p=0.025) and had a tendency to increase insulin (p=0.05). There were no significant FSI × fat (p=0.19) and FSI × protein (p=0.08) interaction effects on glucose AUC. In addition, the changes in RGR per gram of fat (r = -0.05, p = 0.82) or protein (r = -0.08, p = 0.70) were not related to FSI.

Conclusions: The hypoglycemic effect of fat and protein was not blunted by insulin resistance. Protein increased insulin but had no effect on C-peptide and insulin secretion rate, suggesting reduced hepatic insulin extraction or increased C-peptide clearance. However, the exact mechanism for whey protein-induced reduction in hepatic insulin extraction remains to be examined.
4.2 Introduction

It is generally accepted that adding fat and protein to carbohydrate reduces blood glucose compared with carbohydrate alone (32-34, 259); however, the glucose-lowering effect of fat is attenuated or absent in people with diabetes (5, 147, 245). Unlike the effect of fat, the variations in the effect of protein on glucose and insulin responses do not seem to be explained by subject groups, because a variety of effects are seen in both healthy and diabetic subjects (147). The most likely explanations seem to be the different protein source (152-154) and digestion kinetics (149-151). Most of the studies on the metabolic effects of fat and protein focus on healthy and diabetic subjects; very few studies have investigated how non-diabetic, insulin-resistant subjects handle fat and protein.

In a pilot study, we investigated the hypoglycemic effect of fat (corn oil) and protein (soy protein) in healthy and hyperinsulinemic humans, and found that higher fasting insulin was associated with a lower glucose-lowering effect of fat (38). Brand-Miller et al studied the effect of protein (lean beef steak) in lean healthy white subjects, and found a significant inverse relationship between insulin sensitivity and the extent of the decline in plasma glucose (33). These results must be interpreted with caution because they had a wide scatter of data around the line of best fit.

The exact mechanisms associated with the hypoglycemic effect of fat and protein are not clear, although they are suggested to occur through similar mechanisms, such as delayed gastric emptying (35) and/or enhanced insulin secretion through augmented GLP-1 and GIP secretion (36); however, a study in mice found that glucose-induced incretin hormone release was differently modulated by fat and protein (37). The same study also found that the insulin response to glucose was augmented 3-fold by protein and was associated with enhanced oral glucose tolerance, whereas, the addition of fat resulted in only a 1.5-fold increase in insulin with
no accelerated glucose disposal (37). This is consistent with a human study that showed that protein reduced glucose response 2 to 3 times more than fat, and there was no significant fat × protein interaction (38). Taken together, this evidence suggests that fat and protein may modulate postprandial glucose response through different mechanisms.

Therefore, we investigated whether acute postprandial metabolic responses elicited by fat and protein were influenced by the degree of insulin sensitivity of the subjects and examined whether fat and protein modulate postprandial metabolic responses through different mechanisms.

4.3 Subjects and Methods

4.3.1 Subjects and study design

Forty-six healthy, non-diabetic men and women (18-45 y old) were screened by measuring height, weight, waist circumference (WC), hip circumference, blood pressure and fasting serum glucose, insulin, C-reactive protein (CRP), liver enzymes, and creatinine (initial recruitment date: April 30, 2007). The aim was to recruit 8 to 9 non-diabetic subjects in each of the following categories of fasting serum insulin (FSI): low (FSI < 40 pmol/L), medium (40 ≤ FSI < 70 pmol/L) and high (FSI ≥ 70 pmol/L). The rationale of this method of recruitment was based on the fact that FSI is strongly correlated with insulin resistance measured by euglycemic-hyperinsulinemic clamp (260), and the 40 pmol/L cut-off point was chosen because this represents approximately the 67th percentile for non-diabetic subjects in our laboratory (244). In our preliminary study, in which FSI>40 pmol/L was used to define hyperinsulinemia (38), subjects with FSI>40 pmol/L had significantly greater WC, BMI, HOMA insulin resistance index, total and LDL cholesterol and triglycerides, and lower HDL cholesterol than those with
FSI<40 pmol/L (38). We divided subjects with FSI>40 pmol/L into medium (FSI<70 pmol/L) and high (FSI ≥ 70 pmol/L) groups because people with newly diagnosed diabetes have mean FSI>70 pmol/L (261, 262). Two subjects from the high-FSI group had impaired glucose tolerance (IGT) (2-h postprandial glucose: 7.8-11.1 mmol/L) (1). Data from the 2 IGT subjects was still used for analysis because even after excluding the 2 IGT subjects, the results did not change. Subjects were not eligible if they had BMI>35 kg/m², type 2 diabetes (fasting glucose ≥ 7.0 mmol/L), serum triglycerides ≥ 10.0 mmol/L, impaired renal function (serum creatinine >1.2 times upper limit of normal), or liver function (aspartate transaminase, alanine transaminase, or gamma-glutamyl transpeptidase >2 times upper limit of normal). Other exclusion criteria were: smoking, use of diuretics, corticosteroids or β-blockers, or presence of medical events or chronic conditions influencing gastrointestinal function or glucose metabolism.

Twenty-five eligible subjects were enrolled in the study; n=9 with a low FSI, n=8 with a medium FSI, and n=8 with a high FSI (the screened subjects were excluded because we only needed certain number of people with low, medium or high FSI). The research protocol was reviewed and approved by Research Ethics Boards at the University of Toronto and St.Michael’s Hospital. All subjects gave written informed consent.

The subjects were instructed to maintain their usual daily routine between study days and refrain from exercise on the morning of the test. After an overnight fast (10-14h), they came to the Risk Factor Modification Center at St. Michael’s hospital on 11 separate mornings (over a three month period) between 7:30 and 9:30. Venous blood samples were drawn before and at 15, 30, 45, 60, 90 and 120 min after starting to ingest test meals.
4.3.2 Test drinks

Nine test drinks, consisting of 50g anhydrous glucose (Grain Processing Enterprising LTD, Scarborough, On, Canada), 250ml water, 0g, 5g or 30g fat (Canola oil, Sardo Foods, Brampton, Ontario, Canada), and 0g, 5g or 30g protein (whey protein concentrate 392, Fonterra Inc. Camp Hill, PA, USA), were blended to smooth drink prior to consumption. The composition and caloric content of the test meals are shown in Table 4.1. The test meals are not calorically equal meals, with 50g glucose + 50g fat + 50g protein containing the highest calorie (Table 4.1). The test meals were administered in a random order. The choices of 5g and 30g of fat or protein are based on the results of preliminary study (38) that the difference in the effect of fat on glycemic responses between healthy and hyperinsulinemic subjects (FSI>40pmol/L) was greatest at the highest level of fat (30g). In contrast, a difference in the effect of protein was evident only at the lowest dose (5g). The control drink was 50g glucose alone, and was tested on each subject three times at the beginning, middle and end of the study. This is because relative glycemic response (RGR) and relative insulin response (RIR) to the test meals were calculated as AUC after each test meal divided by the mean AUC of the 3 glucose controls in each subject. The use of the average AUC of the 3 oral glucose tests in each subject results in a greater probability that the glucose is representative of the subject’s true response to oral glucose than a single determination of AUC. In addition, use of the mean of the 3 glucose control tests reduces within-subject variability and increases the power of the study (263).

4.3.3 Assessment of habitual dietary intake

Habitual nutrient intakes were estimated using two 3-day food records (at the beginning and end of the study period), which will correctly classify 70-80% of subjects in the top or bottom tertile of intake for percentage (%) energy from fat, carbohydrate and dietary fibre (264),
the main nutrients of interest. Nutrient analysis was done using Food Processor SQL edition, version 9.3.1m (ESHA Research, Salem, OR).

4.3.4 Assessment of physical activity

Because exercise improves insulin sensitivity (265, 266), the subjects’ physical activities including work-, sports-, and leisure-time were assessed using Baecke Physical Activity Questionnaire (267), which was administered at the beginning and the end of the study.

4.3.5 Blood analysis

Venous blood samples for the measures of glucose, insulin, and C-peptide were collected in BD Vacutainer™ SST™ tubes (BD, Franklin Lakes, NJ, USA). Serum glucose was measured by a glucose oxidase method (SYNCHRON LX Systems, Beckman Coulter, Brea, California, USA), with inter-assay coefficient of variation (CV) of 1.9%. Insulin was measured using one-step immunoenzymatic (“sandwich”) assay (Beckman Access Ultrasensitive Insulin Assay, Beckman Coulter, Brea, California, USA), with inter-assay CV of 2.5 to 4.3 %. Insulin has no cross-reactivity with proinsulin. C-peptide was measured using double antibody competitive radioimmunoassay (Siemens Medical Solutions Diagnostics, Los Angeles, CA), with inter-assay precision of 10% or less.

Venous blood samples for GLP-1 were collected in BD Vacutainer™ EDTA™ tubes (BD, Franklin Lakes, NJ, USA). The dipeptidyl peptidase-4 inhibitor (Linco Research, St. Charles, Missouri) was added immediately (less than 30 seconds) after collection. Plasma GLP-1 was measured by capturing the active GLP-1 from the sample by a monoclonal antibody (specific binding to the N-terminal region) using GLP-1 (active) ELISA Kit (Linco Research, St.
Charles, Missouri, USA), with inter-assay CV ranges from 1-13%. All the samples were stored at -70°C before analysis.

4.3.6 Calculation and statistical analysis

Incremental areas under the curve (AUC), ignoring area below baseline, for glucose, insulin, C-peptide and GLP-1 were calculated using the trapezoid rule (31). The net AUC, including the areas falling below the baseline was also calculated using the trapezoid rule (31). The endpoints analyzed from the AUC and net AUC were similar; therefore, only results from AUC were presented. Total AUC_{ins/glu} was calculated using the trapezoidal rule applied to the insulin and glucose curves from 0 to 120 mins. The same method was used to calculate total AUC of insulin secretion rate (ISR). Basal hepatic insulin extraction (HIE_{basal}) was calculated as fasting C-peptide divided by fasting insulin and postprandial hepatic insulin extraction (HIE_{auc}) was determined by the AUC of C-peptide divided by that of insulin (268). Insulin secretion rate was calculated from deconvolution of the plasma C-peptide concentration using ISEC software package developed by Hovorka et al (269). The relative glycemic response (RGR) and relative insulin response (RIR) to the test meals were calculated as AUC after each test meal divided by the mean AUC of the 3 glucose controls in each subject.

The means of 3 OGTT were used to calculate insulin sensitivity index. The validated insulin sensitivity index of Matsuda and DeFronzo (173) was used as an index of whole-body insulin sensitivity; it was calculated as 10,000/(fasting glucose × fasting insulin × mean glucose × mean insulin )^{0.5} (173). Mari’s oral glucose insulin sensitivity index, a glucose-insulin model constructed on established principles of glucose kinetics and insulin action was also calculated (174). The oral glucose insulin sensitivity has been validated against the euglycemic-hyperinsulinemic clamp in healthy, obese and type 2 diabetic subjects (174).
The hepatic insulin resistance was calculated as the product of total AUC of glucose and insulin during the first 30 minutes \((\text{glucose}_{0-30}[\text{AUC}] \times \text{insulin}_{0-30}[\text{AUC}])\) (172) and muscle insulin sensitivity was calculated as the rate of decay of plasma glucose concentration from its peak value to its nadir divided by the mean insulin concentration (172). Both indices of hepatic insulin resistance and muscle insulin sensitivity derived from OGTT have been validated using hyperinsulinemic-euglycemic clamp (172).

The \(\beta\)-cell compensation for insulin resistance was estimated using the insulin secretion/insulin resistance (disposition) index derived from OGTT (270), which was shown to be the best predictor of future development of type 2 diabetes in subjects with normal glucose tolerance (NGT) compared with other predictive models such as San Antonio Diabetes Prediction Model (271) (including age, sex, ethnicity, BMI, blood pressure, fasting plasma glucose, triglycerides, and HDL) and 2-hour plasma glucose concentration. To calculate insulin secretion/insulin resistance (disposition) index, first, insulin secretion was expressed as the changes in AUC of insulin secretion rate \((\Delta\text{ISR}_{\text{AUC}})\) relative to changes in AUC of plasma glucose response \((\Delta\text{ISR}_{\text{AUC}}/\Delta\text{G}_{\text{AUC}})\), then \(\Delta\text{ISR}_{\text{AUC}}/\Delta\text{G}_{\text{AUC}}\) was divided by the severity of insulin resistance \((\Delta\text{ISR}_{\text{AUC}}/\Delta\text{G}_{\text{AUC}}/\text{IR})\), as measured by the inverse of Matsuda’s insulin sensitivity index.

Data were expressed as mean ± SEM for normally distributed variables or median (interquartile range) for non-normally distributed variables. Normality was assessed using the Shapiro and Wilk statistic and the normality plots (PROC UNIVARIATE procedure of SAS). Skewed variables were log-transformed prior to analysis. The AUCs of the biomarkers (glucose, insulin, C-peptide, insulin secretion rate, GLP-1) and hepatic insulin extraction were subjected to the repeated measures of analysis of variance (ANOVA) (PROC MIXED) to test for the main
effects of FSI (low vs. medium vs. high), fat dose, protein dose and fat × protein, FSI × fat, FSI × protein, and FSI × fat × protein interactions. Since the design is not balanced and the sample size is relatively small, the correlation of FSI× fat × protein term with FSI × fat and FSI × protein terms might obscure the significance of an effect; therefore, the FSI × fat × protein term was omitted when it was not significant, and the reduced model was reanalyzed to see if there were any significant FSI × fat or FSI × protein interaction effects. Age and BMI were included in the model as covariates to control for the differences in these variables between subjects. Tukey’s post hoc test was performed to compare treatments if the treatment effects were statistically significant.

The effect of fat or protein on relative glycemic response and relative insulin response in each subject was defined as the slope of the regression line of relative glycemic response or relative insulin response on doses of fat (or protein). The regression equations were based on 9 points in each subject (3 × 3 levels of fat and protein). Before pooling the data for each subject to calculate the slopes, the relative glycemic response and relative insulin response were subjected to the repeated measures ANOVA to test whether there were significant fat × protein and FSI × fat × protein interaction. This is because it is only valid to pool data in this way if there was no significant fat × protein or FSI × fat × protein interaction and no significant evidence of a nonlinear dose-response relation. The results showed no significant fat × protein (relative glycemic response, p=0.72; relative insulin response, p=0.55) or FSI × fat × protein interaction (relative glycemic response, p=0.47; relative insulin response, p=0.20). The correlations between changes in relative glycemic response and relative insulin response per g of fat (or protein) and the variables (FSI, habitual dietary fat and fiber intakes) were determined by
simple linear regressions. All analyses were done using SAS 9.1, (SAS Institute Inc, Cary). Differences were considered significant if 2-tailed p<0.05.

4.4 Results

The anthropometric and metabolic characteristics of subjects with a low, medium or high FSI are shown in Table 4.2. Compared with those with a low- or medium-FSI, subjects with a high-FSI were older and had a significantly higher waist circumference, waist-to-hip ratio, systolic & diastolic blood pressure and total cholesterol, triglycerides and LDL cholesterol (Table 4.2). Subjects with a high-FSI also had a significantly higher BMI and total:HDL cholesterol ratio than did those with a low-FSI. Fasting glucose, HDL cholesterol and C-reactive protein were not significantly different between the 3 FSI groups.

Both HIE_{basal} and postprandial HIE_{auc} in those with a medium- or high-FSI were significantly lower than those of the low-FSI (Table 4.2). In fact, a significant inverse relation was observed between FSI and basal (r=-0.70, p<0.0001) and postprandial hepatic insulin extraction (r=-0.72, p<0.0001) (Appendix 1). In addition, postprandial insulin response was also inversely associated with postprandial HIE_{auc} (r=-0.80, p<0.0001) (Appendix 1). These results are consistent with Meier et al’s observation that plasma insulin concentration is inversely related to liver insulin clearance (208). Higher insulin concentrations at both basal and postprandial were associated with lower liver insulin clearance, which conserves insulin and contributes to elevated insulin concentrations.

Mari’s oral glucose insulin sensitivity, Matsuda’s whole body insulin sensitivity indices (ISI) and muscle insulin sensitivity index decreased in a step-wise fashion across the groups; subjects with a high-FSI were the least insulin sensitive (Table 4.2). Subjects with a medium- or high-FSI had a significantly higher hepatic insulin resistance than did those with a low-FSI
(Table 4.2). In fact, an inverse association was observed between hepatic insulin resistance and hepatic insulin extraction \( (r=-0.61, p=0.001) \) (Appendix 2), showing that insulin clearance is inextricably linked to hepatic insulin action. The more insulin resistant the liver becomes, the lower the liver insulin clearance.

Insulin secretion/insulin resistance (disposition) index \( (\Delta ISR_{AUC}/\Delta G_{AUC} + IR) \) was significantly lower in subjects with a high-FSI than those with a low- or medium-FSI (Table 4.2). Subjects with a medium-FSI had lower disposition index than those with a low-FSI, albeit it was not significant (Table 4.2).

Neither the individual physical activity indices (work-, sports-, and leisure-time) nor were the mean physical activity indices differ significantly among the subject groups (Appendix 3). This lack of significant difference may be attributable to the fact that all the subjects were relatively young (18-45y) and healthy.

The mean AUC of glucose, insulin, C-peptide, and GLP-1 responses after 3 separate OGTTs and their inter- and intra-subject coefficients of variations (CV) are shown in Table 4.3. As the subjects’ fasting insulin increased, the AUC of glucose and insulin increased. There was also a tendency towards an increased C-peptide AUC with increasing fasting insulin, although it was not significant \( (p=0.06) \). The AUC of GLP-1 was not significantly different among the three subject groups \( (p=0.995) \). In general, for all the biomarkers, the inter-subject variation was higher than intra-subject variation in the 3 FSI groups (Table 4.3).

\( P \) values for the main and interaction effects of FSI group, fat, and protein on the AUCs of the biomarkers (glucose, insulin, C-peptide, insulin secretion rate, and GLP-1) and hepatic insulin extraction are shown in Table 4.4. Fat had a significant main effect only on the GLP-1 response. Protein significantly influenced glucose, insulin, GLP-1 responses, and hepatic insulin
There were significant FSI group effects on glucose, insulin, the insulin secretion rate, GLP-1 and hepatic insulin extraction. There was no fat × protein interaction effect on any of the biomarkers. The FSI × fat interaction effect was observed only for the insulin response, which suggested that the ability of fat to influence the insulin response depended on FSI of the subjects. There were significant FSI × protein interaction effects on insulin, GLP-1, and hepatic insulin extraction, which suggested that the effects of protein on these biomarkers depended on FSI of the subjects. The FSI × fat × protein interaction effects were only observed on insulin and hepatic insulin extraction, which suggested that the effect of fat and protein on insulin and hepatic insulin extraction depended on FSI of the subjects.

The 2-hour responses of glucose, insulin, C-peptide, and GLP-1 after 50g oral glucose plus 0, 5g or 30g protein in non-diabetic humans with a low, medium or high FSI are shown in Figure 4.1. In the 3 FSI groups, 30g protein significantly reduced glucose between 30 to 90 min compared to 0 or 5g protein. There was no protein dose effect on insulin in low-FSI group; however, 30g protein significantly increased insulin at 30 min in the medium-FSI group and at 30 and 45 min in the high-FSI group. Protein had no significant effect on C-peptide and GLP-1 at any of the time points or in any of the FSI groups. The 2-hour postprandial responses (glucose, insulin, C-peptide, and GLP-1) after 50g oral glucose plus 0, 5g or 30g fat showed a trend similar to that of protein, albeit not significant (Appendix 4).

The main and interaction effects of FSI group, fat, and protein on the AUCs of the biomarkers (glucose, insulin, C-peptide, insulin secretion rate, and GLP-1) and hepatic insulin extraction are illustrated in Figure 4.2 and Figure 4.3. The main effects of fat or protein on glucose, insulin, C-peptide, insulin secretion rate, and GLP-1 responses expressed as AUC and hepatic insulin extraction are shown in Figure 4.2. Protein significantly decreased glucose
(p<0.0001), increased insulin (p<0.0001), and had no significant effect on C-peptide (p=0.69) and insulin secretion rate (p=0.13); however, protein significantly decreased hepatic insulin extraction (p<0.0001) and increased GLP-1 (p=0.004). Fat (30g) significantly increased only the GLP-1 response (p=0.025) and had a tendency to increase insulin (p=0.05).

The main and interaction effects of FSI group, fat, and protein on glucose, insulin, C-peptide, the insulin secretion rate, and hepatic insulin extraction shown in Figure 4.3 are consistent with those shown in Table 4.4. Fat had no significant effect on glucose, insulin, C-peptide, insulin secretion rate, or hepatic insulin extraction in any of the FSI groups; however, there was a significant FSI × fat interaction effect on the insulin response (Figure 4.3). For instance, insulin responses to 30g fat intake were significantly higher in the high-FSI group than in the low- and medium- FSI groups. Unlike fat, 30 g protein significantly decreased the glucose response in all FSI groups, to a similar magnitude. Protein at 30g significantly increased the insulin responses in the medium- and high-FSI groups, but exerted no effect on C-peptide or the insulin secretion rate; however, 30g protein significantly decreased hepatic insulin extraction in the low- and medium-FSI groups, but had no effect on the high-FSI group. This may have been because the high-FSI group already had a reduced hepatic insulin extraction (Table 4.2).

Significant FSI ×protein interaction effects were shown for the insulin response and hepatic insulin extraction: with the 30g protein intake, the insulin response was significantly higher in the high-FSI group than in the low-FSI group, and hepatic insulin extraction was significantly lower in the medium- and high-FSI groups than in the low-FSI.

The changes in relative glycemic response per gram of fat (r = -0.05, p = 0.82) or protein (r = -0.08, p = 0.70) were not related to FSI (Appendix 5). Similarly, the changes in relative
insulin response per gram of fat ($r = -0.06, p = 0.77$) or protein ($r =0.04, p =0.86$) were also not related to FSI (Appendix 5).

For habitual dietary fat and fiber intake, the changes in relative glycemic response per gram of fat ($r = -0.12, p = 0.58$) or protein ($r = 0.06, p = 0.76$) were not related to habitual dietary fat intake. Similarly, the changes in relative insulin response per gram of fat ($r = -0.03, p = 0.89$) or protein ($r =-0.12, p =0.58$) were also not related to habitual dietary fat intake. Contrary to previous finding (38), the changes in relative glycemic response per gram of protein ($r = 0.29, p = 0.16$) was not related to habitual dietary fiber intake.
Table 4.1 Composition of the 9 test meals, their total energy content, and the percentage of energy from carbohydrate, fat, and protein.

