EFFECTS OF GLUCOSE, FRUCTOSE AND SUCROSE ON POSTPRANDIAL GLUCOSE AND INSULIN RESPONSES

by

Brenda Minfei Lee

A thesis submitted in conformity with the requirements for the Degree of Master of Science, Graduate Department of Nutritional Sciences, University of Toronto.

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<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
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<tr>
<td>CHO</td>
<td>Carbohydrate</td>
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<tr>
<td>g</td>
<td>Grams</td>
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<tr>
<td>GI</td>
<td>Glycemic Index</td>
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<td>GR</td>
<td>Glucose Response</td>
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<tr>
<td>II</td>
<td>Insulinemic Index</td>
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<tr>
<td>IAUC</td>
<td>Incremental Area Under the Curve</td>
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<tr>
<td>kg</td>
<td>Kilogram</td>
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<tr>
<td>L</td>
<td>Litre</td>
</tr>
<tr>
<td>LDL</td>
<td>Low Densisty Lipoprotein</td>
</tr>
<tr>
<td>min</td>
<td>Minute</td>
</tr>
<tr>
<td>mmol/L</td>
<td>Millimole per Litre</td>
</tr>
<tr>
<td>nmol/L</td>
<td>Nanomole per Litre</td>
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<tr>
<td>SEM</td>
<td>Standard Error of the Mean</td>
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<tr>
<td>VLDL</td>
<td>Very Low Density Lipoprotein</td>
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<tr>
<td>x</td>
<td>Dose of Carbohydrate</td>
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EFFECTS OF GLUCOSE, FRUCTOSE AND SUCROSE ON POSTPRANDIAL GLUCOSE AND INSULIN RESPONSES

Master of Science, 1997
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ABSTRACT

To investigate effects of glucose, fructose and sucrose on postprandial glucose and insulin responses, eight healthy subjects consumed 25, 50 and 100 g of glucose, sucrose and white bread and 25 and 50 g of fructose on separate occasions following overnight fasts.

Postprandial plasma glucose responses areas (AUC) were significantly influenced by carbohydrate type (p<0.008) and dose (p<0.003). Mean glucose AUC for glucose, white bread, sucrose and fructose (all doses combined) were 219.6, 156.0, 125.7 and 11.3 mmol/min/L. Mean glucose AUC for 25, 50 and 100 g doses (glucose, sucrose and white bread combined) were 105.5, 166.8 and 228.9 mmol/min/L. Postprandial plasma insulin AUC was significantly influenced by dose (p<0.002). Mean insulin AUC for 25, 50 and 100 g doses (glucose, sucrose and white bread combined) were 6.6, 12.3 and 25.5 nmol/min/L.

It was concluded that glucose, fructose and sucrose did not produce inappropriate insulin responses in normal subjects.
CHAPTER ONE

Introduction and Literature Review
1. INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

The role that sugars play in the diet and their relationship to a range of health concerns is an area of tremendous interest in nutritional sciences (1). There is a popular conception that sugar consumption is harmful for health. Consequently, there are many people who would advocate the restriction of sugars in the diet.

The belief that sugars uniquely raise blood glucose levels is the basis for many clinical interventions and medical hypotheses (2). For instance, the use of glucose and glucose-containing simple sugars has been restricted in the diets of patients with diabetes (3). This is grounded primarily on the premise that sugars cause rapid rises in blood glucose concentrations (3, 4). The rationale is that mono- and disaccharide sugars such as glucose and sucrose are more readily available for immediate absorption, thus producing a greater and faster rise in postprandial plasma glucose and insulin responses, in contrast to the supposedly slower and more gradual absorption of more complex carbohydrates such as starch (5, 6). This is particularly relevant in the management of diabetes where there is an important need to prevent rapid fluctuations in blood glucose concentrations for the purpose of reducing acute and long term complications (3). In addition, elevated plasma insulin levels are associated with an increased incidence of cardiovascular disease in both normal and diabetic individuals (7).
However, many of the studies which support the belief that simple sugars are more rapidly digested and absorbed than complex carbohydrates have limitations. Furthermore, a number of more recent studies have shown that even some cooked starches cause glycemic responses that are similar to, or only slightly less than the glycemic response to glucose, and that are the same or greater than that observed with sucrose (8).

The aim of the present work is to compare the glucose and insulin responses of different doses of glucose, fructose and sucrose relative to a starchy carbohydrate such as white bread.
1.2 LITERATURE REVIEW

1.2.1 Trends in Sugar Consumption

The optimum level of sugar in the diet is unknown. Sugars refers to the mono- and disaccharides such as glucose, fructose and sucrose, which are both added and naturally occurring in the human diet. A major issue of debate in human nutrition is the effect of the level of sugar intake on certain aspects related to health (9). In western populations, sugar intake is considered to be quite high and shows little inclination to decrease (9, 10). In developing populations, sugar intake is increasing considerably (11).

According to data from the 1987-1988 US Department of Agriculture Nationwide Food Consumption Survey, the mean percent of energy from total sugars (mono- and disaccharides) not including lactose is 18% in the United States. Amongst nations of the European Union, energy from total sugars ranges from 8.0% up to 21.2%, with a mean value of 15.2% (12).

The profile of sugars consumption is also undergoing some transformation. The development of new types of commercial sweeteners is rapidly changing the pattern of sugar usage (13). One of the most important changes has been the amount of free fructose in the diet. Until more recently, free fructose was found in relatively small amounts in the diet, with most fructose coming in glycosidic linkage with glucose to make up sucrose (14, 15), the main sugar in our diet. However, due to the introduction of high-fructose corn sweeteners in manufactured foods and for home use, fructose is quickly becoming a major dietary component (13, 16).
1.2.2 Digestion and Absorption of Carbohydrate

Dietary carbohydrate is a major component of the human diet and thus, the alimentary tract is well adapted for carbohydrate digestion and the subsequent absorption of the resulting end products (17). In general, the diet provides a wide assortment of carbohydrate species, ranging from mono-, di- and oligosaccharides to polysaccharides (18). The monosaccharide is generally believed to be the fundamental unit of carbohydrate absorption. Consequently, a major function of the small intestine is to hydrolyze those components of the carbohydrate in the diet into their constituent absorbable monosaccharide units (19).

Digestion of available polysaccharides such as starch begins in the mouth with the action of salivary amylase (18, 20). Further hydrolysis is extremely limited in the stomach (17, 18). The majority of the hydrolysis of starches however, takes place in the small intestine by the action of pancreatic α-amylase, producing a whole range of molecular fragments (18). The continued breakdown of these newly formed fragments occurs on the enterocytes’ brush borders where surface enzymes are located. These carbohydrases are built right into the surface membranes of the microvilli of mature enterocytes, effectively breaking down the larger dietary molecules into absorbable monosaccharides (17).

Disaccharides are generally broken down into their constituent monosaccharide units prior to absorption (18). Sucrose, a major disaccharide in the human diet, is efficiently hydrolyzed by the disaccharidase sucrase, which is found
on the brush border of the intestinal mucosa, into its component fructose and glucose moieties (19).

Under normal circumstances the ingestion of monoaccharides usually accounts for only a small portion of dietary sugars intake (21). As monosaccharides are of a molecular size that permits them to cross the mucosal cell membranes by simple diffusion, they do not undergo further hydrolysis prior to absorption (18).

The rationale for the restriction of simple sugars from the diet in favour of starchy carbohydrates is based largely on the belief that sugars such as glucose and sucrose produce a greater and faster rise in postprandial glucose and insulin responses because they are more readily available for absorption. In contrast, starches were thought to produce more gradual postprandial glucose and insulin responses because they were considered to be more slowly digested and absorbed (5). However, this rationale is rather problematic since other lines of investigation have found that starch and glucose are actually assimilated at a similar rate (5). In fact, studies such as one by Dahlqvist and Borgstrom in 1961 found that sufficient amounts of intraluminal amylase were present to rapidly hydrolyze ingested starch (22). A further study by Fogel and Gray in 1973 concluded that it was absorption, and not intraluminal digestion that was the rate-limiting step in the starch assimilation process (23).

The products of carbohydrate hydrolysis can then be absorbed across the epithelial lining of the small intestine via three processes: simple diffusion, facilitated diffusion and active transport. Absorption of monosaccharides into the
blood stream involves movement from the bulk phase across the “unstirred layer” to the enterocyte’s surface, movement across the brush border membrane, movement across the cytoplasm and finally, exit via the basolateral membrane (17). The general consensus is that the movement of monosaccharides that form in the bulk phase of the luminal fluid occurs via simple diffusion by way of accordance with their concentration gradient (17). Once at the surface of the enterocyte, the hexoses can cross the membrane by simple diffusive movement. However, since this alone would be inefficient for the assimilation of large amounts of hexoses produced form a carbohydrate-rich meal, specific transfer mechanisms of facilitated diffusion and active transfer are thought to come into play (24).

Although free glucose is not the primary component of sugars entering the small intestine, the production of glucose through hydrolysis of various disaccharides means that glucose is the major monosaccharide entering the enterocyte and eventually into the circulation (18). Glucose is believed to move across the enterocyte brush border by active transport via a carrier-mediated cotransporter system that is sodium-dependent (17, 18, 24, 25, 26). It has been established that glucose and galactose are absorbed by a shared active transport system located at the brush border membrane of the enterocyte (21).

