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DISCOVERY OF A CARBOHYDRATE SYSTEM THAT DOES NOT EXACERBATE POSTPRANDIAL GLYCEMIA

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By

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* * * * *

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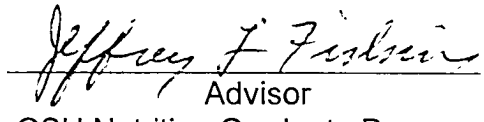

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ABSTRACT

Controlling blood glucose concentration is the primary medical concern in treating people with diabetes mellitus. Postprandial glycemia is directly related to rate of starch digestion. In the first study, chemical modifications of starch were evaluated. Rate of *in vitro* starch hydrolysis was slow for raw cornstarch (RCS) but was not affected by chemical modification; furthermore, for most modified starches, as the degree of modification increased, extent of hydrolysis decreased, suggesting an increase in the amount of resistant starch.

In the second and third studies, the postprandial glycemic responses to RCS and to chemically modified cornstarch esterified with 1-octenyl succinic anhydride (OSA), respectively, were evaluated in humans. Net incremental area under the glucose curve (AUC) was reduced 53% ($P < 0.05$) by RCS and 16% ($P < 0.05$) by OSA compared with a glucose control. These ingredients may be beneficial carbohydrate sources in the development of nutritional products for individuals with diabetes.

In the fourth study, a novel acid induced-viscosity (I-V) complex was evaluated in humans. When incorporated into a glucose-based beverage, the acid I-V complex reduced net incremental AUC for serum glucose by 75% ($P <$

0.01). The postprandial peak glucose concentration was lower than predicted from published literature and led to the hypothesis that supplemental fructose, which was present in the glucose-based beverages, attenuates postprandial glycemia.

In the fifth study, supplemental fructose was evaluated. In a series of experiments using the fatty Zucker *fa/fa* rat model of type 2 diabetes, supplemental fructose (or sucrose) was found to reduce the postprandial glycemic response to glucose or rapidly digested starch. These findings support the hypothesis that supplemental fructose may have clinical benefit in the normalization of postprandial glycemia in people with diabetes.

In the sixth study, a novel amylase I-V complex, supplemental fructose, or their combination were evaluated in humans. Products containing the amylase I-V complex reduced ($P < 0.05$) the incremental AUC by ~18% compared with the control; however, supplemental fructose had no effect. The acid I-V and amylase I-V concepts may provide a means for developing liquid, ready-to-feed nutritional formulas for people with diabetes.

Dedicated to my Lord and Savior, Jesus Christ

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FIELDS OF STUDY

Major Field: Nutrition

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LIST OF ABBREVIATIONS

ADA	American Diabetes Association
AUC	Area under the curve
BMI	Body mass index
CHO	Carbohydrate
d	Days
DE	Dextrose equivalence
DM	Diabetes mellitus
DS	Digestible starch
Exp	Experiment
GI	Glycemic index
h	Hours
Hb A_{1c}	Glycosylate hemoglobin A_{1c}
HDL	High density lipoprotein
IDDM	Insulin-dependent diabetes mellitus
I-V	Induced-viscosity
LDL	Low density lipoprotein
min	Minutes
MGTT	Meal glucose tolerance test
NIDDM	Non-insulin-dependent diabetes mellitus
OGTT	Oral glucose tolerance test
OSA	1-octenyl succinic anhydride
RDS	Rapidly digested starch
RCS	Raw cornstarch
RS	Resistant starch
RTF	Ready-to-feed

SDS	Slowly digested starch
SEM	Standard error of the mean
y	Years

CHAPTER 1

INTRODUCTION

The control of blood glucose in people with diabetes mellitus is of paramount importance for the long-term management of this disease (DCCT Research Group, 1993; UKPDS Group, 1998). Although intensive treatment with insulin or oral glucose-lowering agents is of great benefit, it is associated with an increased risk of hypoglycemia and weight gain (DCCT Research Group, 1993; UKPDS Group, 1998). The development of novel products that attenuate the postprandial glycemic excursion should enhance the use of nutrition as adjunctive therapy for people with diabetes mellitus.