<table>
<thead>
<tr>
<th>Test meals</th>
<th>Total energy (Calories)</th>
<th>Carbohydrate % of energy</th>
<th>Fat % of energy</th>
<th>Protein % of energy</th>
</tr>
</thead>
<tbody>
<tr>
<td>50g glucose + 0g fat + 0g protein (control)</td>
<td>200</td>
<td>100</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>50g glucose + 0g fat + 5g protein</td>
<td>220</td>
<td>90.9</td>
<td>0.0</td>
<td>9.1</td>
</tr>
<tr>
<td>50g glucose + 0g fat + 30g protein</td>
<td>320</td>
<td>62.5</td>
<td>0.0</td>
<td>37.5</td>
</tr>
<tr>
<td>50g glucose + 5g fat + 0g protein</td>
<td>245</td>
<td>81.6</td>
<td>18.4</td>
<td>0.0</td>
</tr>
<tr>
<td>50g glucose + 5g fat + 5g protein</td>
<td>265</td>
<td>75.5</td>
<td>17.0</td>
<td>7.5</td>
</tr>
<tr>
<td>50g glucose + 5g fat + 30g protein</td>
<td>365</td>
<td>54.8</td>
<td>12.3</td>
<td>32.9</td>
</tr>
<tr>
<td>50g glucose + 30g fat + 0g protein</td>
<td>470</td>
<td>42.6</td>
<td>57.4</td>
<td>0.0</td>
</tr>
<tr>
<td>50g glucose + 30g fat + 5g protein</td>
<td>490</td>
<td>40.8</td>
<td>55.1</td>
<td>4.1</td>
</tr>
<tr>
<td>50g glucose +30g fat +30g protein</td>
<td>590</td>
<td>33.9</td>
<td>45.8</td>
<td>20.3</td>
</tr>
</tbody>
</table>

The test meals were randomly administered. The control glucose drink was tested at the beginning, middle, and end of the study.
Table 4.2 Anthropometric and metabolic characteristics of subjects by fasting serum insulin

<table>
<thead>
<tr>
<th></th>
<th>Low</th>
<th>Medium</th>
<th>High</th>
<th>P²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(FSI&lt;40pmol/L)</td>
<td>(40 ≤ FSI &lt;70pmol/L)</td>
<td>(FSI ≥ 70 pmol/L</td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>27±3&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;-&lt;/sup&gt;&lt;sup&gt;c&lt;/sup&gt;</td>
<td>26±2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38±2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.003</td>
</tr>
<tr>
<td>Ethnicity (n)</td>
<td>6:0:2:0:0:1</td>
<td>2:2:2:1:1:0</td>
<td>3:4:0:1:0:0</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>24±1.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27±1.5&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>31±1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.001</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>82±3.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85±5.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>102±3.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.003</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.79±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.8±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.91±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.01</td>
</tr>
<tr>
<td>Fasting insulin (pmol/L)</td>
<td>22(21-30)&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;-&lt;/sup&gt;&lt;sup&gt;5&lt;/sup&gt;</td>
<td>53(50-54)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>88(76-123)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>4.6±0.1</td>
<td>5.0±0.1</td>
<td>5.0±0.2</td>
<td>0.05</td>
</tr>
<tr>
<td>HIE&lt;sub&gt;basal&lt;/sub&gt;</td>
<td>16.2±0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.6±0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.8±0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HIE&lt;sub&gt;auc&lt;/sub&gt;</td>
<td>6.6±0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.3±0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.8±0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mari OGIS (ml/min/m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>507±7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>458±10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>377±10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Matsuda Whole-body ISI&lt;sup&gt;6&lt;/sup&gt;</td>
<td>36.7(25.1-41.8)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.8(15.5-20.5)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.5(8.3-10.9)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Muscle ISI&lt;sup&gt;6&lt;/sup&gt;</td>
<td>3.7(3.6-6.3)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.7(1.4-2.0)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.8(0.6-1.8)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.003</td>
</tr>
<tr>
<td>Hepatic insulin resistance index&lt;sup&gt;6&lt;/sup&gt;</td>
<td>2.9(2.3-4.2)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.4(4.4-9.9)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.7(5.2-11.5)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.01</td>
</tr>
<tr>
<td>∆ISR&lt;sub&gt;AUC&lt;/sub&gt;/ΔG&lt;sub&gt;AUC&lt;/sub&gt; ÷ IR&lt;sup&gt;6&lt;/sup&gt;</td>
<td>102±8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>81±12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27±3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.008</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>107±3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>102±4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>127±5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.001</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>64±2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>62±3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85±3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.1±0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4±0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.5±0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.001</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.77(0.5-1)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.78(0.5-1.23)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.72(1.59-1.93)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.0004</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.3±0.1</td>
<td>1.0±0.1</td>
<td>1.1±0.2</td>
<td>0.18</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>2.4±0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.6±0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.5±0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.01</td>
</tr>
</tbody>
</table>
Table 4.2 (Continued)

<table>
<thead>
<tr>
<th></th>
<th>Low</th>
<th>Medium</th>
<th>High</th>
<th>P²</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>(FSI&lt;40pmol/L)</td>
<td>(40 ≤ FSI &lt;70pmol/L)</td>
<td>(FSI ≥ 70 pmol/L)</td>
<td></td>
</tr>
<tr>
<td>Total: HDL cholesterol</td>
<td>3.2±0.2ᵃ</td>
<td>4.2±0.5ᵃᵇ</td>
<td>5.4±0.6ᵇ</td>
<td>0.01</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>0.33(0.19-1.21)</td>
<td>0.4(0.19-4.1)</td>
<td>2.91(1.08-5.81)</td>
<td>0.13</td>
</tr>
</tbody>
</table>

¹ n = 25 except where otherwise noted. FSI, fasting serum insulin; HIE, hepatic insulin extraction; OGIS, oral glucose insulin sensitivity; ISI, insulin sensitivity index; AUC, area under the curve; IR, insulin resistance; BP, blood pressure. Values in the same row with different superscript letters are significantly different, P<0.05 (Tukey’s post hoc test).

² P represents overall significant differences across groups by one-factor ANOVA. The p-values for HIEbasal, HIEauc, OGIS, ISI and disposition index were derived from repeated measures ANOVA.

³ Mean±SEM (all such values).

⁴ C. Caucasian; SA, South Asian; EA, East Asian; Af, African; ME, Middle Eastern.

⁵ Median; interquartile range in parentheses (all such values).

⁶ Calculated by using the mean of 3 oral-glucose-tolerance test results. PROC MIXED repeated measures ANOVA, adjusted for age, BMI and WC.
Table 4.3  Mean areas under the curve (AUCs) for glucose, insulin, C-peptide, and glucagon-like peptide 1 (GLP-1) responses after 3 separate oral-glucose-tolerance tests and their inter- and intra-subject CVs by fasting serum insulin (FSI)\(^1\)

<table>
<thead>
<tr>
<th></th>
<th>Low</th>
<th>Medium</th>
<th>High</th>
<th>(P^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(FSI&lt;40pmol/L)</td>
<td>(40 (\leq) FSI &lt; 70pmol/L)</td>
<td>(FSI (\geq) 70 pmol/L)</td>
<td></td>
</tr>
<tr>
<td>Glucose AUC (mmol×min/L)</td>
<td>206(184-228)(^{a,b})</td>
<td>197(137-236)(^a)</td>
<td>322(257-377)(^b)</td>
<td>0.004</td>
</tr>
<tr>
<td>Intersubject CV</td>
<td>35</td>
<td>45</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>Intrasubject CV</td>
<td>35</td>
<td>32</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Insulin AUC (nmol×min/L)</td>
<td>13(11-17)(^a)</td>
<td>34(27-39)(^b)</td>
<td>45(35-79)(^b)</td>
<td>0.001</td>
</tr>
<tr>
<td>Intersubject CV</td>
<td>60</td>
<td>47</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Intrasubject CV</td>
<td>29</td>
<td>27</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>C-peptide AUC (nmol×min /L)</td>
<td>99±11(^d)</td>
<td>131±16</td>
<td>150±16</td>
<td>0.06</td>
</tr>
<tr>
<td>Intersubject CV</td>
<td>46</td>
<td>47</td>
<td>63</td>
<td></td>
</tr>
<tr>
<td>Intrasubject CV</td>
<td>37</td>
<td>40</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>GLP-1 AUC (pmol×min /L)</td>
<td>182(83-285)</td>
<td>177(75-352)</td>
<td>156(79-235)</td>
<td>0.995</td>
</tr>
<tr>
<td>Intersubject CV</td>
<td>99</td>
<td>83</td>
<td>116</td>
<td></td>
</tr>
<tr>
<td>Intrasubject CV</td>
<td>66</td>
<td>51</td>
<td>55</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) \(n = 25\). Values in the same row with different superscript letters are significantly different, \(P < 0.05\) (Tukey’s post hoc test).

\(^2\) \(P\) represents overall significant differences across groups by one-factor ANOVA.

\(^3\) Median; interquartile range in parentheses (all such values).

\(^4\) Mean ± SEM (all such values).
Table 4.4  *P* values for main and interaction effects of fasting serum insulin (FSI) group, fat, and protein on glucose, insulin, C-peptide, insulin secretion rate (ISR), and glucagon-like peptide 1 (GLP-1) responses expressed as area under the curve (AUC) and hepatic insulin extraction (HIE)\(^1\)

<table>
<thead>
<tr>
<th></th>
<th>Glucose</th>
<th>Insulin</th>
<th>C-peptide</th>
<th>ISR</th>
<th>GLP-1</th>
<th>HIE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Main effects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td>0.53</td>
<td>0.05</td>
<td>0.33</td>
<td>0.16</td>
<td>0.025</td>
<td>0.21</td>
</tr>
<tr>
<td>Protein</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.69</td>
<td>0.13</td>
<td>0.004</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>FSI(^2)</td>
<td>0.02</td>
<td>&lt;0.0001</td>
<td>0.08</td>
<td>0.03</td>
<td>0.009</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Interactions</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat × Protein</td>
<td>0.50</td>
<td>0.06</td>
<td>0.49</td>
<td>0.18</td>
<td>0.18</td>
<td>0.28</td>
</tr>
<tr>
<td>FSI × Fat</td>
<td>0.19</td>
<td>0.02</td>
<td>0.61</td>
<td>0.53</td>
<td>0.12</td>
<td>0.32</td>
</tr>
<tr>
<td>FSI × Protein</td>
<td>0.08</td>
<td>0.02</td>
<td>0.69</td>
<td>0.48</td>
<td>0.03</td>
<td>0.04</td>
</tr>
<tr>
<td>FSI × Fat × Protein</td>
<td>_</td>
<td>0.04</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>0.01</td>
</tr>
</tbody>
</table>

\(^1\) *P* values were derived from repeated-measures ANOVA (PROC MIXED; SAS Institute, Cary, NC); *P* < 0.05 indicates significance. Age and BMI were included in the model as covariates.

\(^2\) Low compared with medium compared with high.
Figure 4.1 Mean (±SEM) 2-h postprandial plasma glucose, insulin, C-peptide, and glucagon-like peptide 1 (GLP-1) concentrations after 50 g oral glucose plus 0, 5, and 30 g protein in nondiabetic humans with different concentrations of fasting serum insulin (FSI): Low (FSI < 40 pmol/L), Medium (40 ≤ FSI < 70 pmol/L), and High (FSI ≥ 70 pmol/L). *Significantly different from 0 and 5 g protein, P < 0.05 (2-factor ANOVA). n = 25. The error bars are not shown if they overlap or are smaller than the symbol.
Figure 4.2  Mean (±SEM) main effects of fat and protein on glucose, insulin, C-peptide, insulin secretion rate (ISR), and glucagon-like peptide 1 (GLP-1) responses expressed as area under the curve (AUC) and hepatic insulin extraction (HIE) in nondiabetic humans by combined fasting serum insulin (FSI) groups: low (FSI < 40 pmol/L), medium (40 ≤ FSI <70 pmol/L), and high (FSI ≥ 70 pmol/L). Means not sharing a common letter (a and b are for the comparisons of different levels of fat and A and B are for the comparison of different levels of protein) are significantly different [repeated-measures ANOVA, PROC MIXED procedure (SAS Institute,
Cary, NC). Age and BMI were included in the model as covariates (Tukey’s post hoc test, $P < 0.05$). $n = 25$. The error bars are not shown if they overlap or are smaller than the symbol.
Figure 4.3  Mean (±SEM) effects of 50 g glucose plus 0, 5, or 30 g fat or protein on glucose, insulin, C-peptide, the insulin secretion rate (ISR) expressed as the area under the curve (AUC), and hepatic insulin extraction (HIE) in healthy nondiabetic subjects with different levels of fasting serum insulin (FSI): low (FSI < 40 pmol/L, white bars), medium (40 ≤ FSI < 70 pmol/L,
grey bars), and high (FSI ≥ 70 pmol/L, black bars). Means not sharing a common letter are significantly different [repeated measures ANOVA, PROC MIXED procedure (SAS Institute, Cary, NC)]. Age and BMI were included in the model as covariates (Tukey’s post hoc test, P < 0.05). n = 25.
4.5 Discussion and conclusions

We found that the ability of fat and protein to lower glucose responses was not influenced by the degree of insulin sensitivity of the subjects. Protein significantly decreased the glucose response regardless of whether the subjects had a low, medium or high FSI. We also found that protein increased the insulin response but had no effect on the C-peptide response or insulin secretion rate, which may be explained by either a decreased insulin clearance or increased rate of C-peptide clearance.

Previous studies suggest that the ability of fat (38) and protein (33) to lower glucose response was attenuated in subjects with insulin resistance. In these studies, the correlation between measures of insulin sensitivity and the ability of fat or protein to lower the glucose response was weak because of the wide scatter of data around the line of best fit. Nevertheless, a few possible reasons may contribute to the discrepancy between our study and previous studies. First, different sources of fat and protein were used. Moghaddam et al (38) used corn oil and soy protein and Brand-Miller et al (33) used lean beef steak, whereas we used canola oil and whey-protein. It is known that the fat source (272, 273) and protein source (274-277) influence postprandial glucose responses differently. In particular, whey-protein is considered a “fast” protein, which is digested and absorbed rapidly (278-282). Second, Moghaddam et al (38) used capillary blood for the measurement of glucose, whereas we used venous blood. The glucose responses measured in venous plasma were shown to be lower and more variable than those in capillary blood (283). Finally, the variations may be due to the type of subjects studied. Brand-Miller et al (33) studied lean healthy male white subjects with very small differences in insulin sensitivity (assessed by euglycemic-hyperinsulinemic clamp), whereas we studied multiethnic subjects with a much wider range of insulin sensitivity, as evidenced by their significantly different oral glucose insulin sensitivity and whole-body insulin sensitivity (Table 4.2).
We found that protein decreased the glucose response, whereas fat had no such effect. This was unexpected because, usually, when fat is added to carbohydrate, it results in a lower glucose response (32, 147, 246). This may be due to the difference between solid and liquid meals — if fat delays gastric emptying, it may have more of an effect with solid than with liquid meals. Another reason is that the increase in insulin after fat intake may be too small to result in a decreased glucose response (Figure 4.2). It is also possible that fat and protein may differentially modulate the release of cholecystokinin (CCK) and peptide YY (PYY), the 2 gut hormones that delay gastric emptying (284, 285). It is known that the rate of gastric emptying is a major determinant of postprandial glycemia; even modest changes may have a substantial effect on the magnitude of postprandial increases in glucose and insulin (286, 287). Although CCK and PYY were not measured in this study, a previous study showed that protein is more effective than fat at delaying gastric emptying (37), and, in normal-weight and obese humans, protein intake induces a greater release of PYY than does fat (288).

Gram per gram, the insulin-raising effect of protein was 2 times that of fat. This may be because hepatic insulin extraction was not affected by fat, whereas it was significantly decreased with protein intake (Figure 4.2). It is also possible that fat and protein may differentially modulate incretin hormone release and inactivation. We found that both 30g fat and protein significantly increased the GLP-1 response; however, we do not know whether GIP increased in a similar fashion. We did not measure GIP, but it is known that whey protein is a potent stimulator of GIP release (289), and it was previously shown that the early (30 min) GIP response was higher after protein ingestion than after fat ingestion (290). In terms of incretin inactivation, a rodent model showed that whey protein inhibits proximal small intestine dipeptidyl peptidase-4 activity (the enzyme deactivates GLP-1), thus prolonging the action of
GLP-1, whereas fat has no such effect (37). It is not known whether such effect also exists in the small intestine of humans.

We found that adding whey protein to 50g oral glucose had no effect on C-peptide or the insulin secretion rate, whereas a previous study showed that feeding protein (beef) alone increased the C-peptide response (291). This discrepancy is unlikely because of a slower gastric emptying time and lower glycemic response to oral glucose because the glucose response curves were identical over the first 15 minutes, regardless of whether whey protein was added to oral glucose or not (Figure 4.1). Nevertheless, why did whey protein dose-dependently increase the insulin concentration but have no effect on C-peptide or insulin secretion rate? The elevation of peripheral insulin concentrations after meal ingestion is due to both the stimulation of pancreatic \( \beta \)-cell insulin secretion and a reduction in hepatic insulin clearance (201, 202); therefore, one possible mechanism is that the increased insulin after whey protein may be due to reduced liver insulin clearance. Indeed, our data show that, although whey protein had no effect on the insulin secretion rate, it dose-dependently reduced hepatic insulin extraction (Figure 4.2). A previous study described a close relation between whey protein induced insulin response and a concomitant increase in postprandial amino acids (277), and leucine, isoleucine, valine, lysine and threonine are the major amino acids responsible for increased insulinemia (289). Beside amino acids, milk proteins are a highly abundant source of bioactive peptides (292). We postulate that certain branched-chain amino acids (i.e. leucine, isoleucine, valine, lysine and threonine) and/or whey-derived bioactive peptides (Appendix 7) may act as competitive inhibitors and that they compete with insulin by binding to insulin receptors on hepatocytes; thus less insulin is cleared by the liver, and peripheral insulin concentrations increase. The other possible mechanism for the unaltered C-peptide and insulin secretion rate is that whey protein
may affect the C-peptide metabolic clearance rate. C-peptide is susceptible to renal uptake, with >85% the amount primarily metabolized by the kidney and only a small proportion of C-peptide being excreted intact in urine (293). It is known that protein intake increases urea nitrogen production, and a strong positive correlation was observed between the changes in urea nitrogen excretion and the changes in creatinine clearance in humans (the measure of glomerular filtration rate), and also between protein intake and creatinine clearance (294). Therefore, it is possible that protein may increase glomerular filtration rate via increased urea nitrogen excretion, as the result of which more C-peptide may end up cleared by the kidney, causing no change in plasma C-peptide to be observed.

In conclusion, we found that the glucose-lowering effect of fat and protein was not blunted by insulin resistance. Fat has no effect on liver insulin clearance, whereas protein significantly decreased it. Protein significantly increased peripheral insulin concentration without any effect on C-peptide and insulin secretion rate, which may be explained by either decreased insulin clearance or increased rate of C-peptide clearance.
CHAPTER 5

ARE THE GLYCEMIC AND INSULINEMIC INDEX VALUES OF CARBOHYDRATE FOODS SIMILAR IN HEALTHY CONTROL, HYPERINSULINEMIC AND TYPE 2 DIABETIC PATIENTS?

EFFECTS OF METABOLIC STATUS ON GI AND INSULINEMIC INDEX

A partial content of this chapter is published in European Journal of Clinical Nutrition (370).
5.1 Abstract

Background: A criticism of Glycemic Index (GI) is that it does not indicate the insulin response of foods (Insulinemic Index). However, it is unknown if the GI and Insulinemic Index of foods are equivalent in all subjects, a necessary criterion for clinical utility.

Objective: To compare GI and Insulinemic Index in non-diabetic subjects with fasting-serum-insulin (FSI) <40pmol/L (Healthy Control) or with FSI≥ 40pmol/L (Hyperinsulinemic) and subjects with type 2 diabetes (T2DM), and to see whether GI and Insulinemic Index were related to the serum-glucose concentrations, insulin sensitivity, β-cell function, and hepatic insulin extraction of the subjects.

Design: Serum-glucose, -insulin and -C-peptide responses after 50g available-carbohydrate portions of glucose (tested 3 times by each subject), sucrose, instant mashed-potato, white-bread, polished-rice and pearled-barley were measured in Healthy Control (n=9), Hyperinsulinemic (n=12) and T2DM (n=10) subjects using standard GI methodology.

Results: Food GI values did not differ significantly among the 3 subject-groups, whereas Insulinemic Index values were higher in T2DM (100±7) than Healthy Control (78±5) and Hyperinsulinemic subjects (70±5) (mean ±SEM, p=0.05). Insulinemic Index was inversely associated with insulin sensitivity (r=−0.66, p<0.0001) and positively related to fasting- and postprandial-glucose (both r=0.68, p<0.0001) and hepatic insulin extraction (r=0.62, p=0.0002). In contrast, GI was not related to any of the biomarkers (p>0.05).

Conclusion: The GI is a valid property of foods because its value is similar in Healthy Control, Hyperinsulinemic and T2DM subjects, and is independent of subjects’ metabolic status. However, Insulinemic Index may depend upon the glycemic control, insulin sensitivity and hepatic insulin extraction of the subjects.
5.2 Introduction

The blood glucose raising potential of carbohydrate in foods is numerically classified as Glycemic Index (GI), which is the incremental area under the glycemic response curve (AUC) elicited by a 50g available-carbohydrate portion of a food expressed as a percentage of that after 50g glucose in the same subject (15). Many foods have been tested for their GI values in healthy or diabetic subjects (17). Since the GI values of foods are similar in healthy and diabetic subjects (19-21), GI values tested in healthy subjects can be applied in the nutritional management of diabetes (295). However, because GI has never been tested in non-diabetic subjects with insulin resistance/hyperinsulinemia, it is not known whether GI is valid in this population.