In contrast, the absorption of fructose is believed to be via carrier-mediated facilitated diffusion (18, 19, 27, 28, 29). Although there is also some speculation that in some species fructose is actively transported by an energy- and sodium-
dependent mechanism separate from the glucose-galactose cotransporter. At present there is no evidence to support the existence of the active transport of fructose in humans (18, 28). Fructose absorption from sucrose or in the presence of glucose appears to be more complete than absorption of fructose alone (14, 29, 30, 31). The exact mechanism by which the addition of glucose enhances fructose absorption is still under investigation. A number of possibilities have been postulated, namely solvent drag induced by the water absorption stimulated by glucose (19), a direct stimulating effect of glucose on the intracellular conversion of fructose in the mucosa, or the possible activation of the fructose carrier (29).

Once the hexose enters the enterocyte, either by facilitated diffusion or active transport, it is believed to remain free and osmotically active, able to diffuse across to the basolateral membrane. Hexoses in the enterocytes move across and enter the blood circulation by diffusion and a facilitated transfer process that is sodium-independent (24, 25 26). The glucose transporters located at the basolateral membrane are part of a family of facilitated glucose transporters of which there are five different isoforms. These molecules transport glucose and closely related sugars down their concentration and into the circulation (26).

### 1.2.3 Effects of Sugars on Plasma Glucose

Sugar is believed to cause rapid rises in blood sugar concentrations and for this reason, sugars of all kinds have been restricted in the diets of most diabetic patients. The primary goal in the dietary management of diabetes mellitus is to
optimize glycemic control, while minimizing the risk of hypoglycemia in people treated with insulin. Tight regulation of blood glucose within narrow limits reduces the risk of complications, both acute and long-term (3). This belief that sugars rapidly raise blood glucose concentrations is based largely on Allen's work in the 1920s, which showed that pancreatectomized dogs exhibited greater amounts of glucose in the urine after ingestion of glucose than after starch ingestion (4). The conclusion was that glucose, when compared with the complex carbohydrate starch, results in a more rapid rise in blood glucose levels and was subsequently expanded to include all simple sugars, including table sugar, sucrose. This belief continues to persist even today. Yet, one might argue that it is not possible to extrapolate the results from glucose to other sugars such as sucrose or fructose.

A study by Macdonald et al. in 1978 looked at the blood metabolites of nine healthy young men after glucose, sucrose, fructose, and sorbitol were given at 0.25, 0.5, 0.75 or 1.0 g/kilogram of body weight. The results showed that the mean increase in blood glucose following fructose ingestion was much lower than that of glucose or sucrose, only approaching significance at doses after 0.5 and 0.75 g/kg of body weight, but not at the 0.25 g/kg of body weight dose level. The mean change in serum glucose following sucrose ingestion was slightly, though not significantly, less than that following glucose ingestion, despite the fact that only half the amount of glucose is present in sucrose when compared to an equal weight of glucose (32). However, the volunteers in this study were the same within, but not between the carbohydrates. Given the high variation in plasma glucose response within an
individual, there is some difficulty in making comparisons of responses of different sugars between different groups of subjects. This further emphasizes the need to examine the responses to different doses of different sugars while maintaining the same block of subjects. Nonetheless, other studies have also shown that fructose tends to produce much lower blood glucose and insulin responses in comparison to glucose, with the response to sucrose, which consists of glucose plus fructose, being intermediate (8, 33, 34).

In addition, many of the studies which support the belief that simple sugars are more rapidly digested and absorbed than complex carbohydrates have limitations. For instance, a study by Crapo et al. in 1976 compared the plasma glucose and insulin responses of healthy subjects to oral loads of glucose, sucrose and starch. The conclusion from the study was that starch elicited much lower glycemic responses in comparison to glucose or sucrose (5). However, the use of uncooked starch in this study may account for the results seen (3). Raw starch tends not to be as readily hydrolyzed and is thus, more slowly absorbed than cooked starch (35).

More current studies have actually shown that even some cooked starches such as bread, rice and potato cause glycemic responses that are similar to, or only slightly less than the glycemic response to glucose, and that are frequently the same or greater than that observed with sucrose (8). In 1981, the glycemic index as a system of classification of foods according to their blood glucose responses was proposed. Since then the glycemic responses of approximately 200 foods have been
classified, with a wide range of glycemic responses observed for both starchy foods and foods containing sugars (8, 36, 37, 38).

1.2.4 Effects of Sugars on Plasma Insulin

Insulin is the main pancreatic hormone which tightly regulates glucose in the blood. In normal individuals, the response to increased plasma glucose levels is an increase in the secretion of insulin from β-cells of the pancreatic islets. This increase in circulating insulin levels leads to stimulation of glucose transport into peripheral tissues, while inhibiting hepatic gluconeogenesis (39).

The preconceived belief is that sugars cause greater postprandial insulin responses. Looking at the effects of sugars on blood insulin is especially important because elevated plasma insulin levels have been implicated in the pathogenesis of atherosclerosis. Furthermore, high insulin may also play a role in exacerbating insulin resistance, which is a central feature of diabetes, abdominal obesity, hypertriglyceridemia and hypertension (7). Evidence suggests that for starchy foods, the plasma insulin response is related to the glycemic response (40, 41), and that in fact, the consumption of low glycemic index starchy foods appears to reduce insulin secretion in normal and diabetic subjects (42, 43). This relationship, however, may not hold true for sugars because it has been suggested that fructose and sucrose, which contains fructose, may cause high insulin responses (44). Elevated insulin may, in turn, stimulate hepatic lipid synthesis and thus, be one of the mechanisms by
which fructose and sucrose increase serum cholesterol and triglycerides in susceptible populations (45, 46).

There is evidence under certain experimental conditions that fructose and sucrose do increase blood insulin concentrations. Reiser's group has shown that consuming moderate amounts of fructose or sucrose for 5-6 weeks increases insulin responses to a sucrose load (45, 47). However, it is crucial to point out that in these studies subjects consumed 90% of daily energy in one meal in the evening. This type of gorging meal pattern is associated with impairment of glucose tolerance (48) and may not be an ideal model for studying the effects of sucrose or fructose. Also, a nibbling paradigm, as opposed to the gorging meal pattern, has been linked with a reduction in blood lipids (49). In both studies, the oral sucrose tolerance tests were done in the evening after an 8 hour fast during the day. As this is not a typical eating pattern, it may result in different effects than those observed for tests done in the morning after an overnight fast, which is generally the time most investigators study postprandial effects.

Reiser's group has also tested the effect of consuming a very large amount of fructose (1.75 g/kg body weight) 20 minutes following initial test meals of 1 g/kg glucose or 0.9 g/kg starch (44). This study showed that consuming fructose, compared to the same premeal without fructose, raised serum insulin responses without raising blood glucose. It was concluded that fructose can be insulinogenic in humans. However, as all carbohydrates are insulinogenic, this study does not allow
the determination of whether fructose is more insulinogenic than other carbohydrates fed in the same manner. Most studies investigating the effects of equal amounts of different sugars on postprandial glucose and insulin have shown that fructose tends to produce lower glucose and insulin responses than glucose and sucrose (33, 36). The only exception was in patients with poorly controlled diabetes where no difference between fructose and sucrose was seen (50). In this situation it is possible that there is increased hepatic glucose output since fructose would provide a good substrate for hepatic glucose production (51).

1.2.5 Effects of Amount and Type of Carbohydrate on Plasma Glucose and Insulin

Glucose and insulin responses are dependent upon the type and dose of carbohydrate. Thus, increasing the amount of carbohydrate consumed increases blood glucose and insulin responses. The exact shape of the dose-response curve depends upon a number of methodological variables including, for example, how the area under the curve is calculated (52). Gannon et al. tested various doses of glucose up to 50 g and found nearly linear increases in glucose and insulin responses (53). However, normal meals commonly contain more than 50 g of carbohydrate. Therefore, Jenkins et al. examined the effects of 0-100 g doses of carbohydrate from glucose, bread and lentils on blood glucose response and discovered that the glycemic response tends to increase nearly linearly up to 50 g of carbohydrate, but at
a dose level between 50 and 100 g carbohydrate there was only a small additional increase in the blood glucose response area (8).

A study by Claudia Bolognesi in 1995 formulated mathematical models for the prediction of glucose and insulin responses for different starchy carbohydrate foods, based on the dose and glycemic index of the carbohydrate. These mathematical models were derived from the glucose and insulin responses of different starchy carbohydrate foods in normal subjects. The mathematical model for the prediction of relative glucose responses was determined to be $GR=1.5\times GI(1-e^{-0.018x})$, where $x$=dose of the carbohydrate consumed and $GI$=glycemic index. This model accounted for 94% of the variance of the mean relative glucose responses for the fourteen different test meals ($p<0.0001$). The mathematical model for the prediction of relative insulin responses was determined to be $IR=2.9\times II(1-e^{-0.0078x})+5$, where $x$=dose of the carbohydrate consumed and $II$=insulinemic index. This mathematical model accounted for 95% of the variance of the mean relative insulin responses for the fourteen test meals ($p<0.0001$). An equation to describe insulin responses based on the glycemic responses was obtained by multiple linear regression: $II=GI\times 0.6+GI^2\times 0.003$. Thus, by substituting for the term $II$, the equation used to predict the relative insulin response as a function of the glycemic index of the food was: $IR=2.9\times(GI\times 0.6+GI^2\times 0.003)\times (1-e^{-0.0078x})+5$. This equation accounted for 91% of the variance of the mean relative insulin responses for the fourteen test meals ($p<0.0001$) (54).
1.2.6 Health Aspects Related to Sugars Intake

1.2.6.1 Introduction

Dietary sugars are normally a significant source of our daily energy intake, thus highlighting the importance of examining their effects on various aspects of health (15). The popular consensus is that sugars consumption is harmful to health. Many studies have been done to look at possible associations between sugar intakes and certain health concerns such as the causation of dental caries and behavioural disturbances in children, reduction of nutrient intakes, and the development of conditions such as obesity, diabetes and heart disease (15).