Because of the divergence in the glycemic response to different foods, especially high carbohydrate foods (Brand-Miller et al., 1999), special care must be taken when making dietary recommendations. Historically, high fat (i.e., low carbohydrate) diets were recommended because of their ability to control postprandial blood glucose excursions (American Diabetes Association, 2001). Franz et al. (1994) recommended decreasing fat intake (specifically saturated fat), which is in agreement with the recommendations made by the American Heart Association (Krauss et al., 2001) and the food guide pyramid for the general population (total fat < 30% of kcal, saturated fat < 10% of kcal). In

order to fulfill these recommendations, dietary carbohydrate should substitute for the calories associated with the reduction in fat. In Chapter 2, I will review the literature related to the role of dietary carbohydrates (e.g., starch, fructose, dietary fiber) in the management of postprandial glycemia.

Convenience items such as snack bars and liquid, shelf-stable, ready-to-feed (RTF) nutritional supplements are practical approaches to provide supplemental nutrition. The use of liquid RTF nutritional products provides convenience to all consumers, and these products are especially advantageous for people who have difficulties meeting nutritional needs through usual dietary intake. The focus of my dissertation was to identify and test novel approaches to develop a carbohydrate system for incorporation into a liquid, shelf-stable, RTF product that does not exacerbate postprandial glycemia. Two separate and distinct approaches to develop and test this concept were evaluated: 1) direct modification of the carbohydrate to slow down its rate of digestion (i.e., chemical modification of rapidly digested starch), and 2) direct modification of the gastrointestinal environment through the addition of viscous dietary fiber to reduce the rate of digestion/absorption of the carbohydrate (i.e., evaluate the application of novel methods of incorporating soluble viscous fiber into liquid RTF nutritional products). Each approach should be considered as a separate platform that was evaluated independently of the other in a parallel path. However, the ultimate application of both technologies could be incorporated together to obtain a synergistic effect.

A number of groups have developed *in vitro* methods to predict the digestion of starch in the small intestine (Englyst et al., 1992; Muir and O'Dea, 1992, 1993; Brighenti et al., 1995; Wolf et al., 1999). Methods have been adapted to predict the amounts of starch that would be rapidly digested, slowly digested, or not digested (resistant) in the small intestine. In Chapter 3, I will describe an *in vitro* starch hydrolysis method that I used to evaluate the effects of chemical modification on the extent of starch hydrolysis over time. The purpose of this research was to screen potential starch ingredients that may serve as a source of slowly digested starch (SDS) in liquid enteral formulas.

Raw (uncooked) cornstarch (RCS) has been shown to serve as an effective oral therapy for the prevention of nighttime hypoglycemic episodes in patients with type I glycogen storage disease (Wolfsdorf and Crigler, 1997). Chen et al. (1984) made similar observations; however, ingestion of cooked cornstarch sharply increased blood glucose concentrations, followed by a rapid fall to hypoglycemic levels. These results suggest that RCS is a SDS. Considering these data, RCS was chosen as a positive SDS control. Unfortunately, RCS cannot be added to a liquid RTF product because product retort (i.e., sterilization at high temperature) cooks the starch. As shown by Chen et al. (1984) and Collings et al. (1981), cooking (which allows complete gelatinization) increases the starch digestion rate. In Chapter 4, I will validate the use of finger-pricking methodology for the clinical evaluation of glycemic response by evaluating the glycemic response of RCS compared with glucose in a meal glucose tolerance test (MGTT).

Chemical modification of starch may allow for the production of a SDS that could be used for the treatment of certain medical modalities. Numerous chemically modified food starches are available as ingredients for processed foods. I postulated that the use of these modifications might allow for the production of a SDS. Food starch esterified by 1-octenyl succinic anhydride (OSA) has been demonstrated to be compatible with liquid nutritional formulas (Mahmoud, 1987). In Chapter 5, I will describe an experiment that evaluates the postprandial glycemic response of food starch esterified with OSA.