Because hyperinsulinemia may play a role in the pathogenesis of insulin resistance and associated chronic diseases (22-27), a major concern about the GI concept is that it does not consider concurrent insulin response. Thus, some investigators have begun to report values for Insulinemic Index (28-30). Insulinemic Index is calculated in a similar way to GI to measure the extent to which a food raises plasma insulin (31). However, for Insulinemic Index to be a valid property of a food, its value should be similar in different subjects regardless of their degree of insulin sensitivity, β-cell function or glucose tolerance status. There is evidence that relative insulin responses differ in lean and obese subjects with normal glucose tolerance (NGT), subjects with impaired glucose tolerance (IGT) and subjects with type 2 diabetes (296). In addition, relative insulin responses were inversely related to subjects’ fasting insulin, suggesting that Insulinemic Index may dependent on subject’s insulin sensitivity (297). However, these studies used mixed meals rather than individual foods and did not use standard GI methodology; thus,
their results cannot be used to draw conclusions about the validity of the Insulinemic Index of carbohydrate foods.

Therefore, we investigated whether the GI and Insulinemic Index of a variety of carbohydrate foods are similar in healthy control, hyperinsulinemic and type 2 diabetic subjects, and whether metabolic status such as insulin sensitivity, β-cell function, severity of glycemia (fasting- and postprandial-glucose), hepatic insulin extraction and plasma GLP-1 response of the subjects were correlated with the GI and Insulinemic Index obtained.

5.3 Subjects and Methods

5.3.1 Subjects and study design

We recruited male and non-pregnant, non-lactating female subjects aged 18-70 yr (BMI < 35kg/m²) with and without T2DM. Subjects were excluded for any of the following: history of gastrointestinal disease or gastroparesis, evidence of liver disease (aspartate transaminase, alanine transaminase, and gamma glutamyl transpeptidase > 2 times upper limit of normal) or kidney disease (creatinine > 1.2 times upper limit of normal), use of α-glucosidase or lipase inhibitors or insulin, or any acute medical or surgical event requiring hospitalization within 6 months. Subjects without diabetes had fasting glucose <7.0mmol/L and were divided prospectively into those with normal fasting serum insulin (FSI) (Healthy Control, n=9, FSI < 40 pmol/L), or high-FSI (Hyperinsulinemic, n=12, FSI ≥ 40 pmol/L). Currently, there are no criteria by which an individual could be classified as insulin sensitive or resistant, the rationale for using FSI is the fact that FSI is strongly correlated with insulin resistance measured by euglycemic- hyperinsulinemic clamp (260), and the 40 pmol/L cut-off point was chosen because this represents approximately the 67th percentile for non-diabetic subjects in our laboratory (244).
In previous studies, non-diabetic subjects with FSI > 40 pmol/L had significantly greater waist circumference (WC), BMI, homeostasis model assessment (HOMA) insulin resistance index, total and LDL cholesterol and triglycerides, and lower HDL cholesterol than those with FSI < 40 pmol/L (38).

Subjects with T2DM (n=10) had fasting glucose > 7.0 mmol/L or HbA1C > 6.0% and were treated by diet alone or any combination of sulfonylurea, metformin, thiazolidinedione and/or meglitinide. After screening 25 subjects with T2DM, 10 were recruited who had HbA1C of 7.3 ± 0.3% (mean±SEM) and duration of diabetes of 7.0±1.0 y (mean±SEM). Subjects were excluded for any of the following: history of gastrointestinal disease or gastroparesis, evidence of liver disease (aspartate transaminase, alanine transaminase, and gamma glutamyl transpeptidase > 2 times upper limit of normal) or kidney disease (creatinine > 1.2 times upper limit of normal), use of α-glucosidase or lipase inhibitors or insulin, or any acute medical or surgical event requiring hospitalization within 6 months. Eight patients were on metformin alone, 1 on metformin and pioglitazone and 1 on metformin and sulfonylurea. The patients took their usual medication on study days after the fasting blood sample and prior to starting the test meal. None of the patients had a history of micro- or macro-vascular complications.

There were originally 13 Hyperinsulinemic subjects, one subject missed 2 test meals; thus her data were excluded from the statistical analysis. In a T2DM patient, the AUC of insulin to one of the oral glucose tests was exceedingly low; therefore, the data from that oral glucose test were not used in calculating the reference AUCs for GI, Insulinemic Index and C-peptide index.

The protocol was designed to conform to standard GI testing methodology for subjects with and without diabetes. Subjects were instructed to maintain their usual daily routine and
food intake patterns between study days and refrain from exercise on the mornings of the test. After an overnight fast (10-14hr), they came to the Risk Factor Modification Center at St. Michael’s hospital on 8 separate mornings between 7:30-9:30 am. Healthy controls and Hyperinsulinemic subjects had venous blood samples drawn just before and at 15, 30, 45, 60, 90 and 120 min after starting to eat. T2DM subjects had venous blood samples drawn fasting and at 30, 60, 90, 120 and 180 min after starting to eat. The samples were used to measure plasma glucose, insulin, C-peptide and GLP-1. The protocol was reviewed and approved by Research Ethics Boards at the University of Toronto and St. Michael’s Hospital. All subjects gave written informed consent.

5.3.2 Test foods

Subjects were fed test meals consisting of 50g available carbohydrate (defined as total carbohydrate minus dietary fibre) as 50g anhydrous glucose (Grain Processing Enterprising LTD, Scarborough, Ontario, Canada), 50g sucrose (Redpath brand, Toronto, Ontario, Canada), 71.8 g instant mashed potato (General Mills, Mississauga, Ontario, Canada), 107g white bread (Weston Bakeries LTD, Toronto, Ontario, Canada), 62.5 g polished rice (Dainty brand, Windsor, Ontario, Canada) and 80.6 g pearled barley (Quik kook brand, JVF Canada Inc, Toronto, Ontario, Canada). The nutrient composition of the test foods was shown in Table 5.1. Sugars (glucose + sucrose) were dissolved in 250ml water. Instant potato was weighed dry, and boiling water added according to package directions. Two to four portions of rice and barley were weighed dry, boiled in salted water according to package directions on the morning of the test. The resulting cooked amount was weighed and divided into portions. The test meals were served
with a glass of water. Each subject tested glucose 3 times (first, fourth and last tests) and the other 5 test meals were once each in randomized order.

5.3.3 Blood analysis

Venous blood samples for glucose, insulin and C-peptide were collected in BD Vacutainer™ SST™ tubes (BD, Franklin Lakes, NJ, USA). Serum glucose was measured by a glucose oxidase method (SYNCHRON LX Systems, Beckman Coulter, Brea, California, USA), with inter-assay coefficient of variation (CV) of 1.9%. Insulin was measured using one-step immunoenzymatic ("sandwich") assay (Beckman Access Ultrasensitive Insulin Assay, Beckman Coulter, Brea, California, USA), with inter-assay CV of 2.5 to 4.3%. Insulin has no cross-reactivity with proinsulin. C-peptide was measured using double antibody competitive radioimmunoassay (Siemens Medical Solutions Diagnostics, Los Angeles, CA), with inter-assay precision of 10% or less.

Venous blood samples for GLP-1 were collected in BD Vacutainer™ EDTA™ tubes (BD, Franklin Lakes, NJ, USA). The dipeptidyl peptidase-4 inhibitor (Linco Research, St. Charles, Missouri) was added immediately (less than 30 seconds) after collection. Plasma GLP-1 was measured by capturing the active GLP-1 from the sample by a monoclonal antibody (specific binding to the N-terminal region) using GLP-1 (active) ELISA Kit (Linco Research, St. Charles, Missouri, USA), with inter-assay CV ranges from 1-13%. All the samples were stored at -70°C before analysis.
5.3.4 Calculations and statistical analysis

The sample size was determined based on previous studies (296, 298), with a power of 80% and p<0.05 (with GI and Insulinemic Index as the dependent measures). Incremental areas under the curve (AUC), ignoring area below baseline, for glucose, insulin, C-peptide and GLP-1 were calculated using the trapezoid rule (31). Hepatic insulin extraction (HIE\textsubscript{auc}) was determined by the AUC of C-peptide divided by that of insulin (268). Insulin secretion rate (ISR) was calculated from deconvolution of the plasma C-peptide concentration using ISEC software package developed by Hovorka et al (269). Glycemic Index (GI) was calculated as the AUC of the test food expressed as a percentage of the mean AUC of 3 tests of oral glucose in the same subject (15); the mean of the resulting values was the GI of the food. Insulinemic Index was calculated in a similar fashion as GI. C-peptide is co-secreted with insulin in equimolar amounts, but is not subjected to hepatic insulin clearance, which varies considerably; thus, C-peptide is regarded as a much better estimate of insulin secretion than levels of insulin itself (239). Therefore, C-peptide index was also calculated using the same method for GI and Insulinemic Index calculation.

The mean glucose and insulin values of the 3 oral glucose tests were used to calculate insulin sensitivity index, which was calculated using two validated OGTT-derived indices of insulin sensitivity: 1) Matsuda’s insulin sensitivity index, which incorporates both hepatic and muscle components of insulin resistance, correlates well with euglycemic-hyperinsulinemic clamp, and was calculated as 10,000/(fasting glucose × fasting insulin × mean glucose × mean insulin )\textsuperscript{0.5} (173); 2) Mari’s glucose-insulin model-based oral glucose insulin sensitivity index constructed on established principles of glucose kinetics and insulin action (174). Oral glucose
insulin sensitivity has been validated against the euglycemic-hyperinsulinemic clamp in healthy, obese and type 2 diabetic subjects (174).

The β-cell compensation for insulin resistance was estimated using the insulin secretion/insulin resistance (disposition) index derived from OGTT(270), which was shown to be the best predictor of future development of type 2 diabetes in subjects with NGT compared with other predictive models such as San Antonio Diabetes Prediction Model (271) (including age, sex, ethnicity, BMI, blood pressure, fasting plasma glucose, triglycerides, and HDL) and 2-hour plasma glucose concentration. To calculate insulin secretion/insulin resistance (disposition) index, first, insulin secretion was expressed as the changes in AUC of insulin secretion rate ($\Delta$ISR$_{AUC}$) relative to changes in AUC of plasma glucose response ($\Delta$ISR$_{AUC}$/ΔG$_{AUC}$), then $\Delta$ISR$_{AUC}$/ΔG$_{AUC}$ is divided by the severity of insulin resistance ($\Delta$ISR$_{AUC}$/ΔG$_{AUC}$/IR), as measured by the inverse of Matsuda’s insulin sensitivity index.

Data were expressed as mean ± SEM for normally distributed variables or median (interquartile range) for non-normally distributed variables. Normality was assessed using the Shapiro and Wilk statistic and the normality plots (PROC UNIVARIATE procedure of SAS). Skewed variables were log-transformed prior to analysis. The values of GI, Insulinemic Index and C-peptide index were subjected to repeated measures ANOVA (PROC MIXED) to test for the main effects of food and subject group (Healthy Control, Hyperinsulinemic and T2DM) and the food×group interaction. Age, BMI and WC were included in the model as covariates to control for the differences in these variables between subjects. Tukey’s post hoc test was performed to compare individual means if the main effects or interactions were statistically significant.
The correlations between GI, Insulinemic Index and the metabolic indices [insulin sensitivity, β-cell function, hepatic insulin extraction, severity of glycemia (fasting glucose, mean postprandial glucose and glucose AUC), and GLP-1 response] were determined by simple linear regressions. Step-wise multiple regression analysis was used to examine the extent to which the different variables accounted for the variability of GI or Insulinemic Index. When GI is the dependent variable, the independent variables are age, BMI, oral glucose insulin sensitivity, insulin secretion/insulin sensitivity (disposition index), hepatic insulin extraction, and the AUC of glucose and GLP-1. When Insulinemic Index is the dependent variable, the independent variables are GI, age, BMI, oral glucose insulin sensitivity, insulin secretion/insulin sensitivity (disposition index), hepatic insulin extraction, and the AUC of glucose and GLP-1. Collinearity was determined by including variance inflation factor in the model, with variance inflation factor of 5 or 10 and above indicates a multicollinearity problem (299). No collinearity was apparent for the variables included in the regression analysis. All analyses were done using SAS 9.2, (SAS Institute Inc, Cary). Differences were considered significant if 2-tailed p<0.05.

5.4 Results

T2DM subjects were significantly older and had higher BMI and WC than both Healthy Control and Hyperinsulinemic subjects (Table 5.2). Fasting glucose and postprandial glucose responses (glucose AUC) were not significantly different between Healthy Control and Hyperinsulinemic subjects but were significantly higher in T2DM (Table 5.2). The intra-individual coefficient of variations (CV) of blood glucose AUC for repeated tests of oral glucose for Healthy Control, Hyperinsulinemic and T2DM subjects are 24±5, 25±3 and 17±3 (mean±SEM) respectively. Fasting insulin was significantly higher in Hyperinsulinemic and T2DM than Healthy Control subjects. Fasting C-peptide increased in a step-wise fashion from
Healthy Control to Hyperinsulinemic to T2DM (Table 5.2). Hepatic insulin extraction was significantly higher in T2DM than Hyperinsulinemic subjects. Matsuda’s insulin sensitivity index was significantly lower in Hyperinsulinemic and T2DM than Healthy Control subjects, while oral glucose insulin sensitivity and disposition index decreased step-wisely from Healthy Control to Hyperinsulinemic to T2DM (Table 5.2). Systolic and diastolic blood pressure, total cholesterol, triglyceride, total:HDL cholesterol were similar in Healthy Control and Hyperinsulinemic subjects, but were significantly higher in T2DM. Fasting and postprandial GLP-1, HDL, LDL and CRP were not significantly different among the 3 subject groups.

Figure 5.1 shows the 2-3hr postprandial glucose, insulin, C-peptide and GLP-1 responses elicited by different foods in Healthy Control, Hyperinsulinemic and T2DM subjects. For all foods, the general trend was that serum glucose was elevated in T2DM, whereas it was similar in Healthy Control and Hyperinsulinemic; serum insulin was much higher in Hyperinsulinemic than Healthy Control and T2DM subjects; C-peptide was elevated in both Hyperinsulinemic and T2DM subjects; GLP-1 was lower in T2DM but similar in Healthy Control and Hyperinsulinemic subjects.

Table 5.3 shows that the mean AUC for glucose and insulin differed significantly across subject-groups for each individual food as well as for the mean of all carbohydrate foods. C-peptide AUC was significantly higher in Hyperinsulinemic than Healthy Controls for oral glucose, rice and the mean of all foods (Table 5.3). GLP-1 AUC was not significantly different among subject groups for any food individually nor for the mean of all carbohydrate foods (Table 5.3). The AUCs of ISR were significantly higher in Hyperinsulinemic subjects than Healthy Control and T2DM subjects except for white bread and barely (Table 5.3). Hepatic
insulin extraction in response to oral glucose was significantly higher in T2DM than Hyperinsulinemic subjects (Table 5.3).

There were significant food effects on GI, Insulinemic Index and C-peptide index (p<0.0001) (Table 5.4), demonstrating that different carbohydrate foods produced different GI, Insulinemic Index or C-peptide index. For GI values, there was neither significant subject-group effects (p=0.20) nor significant food × subject-group interactions (p=0.26) (Table 5.4); thus, the GI values were not significantly different among Healthy Control, Hyperinsulinemic and T2DM for any food individually, nor for the mean of all carbohydrate foods (Table 5.5). For Insulinemic Index, there was a tendency of significant subject-group effects (p=0.05) and food × subject-group interaction effects (p=0.09); thus the Insulinemic Index of white bread was significantly higher in T2DM than Healthy Control and Hyperinsulinemic subjects (p=0.01) and the mean Insulinemic Index of all carbohydrate foods was much higher in T2DM than Healthy Control and Hyperinsulinemic subjects (p=0.05) (Table 5.5). For C-peptide index, there was a significant food × subject-group interactions (p=0.03), but no significant subject-group effects (p=0.40) (Table 5.4). The values of C-peptide index were not significantly different among Healthy Control, Hyperinsulinemic and T2DM for any food individually, nor for the mean of all carbohydrate foods (Table 5.5).

The GI and Insulinemic Index were well correlated in Healthy Control (r=0.61, p<0.0001), Hyperinsulinemic subjects (r=0.62, p<0.0001) and T2DM patients (r=0.29, p=0.04) respectively (Figure 5.2); similarly, the GI and C-peptide index were also correlated in Healthy Control (r=0.36, p=0.02), Hyperinsulinemic subjects (r=0.48, p=0.0001) and T2DM patients (r=0.46, p=0.0009) respectively (Figure 5.2). The regression lines of GI vs. Insulinemic Index
C-peptide index were nearly identical for Healthy Control, Hyperinsulinemic and T2DM patients (Figure 5.2).

The GI was not related to any of the anthropometric (age and BMI) or metabolic indices (oral glucose insulin sensitivity, hepatic insulin extraction, disposition index and GLP-1 response) for each food individually (p>0.05), nor for the mean of all carbohydrate foods (p > 0.05) (Figure 5.3). Multiple regression analysis using GI as dependent variable and age, BMI, oral glucose insulin sensitivity, disposition index, hepatic insulin extraction and AUC of glucose and GLP-1 as independent variables found that none of the variables met the 0.05 significance level for entry into the model.

Insulinemic Index was inversely associated with oral glucose insulin sensitivity (r = -0.66, p<0.0001) and positively related to hepatic insulin extraction (r = 0.62, p=0.0002). GI was not related to either oral glucose insulin sensitivity (r = -0.07, p=0.73) or hepatic insulin extraction (r=-0.09, p=0.65) (Figure 5.3). C-peptide index was not significantly related to oral glucose insulin sensitivity (r=-0.17, p=0.37) and hepatic insulin extraction (r=0.35, p=0.057). The GI, Insulinemic Index, and C-peptide index were not related to either GLP-1 response or disposition index (p>0.05). Multiple regression analysis using Insulinemic Index as dependent variable and age, BMI, GI, oral glucose insulin sensitivity, disposition index, hepatic insulin extraction and the AUC of glucose and GLP-1 as independent variables showed that oral glucose insulin sensitivity and hepatic insulin extraction together predicted Insulinemic Index (Insulinemic Index =106-0.09×oral glucose insulin sensitivity +2.75×hepatic insulin extraction), and explained approximately 51% of the variation in Insulinemic Index ($r^2=0.51$, p<0.0001). In particular, oral glucose insulin sensitivity alone explained 43% of the variation in Insulinemic Index ($r^2=0.43$, p<0.001).
GI was not related to any of the markers of the severity of glycemia (p>0.05) (Figure 5.4) whereas Insulinemic Index was positively associated with fasting glucose (r=0.68, p<0.0001), mean postprandial glucose (r=0.68, p<0.0001) and glucose AUC (r=0.46, p=0.009).

Figure 5.5 shows the comparison of the overall means of GI, Insulinemic Index and C-peptide index for each carbohydrate food for all 31 subjects. Barley, rice, sucrose and the mean of all carbohydrate foods had similar GI, Insulinemic Index and C-peptide index; however, mashed potato had disproportionately higher Insulinemic Index and C-peptide index than GI and white bread had higher Insulinemic Index than GI (Figure 5.5).
Table 5.1  Nutrition composition of the test foods

<table>
<thead>
<tr>
<th></th>
<th>Weight (g)</th>
<th>Total CHO (g)</th>
<th>Fat (g)</th>
<th>Protein (g)</th>
<th>Dietary fiber (g)</th>
<th>Available CHO (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral glucose</td>
<td>50.0</td>
<td>50.0</td>
<td>0</td>
<td>0</td>
<td>0.0</td>
<td>50.0</td>
</tr>
<tr>
<td>Sucrose</td>
<td>50.0</td>
<td>50.0</td>
<td>0</td>
<td>0</td>
<td>0.0</td>
<td>50.0</td>
</tr>
<tr>
<td>Mashed potato</td>
<td>71.8</td>
<td>56.3</td>
<td>0.9</td>
<td>6.2</td>
<td>6.3</td>
<td>50.0</td>
</tr>
<tr>
<td>White bread</td>
<td>107</td>
<td>51.4</td>
<td>2.9</td>
<td>10</td>
<td>1.4</td>
<td>50.0</td>
</tr>
<tr>
<td>Rice</td>
<td>62.5</td>
<td>50.0</td>
<td>0</td>
<td>5.6</td>
<td>0.0</td>
<td>50.0</td>
</tr>
<tr>
<td>Barley</td>
<td>80.6</td>
<td>62.5</td>
<td>0.9</td>
<td>9.0</td>
<td>12.5</td>
<td>50.0</td>
</tr>
</tbody>
</table>

Source: the information on carbohydrate, fat, protein and dietary fiber are from the nutrient composition table on the package of the foods. Available CHO = total CHO - dietary fiber.
<table>
<thead>
<tr>
<th>Table 5.2   Anthropometric and metabolic characteristics of the study groups&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Control (n=9)</th>
<th>Hyper[1] (n=12)</th>
<th>T2DM (n=10)</th>
<th>( P^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>24(23-29)&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>26(23-37)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>57(53-58)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI (kg/m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>22(21-22)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25(22-30)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>32(29-34)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>72(70-76)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>88(77-95)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>102(94-115)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>4.6 (4.5-4.7)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.9 (4.7-5.2)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.8 (7.2-11.3)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Glucose AUC (mmol×min/L)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>198(138-291)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>247(186-278)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>831(699-978)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.0002</td>
</tr>
<tr>
<td>Fasting insulin (pmol/L)</td>
<td>29 (24-31)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51 (47-57)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>51 (42-63)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.0002</td>
</tr>
<tr>
<td>Fasting C-peptide (pmol/L)</td>
<td>231 (213-241)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>412 (345-445)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>784 (711-878)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fasting GLP-1 (pmol/L)</td>
<td>6.1±2.2</td>
<td>6.5±2.2</td>
<td>4.4±0.6</td>
<td>0.70</td>
</tr>
<tr>
<td>2hr-postprandial GLP-1 (pmol ×min/L)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>211(94-340)</td>
<td>183(113-294)</td>
<td>281(133-420)</td>
<td>0.64</td>
</tr>
<tr>
<td>Hepatic insulin extraction&lt;sup&gt;4&lt;/sup&gt;</td>
<td>3.8(3.3-6.4)&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.4(2.7-3.8)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.8(5.7-10.4)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.002</td>
</tr>
<tr>
<td>Matsuda’s insulin sensitivity index&lt;sup&gt;4&lt;/sup&gt;</td>
<td>28 (27-32)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14 (12-17)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12 (9-15)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Oral glucose insulin sensitivity&lt;sup&gt;4&lt;/sup&gt;</td>
<td>497 (478-521)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>459 (424-485)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>280 (259-362)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.001</td>
</tr>
<tr>
<td>Disposition index&lt;sup&gt;4&lt;/sup&gt;</td>
<td>63±5&lt;sup&gt;a,5&lt;/sup&gt;</td>
<td>38±4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29±4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.0008</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>101±2.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>109±2.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>130±4.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>61±2.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>67±2.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>79±2.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.0001</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.1±0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.4±0.3&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>5.5±0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.02</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.7 (0.5-0.9)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.9 (0.8-1.1)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.5 (1.2-1.9)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.02</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.4±0.1</td>
<td>1.2±0.1</td>
<td>1.2±0.1</td>
<td>0.41</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>2.4±0.3</td>
<td>2.7±0.2</td>
<td>3.1±0.3</td>
<td>0.27</td>
</tr>
<tr>
<td>Total:HDL cholesterol</td>
<td>2.8 (2.7-3.8)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.6 (3.2-3.9)&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.9 (3.3-6.6)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.02</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>0.7 (0.2-1.2)</td>
<td>1.5 (0.5-3.8)</td>
<td>3.7 (1.1-5.4)</td>
<td>0.10</td>
</tr>
</tbody>
</table>
Healthy, non-diabetic subjects with fasting serum insulin (FSI) <40pmol/L; Hyperinsulinemic, non-diabetic subjects with FSI≥40pmol/L; T2DM, subjects with type 2 diabetes; BP, blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

2 P represents overall significant differences across groups. P-values were derived from one-way ANOVA except those for 2-hour postprandial GLP-1 response, hepatic insulin extraction, Matsuda’s insulin sensitivity index, oral glucose insulin sensitivity and disposition index.