1.2.6.2 Intake and Nutrient Content in the Diet

One of the major concerns of a high sugar intake is that it may displace important nutrients, vitamins and minerals from the diet. Some recent studies have been carried out to address this concern. The general conclusion was that high sugar intakes were not consistently associated with lower intakes of vitamins and minerals. For instance, a study on English adolescents found that when sugars intake was expressed as weight, energy intake and consumption of most nutrients were considerably higher in those eating high levels of added sugars (11). Other studies have also found similar results (55, 56, 57). It seems that the dilution effect of sugars in the diet is only a more relevant concern in those individuals with very low energy intakes who choose to eat a lot of sugars (9). The 1987-1988 US Department of Agriculture Nationwide Food Consumption Survey found that moderate consumers
of sugars tended to have the most adequate micronutrient profiles. Hence, consuming lower levels of sugars did not necessarily guarantee a more adequate nutrient profile, nor did high levels of sugars consumption automatically result in inadequate micronutrient intakes (12).

1.2.6.3 Sugars Intake and the Development of Obesity

Another of the health concerns of high sugars intake is the belief that it may contribute to the development of obesity. However, research has provided little evidence to substantiate this claim. In fact, obesity appears to be less prevalent amongst individuals with higher levels of sugar consumption. A study by Lewis et al. in 1992 failed to find a connection between increases in body weight and high intakes of added sugars (58). This result was in agreement with observations of other researchers who had previously shown little association between high sugars intake and increased body weights (59, 60).

1.2.6.4 Sugars Intake and Diabetes, Glucose Tolerance

Diets high in sugars have also been thought to play a direct role in the causation of diabetes. There is no strong evidence to support such a claim (15, 61). However, there is a growing concern with regard to diets high in sugars and glycemic regulation. This is primarily due to the fact that one of the principal aims in the dietary management of diabetes mellitus is to enhance blood glucose control in order to reduce the risk of development of acute and long-term complications (3, 61).
Consequently, the potential for negative chronic effects on glycemic regulation is a frequently expressed concern, as well as concurrent changes in insulin and other glucoregulatory hormone response patterns (15). Impairment of glucose tolerance is a major risk factor for the development of diabetes mellitus. In addition, an abnormal insulin response to a glycemic stress is considered to be one of the earliest detectable signs associated with diabetes (62).

Thus, a high sugars consumption level has been rigorously studied as a possible contributor to impairment of glucose tolerance. Studies of the effects of dietary sucrose on glucose tolerance in humans have been inconclusive (62). Some feeding studies have demonstrated a negative effect on glucose tolerance when sucrose replaces starch in the experimental diet (62, 63). Yet, other studies have shown no significant effect on glucose tolerance when the dietary carbohydrate starch is exchanged for sucrose (64, 65). These contradictory findings are probably due in part to the differences in the experimental conditions (66).

In addition, fructose has often been suggested as a possible sugar that may be safely consumed by diabetics (66). According to a review by Bantle (67), a number of studies assessing the use of dietary fructose in diabetic patients have shown no adverse effects on glycemic control (68, 69, 70). With respect to sucrose, the exchange for starch in the diet in diabetic patients did not result in significant differences in any of the measures of glycemic control (69, 70).

Thus, according to the Sugars Task Force Report, 1986, human studies have shown that currently, there is no persuasive scientific evidence that sugars at current
consumption levels in the U.S. population are an independent risk factor for the development of glucose tolerance (15). Although there is some evidence to suggest the existence of a carbohydrate-sensitive subset of individuals that can demonstrate impaired glucose tolerance and adverse effects on insulin metabolism, feeding sugars to normal human volunteers at levels equivalent to those currently consumed by the U.S. population failed to produce any detectable adverse effects on glucose or insulin responses (15, 64). Furthermore, feeding normal human subjects fructose at levels approximating the 90th percentile intake levels of the U.S. population failed to demonstrate adverse effects on insulin sensitivity or glucose tolerance (15).

1.2.6.5 Sugars Intake and Serum Lipid Concentrations Associated with Coronary Heart Disease

Currently, there is no conclusive evidence that dietary sugars are an independent risk factor for coronary artery disease in the general population (15). However, a review of previous human studies reveals considerable controversy regarding the effects of dietary fructose and sucrose on serum lipid concentrations. For instance, there are some studies looking specifically at sucrose which demonstrate deleterious metabolic effects that are associated with an increased risk for the development of coronary artery disease (71, 72, 73). Some of these negative effects on lipid metabolism may include increased plasma concentrations of triglycerides, and VLDL- and LDL-cholesterol fractions (74). However, there are also a number of other studies which have found opposing outcomes (65, 75, 76).
First, looking at the effects of sugars on plasma triglycerides is difficult because there are many confounding variables which may effect the results. Some of these factors include obesity, excessive alcohol consumption, and in more rare cases, genetic abnormalities, or renal failure. A diet high in carbohydrates may also raise fasting plasma triglycerides in the short term (77). Nevertheless, some feeding studies looking at diets including dietary sugars such as fructose and sucrose appear to elevate plasma triglycerides levels (45, 78, 79, 80). In contrast, other studies have demonstrated little rise in plasma triglycerides (65, 75, 81, 82). Furthermore, there is little evidence that supports that sucrose and fructose, when consumed at levels approximating typical Western diets, influences plasma triglyceride concentrations (77). Still, as most of these studies are of short duration, there is some speculation that any sucrose- or fructose-induced increase in plasma triglycerides observed may be transient (75).

With respect to serum cholesterol, an analysis of past study results have been somewhat contradictory. Dietary fructose, when substituted for starch, has been shown to lead to rises in plasma total cholesterol and LDL in normal healthy subjects (45, 46, 83). However, a study by Bossetti et al. failed to come to the same conclusion (82). Another study by Crapo and Kolterman examining metabolic effects of two-week fructose feeding in normal subjects also found no increases in serum cholesterol levels (84).

Studies examining the effects of dietary sucrose on plasma cholesterol concentrations have been very equivocal. A number of studies did not report any
changes in plasma cholesterol levels (65, 82, 85). In comparison to those results, other studies have observed rises in plasma cholesterol concentrations (78, 79, 86). When looking at specific lipoprotein fractions, a study by Reiser et al. on hyperinsulinemic subjects fed three levels of sucrose found increases in VLDL-, LDL- and HDL-cholesterol fractions. There was also a decline in the ratio of HDL-to total cholesterol (79). Several studies have suggested that a high-sucrose diet may decrease HDL-concentrations (87, 88), but this effect may be independent of the effect of sucrose since HDL-cholesterol levels tend to fall in response to high-carbohydrate, low-fat diets (77).

The interest in the roles that sucrose and fructose play in the diet is of particular interest to people with diabetes. The incidence of macrovascular disease such as atherosclerosis of the coronary vessels in diabetes is very high (77). Thus, any potential for deleterious metabolic effects of high sucrose and fructose diets are a special concern.

1.2.6.6 Sugar Intake and Other Related Health Concerns

Other health conditions which are believed to be associated with high consumption of added sugars are behavioural disturbances in children and the causation of dental caries. Nonetheless, according to the 1986 report by the Sugars Task Force, there is no substantive evidence to suggest that current dietary intake of sugars contributes to hypertension. There is also no evidence to support that sugars consumption is responsible for behavioural changes, except only in those rare cases
of hypoglycemia that are present in the population (15). The causation of dental caries is multifactorial (9, 15). Although the consumption of sucrose and fermentable carbohydrates facilitates the development of plaque, dental caries and periodontal disease, there is no apparent simple relationship between sugars content and cariogenic potential (15). Despite the fact that the 1990 COMA Report states that extensive evidence suggests that sugars are the single most important dietary factor in the cause of dental caries, it is important not to overlook the impact of preventative dental methods such as fluoridation, topical fluoride treatments, dental sealants, vaccines against cariogenic bacteria and programs initiated to improve oral hygiene on the declining incidence of dental caries (9, 15 41).

1.2.7 Conclusion to Literature Review

The level of sugar intake in the diet is an important concern for many investigators in nutritional sciences. A review of past literature shows that a significant amount of research has been carried out to study various health aspects related to dietary sugars. Currently, there is little concrete evidence to suggest that sugars consumption causes deleterious health effects in normal, healthy individuals. The investigation of postprandial glucose and insulin responses to certain loads of mono- and disaccharides has attracted particular interest, with many studies looking specifically at the postprandial responses to different doses of the common dietary sugars glucose, fructose and sucrose. However, a number of these studies have
limitations, making it difficult to compare postprandial glucose and insulin responses between different sugars and starchy carbohydrate foods.

One prominent study by Macdonald et al. in 1978 studied the effects, in male healthy subjects, of varying doses of different sugars (32). However, a notable feature of this experimental design was that the same block of volunteers was maintained within, but not between carbohydrates, which introduces difficulties in making comparisons between sugars.

A study by Crapo et al. compared the postprandial responses of glucose, sucrose and wheat starch (5), concluding that starch produced significantly lower plasma glucose and insulin responses than either glucose or sucrose. Yet, the use of raw starch is a questionable feature of this study due to the fact that raw starch, in comparison to a cooked starch, is less readily hydrolyzed and more slowly absorbed (35). Another well known study by Reiser et al. in 1987 looked at the insulinogenic properties of fructose during postprandial hyperglycemia. However, the study design did not allow comparison of fructose to any other carbohydrate.