Viscous soluble fiber (e.g., guar gum, psyllium, oat β-glucan) supplementation to test meals has been shown to effectively blunt postprandial glycemia (Braaten et al., 1991; Jenkins et al., 1978; Pastors et al., 1991). Despite the existence of *in vivo* evidence, however, there is still considerable doubt about the efficacy of dietary fiber in the treatment of hyperglycemia (Franz et al., 1994). This doubt may exist because different types of dietary fibers have different physiological effects. For example, soluble viscous fibers generally have a greater effect on carbohydrate metabolism. Unfortunately, foodstuffs containing viscous fibers (e.g., guar gum) usually exhibit slimy mouth-feel, tooth packing, and poor palatability (Ellis et al., 1991). The overall hedonic quality of guar-containing foods can be improved by reducing the average molecular weight (e.g., through chemical hydrolysis) of the galactomannan in guar gum (Ellis et al., 1991); however, this results in a concurrent loss in clinical efficacy (Jenkins et al. 1978). In Chapter 6, I will

describe the clinical evaluation of a novel, low viscosity, liquid product that becomes viscous under acidic conditions (e.g., within the gastric environment).

Fructose alone increases the postprandial blood glucose levels less than isocaloric amounts of glucose (Crapo et al., 1980; Jenkins et al., 1981). Shiota et al. (1998) found that intraportal infusion of small amounts of fructose augment net hepatic glucose uptake during hyperglycemic hyperinsulinemia in dogs. I hypothesized that the blunted postprandial glycemic response found in the clinical trial described in Chapter 6 was partly attributed to the presence of fructose in the glucose challenge. I serendipitously found that fructose supplementation to a glucose challenge appears to attenuate the glycemic response in healthy nondiabetic adult subjects. In Chapter 7, I will describe a series of animal experiments in which I evaluated the effects of supplemental fructose on postprandial glycemia.

A second novel approach to incorporate viscous fiber into a RTF nutritional product was developed in which viscosity increases as salivary and pancreatic amylase initiate starch hydrolysis. In Chapter 8, I will describe the clinical evaluation of this induced-viscosity concept, supplemental fructose, and their combined effects on postprandial glycemia.

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CHAPTER 2

LITERATURE REVIEW – MODERATION OF THE GLYCEMIC RESPONSE AND THE ROLE OF DIETARY CARBOHYDRATE

Introduction

The use of liquid ready-to-feed (RTF) nutritional products provides convenience to all consumers, and these products are especially advantageous for people who are unable to obtain adequate nutrition by usual dietary intake. The focus of this dissertation was to identify and test novel approaches for developing a carbohydrate (CHO) system for incorporation into a liquid, shelf-stable, RTF product that does not exacerbate postprandial glycemia. The findings of this dissertation may allow the development of a product that could be used as a therapeutic adjunct in treating diabetes, hypoglycemia, and glycogen storage disease; suppressing appetite; and assisting in the performance of sustained physical activity.

Background

Diabetes mellitus (DM) is a syndrome characterized by chronic hyperglycemia and disturbances of CHO, fat, and protein metabolism that are associated with absolute or relative deficiencies in insulin production by the beta cells of the pancreas and/or insulin action at target tissues (Bennett, 1994).

The criteria for the diagnosis and classification of DM were recently revised by the American Diabetes Association (1997). These criteria emphasize classification based upon etiology rather than clinical characteristics (e.g., polyuria, polydipsia, unexplained weight loss), which had been previously emphasized (National Diabetes Data Group, 1979). There are several classifications of people with glucose intolerance, the majority of which fall into one of two types: type 1 DM (historically called insulin-dependent DM; IDDM) or type 2 DM (historically called non-insulin-dependent DM, NIDDM). Type 2 DM accounts for approximately 90% of the cases, most of whom are over the age of 60 years (Warram et al., 1994).