3 Median; interquartile range in parentheses (all such values). Values in the same row with different superscript letters differ significantly, P<0.05 (Tukey’s post hoc test).

4 Calculated by using the mean of 3 oral-glucose-tolerance test results. PROC MIXED repeated measures ANOVA, adjusted for age, BMI and WC. The units for Matsuda’s insulin sensitivity index, oral glucose insulin sensitivity and disposition index are (mmol ×pmol)^{-1}×L, (ml×min^{-1} ×m^{-2}), and (mmol)^{-2}×kg^{-1}×min^{-1}×L respectively. Hepatic insulin extraction doesn’t have units.

5 Mean±SEM (all such values).
Table 5.3  The glucose, insulin, C-peptide, GLP-1 and insulin secretion rate (ISR) expressed as area under the curve (AUC) and hepatic insulin extraction (HIE) for each individual food and the mean of all carbohydrate foods in the study groups:

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Glucose AUC (mmol ×min/L)</th>
<th>Sucrose</th>
<th>Potato</th>
<th>Bread</th>
<th>Rice</th>
<th>Barley</th>
<th>Mean ²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control FSI&lt;40</td>
<td>198(138-291) a, b</td>
<td>123(84-165) a</td>
<td>256(139-265) a</td>
<td>125(66-149) a</td>
<td>134(82-151) a</td>
<td>140(69-175) a</td>
<td>139(92-200) a</td>
</tr>
<tr>
<td>Hyper[I] FSI≥40</td>
<td>247(186-278) a</td>
<td>163(118-213) a</td>
<td>163(105-356) a</td>
<td>173(126-258) b</td>
<td>212(119-232) b</td>
<td>111(58-161) a</td>
<td>174(120-239) b</td>
</tr>
<tr>
<td>Type 2 Diabetes</td>
<td>831(699-978) b</td>
<td>473(402-747) b</td>
<td>708(562-1145) b</td>
<td>651(436-818) c</td>
<td>609(403-851) c</td>
<td>468(255-564) b</td>
<td>609(437-826) c</td>
</tr>
<tr>
<td></td>
<td>P 0.0002</td>
<td>0.002</td>
<td>0.0004</td>
<td>0.0004</td>
<td>&lt;0.0001</td>
<td>0.01</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Insulin AUC (nmol×min/L)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Control FSI&lt;40</td>
<td>16(12-18) a</td>
<td>8(8-14) a</td>
<td>20(15-24) a</td>
<td>11(10-17) a</td>
<td>9(7-11) a</td>
<td>6(4-9) a</td>
</tr>
<tr>
<td>Hyper[I] FSI≥40</td>
<td>33(29-49) b</td>
<td>23(19-37) b</td>
<td>39(23-60) b</td>
<td>25(16-46) b</td>
<td>19(14-22) b</td>
<td>12(8-20) b</td>
</tr>
<tr>
<td>Type 2 Diabetes</td>
<td>14(10-25) a</td>
<td>12(7-17) a</td>
<td>17(14-29) ab</td>
<td>18(11-25) ab</td>
<td>11(8-19) ab</td>
<td>9(6-11) ab</td>
</tr>
<tr>
<td></td>
<td>P &lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.002</td>
<td>0.03</td>
<td>0.002</td>
<td>0.04</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Subjects</th>
<th>C-peptide AUC (nmol×min/L)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Control FSI&lt;40</td>
<td>64±4 a</td>
<td>42±7 a</td>
<td>87±10</td>
<td>56±8</td>
<td>28±7 a</td>
<td>30±4</td>
</tr>
<tr>
<td>Hyper[I] FSI≥40</td>
<td>117±9 b</td>
<td>82±8</td>
<td>108±12</td>
<td>92±12</td>
<td>89±12 b</td>
<td>50±6</td>
</tr>
<tr>
<td>Type 2 Diabetes</td>
<td>111±15 ab</td>
<td>93±14</td>
<td>139±20</td>
<td>122±15</td>
<td>85±15 ab</td>
<td>62±9</td>
</tr>
<tr>
<td></td>
<td>P 0.0009</td>
<td>0.049</td>
<td>0.37</td>
<td>0.11</td>
<td>0.01</td>
<td>0.19</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Subjects</th>
<th>GLP-1 AUC (pmol×min/L)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Control FSI&lt;40</td>
<td>211(94-340)</td>
<td>66(46-217)</td>
<td>169(90-329)</td>
<td>66(49-184)</td>
<td>59(26-87)</td>
<td>52(34-71)</td>
</tr>
<tr>
<td>Hyper[I] FSI≥40</td>
<td>183(113-294)</td>
<td>94(40-151)</td>
<td>159(99-281)</td>
<td>80(8-213)</td>
<td>33(18-184)</td>
<td>23(1-89)</td>
</tr>
<tr>
<td>Type 2 Diabetes</td>
<td>281(133-420)</td>
<td>62(20-227)</td>
<td>216(90-270)</td>
<td>169(34-259)</td>
<td>102(70-186)</td>
<td>72(21-111)</td>
</tr>
<tr>
<td></td>
<td>P 0.64</td>
<td>0.85</td>
<td>0.99</td>
<td>0.91</td>
<td>0.60</td>
<td>0.95</td>
</tr>
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</table>
Table 5.3 (continued)

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Glucose</th>
<th>Sucrose</th>
<th>Potato</th>
<th>Bread</th>
<th>Rice</th>
<th>Barley</th>
<th>Mean^\text{*}</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISR AUC (pmol/kg)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Control FSI&lt;40</td>
<td>404±32^a</td>
<td>281±45^a</td>
<td>546±63^a</td>
<td>395±55</td>
<td>233±46^a</td>
<td>225±32</td>
<td>361±21^a</td>
</tr>
<tr>
<td>Hyper[I] FSI≥40</td>
<td>641±44^b</td>
<td>504±60^b</td>
<td>692±61^b</td>
<td>540±85</td>
<td>565±75^b</td>
<td>364±53</td>
<td>576±27^b</td>
</tr>
<tr>
<td>Type 2 Diabetes</td>
<td>559±29^a</td>
<td>498±50^ab</td>
<td>568±62^ab</td>
<td>555±43</td>
<td>456±52^ab</td>
<td>419±47</td>
<td>522±18^b</td>
</tr>
<tr>
<td>*P</td>
<td>0.001</td>
<td>0.02</td>
<td>0.04</td>
<td>0.24</td>
<td>0.003</td>
<td>0.14</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Hepatic insulin extraction

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Control FSI&lt;40</th>
<th>Hyper[I] FSI≥40</th>
<th>Type 2 Diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.8(3.3-6.4)^ab</td>
<td>3.4(2.7-3.8)^a</td>
<td>6.8(5.7-10.4)^b</td>
</tr>
<tr>
<td></td>
<td>5.4(2.0-7.0)</td>
<td>3.2(2.3-4.2)</td>
<td>7.3(5.3-8.4)</td>
</tr>
<tr>
<td></td>
<td>4.4(3.8-5.5)</td>
<td>3.1(2.3-3.7)</td>
<td>6.4(4.7-8.4)</td>
</tr>
<tr>
<td></td>
<td>5.1±0.8</td>
<td>3.3±0.5</td>
<td>6.9±0.9</td>
</tr>
<tr>
<td></td>
<td>3.0(1.7-4.5)</td>
<td>4.0(2.8-6.6)</td>
<td>6.9(5.7-7.7)</td>
</tr>
<tr>
<td></td>
<td>5.2(2.6-7.6)</td>
<td>3.9(2.7-6.1)</td>
<td>6.4(5.0-9.7)</td>
</tr>
<tr>
<td></td>
<td>4.9(2.8-6.6)</td>
<td>3.3(2.5-4.4)</td>
<td>6.8(5.4-9)</td>
</tr>
<tr>
<td>*P</td>
<td>0.002</td>
<td>0.26</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>0.07</td>
<td>0.24</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Healthy control subjects with fasting serum insulin (FSI) <40pmol/L (n=9); Hyperinsulinemic, non-diabetic subjects with FSI≥40pmol/L (n=12); T2DM, subjects with type 2 diabetes (n=10).

2 Refers to the mean of all carbohydrate foods.

3 Median; interquartile range in parentheses (all such values). Values in the same column with different superscript letters are significantly different, *P* < 0.05 (Tukey’s *post hoc* test).

4 Mean±SEM (all such values).

5 *P* refers to overall significant differences across subject-groups (repeated measures ANOVA, PROC MIXED, Tukey’s *post hoc* test, adjusted for age, BMI and WC).
Table 5.4  Main and interaction effects of food, subject group on GI, Insulinemic Index and C-peptide index

<table>
<thead>
<tr>
<th></th>
<th>GI$^2$</th>
<th>Insulinemic Index$^2$</th>
<th>C-peptide index$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p-value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Subjects group$^1$</td>
<td>0.20</td>
<td>0.05</td>
<td>0.40</td>
</tr>
<tr>
<td>Food×subjects group</td>
<td>0.26</td>
<td>0.09</td>
<td>0.03</td>
</tr>
</tbody>
</table>

$^1$Healthy Control vs. Hyper [I] vs. T2DM subjects. The p-values are derived from repeated measures of ANOVA (PROC MIXED). Age, BMI and WC are included in the model as covariates.

$^2$GI was normally distributed. Insulinemic Index and C-peptide index were not normally distributed and were log transformed prior analysis.
Table 5.5  The GI, Insulinemic Index and C-peptide index of different carbohydrate foods in the study groups

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Glucose</th>
<th>Sucrose</th>
<th>Potato</th>
<th>Bread</th>
<th>Rice</th>
<th>Barley</th>
<th>Mean¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycemic Index (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>100±0</td>
<td>68±9</td>
<td>101±7</td>
<td>63±11</td>
<td>61±7</td>
<td>58±6</td>
<td>70±4</td>
</tr>
<tr>
<td>Hyper[I]</td>
<td>100±0</td>
<td>69±6</td>
<td>81±10</td>
<td>70±6</td>
<td>72±7</td>
<td>44±6</td>
<td>67±3</td>
</tr>
<tr>
<td>T2DM</td>
<td>100±0</td>
<td>68±5</td>
<td>98±5</td>
<td>71±6</td>
<td>70±10</td>
<td>47±5</td>
<td>70±4</td>
</tr>
<tr>
<td>P³</td>
<td>0.95</td>
<td>0.20</td>
<td>0.13</td>
<td>0.10</td>
<td>0.55</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td>Insulinemic Index (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>100±0</td>
<td>70±8</td>
<td>128±10</td>
<td>86±9²</td>
<td>60±6</td>
<td>46±5</td>
<td>78±5</td>
</tr>
<tr>
<td>Hyper[I]</td>
<td>100±0</td>
<td>74±8</td>
<td>114±10</td>
<td>74±7²</td>
<td>56±7</td>
<td>35±4</td>
<td>70±5</td>
</tr>
<tr>
<td>T2DM</td>
<td>100±0</td>
<td>81±10</td>
<td>137±12</td>
<td>138±13³</td>
<td>73±7</td>
<td>70±16</td>
<td>100±7</td>
</tr>
<tr>
<td>P</td>
<td>0.85</td>
<td>0.28</td>
<td>0.01</td>
<td>0.81</td>
<td>0.31</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>C-peptide index (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>100±0</td>
<td>65±11</td>
<td>142±18</td>
<td>85±10</td>
<td>45±11</td>
<td>47±7</td>
<td>77±7</td>
</tr>
<tr>
<td>Hyper[I]</td>
<td>100±0</td>
<td>74±9</td>
<td>96±12</td>
<td>77±8</td>
<td>77±10</td>
<td>44±6</td>
<td>74±4</td>
</tr>
<tr>
<td>T2DM</td>
<td>100±0</td>
<td>83±9</td>
<td>123±14</td>
<td>113±11</td>
<td>72±8</td>
<td>57±6</td>
<td>90±6</td>
</tr>
<tr>
<td>P</td>
<td>0.44</td>
<td>0.07</td>
<td>0.14</td>
<td>0.24</td>
<td>0.70</td>
<td>0.40</td>
<td></td>
</tr>
</tbody>
</table>

¹ Healthy control subjects with fasting serum insulin (FSI) <40pmol/L (n=9); Hyperinsulinemic, non-diabetic subjects with FSI≥40pmol/L (n=12); T2DM, subjects with type 2 diabetes (n=10).

² Values (Mean±SEM) in the same column with different superscript letters are significantly different, P < 0.05 (Tukey’s post hoc test).

³ Refers to the mean of all carbohydrate foods.
\textsuperscript{3} \textit{p}-values refer to overall significant differences across subject-groups, and were derived from repeated measures of ANOVA (PROC MIXED, Tukey’s \textit{post hoc} test). Age, BMI and WC were included in the model as covariates.
Figure 5.1 The 2-3hr postprandial glucose, insulin, C-peptide and GLP-1 responses (Mean±SEM) to different carbohydrate foods in Healthy Control (FSI < 40pmol/L, open circle, dotted line), Hyperinsulinemic (FSI ≥ 40pmol/L, grey circle, solid line) and T2DM subjects (black circle, solid line).
Figure 5.2 The linear correlation between Glycemic Index (GI) and Insulinemic Index (top) and C-peptide index (bottom) respectively in Healthy Control (FSI< 40pmol/L, open circle and solid line), Hyperinsulinemic (FSI ≥ 40pmol/L, grey circle and dotted line) and T2DM (black circle and dashed line). The lines are regression line.
Figure 5.3  The linear correlation between GI (top) or Insulinemic Index (middle) or C-peptide index (bottom) and oral glucose insulin sensitivity (OGIS) (left) and hepatic insulin extraction (HIE) (right) respectively in all subject-groups (Healthy Control, FSI < 40pmol/L, white circle; Hyperinsulinemic, FSI ≥ 40pmol/L, grey circle; and T2DM, black circle). r: Pearson’s correlation coefficient. The values of GI, Insulinemic Index and C-peptide index are the mean of five carbohydrate foods for each subject (n=31). Oral glucose insulin sensitivity and hepatic
insulin extraction are calculated from the mean of 3 oral-glucose-tests. The lines are regression line.
Figure 5.4 The linear correlation between GI (top) or Insulinemic Index (bottom) and fasting glucose (left) and mean postprandial glucose (middle) and glucose AUC (right) respectively in all subject-groups (Healthy Control, FSI < 40pmol/L, white circle; Hyperinsulinemic, FSI ≥ 40pmol/L, grey circle; and T2DM, black circle). r: Pearson’s correlation coefficient. The values of GI and Insulinemic Index are the mean of five carbohydrate foods for each subject (n=31). Fasting glucose, mean postprandial glucose and glucose AUC are calculated from the mean of 3 oral-glucose-tests. The lines are regression line.
Figure 5.5  The comparison of overall mean±SEM of GI (white bar), Insulinemic Index (grey bar) and C-peptide index (black bar) for each food and all carbohydrate foods. Data are from all subject groups (Healthy Control, Hyperinsulinemic and T2DM). The p-values were derived from repeated measures of ANOVA (PROC MIXED, Tukey’s post hoc test). Age, BMI and WC were included in the model as covariates.
5.5 Discussion and conclusions

The results of this study confirm that the GI values of carbohydrate foods are similar in all subjects regardless of the severity of glycemia or degree of insulin resistance. This shows that GI is a property of foods and affirms its clinical utility in a broad population. However, the Insulinemic Index values of carbohydrate foods were inversely associated with insulin sensitivity but positively related to the severity of glycemia and hepatic insulin extraction of the subjects, suggesting that Insulinemic Index is not solely a property of foods but also depends on the metabolic status of the subjects in whom it is measured.

We found that between-subject variation of GI values was not explained by any demographic, anthropometric or metabolic variable measured. Previous studies have shown that GI values are similar in healthy controls vs. T2DM (241), individuals with type 1 diabetes (T1DM) vs. individuals with T2DM (242), adults vs. children with T1DM (21), T2DM subjects on oral agents vs. insulin (300), and T2DM subjects in good vs. poor metabolic control (301). We showed here that the GI values of foods in hyperinsulinemic subjects were similar to those in healthy control and T2DM subjects. While not unexpected, this is important because of evidence that GI may be particularly useful for obese and/or insulin resistant subjects to assist with weight management (302) and/or the prevention of T2DM (104) and stroke (303). Thus, it is valid to utilize the GI values of foods tested in healthy control subjects in the dietary management of hyperinsulinemic/insulin resistant subjects.

The GI values of carbohydrate foods depend on differences in their relative rates of digestion and absorption (that is, the rate of glucose appearance from the gut) (31) which, presumably, do not differ in healthy, hyperinsulinemic and diabetic subjects. We included sucrose as a test meal because its glycemic response depends, at least in part, on the hepatic
metabolism of fructose which, in turn, may depend on insulin sensitivity. We previously showed that the GI of fruit leather, over 50% of the available-carbohydrate of which consisted of fructose, was inversely related to fasting insulin and to waist circumference (304). However, the present results are not consistent with this, in that the mean GI of sucrose in Hyperinsulinemic subjects was, if anything, slightly higher than in the healthy control.

One of the major criticisms of GI is that it does not take into account the concurrent insulin response (34); thus, some researchers have advocated using Insulinemic Index in the treatment of diabetes (30, 131); however, for Insulinemic Index to have clinical utility, it must be applicable to a broader population and be similar in all subject groups regardless of their degree of insulin sensitivity and glucose tolerance status. We found that the mean Insulinemic Index values of all 5 carbohydrate foods were higher in T2DM than healthy control and hyperinsulinemic subjects (p=0.05). Furthermore, Insulinemic Index values were inversely related to oral glucose insulin sensitivity and positively related to hepatic insulin extraction and the severity of glycemia. In addition, oral glucose insulin sensitivity alone explained 43% of the variation in Insulinemic Index. These results suggest that the Insulinemic Index values of carbohydrate foods vary depending on the metabolic status, thus limiting its clinical utility.

There is a physiological basis for the observed higher mean Insulinemic Index of the 5 carbohydrate foods for T2DM (100±7) than healthy control (78±5) and hyperinsulinemic subjects (70±5) (p=0.05). Glucose is not the only stimulus for insulin secretion, gastrointestinal hormones, mainly gastric inhibitory polypeptide (GIP) and glucagon-like peptide 1 (GLP-1) are known to potentiate the stimulatory effect of glucose and mediate postprandial insulin secretion (305-308). In addition, the activity of the entero-insulin axis and hepatic insulin extraction all play a role in modulating postprandial insulin concentration. We found that oral glucose insulin
sensitivity and β-cell function decreased in a step-wise fashion from healthy control to hyperinsulinemic to T2DM and hepatic insulin extraction was much higher in T2DM than healthy and hyperinsulinemic subjects. These metabolic aberrations, especially reduced β-cell function (less insulin secretion) and increased hepatic insulin extraction may reduce plasma insulin response to oral glucose (the reference meal) in T2DM patients; thus other components of foods (the test meals) on insulin secretion become more prominent, which resulted in increased Insulinemic Index in T2DM subjects.

Though the mean Insulinemic Index of the 5 carbohydrate foods was higher in T2DM than healthy and hyperinsulinemic subjects, for each individual food, only in white bread a significant difference in Insulinemic Index was observed. How can the fact that Insulinemic Index was correlated with metabolic status (oral glucose insulin sensitivity and the severity of glycemia) be reconciled with the lack of significant difference in Insulinemic Index among the subject groups for each individual food (except white bread)? This may be because Insulinemic Index, oral glucose insulin sensitivity and markers of the severity of glycemia are continuous variables, whereas the subject groups (healthy control, hyperinsulinemic, and T2DM) are categorical variables. It has been demonstrated that continuous variables (regression analysis) has greater statistical power than categorical variables (ANOVA analysis) due to increased precision, a simpler and more informative interpretation of the results, and greater parsimony (309); therefore, it is possible that categorical variables analysis (ANOVA) run the risk of missing significant effects.

We found that the GI and Insulinemic Index/C-peptide index are highly correlated in healthy control, hyperinsulinemic subjects and type 2 diabetic patients respectively, and the regression lines are nearly identical (Figure 5.2); however, the Insulinemic Index values of white
bread and mashed potato were much higher than GI (Figure 5.5). We are not the only group to show the dissociation between insulin and glucose responses in certain foods. Dissociation of the glycemic and insulinemic response has been found in whole and skimmed milk, and the protein fraction in milk was suggested to be responsible for milk’s insulinotropic effect (126). Also it is known that coingestion of fat and protein can evoke additional or synergistic insulin secretion (249, 310, 311); however, our finding of the higher Insulinemic Index than GI for white bread and mashed potato but similar GI and Insulinemic Index for rice and barley is hard to explain because white bread and mashed potato had fat and protein content similar to those of rice and barley (Table 5.1). The mostly likely explanation may be that both mashed potato and white bread are highly processed and refined carbohydrate foods. There is evidence that bakery products (rich in fat and refined carbohydrate) elicited insulin responses that were disproportionately higher than their glycemic responses (30).