Since the consumption of dietary sugars has often been discouraged because of their supposedly high postprandial glucose and insulin responses, there is a need for a study which permits the evaluation of the glycemic and insulinemic effects of common dietary sugars glucose, fructose and sucrose relative to a starchy carbohydrate food such as white bread.
1.3 Research Hypothesis and Objectives

The hypothesis is that glucose, fructose and sucrose do not “inappropriately” raise plasma insulin responses in healthy subjects. An “inappropriate” response is defined as one which differs from that expected from an equivalent amount of carbohydrate from a starchy carbohydrate food which produces a similar glycemic response.

The main objectives are:

1. To compare the plasma glucose and insulin responses of various doses of sugars relative to white bread in normal, healthy human subjects.
2. To compare the responses of different doses of sugars to the responses predicted from mathematical model equations derived from responses of carbohydrate from starchy carbohydrate foods.
3. To compare the plasma glucose responses against plasma insulin responses of different doses of sugars, when expressed as a percentage of the responses to equivalent amounts of carbohydrate from white bread.

The secondary objective is to examine the plasma glucose and insulin responses of fructose in comparison to glucose when added to 50 g of glucose.
CHAPTER TWO

Materials and Methods
2. MATERIALS AND METHODS

2.1 Subjects

Eight normal, healthy human subjects (4 male, 4 female) were recruited for the study. Subjects ranged in age from 21 to 33 years, with an average age of 24.4 ± 4.5 years. The mean BMI was 22.1 ± 2.0 kg/m². All were non-smokers and not taking any medications. Informed written consent was obtained from all subjects.

2.2 Test Meals

All test meals were prepared in the metabolic kitchen on the morning of each test day. White bread was baked in 250 g carbohydrate loaves containing 334 g all purpose flour (Robinhood, Maple Leaf Mills, Toronto, Ontario), 7 g sucrose, 6 g yeast, 4 g salt and 250 ml warm water. The ingredients were placed into an automatic bread maker (model SD-BT2P, Matsushita Electronics Industries Co. Ltd, Japan) according to instructions and mixed, kneaded and baked over a 4 hour period. Loaves were cooled at room temperature for 1 hour and weighed. Crust ends were sliced off and discarded. The remainder of the loaf was cut into 50 g carbohydrate portions, packed into plastic freezer bags and frozen. Prior to consumption, bread was thawed in a microwave oven.

For the experiment, tea was prepared by steeping a single tea bag (Orange pekoe blend, Tetley Canada Inc., Mississauga, Ontario) per 500 ml of boiled water for approximately 2 minutes. Glucose (Bio-Health, Dawson Traders Ltd., Toronto,
Ontario), fructose (Sweeten Less, Maximum Nutrition Inc., Toronto, Ontario) and sucrose (Redpath Sugars, Division of Redpath Industries Ltd., Toronto, Ontario) were dissolved in 500 ml of either tea or water, according to subject preference. The beverage was kept constant for each subject for the duration of the study. An additional 150 ml of water was given following consumption of test meals.

Standard white bread test meals, which consisted of 25, 50, 75 or 100 g of available carbohydrate from white bread, were served with 500 ml of tea or water. Depending on the dose of white bread, an additional 150, 100, 50 or 0 ml of water was given, respectively, following consumption of the test meal.

2.3 Protocol

Subjects were evaluated on 14 separate occasions after a 10 to 12 hour overnight fast. On the first day of the study, subjects chose to drink either 500 ml of tea or water with the test. The drink chosen remained the same for each subject for all subsequent tests for the remainder of the study. Glucose, fructose and sucrose sugars were tested at three doses (25, 50 and 100 g), except for fructose 100 g, which was excluded for reasons related to the potential for malabsorption and associated symptoms (14, 29, 30, 31).

The standard white bread was tested at four different doses (25, 50, 75 and 100 g available carbohydrate portions). In addition, on one occasion, only the standard drink was given and on another separate occasion, a combination of 50 g
fructose and 50 g glucose was consumed. Thus, in total there were 14 different tests (Table 2-1).

Venous blood samples were drawn just prior to consumption of the test meal, and then at 15, 30, 45, 60, 90 and 120 minutes after beginning to eat. All the tests were grouped into 5 blocks: fasting, glucose, fructose, sucrose and bread. The order of these blocks was randomized and the sequence of the test doses within each of the blocks alternated between ascending or descending order of carbohydrate dose. Venous blood samples were prepared and analyzed for plasma glucose and insulin. This protocol was approved by the Human Subjects Review Committee of the University of Toronto.

2.4 Blood Sample Collection

Before blood sampling, the subject’s hand was warmed with an electrically heated pad for approximately 5 minutes. The venous blood samples were collected by placing an indwelling catheter in a forearm vein and kept open by flushing 2 to 3 ml of normal saline after each blood sample. The saline was cleared in advance to each blood sample collection by withdrawing and discarding 1 ml. Blood samples were drawn into fluoro-oxalate tubes (3 ml grey Vacutainer, Beckton-Dickenson, Rutherford, NJ) and refrigerated prior to centrifugation (2000 rpm for 10 minutes) in order to separate the plasma from the blood cells. Plasma aliquots were frozen at -20°C prior to glucose and insulin analysis.
Table 2-1  A summary of all the test meals

<table>
<thead>
<tr>
<th>Glucose (g)</th>
<th>Fructose (g)</th>
<th>Sucrose (g)</th>
<th>Available Carbohydrate from White Bread (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>75</td>
</tr>
<tr>
<td>100</td>
<td>50g fructose</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>+ 50g glucose</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Zero dose was also done
2.5 Plasma Glucose Analysis

Plasma glucose measurements were obtained using an automatic glucose analyzer (2300 Stat Glucose Analyzer, Yellow Springs Instruments, Yellow Springs, OH), which utilizes the glucose oxidase technique (89). A standard glucose solution (10 mmol/L) was used to calibrate the equipment. All the samples were analyzed only after two successive readings of the standard solution gave values of 10.0 or 10.1 mmol/L. When α-D-glucose in a sample of blood makes contact with the immobilized enzyme glucose oxidase, it is rapidly oxidized producing hydrogen peroxide. The hydrogen peroxide (H₂O₂) is, in turn, oxidized at the platinum anode, producing electrons. Thus, a dynamic equilibrium is achieved when the rate of hydrogen peroxide production and the rate at which hydrogen peroxide leaves the immobilized enzyme layer are equivalent and is indicated by a steady state response. The electron flow is linearly proportional to the steady state of hydrogen peroxide concentration and therefore, also to the concentration of glucose.

2.6 Plasma Insulin Analysis

Plasma insulin was measured by radioimmunoassay (Pharmacia Insulin RIA, Dorval, Quebec) at the Banting and Best Core Laboratory, University of Toronto. The insulin in the plasma sample competes with a known added amount of ¹²⁵I-labeled insulin for the binding sites of the specific antibodies. Bound and free insulin are then separated by the addition of a second antibody immunoadsorbent followed by centrifugation and decanting. Radioactivity in the pellet is then measured. The
measurement of the radioactivity is inversely proportional to the amount of insulin that is actually present in the plasma sample (90).

2.7 Statistical Analysis

Results were expressed as mean ± SEM. Incremental areas under the glucose and insulin curves, ignoring any areas below the fasting level, were calculated geometrically (91) and expressed as a percentage of that after 50 g of carbohydrate from white bread.

Observed relative glucose and insulin responses were compared with expected values as predicted from the mathematical model equations obtained from the 1995 study by C. Bolognesi (54). Multiple comparisons were done using one or two-way analysis of variance (92). The Newman-Keuls method was used to adjust the individual means for multiple comparisons (93). Least squares linear regression analysis was performed to evaluate possible correlations between predicted and observed plasma glucose and insulin responses. Differences were considered statistically significant when p<0.05.

The mean glucose responses to 50 g of carbohydrate from white bread for each subject was determined by fitting exponential association curves for each subject's plasma glucose responses to the 4 doses (25, 50, 75 and 100 g) of white bread tested using the following model:  

\[ y = A(1 - e^{-bx}) + C \]  

(54). This regression equation allowed the determination of the 3 constants A, B, and C. Thus, given \( x = 50 \) g (dose of the carbohydrate), the mean glycemic response to bread (y) was calculated.
An example of this calculation is shown in Figure 2-1. Similarly, the mean insulin response to 50 g of carbohydrate from white bread was also determined for each subject. After calculation of each subject’s mean glucose and insulin responses to 50 g carbohydrate from white bread, all plasma glucose and insulin responses to each of the 14 different tests for each subject were expressed as a percentage of the response to 50 g carbohydrate from white bread. The resulting values were called “relative responses.”

2.8 Rationale

As stated in section 1.3, the hypothesis is that the sugars glucose, fructose and sucrose do not “inappropriately” raise plasma insulin responses in healthy subjects, where an “inappropriate” response is defined as one which is more or less than would be expected from an equivalent amount of carbohydrate from a starchy carbohydrate food with a similar glycemic index. Thus, using the mathematical models established by C. Bolognesi in 1995 to predict the glucose and insulin responses for a starchy carbohydrate food (54), the expected glucose and insulin responses for glucose, fructose, sucrose and white bread were calculated. If the hypothesis is correct, the observed glucose and insulin responses for the sugars would be equal to the predicted responses, based on the dose and their glycemic index, calculated from the mathematical models that were derived from the glucose and insulin responses of starchy carbohydrate foods. However, if the hypothesis is incorrect, then the observed glucose and insulin responses would differ from the predicted responses.
Figure 2-1  Example of the calculation for the mean plasma glucose response of one subject to 50 g of carbohydrate from white bread.
Model:  \( y = A(1-e^{-BX})+C; \)
Fitted regression equation:  \( y = 355.96(1-e^{-0.026X})+2.625; \)
for \( x \) (dose)=50 g, \( y \) (glycemic response)=262.3
By evaluating the mean differences between predicted and observed glucose and insulin responses by a two-way analysis of variance, the effects of food, dose and food×dose interaction can be tested.