Harris et al. (1998) determined the prevalence of diabetes in U.S. adults (≥ 20 years of age) based on data from the Third National Health and Nutrition Examination Survey (NHANES III). The prevalence of physician-diagnosed diabetes in this population was 5.1% and an additional 2.7% was determined to have undiagnosed diabetes utilizing the 1997 American Diabetes Association diagnostic criteria (i.e., fasting plasma glucose ≥ 126 mg/dL; American Diabetes Association, 1997). Similar rates of diabetes were found between men and women, but the rates for non-Hispanic blacks and Mexican-Americans was almost twice that for non-Hispanic whites. In general, the prevalence rate increased with age. Harris et al. (1998) concluded that the increasing frequency of obesity and sedentary lifestyles in the population make it likely that diabetes will continue to be a major health problem in the U.S. Using the prevalence rate determined by Harris et al. (1998) and the 1999 resident U.S.

population ≥ 20 years of age (U.S. Census Bureau, 2000), an estimated 15 million Americans have diabetes.

Need for glycemic control

Many medical experts believe that there is potential for the long-term management of the complications associated with diabetes through tight glycemic control (American Diabetes Association, 2001). Intensive therapy to control blood glucose is associated with a lower incidence of microvascular and macrovascular complications in people with type 1 DM (DCCT Research Group, 1993). Strict glycemic control also decreases the risk of microvascular complications in people with type 2 DM (UKPDS Group, 1998). However, intensive treatment was associated with an increased risk of hypoglycemia and weight gain (DCCT Research Group, 1993; UKPDS Group, 1998). These pivotal studies document the improved quality of life in people with diabetes when placed on an intensive blood glucose control program.

The concomitant use of diet as adjunctive therapy for the control of blood glucose may further enhance the quality of life and reduce the incidence of hypoglycemic episodes and weight gain in this population. The formulation of novel products that attenuate the postprandial glycemic excursion (i.e., prevention of hypoglycemia and hyperglycemia) should enhance the use of nutrition as adjunctive therapy for people with diabetes mellitus. Dietary choices can have a profound impact upon glycemic control (Brand et al., 1991). Food properties may be improved by optimizing the type and extent of food processing or making a more careful choice of ingredient materials. To reduce

the postprandial glycemic response to foods, Bjorck et al. (1994) suggest that it is essential to gain more knowledge about the factors responsible for the metabolic features of CHO foods. This review will focus on dietary approaches to glycemic control and specifically the role of dietary CHO.

Dietary intervention

The primary treatment for people with type 2 DM includes: strict adherence to a diet that attenuates the postprandial glycemic excursion, exercise, and, in many cases, use of medications (e.g., insulin or oral glucose-lowering agents). Early dietary recommendations limited CHO, because glycemic control was generally better with a high-fat diet. However, over the years, researchers found that low-CHO, high-fat diets were associated with dyslipidemias and cardiovascular disease because most high-fat diets consumed in industrialized countries were high in saturated fat.

The most recent American Diabetes Association guidelines emphasize individualization of diet strategies (Franz et al., 1994). The purpose is to achieve optimal glycemic and metabolic control by varying the proportion of calories provided by the macro nutrients (i.e., protein, fat, and CHO). The proportion selected is individualized according to dietary preference, treatment goals, metabolic control, and the presence of other medical conditions. The goals of dietary therapy for people with DM should be to provide optimal nutrition with the optimization of blood glucose and lipid levels (Franz et al., 1994). Specifically, the optimal diet should prevent hyperglycemia and hypoglycemia, which may prevent or delay the development of long-term

cardiovascular, renal, retinal, and neurological complications associated with DM.