It is concluded that the GI of carbohydrate foods is not significantly different among healthy control, hyperinsulinemic and T2DM patients, and the GI is not influenced by subject’s metabolic status. This finding supports the clinical utility of GI in the prevention and management of diabetes. On the contrary, Insulinemic Index values were inversely related to insulin sensitivity and positively associated with the severity of glycemia and hepatic insulin extraction of the subjects, suggesting that Insulinemic Index is not a property of food but is subject-dependent. The results suggest that Insulinemic Index may have limited clinical utility.
CHAPTER 6
THE RELATIONSHIP BETWEEN PLASMA GLP-1 RESPONSE AND INDIRECT MEASURES OF INSULIN SENSITIVITY, β-CELL FUNCTION AND HEPATIC INSULIN EXTRACTION
6.1 Abstract

Background: The relationships among insulin resistance, β-cell function and hepatic insulin extraction are well characterized; however, the roles of glucagon-like peptide-1 (GLP-1) in each of these underlying disorders remain unclear.

Objective: To determine whether endogenous postprandial GLP-1 response to oral glucose is related to the indirect measures of insulin sensitivity, β-cell function, and hepatic insulin extraction.

Design: Thirty-eight healthy, non-diabetic subjects classified by their fasting serum insulin (FSI) [13 with low-FSI (FSI < 40 pmol/L); 16 with medium-FSI (40 ≤ FSI < 70 pmol/L); and 9 with high-FSI (FSI ≥ 70 pmol/L)] and 10 type 2 diabetic patients (T2DM) consumed 50g oral glucose on 3 separate occasions for the measurement of fasting and postprandial (2-hours) glucose, insulin, C-peptide and GLP-1. The mean responses from the 3 oral glucose tests were used to calculate indices of insulin sensitivity, β-cell function, and hepatic insulin extraction using validated methods.

Results: Plasma GLP-1 concentration was inversely related to BMI \( r=0.33, p=0.03 \) and waist circumference \( r=-0.28, p=0.05 \), but was not associated with the indirect measures of insulin sensitivity, β-cell function and hepatic insulin extraction \( p>0.05 \).

Conclusion: The endogenous plasma GLP-1 response to oral glucose was not related to the indirect measures of insulin sensitivity, β-cell function and hepatic insulin extraction.
6.2 Introduction

Postprandial glucose homeostasis after a meal is mediated not only by the direct stimulation of insulin but also through the secretion of incretin hormones, namely glucose-dependent insulinotropic peptide (GIP) and GLP-1(305-308). GLP-1 has received much more attention than GIP because, unlike GIP, the insulinotropic effect of GLP-1 is well preserved in type 2 diabetic patients (312, 313). Moreover, in recent years, because of the strong antidiabetic effects of GLP-1, various GLP-1-based pharmacological agents have been developed and are now becoming established as effective therapies for type 2 diabetes (314).

GLP-1 is released from the L-cells of the small intestine in response to nutrient ingestion, particularly glucose and monounsaturated fatty acids (315). The GLP-1 secretion pattern in type 2 diabetic patients is not clear. Some studies have shown that type 2 diabetic patients have reduced plasma GLP-1 response after a mixed meal (251, 316) and decreased insulinotropic potency of GLP-1(317) whereas other studies did not detect any diminished GLP-1 response in diabetic subjects (318, 319).

Patients with type 2 diabetes exhibit a cluster of pathophysiological abnormalities, the major ones are insulin resistance, islet β-cell dysfunction (320), and reduced hepatic insulin extraction (224). Moreover, these parameters are related to each other. For example, insulin resistance is accompanied by compensatory hyperinsulinemia, which is attributed to increased insulin secretion and reduced hepatic insulin extraction (206). The state of insulin resistance is also associated with impaired β-cell function (320). For example, the abnormalities in β-cell function are present in high-risk individuals such as the first-degree relatives of patients with type 2 diabetes (321, 322), women with a history of gestational diabetes (323-325) or polycystic ovarian syndrome (321, 326), and older subjects (327, 328).
Though the relationships among insulin resistance, β-cell function and hepatic insulin extraction are well characterized, the role of GLP-1 in each of these underlying disorders is unclear. First, it is unclear whether insulin sensitivity per se is of regulatory importance for GLP-1 secretion, since both reduced (329, 330) and unaltered (331-333) GLP-1 responses have been reported in non-diabetic insulin resistant subjects. Second, in cultured β-cells and rodent models of diabetes, GLP-1 stimulates pancreatic islet β-cell differentiation (334), proliferation (335, 336), and inhibits apoptosis of β-cells (337, 338); however, whether GLP-1 has similar effects in humans is not known (339). It is not known whether GLP-1 per se exerts direct effects on β-cells in humans or the improvements of β-cell function with GLP-1-based pharmacological agents are secondary effects due to amelioration of hyperglycemia (340). Finally, it is unclear whether GLP-1 has independent effect on liver insulin clearance. Reduced liver insulin clearance was found in mice treated with GLP-1(341) and exenatide (GLP-1 receptor agonist) (342), whereas in humans, both endogenously secreted and exogenously administered GLP-1 had no independent effect on liver insulin clearance (208, 343). These controversies prevent a better understanding of the role of GLP-1 in the three systems (insulin sensitivity, β-cell function and hepatic insulin extraction) important for glucose homeostasis.

Therefore, we utilized the oral-glucose data from two clinical studies (Chapter 4 & 5) to determine whether there is any association between the postprandial endogenous GLP-1 response elicited by oral glucose and indirect measure of insulin sensitivity, β-cell function and hepatic insulin extraction. It is hypothesized that the endogenous plasma GLP-1 response to oral glucose is associated with insulin sensitivity, β-cell function and hepatic insulin extraction.
6.3 Materials & Methods

6.3.1 Subjects

This analysis is a part of two clinical studies comparing the effects of carbohydrate, fat and protein on postprandial metabolic responses in healthy control, hyperinsulinemic and type 2 diabetic patients. In total, there were 48 subjects (38 healthy, non-diabetic subjects, 10 type 2 diabetic patients) who completed 3 separate oral-glucose-tests. We divided the 38 healthy subjects into groups with different levels of fasting serum insulin (FSI): low (n=13, FSI < 40 pmol/L), medium (n=16, 40 ≤ FSI < 70 pmol/L) and high (n=9, FSI ≥ 70 pmol/L). The rationale for this classification was based on the fact that FSI is strongly correlated with insulin resistance measured by euglycemic-hyperinsulinemic clamp (260), and the 40 pmol/L cut-off point was chosen because this represents approximately the 67th percentile for non-diabetic subjects in our laboratory (244). In our preliminary study, in which FSI > 40 pmol/L was used to define hyperinsulinemia (38), subjects with FSI > 40 pmol/L had significantly greater waist circumference (WC), BMI, HOMA insulin resistance index, total and LDL cholesterol and triglycerides, and lower HDL cholesterol than those with FSI < 40pmol/L (38). We divided subjects with FSI > 40pmol/L into medium (FSI < 70 pmol/L) and high (FSI ≥ 70 pmol/L) groups because people with newly diagnosed diabetes have mean FSI > 70 pmol/L (261, 262).

To recruit type 2 diabetic subjects, twenty-five patients were screened by measuring their height, weight, WC, hip circumference, blood pressure, fasting serum glucose and insulin, HbA1C, C-reactive protein, liver enzymes, and creatinine. The inclusion criteria were: 18-70 y, BMI < 35kg/m², fasting glucose > 7.0mmol/L or HbA1C over upper limit or normal, treated by diet alone or with any combination of sulfonylurea, metformin, thiazolidinedione and/or meglitinide. Exclusion criteria: gastrointestinal disease, liver disease (aspartate transaminase,
alanine transaminase, and gamma glutamyl transpeptidase > 2 times upper limit of normal respectively) or kidney disease (creatinine > 1.2 times upper limit of normal), gastroparesis, treated with acarbose, orlistat or insulin and recent acute medical or surgical event. Ten type 2 diabetic patients were recruited, with HbA1C (%) of 7.3± 0.3% (mean ±SEM). On average, the patients had had diabetes for 7.0±3.3 y (mean±SD). Eight patients were on metformin, 1 patient was on metformin + pioglitazone, and 1 patient was on metformin + sulfonylureas. The patients took their usual medication during the study period. None of the patients had a history of micro- or macro-vascular complications.

The research protocol was reviewed and approved by Research Ethics Boards at the University of Toronto and St. Michael’s Hospital. All subjects gave written informed consent.

6.3.2 Study Design

The subjects were instructed to maintain their usual daily routine and dietary pattern between study days and refrain from exercise on the morning of the test. After an overnight fast (10-14hr), they came to the Risk Factor Modification Center at St. Michael’s hospital on three separate mornings between 7:30-9:30am. For healthy, non-diabetic subjects, venous blood samples were drawn just before and at 15, 30, 45, 60, 90 and 120 min after starting to ingest oral glucose (50g anhydrous glucose dissolved in 250ml bottled water). For type 2 diabetic patients, venous blood samples were drawn at 0, 30, 60, 90, 120 and 180 min. The oral glucose test was done 3 times over 3 month in each subject because OGTT-derived indices of β-cell function and insulin sensitivity exhibit high within-subject variability (344). Use of the mean of the 3 tests reduces within-subject variability and increases the power of the study (263).
Blood samples were collected within 2-hours period for the healthy, non-diabetic subjects and 3-hours for type 2 diabetic patients. In order to match the time period, only data from the 0-120 min were used for statistical analysis.

6.3.3 Blood analysis

Venous blood samples for the measures of glucose, insulin and C-peptide were collected in BD Vacutainer™ SST™ tubes (BD, Franklin Lakes, NJ, USA). Serum glucose was measured by a glucose oxidase method (SYNCHRON LX Systems, Beckman Coulter, Brea, California, USA), with inter-assay coefficient of variation (CV) of 1.9%. Insulin was measured using one-step immunoenzymatic (“sandwich”) assay (Beckman Access Ultrasensitive Insulin Assay, Beckman Coulter, Brea, California, USA), with inter-assay CV of 2.5 to 4.3%. Insulin has no cross-reactivity with proinsulin. C-peptide was measured using double antibody competitive radioimmunoassay (Siemens Medical Solutions Diagnostics, Los Angeles, CA), with inter-assay precision of 10% or less.

Venous blood samples for GLP-1 were collected in BD Vacutainer™ EDTA™ tubes (BD, Franklin Lakes, NJ, USA). The dipeptidyl peptidase-4 inhibitor (Linco Research, St. Charles, Missouri) was added immediately (less than 30 seconds) after collection. Plasma GLP-1 was measured by capturing the active GLP-1 from the sample by a monoclonal antibody (specific binding to the N-terminal region) using GLP-1 (active) ELISA Kit (Linco Research, St. Charles, Missouri, USA), with inter-assay CV ranges from 1-13%. All the samples were stored at -70°C before analysis.
6.3.4 Calculations

Pre-hepatic insulin secretion rate (ISR) for each subject during the oral glucose tests was calculated from deconvolution of the plasma C-peptide concentration using ISEC software package developed by Hovorka et al (269). Incremental area under the curve (AUC) for plasma glucose, insulin, C-peptide, insulin secretion rate, and GLP-1 was calculated by applying the trapezoid rule, with determination of the incremental area above baseline (31). Hepatic insulin extraction (HIE_{auc}) was determined by the AUC of C-peptide divided by that of insulin (268).

Insulin sensitivity was calculated using two validated OGTT-derived indices of insulin sensitivity: 1) Matsuda’s insulin sensitivity index, which incorporates both hepatic and muscle components of insulin resistance, correlates well with euglycemic-hyperinsulinemic clamp, and was calculated as 10,000/(fasting glucose × fasting insulin × mean glucose × mean insulin \(0.5\)) (173); 2) Mari’s glucose-insulin model-based oral glucose insulin sensitivity index constructed on established principles of glucose kinetics and insulin action (174). Oral glucose insulin sensitivity has been validated against the euglycemic-hyperinsulinemic clamp in healthy, obese and type 2 diabetic subjects (174).

\(\beta\)-cell function was calculated using three validated methods: 1) the insulin secretion/insulin resistance (disposition) index (270), which was shown to be the best predictor of future development of type 2 diabetes in subjects with NGT compared with other predictive models such as San Antonio Diabetes Prediction Model (271) (including age, sex, ethnicity, BMI, blood pressure, fasting plasma glucose, triglycerides, and HDL) and 2-hour plasma glucose concentration. To calculate insulin secretion/insulin resistance (disposition) index, first, insulin secretion was expressed as the changes in AUC of insulin secretion rate (\(\Delta ISR_{AUC}\)) relative to changes in AUC of plasma glucose response (\(\Delta ISR_{AUC}/\Delta G_{AUC}\)), then \(\Delta ISR_{AUC}/\Delta G_{AUC}\) is divided
by the severity of insulin resistance \((\Delta \text{ISR}_{\text{AUC}}/\Delta \text{G}_{\text{AUC}} \div \text{IR})\), as measured by the inverse of Matsuda’s insulin sensitivity index. 2) Disposition index calculated as total \(\text{AUC}_{\text{ins/gluc}}\times\) Matsuda’s insulin sensitivity index. This method is considered valid for oral glucose based measure of \(\beta\)-cell function because a hyperbolic relationship was demonstrated between OGTT-derived \(\text{AUC}_{\text{ins/gluc}}\) and Matsuda’s insulin sensitivity index across a range of glucose tolerance (345). 3) Disposition index calculated as insulinogenic index \((\Delta \text{I}_{0-30}/\Delta \text{G}_{0-30}) \times 1/\text{fasting insulin}\). The insulinogenic index \((\Delta \text{I}_{0-30}/\Delta \text{G}_{0-30})\) also demonstrated a hyperbolic relation with \(1/\text{fasting insulin}\), and the product of the two variables is predictive of development of diabetes for over 10 years (346).

6.3.5 Statistical Analysis

Data were expressed as mean \(\pm\) SEM for normally distributed continuous variables or median (interquartile range) for non-normally distributed continuous variables. The normality of the variables was assessed using the Shapiro and Wilk statistic and the normality plots (PROC UNIVARIATE procedure of SAS). The skewed variables were transformed into their natural logarithms before being subjected to statistical analysis.

Indices of insulin sensitivity, \(\beta\)-cell function, hepatic insulin extraction and integrated responses of biomarkers were analyzed with an ANCOVA model with the subject group as fixed effect, and age, BMI and WC as the covariates. Tukey’s post hoc correction was performed to compare the variables among the 4 subject groups. Multiple regression analysis was carried out with the plasma GLP-1 concentration as the dependent variable and age, BMI, oral glucose insulin sensitivity, disposition index \((\Delta \text{I}_{0-30}/\Delta \text{G}_{0-30}) \times 1/\text{FSI})\), hepatic insulin extraction, and AUCs of glucose and insulin as independent variables. Collinearity was determined by including
variance inflation factor in the model, with variance inflation factor of 5 or 10 and above indicates a multicollinearity problem (299). No collinearity was apparent for the variables included in the regression analysis. All analyses were done using SAS 9.2, (SAS Institute Inc, Cary, NC, USA). Differences were considered significant if 2-tailed p < 0.05. The graphs were plotted using GraphPad Prim, version 5 (GraphPad Software, Inc, California, USA).

6.4 Results

Type 2 diabetic patients and the subjects with high-FSI were older, and had significantly higher BMI, WC, and waist:hip ratio than their counterparts in the low- and medium-FSI groups (Table 6.1). Among the non-diabetic subjects (low-, medium- and high-FSI groups), fasting insulin and C-peptide increased progressively as the FSI of the subjects increased; however, their fasting and 2-hour glucose were not significantly different (Table 6.1). Type 2 diabetic patients’ fasting insulin and C-peptide were comparable to either the medium or high-FSI group; however, as expected, they had significantly elevated fasting and 2-hour postprandial glucose concentrations (Table 6.1). Basal insulin secretion rate (ISR_{basal}) significantly increased as the fasting insulin of the subject increased, and type 2 diabetic patients had ISR_{basal} comparable to those of the medium- and high-FSI groups. After adjusting for fasting glucose, ISR_{basal} was the highest in the high-FSI group. Fasting GLP-1 was not significantly different among the 4 groups (Table 6.1). Systolic and diastolic blood pressure and triglycerides were not significantly different among the low-, medium- and high-FSI groups; however, these parameters were significantly higher in type 2 diabetic patients (Table 6.1). Total cholesterol, HDL and LDL cholesterol, total:HDL cholesterol ratio and C-reactive protein were not significantly different among the 4 groups (Table 6.1).
The glucose AUC was significantly higher in the high-FSI group and type 2 diabetic patients compared to that of the low- and medium-FSI groups. As expected, the AUC of insulin and C-peptide increased as subjects’ fasting insulin increased, and type 2 diabetic patients had the lowest insulin and C-peptide concentrations (Table 6.2). The postprandial insulin secretion rate (ISR AUC) was significantly higher in the medium and high-FSI groups than that of the low-FSI group and type 2 diabetic patients (Table 6.2). In addition, it was positively related to basal insulin secretion rate (adjusted for fasting glucose) (r=0.70, p<0.0001) (Figure 6.1).

The postprandial GLP-1 response (GLP-1 AUC) was not significantly different among the subject-groups (Table 6.2, Figure 6.2). Gender difference in the GLP-1 response after oral glucose ingestion was observed: plasma GLP-1 concentration was more than 2 times greater in female than that of the male subjects (294±52 pmol × min/L vs. 131±23 pmol × min/L; p<0.0001).

Oral glucose insulin sensitivity decreased in a step-wise fashion across the groups, with type 2 diabetic patients the least insulin sensitive (Table 6.3). All three calculations of β-cell function decreased across the groups, with type 2 diabetic patients exhibiting the most compromised β-cell function (Table 6.3). Even among the healthy, non-diabetic subjects, insulin secretion/insulin resistance (disposition) indices (ΔISR_{AUC}/ΔG_{AUC} ÷ IR) significantly decreased as the subjects’ fasting insulin increased, highlighting the reduced ability of pancreatic β-cells to compensate for insulin resistance even in subjects with normal glucose tolerance.

Type 2 diabetic patients had significantly higher hepatic insulin extraction compared to the low- and medium-FSI subjects (Table 6.3), which partially explains their very low postprandial insulin concentration (Table 6.2). Hepatic insulin extraction was inversely related
to postprandial insulin concentration ($r = -0.76$, $p<0.0001$) but positively associated with age ($r=0.62$, $p<0.0001$) (Figure 6.3).

Plasma GLP-1 response was inversely related to BMI ($r=-0.33$, $p=0.03$) and WC ($r=-0.28$, $p=0.05$) respectively (Figure 6.4). However, no correlation was observed between the GLP-1 response and oral glucose insulin sensitivity and all three calculations of β-cell function (Figure 6.5). There was still no significant correlation even in the non-diabetic subjects alone (Figure 6.6) or in the T2DM subjects alone (Figure 6.7). Plasma GLP-1 response was not associated with hepatic insulin extraction either ($r=-0.02$, $p=0.88$).

Multivariate regression analysis show that age, BMI and disposition index ($\Delta I_{0-30}/\Delta G_{0-30} \times 1/FSI$) explained 16% of the variation in GLP-1 response (GLP-1response $= 218 +5.2\times$age $-10.5\times$BMI $+35.2\times$disposition index, $r^2=0.16$, $p=0.06$) after oral glucose ingestion.
### Table 6.1  Anthropometric and metabolic characteristics of the study groups

<table>
<thead>
<tr>
<th></th>
<th>Low (n=13)</th>
<th>Medium (n=16)</th>
<th>High (n=9)</th>
<th>T2DM (n=10)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>24(23-35)(^a-\textsuperscript{z})</td>
<td>24(22-33)(^a)</td>
<td>40(34-41)(^b)</td>
<td>57(53-58)(^c)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Gender (M:F), n:n</td>
<td>5:8</td>
<td>9:7</td>
<td>4:5</td>
<td>5:5</td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m(^2)</td>
<td>22(21-23)(^a)</td>
<td>24(22-30)(^a)</td>
<td>32(29-32)(^b)</td>
<td>32(29-34)(^b)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>WC, cm</td>
<td>73(71-77)(^a)</td>
<td>82(77-92)(^a)</td>
<td>99(97-108)(^b)</td>
<td>102(94-115)(^b)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Waist:Hip Ratio</td>
<td>0.77±0.02(^a-\textsuperscript{d})</td>
<td>0.80±0.02(^a)</td>
<td>0.90±0.03(^b)</td>
<td>0.93±0.02(^b)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>FSI, pmol/L</td>
<td>27(25-32)(^a)</td>
<td>47(43-55)(^b)</td>
<td>90(75-107)(^c)</td>
<td>62(47-73)(^b)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>FC-peptide, pmol/L</td>
<td>310±33(^a)</td>
<td>511±41(^b)</td>
<td>897±67(^c)</td>
<td>786±33(^c)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>FG, mmol/L</td>
<td>4.5(4.4-4.7)(^a)</td>
<td>4.7(4.7-5.1)(^a)</td>
<td>4.9(4.9-5.4)(^a)</td>
<td>9.8(7.2-11.3)(^b)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>2h glucose, mmol/L</td>
<td>6.1(5.6-6.5)(^a)</td>
<td>6.4(5.7-6.9)(^a)</td>
<td>7.6(7.1-7.8)(^a)</td>
<td>13.1(10.2-15.1)(^b)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ISR(_{\text{basal}}), pmol/kg/min</td>
<td>1.13±0.12(^a)</td>
<td>1.89±0.16(^bc)</td>
<td>2.62±0.17(^d)</td>
<td>2.36±0.10(^d)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ISR(_{\text{basal}})/FG</td>
<td>0.21(0.18-0.29)(^a)</td>
<td>0.37(0.3-0.5)(^bc)</td>
<td>0.50(0.43-0.63)(^b)</td>
<td>0.26(0.19-0.33)(^ac)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fasting GLP-1, pmol/L</td>
<td>2.7(2.2-4.8)</td>
<td>3.2(2.7-5.3)</td>
<td>2.1(1.9-5.1)</td>
<td>3.1(2.4-4.8)</td>
<td>0.67</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>107±4(^a)</td>
<td>110±3(^a)</td>
<td>115±2(^ab)</td>
<td>130±4(^b)</td>
<td>0.0004</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>68±4(^ab)</td>
<td>66±2(^a)</td>
<td>74±3(^ab)</td>
<td>79±3(^b)</td>
<td>0.016</td>
</tr>
<tr>
<td>TC, mmol/L</td>
<td>4.7±0.3</td>
<td>4.4±0.2</td>
<td>4.8±0.4</td>
<td>5.5±0.5</td>
<td>0.09</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>1.00(0.67-1.48)(^ab)</td>
<td>0.88(0.66-1.23)(^a)</td>
<td>1.03(0.74-1.67)(^ab)</td>
<td>1.49(1.20-1.86)(^b)</td>
<td>0.04</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.19±0.08</td>
<td>1.20±0.07</td>
<td>1.13±0.06</td>
<td>1.20±0.10</td>
<td>0.93</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>2.95±0.22</td>
<td>2.74±0.19</td>
<td>3.07±0.32</td>
<td>3.10±0.33</td>
<td>0.70</td>
</tr>
<tr>
<td>TC: HDL</td>
<td>4.5(2.8-4.8)</td>
<td>3.4(3.2-3.8)</td>
<td>4.1(3.1-4.8)</td>
<td>3.9(3.3-6.6)</td>
<td>0.36</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>0.9(0.4-2.3)</td>
<td>1.0(0.3-2.4)</td>
<td>2.3(0.3-3.5)</td>
<td>3.7(1.1-5.4)</td>
<td>0.30</td>
</tr>
</tbody>
</table>
n = 48 except where otherwise noted. low (n=13, FSI < 40 pmol/L), medium (n=16, 40 ≤ FSI < 70 pmol/L), high (n=9, FSI ≥ 70 pmol/L), and type 2 diabetic patients (T2DM) (n=10). WC, waist circumference; FSI, fasting serum insulin; FC-peptide, fasting C-peptide; FG, fasting glucose; ISR, insulin secretion rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglycerides; HDL, high-density-lipoprotein; LDL, low-density-lipoprotein; CRP, C-Reactive Protein. Values in the same row with different superscript letters are significantly different, P < 0.05 (Tukey’s post hoc test). The unit for ISR_{basal}/FG is pmol/mmol/kg/min.