In addition, comparing plasma glucose responses against plasma insulin responses of different doses of glucose, fructose and sucrose, when expressed as a percentage of the responses to equivalent amounts of carbohydrate from white bread, is another way to determine whether sugars produce higher insulin responses than their glycemic responses would suggest. If sugars really do produce higher insulin responses, then insulin responses for the sugars should always be higher than the glucose responses.
CHAPTER THREE

Experimental Results
3. EXPERIMENTAL RESULTS

3.1 Dose-Response of Different Sugars

The eight subjects completed all 14 tests, except for one subject who did not consume the fructose 50 g test due to symptoms of malabsorption, which included gas, cramps and diarrhea, already at the lower 25 g dose level. Also, five of the subjects did experience some mild symptoms of malabsorption at the fructose 50 g dose level.

The mean plasma glucose and plasma insulin response curves are shown in Figure 3-1. Clearly, the glucose and insulin response curves are affected by the type of carbohydrate and the dose level. The incremental areas under the plasma glucose and plasma insulin response curves are summarized in Table 3-1. Fructose, given alone at 25 and 50 g dose levels, produced significantly lower postprandial plasma glucose responses compared to the same doses of glucose, sucrose and white bread. For the combination glucose 50 g + fructose 50 g, the glucose response was significantly lower than for glucose at 100 g, but not different from sucrose or white bread at 100 g. With respect to postprandial plasma insulin response, 50 g fructose produced a significantly lower response than white bread and glucose at the same dose level. The glucose 50 g + fructose 50 g combination produced an insulin response which was significantly lower than glucose 100 g, but not significantly different from sucrose or white bread at 100 g.
Figure 3-1  Mean plasma glucose and plasma insulin response curves of 8 normal, healthy subjects after 0, 25, 50, 75 and 100 g of carbohydrate from white bread, glucose, sucrose and fructose.
Table 3-1  Incremental areas under response curves of plasma glucose (mmol min/L) and plasma insulin (nmol min/L) after different doses of sugars and white bread were consumed by 8 normal subjects.

<table>
<thead>
<tr>
<th>Available Carbohydrate Portion</th>
<th>25g</th>
<th>50g</th>
<th>75g</th>
<th>100g</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PLASMA GLUCOSE</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bread</td>
<td>96.3±8.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>164.0±27.6&lt;sup&gt;de&lt;/sup&gt;</td>
<td>195.9±25.5&lt;sup&gt;e&lt;/sup&gt;</td>
<td>207.6±26.1&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fructose</td>
<td>15.3±4.9&lt;sup&gt;d&lt;/sup&gt;</td>
<td>29.8±11.6&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>—</td>
<td>*207.8±25.3&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glucose</td>
<td>142.2±21.9&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>212.7±37.6&lt;sup&gt;e&lt;/sup&gt;</td>
<td>—</td>
<td>304.0±48.3&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sucrose</td>
<td>78.1±4.5&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>123.8±17.7&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>—</td>
<td>175.2±21.3&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>PLASMA INSULIN</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bread</td>
<td>7.0±1.6&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>13.3±2.3&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>20.0±5.9&lt;sup&gt;d&lt;/sup&gt;</td>
<td>22.3±5.0&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fructose</td>
<td>1.2±0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.4±0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>—</td>
<td>*18.9±3.6&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glucose</td>
<td>8.1±1.6&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>16.3±2.7&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>—</td>
<td>32.0±5.9&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sucrose</td>
<td>4.7±1.0&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.4±1.9&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>—</td>
<td>22.2±3.6&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are means ± SEM.

Comparison of all means: Means sharing same letter superscript are not significantly different. Superscript "a" indicates no significant differences from the response to 0 g carbohydrate. Means with different superscripts are significantly different at p<0.05.

*Indicates 50 g glucose + 50 g fructose.
Two-way ANOVA for plasma glucose and insulin responses for white bread, glucose and sucrose at 25, 50 and 100 g were done and results are shown in Tables 3-2 and 3-3. The zero and fructose-containing doses, since it was given at 25 and 50 g, but not 100 g, were excluded in these analyses in order to retain a balanced 3 foods-by-3 dose levels factorial design, for a total of 9 different treatment combinations. However, fructose at 25 and 50 g consistently produced significantly lower plasma glucose responses than the other tests.

For plasma glucose response, ANOVA showed that there were significant effects of food (p<0.008), dose (p<0.003), but no food×dose interaction. The mean postprandial plasma glucose responses for white bread, glucose and sucrose respectively (all doses combined), were 156.0±15.6, 219.6±24.9 and 125.7±12.2 mmol min/L, with the response for glucose being significantly greater than those for sucrose and white bread. However, the mean responses for white bread and sucrose did not differ significantly. The mean plasma glucose responses for the 25, 50 and 100 g dose levels (glucose, sucrose and white bread combined) were 105.5±9.5, 166±17.6 and 228.9±21.9 mmol min/L, respectively. The response to the 100 g doses was significantly higher than that for the 50 g doses, which, in turn, was significantly higher than the 25 g dose.

For plasma insulin response, there was no effect of food or food×dose interaction. There was, however, an effect of dose (p<0.002). The mean postprandial plasma insulin responses for 25, 50 and 100 g dose levels (glucose, sucrose and
Table 3-2  Two-way analysis of variance of plasma glucose responses for white bread, glucose and sucrose at 25, 50 and 100 g.

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-VAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Condition</td>
<td>303780.0</td>
<td>8</td>
<td>37972.5</td>
<td>11.75</td>
<td>0.0000</td>
</tr>
<tr>
<td>Food</td>
<td>110392.3</td>
<td>2</td>
<td>55196.1</td>
<td>20.74</td>
<td>0.0077</td>
</tr>
<tr>
<td>Dose</td>
<td>82739.9</td>
<td>2</td>
<td>91370.0</td>
<td>34.32</td>
<td>0.0030</td>
</tr>
<tr>
<td>Food×Dose</td>
<td>10647.7</td>
<td>4</td>
<td>2661.9</td>
<td>0.82</td>
<td>0.5157</td>
</tr>
<tr>
<td>Subject</td>
<td>184137.6</td>
<td>7</td>
<td>26305.4</td>
<td>8.14</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>180983.8</td>
<td>56</td>
<td>3231.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>668901.3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3-3  Two-way analysis of variance of plasma insulin responses for white bread, glucose and sucrose at 25, 50 and 100 g.

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-VAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Condition</td>
<td>5399.76</td>
<td>8</td>
<td>674.97</td>
<td>17.27</td>
<td>0.0000</td>
</tr>
<tr>
<td>Food</td>
<td>662.86</td>
<td>2</td>
<td>331.43</td>
<td>6.10</td>
<td>0.0609</td>
</tr>
<tr>
<td>Dose</td>
<td>4519.66</td>
<td>2</td>
<td>2259.83</td>
<td>41.61</td>
<td>0.0021</td>
</tr>
<tr>
<td>Food×Dose</td>
<td>217.24</td>
<td>4</td>
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<tr>
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<td>443.68</td>
<td>11.35</td>
<td></td>
</tr>
<tr>
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<td>2188.82</td>
<td>56</td>
<td>39.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>10694.32</td>
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</tr>
</tbody>
</table>
white bread combined) were 6.6±0.9, 12.3±1.5 and 25.5±2.9 nmol min/L, respectively. There were significant differences between all three dose levels.

Figure 3-2 shows the relationship between mean glucose responses and mean insulin responses. The relationship is nearly linear at the lower glucose response levels. However, at the higher glucose response levels, the insulin response appears to increase at a slightly higher rate. Also, at higher glucose response levels, there was greater variation in the mean insulin responses.

The glycemic indices for glucose, sucrose and fructose were 136±15, 83±17 and 14±20 respectively. None of the values were significantly different from values previously published in literature (p>0.05) (94).

Figure 3-3 shows the mean observed relative glucose responses plotted against the dose of the carbohydrate along with the response lines predicted from the mathematical model. Observed relative glucose responses tended to fall very closely to the predicted response lines with the exception of 25 and 50 g doses of fructose, which were well below the predicted values. Nevertheless, two-way ANOVA showed that there were no significant effects of food, dose, or food×dose interaction on the difference between the observed and predicted relative glucose responses (Table 3-4).

Figures 3-4 shows the correlation between the mean observed and predicted relative plasma glucose responses. There was an extremely strong linear relationship between the observed and predicted responses, where the slope m=1.23±0.05 and the correlation coefficient r=0.99 at p<0.05. Although observed relative glucose
Figure 3-2  Relationship between mean plasma glucose response and mean plasma insulin response.
Figure 3-3  Incremental areas under glycemic response curves of normal subjects after different doses of glucose, white bread, sucrose, and fructose, expressed as a % of the mean response to 50 g of carbohydrate from white bread. Values are means ± SEM. Points represent actual observed responses. Lines represent the equation: \[ GR = 1.5 \times GI \left(1 - e^{-0.0018 \times D}\right) + 13, \] where GR= relative glucose response, GI= glycemic index and D= dose of carbohydrate consumed.
Table 3-4  Two-way analysis of variance of the mean differences between observed and predicted relative plasma glucose responses.