Of the macronutrients, CHO has the largest impact upon the postprandial glycemic excursion. For example, Elliott et al. (1993) fed isoenergetic (375 kcal) meals containing CHO, fat, or protein to healthy volunteers in a meal glucose tolerance test (MGTT). They found a significant rise in plasma glucose and insulin following the CHO meal, whereas plasma glucose and insulin concentrations were unaffected by the fat or protein test meals. As the dose of CHO increases, the net glucose-area response increases (Gannon et al., 1989; Nuttall et al., 1992). Both the amount and type of dietary CHO are important determinants of postprandial glucose responses to mixed meals (Wolever and Bolognesi, 1996; Wolever and Jenkins, 1986). In general, there are several food factors that affect the glycemic response to a meal: nature of starch structure and physical form, cooking and processing procedures, storage procedures, ripeness or maturity, type and amount of fiber content, fat content, and protein/starch interrelationships (Crapo, 1994).

Dietary history and challenges

As alluded to above, the dietary management of people with DM presents major challenges. Prior to 1921, starvation was the only recognized treatment of DM. Recommendations for the distribution of calories from CHO and fat have shifted over the last 75 years. Before 1950, dietary recommendations were for a high fat (70% kcal) diet (American Diabetes Association, 2001), because fat does not result in a postprandial rise in blood

glucose. While high fat diets offer benefits in blood glucose control, they also are associated with dyslipidemia (e.g., elevated low-density lipoprotein cholesterol and/or triacylglycerol), cardiovascular disease, and obesity. Since 1950, dietary recommendations have been made to lower the intake of fat (as a percentage of calories) and concomitantly increase the intake of complex CHO (i.e., starch) and dietary fiber to meet caloric requirements. Specifically, the recommendations state that saturated fat be less than 10% of total kcal. Considerations of fat and protein, which in themselves reduce the glycemic effect of a food, have to be considered from the standpoint of risk factors such as serum lipids and preservation of renal function, respectively (Franz et al., 1994).

On the other hand, reducing the caloric intake of fat (≤ 30% of kcal) and increasing the caloric intake of CHO may, in fact, be detrimental to blood glucose control and the long term outcome of people with diabetes. All CHO are not similar in their effect on glycemia (Jenkins et al., 1988), and the dietary incorporation of high CHO foods is not as simple as it may appear. Because of the discrepancy in the glycemic response of different CHO and high CHO foods, Jenkins et al. (1981) proposed a method of ranking foods on the basis of the incremental blood glucose responses they produce for a given amount of CHO (i.e., the glycemic index). Complex CHO from potatoes, bread, and some cereals may have a high glycemic index (Jenkins et al., 1984), whereas the glycemic index of fructose, a simple sugar, is relatively low.

The disparity between high-CHO foods and the proper control of glycemia is highlighted by a report of Salmeron et al. (1997b). Over 65,000 women (40 - 60 years of age) were followed for a period of 6 years to compare dietary consumption patterns with the onset of type 2 DM. The researchers found that there was a 2.5-fold increase in adult-onset type 2 DM for women whose diets contained large amounts of potatoes, rice and bread (i.e., high glycemic load and low cereal fiber intake compared with low glycemic load and high cereal fiber intake). Glycemic load is the product of glycemic index and CHO intake. Salmeron et al. (1997a) found similar results in a cohort of over 42,000 men (40 – 75 years of age). Considering these data, the simple recommendation for anyone to increase their intake of dietary CHO may be dangerous. These results support the concept of glycemic index, that is, to appropriately choose high CHO foods that allow for optimal glycemic control. The use of low glycemic index foods would not only be appropriate for subjects with DM but also for people at risk for diabetes mellitus (e.g., obese and first degree relatives of people with type 2 DM).

Dietary carbohydrates and glycemic response

Designing a meal low in glucose-containing CHO (e.g., low starch) should improve the postprandial glycemic response. In fact, sugars like sucrose, fructose, and lactose have a reduced postprandial glycemic response compared with many high-starch foods (Jenkins et al., 1984). However, only modest amounts of sugars should be used as sweeteners in the diet (Franz et al., 1994; Jenkins and Jenkins, 1994). Sugars should not be recommended as

a source of calories to increase the CHO composition of the diet. In susceptible individuals, a high fructose intake may raise serum triglyceride concentrations (Hollenbeck, 1993). Similarly, sucrose may increase the serum levels of both cholesterol and triglycerides in susceptible persons (Erkkila et al., 2001). The consumption of too much lactose may result in gastrointestinal intolerance (Hertzler et al., 1996).