2 P represents overall significant differences across groups by one-factor ANOVA.

3 Median; interquartile range in parentheses (all such values).

4 Mean ± SEM (all such values).
Table 6.2  The AUCs of glucose, insulin, C-peptide, insulin secretion rate (ISR) and GLP-1 in the study groups

<table>
<thead>
<tr>
<th></th>
<th>Low (n=13)</th>
<th>Medium (n=16)</th>
<th>High (n=9)</th>
<th>T2DM (n=10)</th>
<th>(P^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose AUC</td>
<td>225(140-272)(^a,3)</td>
<td>209(168-276)(^a)</td>
<td>359(260-387)(^b)</td>
<td>678(597-749)(^c)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Insulin AUC</td>
<td>37±6(^{ab,4})</td>
<td>48±4(^a)</td>
<td>56±8(^a)</td>
<td>12±2(^b)</td>
<td>0.007</td>
</tr>
<tr>
<td>C-peptide AUC</td>
<td>77±8(^a)</td>
<td>116±8(^b)</td>
<td>146±13(^b)</td>
<td>71±9(^a)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ISR AUC</td>
<td>351±41(^a)</td>
<td>485±40(^b)</td>
<td>579±48(^b)</td>
<td>376±24(^a)</td>
<td>0.0002</td>
</tr>
<tr>
<td>GLP-1 AUC</td>
<td>195(82-445)</td>
<td>156(104-365)</td>
<td>163(56-184)</td>
<td>164(80-281)</td>
<td>0.60</td>
</tr>
</tbody>
</table>

\(^{1}\) n = 48 except where otherwise noted. low (n=13, FSI < 40 pmol/L), medium (n=16, 40 ≤ FSI < 70 pmol/L), high (n=9, FSI ≥ 70 pmol/L), and type 2 diabetic patients (T2DM) (n=10). AUC: area under the curve. The units for AUCs of glucose, insulin, C-peptide, ISR and GLP-1 are mmol ×min/L, nmol×min/L, nmol×min/L, pmol/kg, and pmol ×min/L respectively. Values in the same row with different superscript letters are significantly different, \(P < 0.05\) (Tukey’s post hoc test).

\(^{2}\) \(P\) represents overall significant differences across groups by repeated measures ANOVA (PROC-MIXED, adjusted for age, BMI and WC). Data were calculated from the mean of 3 oral glucose tests.

\(^{3}\) Median; interquartile range in parentheses (all such values).

\(^{4}\) Mean±SEM (all such values).
Table 6.3  Insulin sensitivity, β-cell function and hepatic insulin extraction in the study groups

<table>
<thead>
<tr>
<th></th>
<th>Low (n=13)</th>
<th>Medium (n=16)</th>
<th>High (n=9)</th>
<th>T2DM (n=10)</th>
<th>P²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin sensitivity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OGISₐₘᵋᵣ (ml min⁻¹ m⁻²)</td>
<td>505(485-509) ⁴ ⁵</td>
<td>465(433-498) ⁴</td>
<td>375(367-378) ³</td>
<td>294(250-369) ³</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>β-cell function</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔISRₐₚₑₜ/Δ₈₋₃₀ AUC ÷ IR</td>
<td>54.6±4.3  ³ ⁴</td>
<td>38.3±4.4  ⁵</td>
<td>16.8±2.1  ³</td>
<td>13.6±1.7  ³</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>(ΔI₀₋₃₀/Δ₈₋₃₀ × 1/FSI ([mmol]⁻¹)</td>
<td>2.6±0.3  ³</td>
<td>2.9±0.3  ⁵</td>
<td>1.8±0.3  ³</td>
<td>0.4±0.1  ³</td>
<td>0.006</td>
</tr>
<tr>
<td>TAUCᵻⁿₛᵽ⁄ₐₜₐᵢₛᵣᵨₙₛᵽ⁄ₐₜₐᵢₛᵣᵨₙₛᵽₐₛᵣ ([mmol]⁻²)</td>
<td>818±53  ³</td>
<td>807±61  ⁵</td>
<td>536±62  ³</td>
<td>141±28  ³</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hepatic insulin extraction</td>
<td>2.8(1.6-3.3)  ³</td>
<td>3.2(1.7-3.5)  ⁵</td>
<td>2.8(2.5-3.6)  ³</td>
<td>5.7(4.7-8.3)  ³</td>
<td>0.04</td>
</tr>
</tbody>
</table>

¹ n = 48 except where otherwise noted. Low (n=13, FSI < 40 pmol/L), medium (n=16, 40 ≤ FSI < 70 pmol/L), high (n=9, FSI ≥ 70 pmol/L), and type 2 diabetic patients (T2DM) (n=10). OGIS, oral glucose insulin sensitivity; IR, insulin resistance; FSI, fasting serum insulin; TAUC, total AUC; ISI, insulin sensitivity index. The unit for ΔISRₐₚₑₜ/Δ₈₋₃₀ AUC ÷ IR is [mmol]⁻² kg⁻¹ min⁻¹ L⁻¹. Values in the same row with different superscript letters are significantly different, P < 0.05 (Tukey’s post hoc test).

² P represents overall significant differences across groups by repeated measures ANOVA (PROC-MIXED, adjusted for age, BMI and WC). Data were calculated from the mean of 3 oral glucose tests.

³ Median; interquartile range in parentheses (all such values).

⁴ Mean±SEM (all such values).
**Figure 6.1** The correlation between basal insulin secretion rate (ISR$_{\text{basal}}$, adjusted for fasting glucose) and postprandial insulin secretion rate (ISR$_{\text{auc}}$). $r$, Pearson’s correlation coefficient. The line is regression line, $n=48$. 
**Figure 6.2** Mean (±SEM) postprandial plasma GLP-1 response to oral glucose in type 2 diabetic patients (solid circle) and subjects with different levels of fasting serum insulin (FSI): low (open triangle, FSI < 40 pmol/L), medium (open circle, 40 ≤ FSI < 70 pmol/L) and high (open square, FSI ≥ 70 pmol/L). Data were calculated from the mean of 3 oral glucose tests.
Figure 6.3  The correlations between hepatic insulin extraction (ratio of C-peptide AUC and insulin AUC) and postprandial insulin response (top) and age (bottom) respectively.  r: Pearson’s correlation coefficient.  The lines are regression line, n=48.
Figure 6.4 The correlations between GLP-1 response after ingestion of 50g oral glucose and waist circumference (WC) and BMI respectively, in type 2 diabetic patients (solid circle, n=10) and subjects with different levels of fasting serum insulin (FSI): low (open triangle, n=13, FSI < 40 pmol/L), medium (open circle, n=16, 40 ≤ FSI < 70 pmol/L) and high (open square, n=9, FSI ≥ 70 pmol/L). Dashed line indicates the upper and lower 95% CI. r, Pearson’s correlation coefficient. The lines are regression line, n=48.
Figure 6.5  The correlations between GLP-1 response after ingestion of 50g oral glucose and oral glucose insulin sensitivity (OGIS) and markers of β-cell function in type 2 diabetic patients (solid circle, n=10) and subjects with different levels of fasting serum insulin (FSI): low (open triangle, n=13, FSI < 40 pmol/L), medium (open circle, n=16, 40 ≤ FSI < 70 pmol/L) and high (open square, n=9, FSI ≥ 70 pmol/L). Dashed line indicates the upper and lower 95% CI. r, Pearson’s correlation coefficient. The lines are regression lines. n=48.
Figure 6.6  The correlations between GLP-1 response after ingestion of 50g oral glucose and oral glucose insulin sensitivity (OGIS) and markers of β-cell function in non-diabetic subjects with different levels of fasting serum insulin (FSI): low (open triangle, n=13, FSI < 40 pmol/L), medium (open circle, n=16, 40 ≤ FSI < 70 pmol/L) and high (open square, n=9, FSI ≥ 70 pmol/L). Dashed line indicates the upper and lower 95% CI. r, Pearson’s correlation coefficient. The lines are regression lines. n=38.
**Figure 6.7** The correlations between GLP-1 response after ingestion of 50g oral glucose and oral glucose insulin sensitivity (OGIS) and markers of β-cell function in type 2 diabetic patients. Dashed line indicates the upper and lower 95% CI. r, Pearson’s correlation coefficient. The lines are regression lines. n=10.
6.5 Discussion and Conclusion

In this analysis combining 3 separate oral-glucose-test data from two clinical studies (Chapter 4 & 5), we found that plasma intact GLP-1 concentration after oral glucose ingestion was not significantly different among different subject groups. Moreover, no correlation was found between intact GLP-1 response and insulin sensitivity, β-cell function and hepatic insulin extraction, the classic triad of type 2 diabetes.

Our finding that the GLP-1 response after oral glucose in T2DM subjects was not significantly different from that of the healthy, non-diabetic subjects was in agreement with some studies. For example, Theodorakis et al found that plasma GLP-1 concentration actually increased between 20-80 min in newly diagnosed patients with type 2 diabetes (319) and Vollmer et al found no decrease in GLP-1 levels in type 2 diabetic patients after oral glucose or mixed meal ingestion (318). However, Toft-Nielsen et al reported approximately 30% lower postprandial GLP-1 levels in type 2 diabetic patients compared with normal oral glucose tolerant subjects (347), and Vilsboll et al found that both total and intact GLP-1 after a mixed meal were markedly reduced in type 2 diabetic patients (251). The discrepancies in findings may be attributable to the different levels of metabolic aberrations (i.e. degree of hyperglycemia, duration of diabetes) amongst the patients in the different studies. In both Theodorakis and Vollmer’s studies, the patients were either newly diagnosed diabetics or in relatively good glycemic control, with HbA1C of 6.8±0.9% (318) and 7.0±0.2% (mean±SEM) (319) respectively. In contrast, the patients studied by Vilsboll et al and Toft-Nielsen et al exhibited much poorer glycemic control, with HbA1C of 8.4±1.7% (mean±SEM) (347) and 9.2% (range 7.0-12.5) (251). In our study, the patients had had a relatively good glycemic control, with
HbA1C of 7.3± 0.3% (mean ±SEM), which may explain the lack of significant difference in GLP-1 levels between the diabetic patients and non-diabetic subjects.

We also found that the AUCs of postprandial intact GLP-1 response was not significantly different among the low-, medium-, and high-FSI groups and was not related to the insulin sensitivity of the subjects. Previous studies have shown that the postprandial GLP-1 secretion after both meal (331) and oral glucose (332) was not significantly different between first degree relatives of type 2 diabetes and the controls. And in women with a history of gestational diabetes, the GLP-1 response after oral glucose was not different from that of the controls (333). Our data confirms that the degree of insulin sensitivity per se does not determine GLP-1 response, and the reduction in GLP-1 does not precede diabetes. This further suggests that the reduction in GLP-1 response as observed in long-term diabetic patients with poorly controlled glycemia (251, 347) may not be the cause but the consequence of the diabetic state.

The postprandial intact plasma GLP-1 levels were not associated with any of the indirect measures of β-cell function (Figure 6.5). This is not expected because there is a biological basis for the positive effect of GLP-1 on β-cell function. In cell culture studies or rodent models of diabetes, GLP-1 acts on β-cells acutely to enhance insulin biosynthesis and stimulate insulin gene transcription (348) and chronically, stimulates β-cell proliferation (335, 336), and inhibits β-cell apoptosis, thus promoting expansion of β-cell mass (337, 338). In addition, there is some indirect clinical evidence that GLP-1 may have β-cell trophic effect in vivo in humans. For example, patients with clinically significant hypoglycemia after gastric bypass surgery exhibited exaggerated GLP-1 and insulin secretory responses to a mixed meal (349). Since symptomatic hypoglycemia in post-gastric bypass patients only develops after 1-5 y, it was suggested that GLP-1 may have β-cell trophic effects and may increase β-cell mass over time after surgery.
The other clinical evidence is demonstrated in the recent LEAD-3 study, patients with early type 2 diabetes were treated with liraglutide (analogue of human GLP-1, with 97% homology to the endogenous protein) or glimepiride for 52 weeks. Liraglutide treated patients have greater reduction in HbA1C than glimepiride. The most important finding is the sustained reduction in HbA1C at 52 weeks, indicating improved β-cell function.

The lack of association between GLP-1 response and the indirect measures of β-cell function maybe due to the following reasons: first, we only measured the biologically active intact GLP-1-(7-36 amide), which is only a small part of intestinal L-cell secretion, and does not represent GLP-1 secretion. In addition, it does not reflect GLP-1 action since GLP-1 appears to act via sensory afferents before its degradation. A non-specific assay should be used to measure total GLP-1 concentrations [active GLP-1-(7-36) amide + inactive GLP-1-(9-36) amide], which reflects the secretary rate of the L cells. Second, β-cell function was characterized by indirect calculations related to insulin secretion/insulin sensitivity in the basal state and after stimulation with oral glucose. Under these circumstances, changes in glucagon, glucose, free fatty acids, GLP-1, and GIP may determine insulin (and proinsulin) secretion. To better characterize β-cell behavior, tests assessing the influence of single factors (i.e. glucose bolus injection or hyperglycemic clamp) should be used. Third, the data is pooled from two different studies, which are not designed to address the research question (are there any association between GLP-1 response and the indirect measures of β-cell function?). This obviously is the weakness of this analysis.

We did not find any association between hepatic insulin extraction and GLP-1 response (r=-0.02, p=0.88), which is consistent with previous studies in humans, suggesting GLP-1 has no independent effect on liver insulin clearance. In support of this, there is evidence...
that hepatocytes lack GLP-1 receptor expression (353); hence, it is unlikely that GLP-1 can exert any direct influence on liver insulin clearance. However, a significant inverse association was found between hepatic insulin extraction and insulin concentrations after oral glucose ingestion ($r = -0.76$, $p<0.0001$). This result is consistent with our previously published data (354) and Meier et al’s study (208). These data suggest that insulin concentration itself may determine its own rate of clearance from the liver.

Previous literature on hepatic insulin extraction in diabetic patients is inconsistent as both normal (217, 218), decreased (219, 220), or even increased (221) hepatic insulin clearance had been reported. We found that hepatic insulin extraction was significantly increased in type 2 diabetic patients compared to the non-diabetic subjects (Table 6.3). Three possible factors may explain such discrepancies: first, the variations in liver fat content (222, 223) may contribute to this. It is known that increased liver fat content is associated with impaired hepatic insulin clearance (224). Second, the medications the patients took may affect hepatic insulin extraction. For example, all our patients were on metformin. Metformin enhances hepatic insulin extraction in non-diabetic subjects at high-risk of developing diabetes (234). Hence, it is possible that metformin may also enhance hepatic insulin extraction in diabetic subjects. Third, the age of the patients may have played a role. It has been reported that hepatic insulin clearance was increased in elderly men and women (210-212). Indeed, a positive correlation was observed between hepatic insulin extraction and age ($r=0.62$, $p<0.0001$), suggesting that the older the subjects, the higher the liver insulin clearance.

We found that plasma GLP-1 concentration after oral glucose ingestion was 2 times greater in female than male subjects. This finding is consistent with previous results (318, 347). In women, delayed gastrointestinal (GI) motility (355), decreased gall bladder contraction (356),
and increased colonic transit and gastric emptying time (357) have all been reported. Therefore, slower movement of foods through the GI tract may provide more contact time for the food material with intestinal mucosa, which might lead to increased GLP-1 secretion.

We found that intact GLP-1 was inversely associated with the measures of adiposity (BMI and WC); the higher the BMI and WC of the subjects, the lower the GLP-1 response (Figure 6.4). This result is consistent with previous findings. Naslund et al showed that obese subjects had an attenuated GLP-1 release in response to meals (358). Toft-Nielsen et al demonstrated that BMI was negatively related to GLP-1 response (347), and Ranganath et al found a pronounced attenuation of plasma GLP-1 secretion to oral carbohydrate in obese subjects compared to lean subjects (359). It was suggested that increased proximal absorption could hypothetically explain the decreased GLP-1 secretion with increasing obesity (360). The rationale being that obese subjects may have increased proximal absorption rate (361), thus less food will reach the distal intestine, where the GLP-1-producing L cells are most abundant. On the other hand, the presence of nutrients in the proximal small intestine can stimulate GLP-1 release independently of the presence of nutrients within the ileum and colon (362).

In conclusion, the endogenous intact GLP-1 response to oral glucose was not related to indirect measures of insulin sensitivity, β-cell function and hepatic insulin extraction.
CHAPTER 7

GENERAL DISCUSSION
7.1 General discussion

Controlling postprandial glucose excursion is essential in the prevention and management of diabetes. Many substances in foods can affect postprandial glycemia; the major ones are macronutrient (carbohydrate, fat and protein). Numerous studies have compared carbohydrate, fat and protein metabolism in healthy and diabetic subjects; however, there are meager data on the impact of insulin resistance or hyperinsulinemia on macronutrient metabolism. Our studies are the first to address this issue by systematically comparing the postprandial metabolic responses elicited by carbohydrate, fat and protein in healthy control, hyperinsulinemic and type 2 diabetic subjects. The results generated from these studies have important implications for both diabetic patients and people at high risk for diabetes, namely those with insulin resistance/hyperinsulinemia.

We found that the ability of fat and protein to suppress glucose response was not influenced by the degree of insulin sensitivity of the subjects, and the mechanisms through which fat and protein modulate glucose responses differ, specifically, fat had no effect on liver insulin clearance, whereas protein significantly decreased it. For the GI values of various carbohydrate foods, we found that they were similar in healthy control, hyperinsulinemic and type 2 diabetic subjects, suggesting that carbohydrates induce similar increases in relative glycemic response independent of the subjects’ metabolic status. Currently, people with diabetes are the primary users of GI. Our finding suggests that GI is also a useful tool in the dietary management of obese and/or non-diabetic, insulin resistant subjects. For the Insulinemic Index of carbohydrate foods, we found that the mean Insulinemic Index of the 5 carbohydrate foods was much higher for T2DM (100±7) than healthy controls (78±5) and hyperinsulinemic subjects (70±5) (mean±SEM, p=0.05). Moreover, Insulinemic Index was inversely associated with the insulin
sensitivity and positively related to the severity of glycemia and hepatic insulin extraction of the subjects, suggesting that Insulinemic Index is not a property of foods but depends on the metabolic status of the subjects in whom it is measured; therefore, unlike GI, Insulinemic Index may have limited clinical utility.

We hypothesized that adding fat and protein to carbohydrate would reduce glucose responses less in non-diabetic hyperinsulinemic subjects than healthy control subjects, which is likely due to reduced GLP-1 secretion and insulin sensitivity. However, contrary to this hypothesis, the glucose-lowering effect of fat and protein was not dependent on insulin sensitivity of the subjects, and the hypoglycemic effect of protein is equally potent in all subject groups (low-, medium-, and high-FSI). This could be that GLP-1 response to fat or protein was similar in subjects with low, medium or high-FSI (Figure 4.1, Appendix 4 & 6), thus the GLP-1 mediated insulin secretion after fat or protein may be similar as well in all subject groups. Indeed, fat and protein did not appear to affect insulin secretion differentially in the 3 subject groups as there were no significant FSI × fat and FSI × protein interaction effects on both C-peptide (true marker of insulin secretion) and insulin secretion rate (ISR), and C-peptide response and ISR were similar in all 3 subject groups (Figure 4.3).

Besides GLP-1, other gut hormones such as cholecystokinin (CCK) and PYY may also play a role in modulating postprandial glycemia. CCK and PYY were not measured in this study, but it is known that fat and protein are the most important stimulators of CCK secretion (363), and dietary protein is the most potent stimulant of PYY release (288). In subjects with insulin resistance, both CCK and PYY responses to meal may be altered. It was found that healthy female first-degree relatives of people with type 2 diabetes had significantly lower fasting serum PYY levels than controls, but their PYY response to a high fat meal was not
significantly different from the controls (364). For CCK, the plasma CCK response after glucose drink was higher in hyperinsulinemic men compared to the control (365).

The regulation of glucose homeostasis after a meal involves not only β-cell function, hepatic insulin extraction and hormones of the entero-insulin axis, but also the newly discovered pathway of the upper-intestinal-lipids-activated gut-brain-liver neural axis, through which liver insulin sensitivity was enhanced, and hepatic glucose production was reduced (39). However, the gut-brain-liver neural pathway may be impaired in insulin resistant state. Indeed, in high-fat-diet-induced insulin resistant rats, intraduodenal lipid infusion failed to suppress hepatic glucose production, suggesting that insulin resistance may dampen the ability of the gut-brain-liver neuronal network to sense and respond to lipid, thus resulting in impaired glucose regulation (39). Based on these findings, one might logically assume, therefore, that insulin resistant humans may also acquire such defects in hypothalamic nutrient sensing; however, such hypothesis is extremely difficult to prove in humans. This is because the hypothalamus is located deep in the midbrain, and even techniques such as computed tomography scans and magnetic resonance imaging do not have sufficient spatial resolution to detect changes in specific nuclei (366). On the other hand, our finding that the glucose-lowering effect of fat is not influenced by insulin sensitivity of the subjects indirectly suggests that unlike those observed in animal models, the lipid-activated gut-brain-liver neural circuit may not be impaired in insulin resistant humans.