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-VAL</th>
</tr>
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<td>11</td>
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<td>1.10</td>
<td>0.3759</td>
</tr>
<tr>
<td>Food</td>
<td>5388.2</td>
<td>3</td>
<td>1796.1</td>
<td>1.88</td>
<td>0.2335</td>
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<tr>
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<td>0.1096</td>
</tr>
<tr>
<td>Food×Dose</td>
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<td>954.0</td>
<td>0.66</td>
<td>0.6797</td>
</tr>
<tr>
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<td>4.29</td>
<td>0.0005</td>
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</tr>
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<td>Total</td>
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</table>
Figure 3-4  Correlation between the observed and predicted relative plasma glucose responses.
responses were tightly correlated with predicted responses, the slope was significantly different from the line of identity.

Figure 3-5 shows the mean observed relative insulin responses plotted against the dose of the carbohydrate along with the response lines predicted from the mathematical model. There was a tendency for greater variation in mean insulin responses, particularly at the higher carbohydrate dose levels. Observed relative insulin responses tended to fall closely on predicted responses lines with the exception of 100 g dose levels of glucose, sucrose and white bread. Two-way ANOVA showed that there was no significant effect of food, nor a significant food×dose interaction on the mean differences between observed and predicted insulin responses (Table 3-5). However, there was a significant dose effect on the difference between observed and predicted insulin responses (Table 3-5), with the 100 g dose levels of glucose, fructose, sucrose and white bread resulting in significantly higher observed relative plasma insulin responses than predicted (mean difference= 44.9; p<0.05). The mean relative differences for 25 and 50 were -6.26, and -0.71, respectively.

Figure 3-6 shows the correlation between the mean observed and predicted relative plasma insulin responses. There was a very strong linear relationship between the observed and predicted responses, where the slope m=1.28±0.09 and the correlation coefficient r=0.97 at p<0.05. Although the observed and predicted relative insulin responses were highly correlated, the slope was significantly different
Figure 3-5  Incremental areas under insulin response curves of normal subjects after different doses of glucose, white bread, sucrose, and fructose, expressed as a % of mean response to 50 g of carbohydrate from white bread. Values are means ± SEM. Points represent actual observed responses. Lines represent the equation: 
$$IR = 2.9 \times (GI \times 0.6 \times GI^2 \times 0.003) \times (1 - e^{-0.078 \times D}) + 5,$$
where
IR=relative insulin response, GI=glycemic index and D=dose of carbohydrate consumed.
Table 3-5 Two-way analysis of variance of the mean differences between observed and predicted relative plasma insulin responses.

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-VAL</th>
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<td>Total</td>
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</tbody>
</table>
Figure 3-6  Correlation between the observed and predicted relative plasma insulin responses.
from one. However, it seems that at the higher carbohydrate dose levels, there was a tendency for a bit more variation in the mean values.

Figure 3-7 shows a bar graph of the glucose and insulin responses to all the different doses of glucose, fructose and sucrose, expressed as a percentage of the responses to equivalent amounts of available carbohydrate from white bread. Results of ANOVA did not show any significant difference between relative glucose and insulin responses for the sugars tested. The overall mean of the relative glucose and insulin responses, respectively, for glucose were 149±13% and 147±13%, sucrose, 87±7% and 83±8% and fructose, 16±4% and 22±4%.

3.2 Comparison of Glucose 50 g, 100 g and Glucose 50 g + Fructose 50 g

Some of the results from this study can be used to compare the insulinogenic potential of fructose in comparison to another carbohydrate, glucose, when fed in combination with equal 50 g doses of glucose. The incremental areas under the plasma glucose and insulin response for the three tests, glucose 50 g, glucose 100 g and glucose 50 g + fructose 50 g, were shown previously in Table 3-1. The relative incremental areas under the plasma glucose response curves for glucose 50 g, glucose 100 g and glucose 50 g + fructose 50 g tests were 138.7±8.2, 208.1±14.1 and 139.8±5.6, respectively. The relative incremental areas under the insulin response curves for the three tests in the same order were 136.1±20.9, 268.0±40.2 and 164.2±25.6. A bar graph of the relative plasma glucose and insulin responses for the three tests is shown in Figure 3-8 and Figure 3-9. For relative glucose and insulin
Plasma glucose and insulin responses after 25, 50 and 100 g of glucose, fructose and sucrose, expressed as a percentage of the responses to equivalent amounts of available carbohydrate from white bread. * Indicates 50 g glucose + 50 g fructose.
responses, only the glucose 100 g test produced significantly greater responses than the glucose 50 g and glucose 50 g + fructose 50 g tests (p<0.05). The glucose 50 g + fructose 50 g test caused a slightly higher insulin response than the glucose 50 g test, but this was not statistically significant.
Figure 3-8  Relative plasma glucose responses for 3 tests: glucose 50 g, glucose 100 g, glucose 50 g + fructose 50 g. * Indicates significant difference at p<0.05.
Figure 3-9  Relative plasma insulin responses for 3 tests: glucose 50 g, glucose 100 g, glucose 50 g + fructose 50 g. * Indicates significant difference at p<0.05.
CHAPTER FOUR

Discussion and Conclusions
4. DISCUSSION AND CONCLUSIONS

4.1 Dose-Response of Different Sugars

The results of the study demonstrated that in normal, healthy human subjects, the plasma glucose and insulin responses are influenced by the type and amount of carbohydrate tested. On average, glucose produced the highest plasma glucose response, followed by white bread, sucrose and fructose. There was no significant difference between mean plasma glucose responses for white bread and sucrose. This is in agreement with previous studies which found that some cooked starches produced glucose responses that are similar to sucrose, or that are slightly less than glucose (8). This also appears to hold true for plasma insulin responses as well. On average, glucose tended to produce the highest insulin response, followed by white bread, sucrose and fructose. Two-way ANOVA, not including fructose, resulted in a p-value of 0.06, just missing being significant. Statistical significance may have been difficult to attain given the high degree of variation in the plasma insulin response. Also, if fructose could have been included in the analysis, it would have strengthened the argument for a food effect to the point of statistical significance. Considering the results for the individual test meals, the insulin response to 50 g fructose was significantly less than that for 50 g white bread and glucose, and the insulin response to 100 g glucose was greater than that after 100 g bread, 100 g sucrose or 50 g glucose + 50 fructose (Table 3-1).
Two-way ANOVA also demonstrated statistically significant effects of dose level of the carbohydrate on both plasma glucose and insulin responses. Doubling the dose of the carbohydrate from 25 to 50 g resulted in an approximate doubling of the incremental area under the glucose response curve. However, doubling the dose from 50 to 100 g did not result in a subsequent doubling of the glucose response (Figure 3-3). This is similar to what has been seen in past studies (8, 53).

In contrast, the plotting of plasma insulin response against dose for glucose, fructose, sucrose and white bread showed that insulin response increased almost linearly for carbohydrate dose levels up to 100 g with little tendency for the response to flatten off between 50 to 100 g (Figure 3-5).

The glycemic index values for glucose, fructose and sucrose from this study were 136, 14 and 83 respectively. None were significantly different from previously published values (94). Nevertheless, the glycemic index value for fructose was less than half the literature value, and this may be due to the fact that six of the study volunteers experienced mild symptoms of malabsorption during the fructose tests, given that the glycemic index is an indicator of the rate of absorption (8). The high incidence of symptoms suggesting fructose malabsorption was not unusual upon review of previous studies that examined absorptive capacity of fructose in healthy humans. Three different studies which administered 50 g of fructose per 500 ml volume produced malabsorption in 58%, 80% and 37% of the subjects (14, 30, 31).

The results of plotting observed relative plasma glucose responses against predicted responses demonstrated a very strong linear correlation, where 99% of the
variation could be explained. All in all, it appeared that the equation used to predict glucose, which was developed for different doses of starchy carbohydrate foods, was quite effective in predicting the glucose responses of different doses of sugars. Although observed and predicted responses were highly correlated, the slope was significantly different from one, and thus, it was not an exact one-to-one relationship. However, from Figure 3-5 it appears that the observed responses from fructose 25 and 50 g tests may have contributed largely to the slope being significantly different from one. If the predicted responses for the fructose tests had been lower because of malabsorption, then the slope may not have been statistically different from one. Also, the predicted glucose response for the zero dose test was significantly higher than actually observed, which also contributed to the slope being statistically different from the line of identity.

Similarly, the results of plotting observed relative plasma insulin responses against the predicted responses also demonstrated a very strong linear correlation, where 97% of the variation could be accounted for. However, despite the strong correlation between observed and predicted plasma insulin responses, the slope was significantly different from one, suggesting that there was not a one-to-one relationship. Again, this was probably because the predicted values for fructose were too high. If predicted responses for the fructose tests had been lower because of malabsorption, the slope may not have been statistically different from one. Nevertheless, the strong linear correlation indicates that the sugars behaved very much like equivalent doses of carbohydrate from starchy food sources with similar
glycemic indices, in terms of postprandial glucose and insulin responses, given that the predictive models were developed from glucose and insulin responses of starchy carbohydrate foods.

The purpose of this study was to determine if the glucose, fructose and sucrose produced “inappropriate” plasma insulin responses in normal subjects, based on their glycemic index and dose. This was examined in two ways. The first method was by two-way analysis of variance of the mean differences between observed and predicted relative plasma insulin responses for the tests, which found no significant effects of food or food×dose interaction. This means that the differences between observed and predicted insulin responses for the sugars did not differ from those for carbohydrate from starchy white bread. Also, there was no interaction between type or amount of carbohydrate. The sugars, in comparison to carbohydrate from starchy white bread, did not produce inappropriate insulin responses, based on their glycemic index and dose of the carbohydrate. That is, glucose, fructose and sucrose did not produce significantly higher or lower plasma insulin responses when compared to an equivalent dose of starchy carbohydrate with a similar glycemic index. This supports the hypothesis that the sugars glucose, fructose and sucrose produce plasma insulin responses which are equivalent to those which would be expected from an equal amount of carbohydrate from a starchy carbohydrate food with a similar glycemic index.