Then, what is the implication that the ability of fat and protein to suppress the glycemic response to oral glucose is not altered in the presence of insulin resistance/hyperinsulinemia? This suggests that for the dietary management of insulin resistance/hyperinsulinemia, there may not be a need for a specifically designed diet since the glucose-lowering effects of fat and protein
are not influenced by the degree of insulin sensitivity of the subjects. On the other hand, functionality in terms of lowering glucose alone should not be the sole determining factor of macronutrient composition of a diet for insulin resistant humans. The effects on lipids, inflammatory markers (i.e. CRP, TNFα, interleukin-6, etc), food intake, and body weight regulation also need to be taken into consideration.

It is commonly held that the ability of protein to increase insulin concentrations is primarily due to amino acid mediated insulin secretion (127, 156). We have identified a novel, alternative pathway through which protein mediates peripheral insulin concentration. We found that whey protein increased insulin but had no effect on C-peptide or insulin secretion rate, whereas hepatic insulin extraction was decreased. We proposed that whey-induced hyperinsulinemia was probably due to a decrease in hepatic insulin extraction (thus conserves insulin) rather than increased secretion. Then, how does whey protein decrease hepatic insulin extraction? It is known that both amino acids and the biologically active (bioactive) peptides released after protein digestion regulate physiological functions (367) and whey proteins are precursors of many bioactive peptides encrypted in their amino acid sequences (292). Thus, it is hypothesized that the effects on hepatic insulin extraction may be executed by certain branched-chain amino acids (BCAA) (i.e. leucine, isoleucine, valine, lysine and threonine), or whey-derived bioactive peptides (Appendix 7), or synergistic actions among them, which exert their function by acting as competitive inhibitors for insulin receptors on hepatocytes; thus less insulin is cleared by liver.

Proteins are highly heterogeneous in physical-chemical properties and biological functions. For example, varying sources of protein differ in the rate of digestion and absorption (i.e. whey is a fast protein, whereas casein is a slow protein). Moreover, different sources of
protein differ in the composition of amino acids (153) and bioactive peptides (368). All these differences in physical-chemical properties likely will contribute to their different physiological effects. Therefore, our finding of whey-induced reduction in liver insulin clearance does not necessary imply that other proteins such as casein or soy protein also has such an effect.

Previous study has shown that in healthy adults, the GI and Insulinemic Index of carbohydrate foods are well correlated (r=0.69, p<0.001) (29). We found that GI and Insulinemic Index of carbohydrate foods are well correlated not only in healthy subjects, but also in subjects with hyperinsulinemia and type 2 diabetes, suggesting that the ability of GI to reliably predict insulin demand after carbohydrate foods is independent of subject’s metabolic status. This finding expands our knowledge of insulin response to carbohydrate foods in subjects with varying metabolic states.

7.2 Weaknesses

There are a number of limitations to the studies. Due to the expense and technical difficulties associated with performing the euglycemic-hyperinsulinemic clamp, we chose FSI>40pmol/L (based on pilot data) instead as indicative of insulin resistance/hyperinsulinemia. Using fasting insulin as the surrogate measure of insulin sensitivity and arbitrarily defining FSI>40pmol/L as the cut-off point posed the risk of misclassifying subjects. In the future, if similar studies were to be carried out, oral glucose insulin sensitivity would be a better candidate as an indirect measure of insulin sensitivity because it has been found to be the most accurate surrogate of M values derived from the clamp method (369). Nevertheless, this shortcoming does not negate the conclusion that the hypoglycemic effect of fat and protein was not attenuated by insulin resistance. This is because the regression analysis showed that the changes in relative
glycemic response per gram of fat or protein were not related to fasting insulin. Through regression analysis, we treated the subjects as continuous variables instead of as categorical variables (subjects group), thus avoiding the bias that may be brought about by the potential risk of misclassification of subjects.

In the first study (Chapter 4), the subjects with different levels of fasting insulin were not matched for age and adiposity (BMI & WC). In the second study (Chapter 5), the healthy control, hyperinsulinemic and type 2 diabetic subjects were also not matched for age, BMI and WC. This issue was addressed by adjusting the covariates (age, BMI and WC) using analysis of covariance (ANCOVA).

In the second study, the blood sampling schedule for the non-diabetic subjects (healthy controls and hyperinsulinemic subjects) was 2 hours; however, for the type 2 diabetic subjects, it was 3 hours. In future, if a similar study were to be carried out, the blood sampling schedules should be the same for all the subject groups. Blood sampling time should also be extended to 3 hours as it takes longer for insulin and C-peptide to return to baseline.

7.3 Strengths

Our studies systematically investigated whether metabolic responses to macronutrient are different among healthy control, hyperinsulinemic and diabetic subjects, which clearly is a strength as previous studies only focused on the comparison between the healthy and diabetic subjects. In addition, we examined the effects of macronutrient, both singly and in combination, on postprandial glucose and insulin responses. Contrary to the results from animal studies, we found that in humans, the ability of fat to suppress postprandial glycemia after oral glucose ingestion is not influenced by insulin sensitivity of the subjects. Another novel finding is that
protein decreases hepatic insulin extraction whereas fat has no such effect, demonstrating that fat and protein modulate postprandial metabolic responses through different mechanisms.

We are also the first to evaluate both the GI and Insulinemic Index of different carbohydrate foods in healthy control, hyperinsulinemic and type 2 diabetic subjects. These findings affirm the clinical utility of GI in the dietary prevention and management of type 2 diabetes. Furthermore, it suggests that GI is a useful tool for people with insulin resistance/hyperinsulinemia in guiding their choices of carbohydrate foods. However, unlike GI, insulinemic index is not a property of food because it varies depending on insulin sensitivity, the severity of glycemia and the hepatic insulin extraction of the subjects. Thus, it may have limited therapeutic utility. This finding is important as the nutrition community needs this type of information to determine whether food insulin index is a valid and useful tool in clinical settings and in epidemiological research.

In order to evaluate pancreatic β-cell insulin secretion following ingestion of different macronutrient, we measured not only insulin but also C-peptide (the true secretion of insulin) and gut hormone GLP-1. This is an important strength as these biomarkers assist in better understanding of the complex factors involved in altering insulin secretion after food digestion and absorption.
Based on the results of current studies, a few potential promising areas for future studies are identified: first, from a mechanistic point of view, it is necessary to find out how does whey protein, specifically whey-derived amino acids/bioactive peptides influence liver insulin clearance; second, it will be interesting to find out whether fat and protein differentially affect hypothalamic nutrient sensing; third, since Insulinemic Index was inversely associated with oral glucose insulin sensitivity, and oral glucose insulin sensitivity alone explained 43% of the variation in Insulinemic Index ($r^2=0.43$, $p<0.001$), it is necessary to compare Insulinemic Index in different subject groups classified by oral glucose insulin sensitivity. Both mechanism-finding studies (in animal models) and clinical studies are needed to address these issues:

**In animal models:**

1. Test the effects of whey-derived amino acids and bioactive peptides on hepatic insulin extraction. Hepatic insulin extraction can be measured directly using hepatic vein catheterization technique, an invasive method with catheters placed in artery and hepatic veins.

2. The much more potent glucose-lowering effect of protein than fat suggests that other factors besides insulin and gut hormones might play a role. Therefore, in animal models, we can test whether whey protein regulates glucose homeostasis through hypothalamic amino acid sensing (i.e. effect on hepatic glucose production) and compare the effects to those of fat. Further, it is important to explore whether the hypothalamic protein-sensing mechanism is impaired in insulin resistance and diabetes.
In humans:

1. Insulinemic Index is inversely associated with oral glucose insulin sensitivity; therefore, it is important to find out whether Insulinemic Index values were similar in subjects with varying degrees of insulin sensitivity classified by oral glucose insulin sensitivity.

2. In this study, only 5 carbohydrate foods were tested, thus, it is impossible to do a correlation analysis to see if Insulinemic Index of healthy subjects were correlated with those of the hyperinsulinemic or diabetic subjects. Future studies can include more different sources of carbohydrate foods (i.e. 20 or more carbohydrate foods) and to see if there were any correlation between Insulinemic Index derived from the same carbohydrate foods in healthy control vs. hyperinsulinemic, healthy control vs. T2DM, or hyperinsulinemic vs. T2DM. If there were no correlation, this will further support our finding that Insulinemic Index may not have relevance in dietary intervention in hyperinsulinemic and diabetic subjects.
CHAPTER 9

CONCLUSIONS
Conclusions to the specific hypotheses are:

1. Adding fat and protein to oral glucose reduces postprandial glycemic responses to a similar extent in subjects with low-, medium- or high-FSI, suggesting that the hypoglycemic effect of fat and protein is not attenuated by insulin resistance.

2. The effects of macronutrient on postprandial responses are not affected by habitual intakes of fat and dietary fibre. One possible explanation could be that the sample size is most likely not adequate to address habitual diet.

3. The GI values of carbohydrate foods do not differ in healthy control, hyperinsulinemic and type 2 diabetic subjects, which support the clinical utility of GI as a property of the available carbohydrate in foods. The GI values of sucrose are also not significantly different among the subject groups.

4. Unlike GI, Insulinemic Index values are dependent on the metabolic status of the subjects, suggesting that Insulinemic Index may have limited clinical utility.

5. The endogenous GLP-1 response to oral glucose is not related to indirect measures of insulin sensitivity, β-cell function and hepatic insulin extraction.

The overall conclusion of the thesis:

The ability of fat and protein to suppress postprandial glycemia to oral glucose is not influenced by insulin sensitivity of the subjects. Moreover, fat and protein modulate glucose response through different mechanisms. Protein reduces liver insulin clearance whereas fat exhibits no such effect. The insulin-raising effect of protein may be due to reduced liver insulin clearance (thus conserves insulin) rather than increased secretion; however, the exact mechanisms of protein on liver insulin clearance warrant further investigation in animal models.
Carbohydrates induce similar increase in relative glycemic response (GI) in healthy control, hyperinsulinemic and type 2 diabetic subjects. This finding supports the concept that GI is the property of the available carbohydrate in foods rather than the metabolic characteristics of the individual consuming the foods. On the contrary, relative insulin responses (Insulinemic Index) are dissimilar in the three subject groups. Moreover, the associations between Insulinemic Index and the subjects’ metabolic status (insulin sensitivity, severity of glycemia and hepatic insulin extraction) suggest that Insulinemic Index is not a property of foods but is subject-dependent; therefore, unlike GI, Insulinemic Index may have limited clinical utility.

No association was found between endogenous GLP-1 concentration and insulin sensitivity, β-cell function and hepatic insulin extraction. This may be because biologically active GLP-1 was measured instead of total GLP-1 concentration that represents GLP-1 secretion. Moreover, insulin sensitivity, β-cell function and hepatic insulin extraction were characterized using indirect calculations instead of direct measurements (i.e. euglycemic hyperinsulinemic clamp for insulin sensitivity, hyperglycemic clamp for β-cell function, and hepatic vein catheterization for hepatic insulin extraction).
CHAPTER 10

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CHAPTER 11
APPENDICES
Appendix 1. The correlations between fasting insulin and basal hepatic insulin extraction (HIE_{basal}) (top), fasting insulin and postprandial hepatic insulin extraction (HIE_{auc}) (middle), and postprandial insulin response (insulin_{auc}) and postprandial hepatic insulin extraction (HIE_{auc}) (bottom). n=25, the lines are regression lines.
Appendix 2. The correlation between hepatic insulin extraction (C-peptide_{auc}/Insulin_{auc}) and hepatic insulin resistance. n=25, the line is a regression line.
### Appendix 3  Physical activity indices of the subjects by FSI

<table>
<thead>
<tr>
<th></th>
<th>Low (FSI&lt;40pmol/L)</th>
<th>Medium (40 ≤ FSI &lt;70pmol/L)</th>
<th>High (FSI ≥ 70 pmol/L)</th>
<th>P²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Work</td>
<td>2.6±0.2</td>
<td>2.1±0.1</td>
<td>2.5±0.2</td>
<td>0.07</td>
</tr>
<tr>
<td>Sport</td>
<td>2.8±0.2</td>
<td>2.2±0.2</td>
<td>2±0.1</td>
<td>0.44</td>
</tr>
<tr>
<td>Leisure-time</td>
<td>3.3±0.2</td>
<td>3±0.2</td>
<td>2.5±0.1</td>
<td>0.83</td>
</tr>
<tr>
<td>Mean physical activity index³</td>
<td>2.9±0.1</td>
<td>2.4±0.1</td>
<td>2.3±0.1</td>
<td>0.37</td>
</tr>
</tbody>
</table>

¹ n = 25 except where otherwise noted. Values are expressed as mean±SEM.

² P represents overall significant differences across groups by one way ANOVA.

³ Mean physical activity index comprising the work, sport, and leisure-time physical activity indices.
Appendix 4

Mean (±SEM) 2-h postprandial plasma glucose, insulin, C-peptide, and glucagon-like peptide 1 (GLP-1) concentrations after 50 g oral glucose plus 0, 5, and 30 g fat in non-diabetic humans with different concentrations of fasting serum insulin (FSI): low (FSI < 40 pmol/L), medium (40 ≤ FSI < 70 pmol/L), and high (FSI ≥ 70 pmol/L). n = 25. The error bars are not shown if they overlap or are smaller than the symbol.
Appendix 5

Appendix 5. The correlations between relative glucose responses (RGR) per g of fat or protein and fasting insulin (left) and between relative insulin responses (RIR) per g of fat or protein and fasting insulin (right). r: pearson’s correlation coefficient. The lines are regression lines.
Appendix 6

Mean (±SEM) effects of 50 g glucose plus 0, 5, or 30 g fat or protein on 2hr GLP-1 response in healthy nondiabetic subjects with different levels of fasting serum insulin (FSI): low (FSI < 40 pmol/L, white bars), medium (40 ≤ FSI < 70 pmol/L, grey bars), and high (FSI ≥ 70 pmol/L, black bars). Means not sharing a common letter are significantly different [repeated measures ANOVA, PROC MIXED procedure (SAS Institute, Cary, NC)]. Age and BMI were included in the model as covariates (Tukey’s post hoc test, P < 0.05). n = 25.
Appendix 7

Nutrient composition of 30g whey protein

<table>
<thead>
<tr>
<th></th>
<th>whey powder (37g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
<td>153</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>30</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>2.3</td>
</tr>
<tr>
<td>Total CHO (g)</td>
<td>2.6</td>
</tr>
<tr>
<td>Proteose peptones (g)</td>
<td>1.2</td>
</tr>
<tr>
<td>α-lactalbumin (g)</td>
<td>3.3</td>
</tr>
<tr>
<td>β-lactoglobulin (g)</td>
<td>14.3</td>
</tr>
<tr>
<td>Bovine serum albumin (g)</td>
<td>0.4</td>
</tr>
<tr>
<td>Immunoglobulins (g)</td>
<td>1.2</td>
</tr>
<tr>
<td>Glycomacropeptide (g)</td>
<td>4.6</td>
</tr>
<tr>
<td>Lactoferrin (g)</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Source: Product bulletin of Whey protein concentrate 392; Fonterra Inc, Camp Hill, PA.
Appendix 8  Screening form for fat and protein study

XIAPP (CIHR PP Study)

Postprandial responses elicited by fat and protein in normal and hyperinsulinaemic subjects

Contact Person:  Xiaomiao Lan-Pidhainy  xm.lan-pidhainy@utoronto.ca
Contact Phone: 7438
Physician:  Dr. Thomas Wolever, 61 Queen, 6\textsuperscript{th} floor, rm 6-143
Study duration: March-Nov 07

Please enter:

1.  Subject ID: ______  Subject Initial: _______  DOB: ______/_______/ ______  (day) (month) (year)

2.  Enter Doctor Code.  14147 Dr. Wolever

3.  Enter Location Code:  \textbf{XIAPP}

4.  Register the following tests:

- Phlebotomy
- Glucose
- Insulin
- Total cholesterol
- HDL cholesterol
- Triglycerides
- Creatinine
- AST
- CRP
Appendix 9

3-day food records

Initial: ________  Subject ID#: ________  Day of Record: __________

Day of Week (circle): Mon  Tue  Wed  Thu  Fri  Sat  Sun  Date: __________

<table>
<thead>
<tr>
<th>Meal location</th>
<th>Food</th>
<th>Portion</th>
<th>Condiment</th>
<th>Portion</th>
<th>Beverage</th>
<th>Portion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breakfast Time:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Snack Time:</td>
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<td></td>
</tr>
<tr>
<td>Lunch Time:</td>
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<td></td>
</tr>
<tr>
<td>Snack Time:</td>
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<td></td>
</tr>
<tr>
<td>Dinner Time:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Snack Time:</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Portion control guide

- ¼ cup = golf ball
- ½ cup = tennis or racquet ball
- 1 cup = small fist
- 1 oz. = one handful or matchbox
- 4 oz. fish filet = eyeglass case
- 3 oz. portion of cooked meat = a deck of playing cards or cassette tape
- 1 teaspoon = quarter or tip of your thumb
- 3 teaspoons = 1 tablespoon
- 8 fl. oz. = 1 cup

1. Overall, do you feel the food choices and amounts you ate today were typical of your usual diet? Yes____  No____  Somewhat____
2. For the most part, when did you record food items eaten?  Immediately after eating____  A while after eating____  At the end of the day_______
   Other ________________________________
Appendix 10  Consent form for the 1st study: Postprandial responses elicited by fat and protein in normal and hyperinsulinaemic subjects

Risk Factor Modification Center
St. Michael’s Hospital
61 Queen’s St. East
(416) 867-7438

Consent to Participate in a Research Study

Study Title: Postprandial responses elicited by fat and protein in normal and hyperinsulinaemic subjects

Before agreeing to take part in this research study, it is important that you read the information in this research consent form. It includes details we think you need to know in order to decide if you wish to take part in the study. If you have any questions, ask a study doctor or study staff. You should not sign this form until you are sure you understand the information. All research is voluntary. You may also wish to discuss the study with your family doctor, a family member or close friend. If you decide to take part in the study, it is important that you are completely truthful about your health history and any medications you are taking. This will help prevent unnecessary harm to you.

Investigator(s)

INVESTIGATORS: Thomas MS Wolever, PhD, DM,
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Xiaomiao Lan-Pidhainy, Study Coordinator;
MSc, Ph.D student
Department of Nutritional Sciences
University of Toronto
Toronto, ON M5S 3E2
Tel: 416-867-7438 e-mail: xm.lan.pidhainy@utoronto.ca
Doctor of Philosophy Candidate Research Project

This study is to be conducted as part of the study coordinator, Ms Lan-Pidhainy’s Ph. D degree requirements. Ms Lan-Pidhainy has completed her Master’s of Science (MSc) degree at the University of Toronto. Dr. Wolever is Ms Lan-Pidhainy’s advisor and Principle Investigator on this study. This means he will be supervising the conduct of the study.

Conflict of Interest

Dr. Wolever is the president and part owner of Glycemic Index Laboratories (GI Labs), a contract research organization specializing in determining the glycaemic index of foods. However, there is no foreseeable commercial application of the results. Ms Lan-Pidhainy declares no conflicts of interest.

Study Sponsor

Canadian Institutes of Health Research
160 Elgin Street, 9th Floor
Address Locator 4809A
Ottawa, ON, K1A 0W9
CANADA

Purpose of the Research

You are invited to participate in a nutrition research study on the after-meal metabolic responses to fat and protein in normal subjects and subjects with high fasting insulin.

High blood glucose after eating is associated with increased risk of cardiovascular disease, diabetes and cancer. Many studies have compared the effects of different carbohydrate foods on blood glucose but less is known about how fat and protein affect glucose response. We recently found that the glucose-lowering effect of fat was reduced in insulin resistant subjects, and the ability of protein to lower glucose was increased as fiber intake increased. The purpose of this study is to find out why this happened by measuring the effects of fat and protein on insulin and other hormones.

The reason for this study is to test whether the blood glucose lowering effect of fat and protein is reduced in subjects with high fasting insulin compared to the normal subjects. This is likely due to their differences in insulin secretion, hepatic insulin extraction, and gut hormone responses.

Procedure

This study will recruit 8 subjects with high fasting insulin (FPI) (FPI > 80 pmol/L) and 16 subjects with normal insulin level (8 with FPI < 41 pmol/L and 8 with 41 < FPI < 80 pmol/L). Approximately 100 subjects will be screened in order to determine their eligibility. You will be asked to come to the Risk Factor Modification Center, St. Michael’s Hospital (61 Queen’s St.
East) on one morning (between 8:00-9:30am) and give a fasting blood sample (10 ml blood, about 2 tsps). The blood sample will be used to measure insulin and proinsulin levels, and other routine tests (glucose, cholesterol level) to see if you are eligible. You will be asked to fill out a questionnaire only **once** at the beginning of the study; this includes questions about your age, gender, ethnicity and your parents’ ethnicities. You will be asked to have your height, weight, waist, hip circumference and blood pressure measured. You will **not** be eligible for this study if you have the following:

- BMI is >35kg/m² (a ratio of your weight and height)
- Fasting blood sugar >= 7.0mmol/L
- Triglycerides (a measure of fat level in the blood) >= 10.0mmol/L

You will also be asked questions regarding your health status. If you answer ‘yes’ to any of the following questions you will **not** be able to participate in this study:

- Do you have Diabetes?
- Do you have a heart problem such as Congestive Heart Failure (CHF) or a history of Heart Attack?
- Have you had a Stroke or TIA (stroke symptoms that cleared up)?
- Do you have any chronic Liver disease such as Hepatitis C?
- Do you have Kidney Disease?
- Are you HIV positive or diagnosed with AIDS?
- Do you have any chronic stomach or bowel conditions such as Inflammatory Bowel Disease (eg. Crohn’s Disease or Ulcerative Colitis) or Malabsorption (a condition where the body can’t absorb the vitamins and other nutrients eaten)?
- Have you had recent [within 3 months] emergency and surgery?
- Have you been admitted to Hospital urgently for any other medical reasons [within 3 months]?
- Are you pregnant or breast-feeding?
- Are you on Diuretics (drugs that promote water loss from the body, often used for High Blood Pressure and CHF) eg. HCTZ (a thiazide) or Spironolactone.
- Are you on oral Corticosteroids (steroid hormones) or Beta-Blockers (a common heart medication)?