However, there was a significant effect of dose on the mean differences between observed and predicted relative plasma insulin responses. At the 100 g dose
levels of carbohydrate there was a significant difference between the observed plasma insulin responses and the predicted responses. Since this effect was seen in both sugars and the starchy carbohydrate, it seems that the model equation for predicting insulin response tended to underestimate actual insulin response, regardless of whether the carbohydrate came from a sugar source or a starchy food source. If the sugars were to have produced an inappropriate insulin response in comparison to an equivalent amount of carbohydrate from a starchy carbohydrate source, then there would only have been a significant difference between the observed and predicted relative plasma insulin responses in the sugars and not the white bread. Thus, analysis of variance would have revealed a food effect or a food×dose interaction. As neither of these was seen, it is believed that the sugars did not cause inappropriate insulin responses.

The second method used to see if sugars produced “appropriate” insulin responses was to express plasma glucose and insulin responses of glucose, fructose and sucrose as a percentage of the responses to equivalent amounts of available carbohydrate from white bread. If sugars produced inappropriate insulin responses, then insulin responses should be consistently higher than glucose responses for all the sugars. However, the mean glucose response was similar to the mean insulin response for each sugar. This suggests that the relationship between glucose and insulin responses in the sugars tested is similar to the relationship between plasma glucose and insulin responses produced by carbohydrate from starchy white bread. Thus, unlike previous investigators who speculated that fructose or fructose-
containing sugars, unlike a starchy carbohydrate, would produce greater insulin responses than their glucose responses would suggest, the present results show that fructose and sucrose were no more insulinogenic than their glucose responses would indicate.

4.2 Comparison of Glucose 50 g, 100 g and Glucose 50 g + Fructose 50 g

One of the concerns of many investigators is that certain sugars such as fructose are believed to raise plasma insulin levels without similar corresponding rises in the plasma glucose concentrations. For example, a study by Reiser et al. in 1987 fed subjects fructose (1.75 g/kg body weight) 20 minutes following initial test meals of glucose (1.0 g/kg body weight) or starch (0.9 g/kg body weight). Pre-meals followed by fructose in comparison to pre-meals consumed alone did not result in any significant differences in plasma glucose levels. Yet, since the pre-meals followed by fructose produced greater insulin responses in comparison to the pre-meals consumed alone, it was concluded that fructose can be insulinogenic during postprandial hyperglycemia (44).

Since the study design by Reiser et al. made it difficult to make a comparison of the insulinogenic properties of fructose in comparison to any other carbohydrate under the experimental conditions, some results from this thesis were used to compare the insulinogenic potential of fructose in comparison to glucose, when added to 50 g glucose. Figure 3-8 showed that the mean plasma glucose response observed with the glucose 50 g test was not significantly different from the response
seen with the glucose 50 g + fructose 50 g test. This agrees with the results of Reiser et al., where the additional 50 g of fructose did not have any significant effects on the glucose response (44). The glucose 100 g test, which equals 50 g glucose + 50 g glucose, produced a significantly higher glucose response than the 50 g glucose or 50 g glucose + 50 g fructose tests. Hence, it was shown that 50 g fructose, in comparison to 50 g glucose, when added to 50 g glucose, also produced a lower glycemic response than glucose. This is in agreement with the findings in previous studies, which found fructose to produce lower glucose responses (8, 33, 34).

With regard to relative plasma insulin responses in Figure 3-9, in comparison to the glucose 50 g test dose, the glucose 50 g + fructose 50 g combination test increased the response slightly, although this was not statistically significant. Thus, the additional 50 g of fructose did not appear to have a significant effect on the insulin response, unlike that observed in the study by Reiser et al. However, in that study the additional fructose was administered at 1.75 g/kg body, a considerably higher dose level, which might have explained those observations (44).

The glucose 100 g test nearly produced a near doubling of the plasma insulin response in comparison to the glucose 50 g test, increasing by almost 97%. This demonstrates that insulin response, unlike glucose response which tends to flatten off after carbohydrate doses above 50 g, continues to increase when carbohydrate dose levels are above 50 g. Comparing the glucose 100 g test to the glucose 50 g + fructose 50 g combination test showed that under these particular conditions, fructose also produced a lower plasma insulin response than glucose.
To more closely examine the results of Reiser's study, it was helpful to predict the glucose response for the glucose + fructose and glucose alone tests in that study. Based on an average 70 kg subject, the glucose + fructose would consist of 70 g glucose and 122.5 g fructose, with a combined glycemic index of 70.6, calculated according to the proportion of glucose to fructose (54). The glucose alone test would consist of 70 g glucose, with a glycemic index of 138. Using the mathematical model, \( GR=1.5 \times GI \left(1-e^{-0.018X}\right) + 13 \), the glucose + fructose test should result in a 28% decrease in the glucose response in comparison to the glucose alone test. This thesis showed that the mathematical model for predicting insulin response for high doses of carbohydrate was not as good as the mathematical model for glucose response. In fact, the relationship between carbohydrate dose and insulin response was actually closer to linear, instead of the flattening off suggested by the model. Therefore, assuming the relationship remains linear up to a carbohydrate dose of 192.5 g for a 70 kg subject, the insulin response for the glucose + fructose test should result in a 41% increase in comparison to the glucose alone test, after adjusting for the difference in glycemic index (Figure 4-1).

By calculating the incremental area under the glucose response curves in Reiser's study, the glucose + fructose test compared to the glucose alone test resulted in a decrease of 34%. However, calculating the incremental area under the insulin response curves showed that the glucose + fructose test produced a 38% increase in
Figure 4-1  Prediction of insulin response for carbohydrate dose consisting of glucose 1.0 g/kg body weight + fructose 1.75 g/kg body weight, relative to carbohydrate dose of 1.0 g/kg body weight, assuming a 70 kg subject:

if the insulin response to 70 g glucose = 100 arbitrarily,
then the insulin response to 192.5 g glucose = 192.5/70(100) = 275;
thus, insulin response to 70 g glucose + 122.5 g fructose = 275 \times 70.6/138 = 140.7
comparison to the glucose alone test. Therefore, what was observed in Reiser’s study is fairly close to what was expected.

The results of the glucose 50 g + fructose 50 g compared to the glucose 50 g test summarized in this thesis showed that the relative glucose responses for both tests were virtually the same. This is probably due to the addition of only 50 g fructose, as opposed to 122.5 g fructose for a 70 kg subject added in Reiser’s study, which was too little for the detection of any lowering effects on glucose response. However, with respect to insulin response, the glucose 50 g + fructose 50 g test compared to the glucose 50 test resulted in an increase of 21%. This was very similar to what occurred in Reiser’s study.

Thus, it was suggested that since most subjects in the study by Reiser et al. were likely to have consumed carbohydrate intakes greater than 50 g with the glucose or starch pre-meals alone, additional fructose would not have produced increases in the plasma glucose response. However, since plasma insulin responses tends not to flatten off like plasma glucose response, continuing to increase even when carbohydrate dose levels exceed 50 g, increases in plasma insulin concentrations would be observed with the additional fructose.

In conclusion, addition of carbohydrate over 50 g demonstrates a pronounced plateau effect of the plasma glucose response. Plasma insulin response, instead, continues to increase even when carbohydrate dose levels increase above 50 g. Furthermore, the addition of 50 g fructose to 50 g glucose, making up the glucose + fructose combination test, produced lower glycemic and insulin responses when
compared to an addition of 50 g of glucose to 50 g glucose to make up the glucose 100 g dose test. Thus, even under this experimental condition, fructose produced lower glycemic and insulin responses.

4.3 Implications and Future Directions

The belief that mono- and disaccharide sugars such as glucose and sucrose are more readily absorbed, hence causing a greater and faster rise in postprandial plasma glucose and insulin responses than a starchy carbohydrate (5, 6), is the premise for many clinical interventions and medical hypotheses (2). This has led to the perception by the general public that sugars consumption is detrimental to good health and consequently, many people have endorsed the reduction of sugar intake in the diet. However, the results of this study, that glucose, fructose and sucrose do not produce significantly higher or lower postprandial plasma insulin responses when compared with equivalent amounts of carbohydrate from a starchy carbohydrate source with a similar glycemic index, could have a tremendous impact on public health recommendations.

For some time now, the public has been advised of the health benefits of reducing fat in the diet while increasing carbohydrate intake, particularly through higher consumption of starchy carbohydrate foods instead of sugary foods. However, as shown in this study and in previous studies (8), the glycemic response of some starchy carbohydrate foods such as white bread is often similar to, or greater than that of some common dietary sugars. Therefore, the recommendation of certain starchy
carbohydrate foods, some with glycemic indices which are actually higher than those of some dietary sugars, may produce deleterious effects if one is trying to maintain tight regulation of blood glucose and insulin while increasing carbohydrate intake. Also, as a diet low in sugars does require some degree of sacrifice for many people, modest inclusion of certain sugars such as sucrose or fructose may help some individuals adhere to a diet high in carbohydrates and low in fat without harmful effects on glycemic control.