If you are selected for this study, you will be asked to attend 11 clinic visits over a period of 3 months at the Risk Factor Modification Center, St. Michael’s Hospital (61 Queen’s St. East). You will be asked to come on mornings (between 8:00-9:30am) after an overnight fasts (no food or drink for 10-14 hours). Each visit is expected to take about 2 hours of your time.

At the clinic, you will be fed test meals consisting of 50g anhydrous glucose dissolved in 250ml water to which will be added 0, 5 and 30g of whey protein concentrate (Fonterra Inc. USA) and 0, 5 and 30g fat (canola oil). Venous blood samples (blood will be taken from a needle inserted into a vein in your arm) will be collected by the nurse at fasting and at 15, 30, 45, 60, 90 and 120min after starting to eat.
For your each visit, you will give 7 blood samples, and each sample will collect 10 ml of blood; therefore, 70 ml blood for each visit (only require one visit in a week). In total, 770 ml blood will be drawn during approximately 3 month period, which is safe on its own because in blood donation, donors can donate 450 ml blood every 2 month. **You are advised not to donate blood during and 3 months after the study.**

The blood samples will be used to measure the levels of glucose, insulin, and gut hormones.

During the 2 hrs of the test, a short physical activity questionnaire will be administered. It will only take 5 minutes to fill in the form.

To assess dietary nutrient intake, you will be asked to complete two 3-day food records at the beginning and end of the study period (appendix A). Each record will take approximately 20 minutes of your time.

You will be asked to repeat test if the results were poor.

Any abnormal measures (i.e. if the blood pressure was 140/90 or above) will be brought to the attention of Dr. Wolever, a physician at St. Michael's Hospital, who will advise Ms. Lan-Pidhainy as to whether you should be advised to seek follow-up with your family doctor. Dr. Wolever will be available to discuss this with you if you desire, and will arrange appropriate follow-up at St. Michael's Hospital if you does not have a family doctor and desires that course of action.

**Test Meals**

You will be given the test meals in a random order. You will be fed the following test meals during the 11 clinical visits. The control meal will be consumed at the beginning, the middle and the end of the study.

1. control : 50 g glucose alone (0 g fat and protein)
2. 50g glucose + 5g protein
3. 50g glucose + 30g protein
4. 50g glucose + 5g fat
5. 50g glucose + 5g fat + 5g protein
6. 50g glucose + 5g fat + 30 protein
7. 50g glucose + 30g fat
8. 50g glucose + 30g fat + 5g protein
9. 50g glucose + 30g fat + 30g protein

**Risks**

The risks involved in the study are very low. For each test, 7 venous blood samples (70ml blood) will be taken. The risks associated with having venous blood drawn include discomfort, and possible dizziness, fainting or feeling lightheaded, redness, swelling, hematoma (blood
accumulating under the skin), infection (a slight risk any time the skin is broken). Since an experienced registered nurse (RN) will be hired for blood sampling, the risks of these problems are considered extremely low.

**Benefits**

You will not receive any direct benefit by participating in this study. You do not have to participate.

**Alternatives to Participation**

This is not a treatment study. You do not have to participate in this study.

**Protecting Your Health Information**

Confidentiality will be respected and no information that discloses your identity will be released if results of this study are published. Your identity will not be disclosed without your permission unless required by law.

Your blood samples will be sent for biochemical analysis to the Banting and Best Core Diabetes Laboratory, located at the Mount Sinai Hospital in Toronto. The labels on your blood samples will only contain your initial and ID number. The consent forms, data sheets and questionnaires will be kept securely in locked file cabinets in St.Michael’s Hospital. The study co-investigator will require access to your file and identification number and the principle investigator may require access to your personal health information. The likelihood of the principle investigator needing access to your identification information is very low. All data will be stored electronically and will only be identified by initial and ID numbers. And all data will be saved in password protected files.

At the end of the study, the files linking the ID number with the names will be destroyed by the study coordinator. The results will be used in scientific meetings and publications, but subject identity will not be revealed in these presentations.

If you withdraw from the study, your blood samples and any data collected up until the time you decide to withdraw from the study will be destroyed immediately.

**Study Results**

If you were interested in the study results, you may contact either Dr. Wolever or Ms. Lan-Pidhainy. For the published results, you may find in journals such as American Journal of Clinical Nutrition, European Journal of Clinical Nutrition, Journal of Nutrition, etc in the near future.

**Payment for Participation**

For the screening test, you will be paid $15 for your time.
If you are selected for the study, you will be paid $40 for each test completed, and $40 for completing two 3-day food records (at the beginning and end of the study period), for a total of $480 if you complete the entire study.

You may be asked to repeat tests if the results are poor. As long as this is not a result of failure to follow instructions, you will be paid $40 for each test repeated.

**Compensation for Injury**

If you suffer a physical injury as a direct result of the administration of the study procedures, medical care may be obtained by you in the same manner as you would ordinarily obtain any other medical treatment. In no way does this form waive your legal rights nor relieve the investigator, sponsors or involved institutions from their legal and professional responsibilities.

**Participation and Withdrawal**

Participation in this research study is voluntary. If you choose not to participate, you and your family will continue to have access to customary care at St. Michael’s Hospital. If you decide to participate in this study you can change your mind without giving a reason, and you may withdraw from the study at any time without any effect on the care you and your family will receive at St. Michael’s Hospital.

**New Findings or Information**

We may learn new information during the study that you may need to know or that might make you want to stop participating in the study. If so, you will be notified about any new information in a timely manner. You may be asked to sign a new consent form discussing these new findings if you decide to continue in the research study.

**Research Ethics Board Contact**

If you have any questions regarding your rights as a research participant, you may contact Julie Spence, Chair, Research Ethics Board at 416-864-6060 ext. 2557 during business hours.

The study protocol and consent form have been reviewed by a committee called the Research Ethics Board at St. Michael’s Hospital. The Research Ethics Board is a group of scientists, medical staff, individuals from other backgrounds (including law and ethics) as well as members from the community. The committee is established by the hospital to review studies for their scientific and ethical merit. The Board pays special attention to the potential harms and benefits involved in participation to the research participant, as well as the potential benefit to society.
This committee is also required to do periodic review of ongoing research studies. As part of this review, someone may contact you from the Research Ethics Board to discuss your experience in the research study.

**Study Contacts**

If you have any questions concerning your participation in this study or if at any time you feel you have experienced a research-related injury you may contact the study doctor, Dr. Thomas M.S. Wolever at (416) 978-5556 during business hours (Monday to Friday 9:00-5:00) or e-mail him at Thomas.wolever@utoronto.ca.
Consent

The research study has been explained to me, and my questions have been answered to my satisfaction. I have been informed of the alternatives to participation in this study. I have the right not to participate and the right to withdraw without affecting the quality of medical care at St. Michael’s Hospital for me and for other members of my family. As well, the potential harms of participating in this research study have been explained to me. I have been told that I have not waived my legal rights nor released the investigators, sponsors, or involved institutions from their legal and professional responsibilities. I know that I may ask now, or in the future, any questions I have about the study. I have been told that records relating to me and my care will be kept confidential and that no information will be disclosed without my permission unless required by law. I have been given sufficient time to read the above information.

I consent to participate. I have been told I will be given a signed copy of this consent form.

Subject Name: _____________________________________________

Subject Signature: ________________________________ Date: ______________

Name and Position of Person Conducting Informed Consent Decision:

Name: ____________________________________________ Position: _____________

Signature: ________________________________ Date: ______________
Appendix 11  Screening form for carbohydrate study

Patient ID#:__________
Initial:_______
Date of Birth: ____________

Research Study Tel#: 7438
Location: XIAPP
Send results to: DR.WOLEVER 14147
Contact: XIAOMIAO 7438

XIAPP STUDY
CARBOHYDRATE STUDY

☐ PHLEB
☐ GLUF
☐ LIP2F
☐ CRP
☐ INSU
☐ CR
☐ AST
☐ ALT
☐ GGT
☐ HBAIC
Appendix 12  Concent form for the 2nd study: postprandial response to different carbohydrates in normal, hyperinsulinemic and type 2 diabetic subjects

Risk Factor Modification Center
St. Michael’s Hospital
61 Queen’s St. East
(416) 867-7438

Consent to Participate in a Research Study

Study Title: Postprandial responses to different carbohydrates in normal, hyperinsulinaemic and type 2 diabetic subjects

Before agreeing to take part in this research study, it is important that you read the information in this research consent form. It includes details we think you need to know in order to decide if you wish to take part in the study. If you have any questions, ask a study doctor or study staff. You should not sign this form until you are sure you understand the information. All research is voluntary. You may also wish to discuss the study with your family doctor, a family member or close friend. If you decide to take part in the study, it is important that you are completely truthful about your health history and any medications you are taking. This will help prevent unnecessary harm to you.

Investigator(s)
PRINCIPAL INVESTIGATOR:  CO-INVESTIGATOR
Thomas MS Wolever, PhD, DM,  Xiaomiao Lan-Pidhainy,
Staff physician, St. Michael’s Hospital  Study Coordinator;
Professor, Department of Nutritional Sciences  MSc, Ph.D student
Department of Nutritional Sciences
University of Toronto  University of Toronto
Toronto, ON M5S 3E2  Toronto, ON M5S 3E2
Tel: 416-978-5556  Tel: 416-867-7438
e-mail: thomas.wolver@utoronto.ca  e-mail: xm.lan.pidhainy@utoronto.ca

Doctor of Philosophy Candidate Research Project
This study is to be conducted as part of the study coordinator, Ms Lan-Pidhainy’s Ph. D degree requirements. Ms Lan-Pidhainy has completed her Master’s of Science (MSc) degree at the University of Toronto. Dr. Wolever is Ms Lan-Pidhainy’s advisor and Principal Investigator on this study. This means he will be supervising the conduct of the study.

Conflict of Interest
Dr. Wolever is the president and part owner of Glycemic Index Laboratories (GI Labs), a contract research organization specializing in determining the glycaemic index of foods. However, there
is no foreseeable commercial application of the results. Ms Lan-Pidhainy declares no conflicts of interest.

**Study Sponsor**
Canadian Institutes of Health Research
160 Elgin Street, 9th Floor
Address Locator 4809A
Ottawa, ON, K1A 0W9
CANADA

**Purpose of the Research**
You are being asked to consider participating in a nutrition research study on the after-meal physiological responses to different carbohydrate foods in normal subjects, subjects with high fasting insulin, and subjects with type 2 diabetes.

The Glycaemic Index (GI) and Insulinaemic Index (II) measure how much the carbohydrate in foods increases your blood glucose and insulin. For these indexes to have a use in clinics, their values must be the same in different subjects regardless of whether they have normal, high fasting insulin or are diabetic. There is evidence that the GI values of foods are similar in normal and diabetic subjects. However, the GI of foods has not been determined in subjects with high fasting insulin. Thus we do not know if they are similar in normal vs. subjects with high fasting insulin vs. type 2 diabetic subjects.

Because of the uncertainty regarding insulin response to carbohydrate foods, insulin index (II) has also been measured; however, it is not known whether the II values are the same in normal vs. subjects with high fasting insulin vs. type 2 diabetic subjects. Since insulin response is not only glucose-dependent, but also connected with the normal functioning of gut hormones and how insulin is cleared by liver. The II values may be subject-dependent. This means that II may not be a valid measure of food property. Therefore, to see if II is a valid measure of carbohydrate in foods, it is important to use “pure” carbohydrates and carbohydrate foods to measure II values in all conditions.

**Procedure**
This study will recruit 10 subjects with normal insulin level (FPI <40 pmol/L), 10 subjects with high fasting insulin (FPI) (FPI > 40 pmol/L) and 10 subjects with type 2 diabetes. You will be asked to come to the Risk Factor Modification Center, St. Michael’s Hospital (61 Queen’s St. East, the 6th floor) on one morning (between 8:00-9:30am) and give a fasting blood sample (10 ml blood, about 2 tsps). A fasting blood sample means that you cannot have any food or drinks 10-14 hours before your study visit. The blood sample will be used to measure glucose, insulin, and lipids level to see if you are eligible. You will be asked to fill out a questionnaire only once at the beginning of the study; this includes questions about your age, gender, ethnicity and your parents’ ethnicities. You will be asked to have your height, weight, waist, hip circumference and blood pressure measured.
For the non-diabetic healthy subjects, you may not be eligible for this study if you have the following:

- BMI is $>35\text{kg/m}^2$ (a ratio of your weight and height)
- Fasting blood sugar $\geq 7.0\text{mmol/L}$
- Triglycerides (a measure of fat level in the blood) $\geq 10.0\text{mmol/L}$

You will also be asked questions regarding your health status. Your answers to these questions will be used to determine your eligibility to participate in this study:

For subjects with type 2 diabetes, Dr. Wolever will interview you about your health status and medication. You will be eligible for the study if you are:

- 18 - 70 y old
- BMI $<35\text{kg/m}^2$ (a ratio of your weight and height)
- Fasting glucose $>7.0\text{mmol/L}$ or
- HbA1c (a measure of your long-term glucose control) over upper limit or normal or
- Treated with any combination of sulfonylureas, metformin, thiazolidinedione and/or meglitinide.

If you are selected for this study, you will be asked to attend 8 clinic visits over a period of 2 months at the Risk Factor Modification Center, St. Michael’s Hospital (61 Queen’s St. East). You will be asked to come on mornings (between 8:00-9:30am) after an overnight fasts (no food or drink for 10-14 hours). For non-diabetic subject, each visit is expected to take about 2 hours of your time, for diabetic subjects, each visit will take about 3 hours of your time.

At the clinic, you will be fed test meals consisting of 50g available carbohydrate (total carbohydrate minus dietary fibre) as 50g anhydrous glucose (Fisher Scientific, Mississauga, ON), 50g sucrose (Lantic Sugar Ltd, Toronto, ON), 50 instant mashed potato, 104g white bread (Wonder bread, Mississauga, ON), 50g polished rice (Dainty brand, Peterborough, ON) and 50 pearled barley (Gouda brand, Peterborough, ON). Sugars will be dissolved in 250ml bottled water. Instant potato will be weighed dry, and boiling water added according to package directions. Two to four portions of rice and barley will be weighed dry, boiled in salted water according to package directions on the morning of the test and the resulting cooked amount weighed and divided into portions. You are asked to consume the food within 10-15 minutes.

For non-diabetic subjects, venous blood samples (blood will be taken from a needle inserted into a vein in your arm) will be collected by the nurse at fasting and at 15, 30, 45, 60, 90 and 120min after starting to eat. For type 2 diabetic subjects, blood samples will be collected in the same manner at fasting and at 30, 60, 90, 120, 150 and 180min after starting to eat.

For your each visit, you will give 7 blood samples, and each sample will collect 10.5 ml of blood; therefore, 73.5ml (about 5 tablespoons) blood for each visit (only require one visit in a week). In total, 588 ml (about 1¼ pints) blood will be drawn during approximately 2 month period, which is safe on its own because in blood donation, donors can donate 450 ml (about 1
pint) blood every 2 month. **You are advised not to donate blood during the study and for 2 months after the study.**

The blood samples will be used to measure the levels of glucose, insulin, C-peptide, free fatty acids and gut hormone GLP-1.

During the 2 hrs of the test, a short physical activity questionnaire will be administered (at the beginning and end of the study period). It will only take 5 minutes to fill in the form.

You may be asked to repeat some tests if the results that are obtained are poor.

Any abnormal measures (e.g. if the blood pressure was 140/90 or above) will be brought to the attention of Dr. Wolever, a physician at St. Michael's Hospital, who will advise as to whether you should seek follow-up with your family doctor. Dr. Wolever will be available to discuss this with you if you desire, and will arrange appropriate follow-up at St. Michael's Hospital if you does not have a family doctor and desire that course of action.

**Test Meals**
You will be given the test meals in a random order. You will be fed the following test meals during the 8 clinical visits. The control meal will be consumed at the beginning, the middle and the end of the study.

10. control : 50 g glucose alone
11. Sucrose
12. Instant mashed potato
13. White bread
14. Polished rice
15. Pearled barley

**Potential Harms (Injury, Discomforts Or Inconvenience)**
The risks involved in the study are very low. For each test, 7 venous blood samples (70.5ml blood or 5 tablespoons) will be taken. The risks associated with having venous blood drawn include discomfort, and possible dizziness, fainting or feeling lightheaded, redness, swelling, hematoma (blood accumulating under the skin), infection (a slight risk any time the skin is broken). Since an experienced registered nurse (RN) will be hired for blood sampling, the risks of these problems are considered extremely low. With the exception of glucose (approximately 10% of subjects may experience nausea); the likelihood of the other test foods (instant mashed potato, white bread, rice, pearled barley, sucrose) causing nausea is very rare, but you may experience some minor stomach discomfort and bloating.

**Potential Benefits**
You will not receive any direct benefit by participating in this study. You do not have to participate. However, results from this study may further medical or scientific knowledge.

**Alternatives to Participation**
This is not a treatment study. You do not have to participate in this study.
Protecting Your Health Information
The study investigators are committed to respecting your privacy. No other persons will have access to your personal health information or identifying information without your consent, unless required by law. Any medical records, documentation, or information related to you will be coded by study numbers to ensure that persons outside of the study will not be able to identify you. Your blood samples will be sent for biochemical analysis to Mount Sinai Hospital Core laboratory and St. Michael’s Hospital Core Laboratory (both located in Toronto). The labels on your blood samples will only contain your ID number. No identifying information about you will be allowed off site. All information that identifies you will be kept confidential and stored and locked in a secure place that only the study investigators will have access to. In addition, electronic files will be stored on a secure hospital or institutional network and will be password protected. It is important to understand that despite these protections being in place, experience in similar studies indicates that there is the risk of unintentional release of information. The principal investigator will protect your records and keep all the information in your study file confidential to the greatest extent possible. The chance that this information will accidentally be given to someone else is small.

By signing this form, you are authorizing access to your medical records by the study investigators and St. Michael’s Hospital Research Ethics Board. Such access will be used only for purposes of verifying the authenticity of the information collected for the study, without violating your confidentiality, to the extent permitted by applicable laws and regulations.

National and Provincial Data Protection regulations, including the Personal Information Protection and Electronic Documents Act (of Canada) or PIPEDA and the Personal Health Information Protection Act (PHIPA) of Ontario, protect your personal information. They also give you the right to control the use of your personal information, including personal health information, and require your written permission for your personal information (including personal health information) to be collected, used or disclosed for the purposes of this study, as described in this consent form. You have the right to review and copy your personal information. However, if you decide to be in this study or choose to withdraw from it, your right to look at or copy your personal information related to this study will be delayed until after the research is completed.

At the end of this study, the files linking the ID number with the names will be destroyed by the study coordinator.

Study Results
The results of this study will be used in scientific meetings and publications, but your identity will never be revealed in these presentations.

If you were interested in the study results, you may contact either Dr. Wolever or Ms. Lan-Pidhainy. For the published results, you may find in journals such as American Journal of Clinical Nutrition, European Journal of Clinical Nutrition, Journal of Nutrition, etc in the near future.
**Payment for Participation**
For the screening test, you will be paid $15 for your time.

For the non-diabetic subjects, if you are selected for the study, you will be paid $40 ($20/hr, 2 hrs) for each test completed, for a total of $320 if you complete the entire study.

For the diabetic subjects, if you are selected for the study, you will be paid $60 ($20/hr, 3hrs) for each test completed, for a total of $480 if you complete the entire study.

You may be asked to repeat tests if the results are poor. As long as this is not a result of failure to follow instructions, you will be paid $40 or $60 for each test repeated.

If you do not complete the study successfully, you will be compensated on a pro-rated basis at $20/hr for the time you have spent participating in the study.

**Compensation for Injury**
If you suffer an injury from study procedures or taking the study foods or participation in this study, medical care will be provided to you in the same manner as you would ordinarily obtain any other medical treatment. In no way does signing this form waive your legal rights nor release the study doctor(s), sponsors or involved institutions from their legal and professional responsibilities.

**Participation and Withdrawal**
Participation in this research study is voluntary. If you choose not to participate, you and your family will continue to have access to customary care at St.Michael’s Hospital. If you decide to participate in this study you can change your mind without giving a reason, and you may withdraw from the study at any time without any effect on the care you and your family will receive at St. Michael’s Hospital. If you withdraw from the study, your blood samples and any data collected up until the time you decide to withdraw from the study will be destroyed immediately (unless you request otherwise).

**New Findings or Information**
We may learn new information during the study that you may need to know or that might make you want to stop participating in the study. If so, you will be notified about any new information in a timely manner. You may be asked to sign a new consent form discussing these new findings if you decide to continue in the research study.

**Research Ethics Board Contact**
The study protocol and consent form have been reviewed by a committee called the Research Ethics Board at St. Michael’s Hospital. The Research Ethics Board is a group of scientists, medical staff, individuals from other backgrounds (including law and ethics) as well as members from the community. The committee is established by the hospital to review studies for their scientific and ethical merit. The Board pays special attention to the potential harms and benefits involved in participation to the research participant, as well as the potential benefit to society.
This committee is also required to do periodic review of ongoing research studies. As part of this review, someone may contact you from the Research Ethics Board to discuss your experience in the research study.

If you have any questions regarding your rights as a research participant, you may contact Julie Spence, Chair, Research Ethics Board at 416-864-6060 ext. 2557 during business hours.

**Study Contacts**
If you have any questions concerning your participation in this study or if at any time you feel you have experienced a research-related injury you may contact the study doctor, Dr. Thomas M.S. Wolever at (416) 978-5556 during business hours (Monday to Friday 9:00-5:00) or e-mail him at Thomas.wolever@utoronto.ca.
Consent

The research study has been explained to me, and my questions have been answered to my satisfaction. I have been informed of the alternatives to participation in this study. I have the right not to participate and the right to withdraw without affecting the quality of medical care at St. Michael’s Hospital for me and for other members of my family. As well, the potential harms of participating in this research study have been explained to me. I have been told that I have not waived my legal rights nor released the investigators, sponsors, or involved institutions from their legal and professional responsibilities. I know that I may ask now, or in the future, any questions I have about the study. I have been told that records relating to me and my care will be kept confidential and that no information will be disclosed without my permission unless required by law. I have been given sufficient time to read the above information.

I consent to participate. I have been told I will be given a signed copy of this consent form.

Subject Name: _____________________________________________

Subject Signature: ________________________________      Date: ________________

Name and Position of Person Conducting Informed Consent Decision:

Name: _______________________________________           Position: _____________

Signature: _____________________________________          Date: _______________