The possibility of deleterious effects of sugars consumption on plasma lipid metabolism is an area that requires further investigation. If consumption of sugars produces adverse effects on plasma lipid profiles in normal subjects, then it would appear that it is by a mechanism other than inappropriately raised insulin responses specific to sugars, which was found to be untrue. To speculate, since plasma glucose and insulin responses are affected by dose of carbohydrate, in addition to the type, it is possible that increases in plasma triglycerides due to sugars intake may be because of an increase in insulin, which is due to an increase in total carbohydrate intake and not because of an increase in insulin specific to sugars. Moreover, it is generally easier to consume higher levels of carbohydrate when the greater proportion is from sugars rather than other starches or polysaccharides. Perhaps if one can maintain isocaloric carbohydrate consumption from either sugars or starches with similar glycemic indices, there would be no differential effects on plasma triglycerides.

Despite the findings on glucose, fructose and sucrose in healthy human subjects, the same cannot be concluded for all subject groups such as those with
impaired glucose tolerance and/or diabetes. Although it has been shown that glycemic indices of foods are similar for healthy and non-insulin dependent and insulin dependent individuals alike (95, 96, 97, 98), subjects with either of these conditions would demonstrate abnormal glycemic regulation and insulin secretion. Consequently, it may not be possible to extrapolate the results seen in normal healthy subjects to all subject groups and further studies to examine postprandial plasma glucose and insulin effects in these special populations would be necessary.

Another factor which deserves further consideration is the effect of other dietary components such as fat and protein. In this study postprandial glucose and insulin responses of sugars were studied without looking at the possible confounding variable effects of fat and protein. Since under more normal circumstances the consumption of sugars is usually combined with other dietary nutrients within a meal, the conclusions arrived at from this study are not necessarily applicable under meal conditions. For that reason, further studies of sugars under more normal meal conditions are still required.

Nevertheless, under the experimental conditions observed in this study, in normal healthy subjects, the common dietary sugars glucose, fructose and sucrose do not appear to cause significantly higher or lower postprandial plasma glucose and insulin responses than expected from an equivalent dose of carbohydrate from a starchy carbohydrate source with similar glycemic index.
4.4 Conclusions

There were two main objectives outlined in this thesis. The first objective was to analyze and compare the postprandial plasma glucose and insulin responses of different doses of glucose, fructose and sucrose in normal healthy human subjects. The second objective was to determine if the sugars produced inappropriate insulin responses by two methods: (1) By comparing the responses of different doses of the sugars, when expressed relative to the white bread, to the responses of equivalent doses of available starch from starchy carbohydrate foods with similar glycemic indices by way of mathematical models and (2) By comparing the plasma glucose responses against plasma insulin responses of different doses of sugars, when expressed relative to equivalent amounts of carbohydrate from white bread. In addition, the secondary objective was to investigate the plasma glucose and insulin responses of fructose in comparison to glucose when both are given in combination with an equivalent dose of glucose.

When plasma glucose and insulin responses to different doses of glucose, fructose and sucrose were examined, it was found that plasma glucose was significantly affected by carbohydrate type and dose level. Plasma insulin was significantly affected by dose level. Glucose tended to produce the highest glucose and insulin responses, followed by white bread, sucrose and fructose. However, this was not statistically significant at all dose levels.

Relative plasma glucose and insulin responses to different doses of glucose, fructose and sucrose, when compared to equivalent doses of available carbohydrate
from starchy carbohydrate food with a similar glycemic index, did not produce
significantly higher or lower insulin responses. Comparison of plasma glucose and
insulin responses, when expressed relative to equivalent amounts of carbohydrate
from white bread, found glucose responses to be very similar to insulin responses for
each sugar, suggesting that sugars are no more insulin producing than their glucose
response would suggest.

When glucose or fructose is given in combination with an equivalent dose of
glucose, fructose produced lower glycemic and insulin responses in comparison to
glucose.

Thus, the evidence from this study supports the hypothesis that glucose,
fructose and sucrose, when compared to equivalent amounts of available
carbohydrate from a starchy carbohydrate food with the same glycemic index, do not
result in inappropriate plasma insulin responses in normal subjects.
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Appendices
APPENDIX A:

CONSENT FORM

EFFECTS OF SUCROSE, FRUCTOSE AND GLUCOSE ON POSTPRANDIAL PLASMA GLUCOSE AND INSULIN RESPONSE

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Tel: 978-5556
Brenda Lee
Department of Nutritional Sciences, University of Toronto
Tel: 978-0403

Purpose
High blood glucose and insulin levels may increase the risk of developing diabetes, high blood pressure and heart disease. The purpose of this study is to examine the effects of sucrose, fructose and glucose on postprandial plasma glucose and insulin responses.

Procedures
If I agree to take part in this study, I will come to the Clinical Nutrition and Risk Factor Modification Center (61 Queen Street East, 6th Floor) on 14 different mornings after a 10-12 hour overnight fast over a period of 2-4 months. Each visit will take approximately 2 1/2 hours. When I arrive, I will be weighed and then have a small tube inserted into a vein in my arm for taking blood samples. This tube will be kept open by injecting 2-3 ml (1/2 teaspoon) of normal saline (salt solution) after each blood sample. After a fasting blood sample is obtained, I will be given one of 14 different breakfast test meals consisting of coffee, tea or water with glucose, fructose, sucrose or white bread. I will be asked to consume the test meal within 10-15 minutes. Blood samples (3 ml or 1/2 teaspoon) will be taken at 15, 30, 45, 60, 90 and 120 minutes after starting to eat for measurement of blood glucose and insulin responses. The amount of blood taken is minimal for measuring glucose and insulin and any left over blood will be discarded. After the last blood sample, I will be free to leave. The total amount of blood drawn for the whole study will be approximately 300 ml (1 1/5 cup).

Risks/discomforts
There is virtually no risk from taking part in this study. Minor discomfort is expected when the tube is inserted into my vein, and saline injection may be felt as a coolness in the arm, which is normal and harmless. This can be reduced by warming the saline with the heating pads provided. Bruising may occur when the tube is removed, but this can be prevented by keeping pressure on the site for 3 minutes after the tube is removed.
Benefits

I will derive no direct benefit from this study apart from being informed of my results. If I wish, I may request that the results be forwarded to my doctor.

Confidentiality

I understand that the results of this study may be published in a scientific journal as mean results, but individual subjects will not be identified, and my results will be confidential. My results will not be shown to anyone without my written permission, or as required by law.

Payments

As compensation for the time I spend participating in the study and to defray transportation costs, I will be paid $20 for each of the 14 study sessions I complete (total of $280 for the entire study). I understand that the results will not be valuable to the investigators unless I complete all 14 sessions.

Stopping the study

I understand that the investigator may stop my being in the study at any time without my consent. My participation may be discontinued if the investigator judges that it is in my best interests or if I fail to comply with the study procedures.

Qualifications

I understand that I cannot be in this study if I abuse alcohol or drugs, or if I have a serious illness which is not under control. I am above the age of 18 years.

Consent

I have read this consent form and have had all my questions about the study answered. I believe that I know what will happen to me if I agree to participate.

I freely volunteer to take part in this study. I may quit at any time. If I decide not to participate, or quit, I will not be penalized and will not give up any benefits which I had before entering the study. I have received a copy of this consent form.

Volunteer's name:...........................................................................................................

Date of birth:..................Height (cm):...............Weight (kg):..........................

Volunteer's signature:.............................................................Date:..........................

Investigator's signature:.............................................................Date:..........................
APPENDIX B

Relative incremental areas under plasma glucose and insulin response curves after different doses of sugars and white bread were consumed by 8 normal healthy subjects.

<table>
<thead>
<tr>
<th>Available Carbohydrate Portion</th>
</tr>
</thead>
<tbody>
<tr>
<td>25g</td>
</tr>
</tbody>
</table>

**PLASMA GLUCOSE**

<table>
<thead>
<tr>
<th></th>
<th>25g</th>
<th>50g</th>
<th>75g</th>
<th>100g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bread</td>
<td>65.5±1.6</td>
<td>104.4±2.0</td>
<td>129.8±3.7</td>
<td>144.9±8.3</td>
</tr>
<tr>
<td>Fructose</td>
<td>9.7±1.1</td>
<td>16.9±2.1</td>
<td>—</td>
<td>139.8±5.6</td>
</tr>
<tr>
<td>Glucose</td>
<td>95.0±5.4</td>
<td>138.7±8.2</td>
<td>—</td>
<td>208.1±14.1</td>
</tr>
<tr>
<td>Sucrose</td>
<td>55.5±2.3</td>
<td>83.1±4.4</td>
<td>—</td>
<td>117.1±4.2</td>
</tr>
</tbody>
</table>

**PLASMA INSULIN**

<table>
<thead>
<tr>
<th></th>
<th>25g</th>
<th>50g</th>
<th>75g</th>
<th>100g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bread</td>
<td>49.6±5.2</td>
<td>106.1±7.2</td>
<td>139.3±13.2</td>
<td>169.2±15.3</td>
</tr>
<tr>
<td>Fructose</td>
<td>9.4±2.1</td>
<td>21.4±4.5</td>
<td>—</td>
<td>*164.2±25.6</td>
</tr>
<tr>
<td>Glucose</td>
<td>69.1±11.1</td>
<td>136.1±20.9</td>
<td>—</td>
<td>*268.0±40.2</td>
</tr>
<tr>
<td>Sucrose</td>
<td>35.1±3.8</td>
<td>60.4±13.6</td>
<td>—</td>
<td>191.7±25.7</td>
</tr>
</tbody>
</table>

Values are means ± SEM. Superscript "a" indicates no significant differences from the response to 0 g carbohydrate. Means with different superscripts are significantly different at p<0.05. *Indicates 50 g glucose + 50 g fructose.