Worldwide, starch and its products constitute most of the digestible CHO in the human diet. Postprandial blood glucose and insulin responses to dietary starch are directly related to the rate (O'Dea et al., 1981; Jenkins et al., 1984) and extent (Achour et al., 1997; Raben et al., 1994) of starch digestion. Factors affecting starch digestion in the small intestine were reviewed by Englyst et al. (1992) and are briefly summarized below. Incomplete starch digestion in the small intestine may be attributed to one or more of the following intrinsic factors (i.e., related to the starchy food itself): 1) physical entrapment within a cellular or multi-cellular structure (e.g., starch within a whole cereal grain or in pasta), 2) retrogradation (cooking and cooling of a high amylose starch), 3) amylose-lipid complexes, 4) native α-amylase inhibitors, and 5) type of starch granule. Extrinsic factors that may affect the extent of starch digestion include: 1) degree of mastication, 2) concentration of amylase and amount of starch in the gut, 3) transit time, and 4) presence of other food materials. All starch was once assumed to be hydrolyzed and absorbed in the small intestine because pancreatic α-amylase can be produced in ample amounts (Fogel and Gray, 1973). However, a substantial amount of starch is now known to escape

digestion in the small intestine and enter the colon (Englyst and Cummings, 1985; Berry, 1986).

One goal of my research is to identify a starch ingredient that is not resistant to digestion in the small intestine, but rather is slowly digested. Englyst et al. (1992) have defined slowly digested starch (SDS) as starch that is likely to be completely digested in the small intestine but at a slower rate. Jenkins et al. (1990) conducted a clinical study in healthy volunteers to specifically evaluate the rate of glucose absorption on postprandial metabolic effects. Nine subjects consumed a 50-g bolus of glucose or sipped 50 g of glucose (3.57 g/0.25 hours over 3.5 hours) in a crossover design. When subjects sipped the glucose meal (simulating a slow rate of glucose absorption), the early blood glucose excursion was reduced and the later phase blood glucose concentrations (120 and 180 min postprandial) were maintained above basal levels, thus avoiding hypoglycemia. Some of the earliest studies evaluating the nibbling versus gorging paradigm have documented an improvement in blood lipid profiles (e.g., reduced total cholesterol and triglyceride concentrations) when the frequency of meals was increased (reviewed by Jenkins et al., 1994).

Type 1 glycogen storage disease is associated with the absence or deficiency of glucose-6-phosphatase (glucose-6-phosphate → glucose) in glycogenolysis, which results in hypoglycemia during fasting. Raw (uncooked) cornstarch (RCS) has been shown to serve as an effective oral therapy for the prevention of nighttime hypoglycemic episodes in these patients (Wolfsdorf and

Crigler, 1997). Chen et al. (1984) made similar observations; however, ingestion of cooked cornstarch resulted in a sharp rise in blood glucose levels, followed by a rapid fall to hypoglycemic levels. In 11 healthy male medical students, Collings et al. (1981) compared the postprandial glycemic and insulinemic responses of RCS, cooked cornstarch, glucose via an oral glucose tolerance test (OGTT). They found that the glycemic response of cooked cornstarch was similar to glucose; however, RCS had a significantly lower glycemic response over the 90 min test. These results suggest that RCS is a SDS and that cooking disrupts the cornstarch granules, making them more rapidly digested. The granular structure of RCS appears to retard the ability of α-amylase to rapidly hydrolyze the starch molecule, thus slowing its rate of digestion. Unfortunately, RCS cannot be added to a liquid ready-to-feed (RTF) product because product retort (i.e., sterilization at high temperature) results in the cooking of the starch. As shown by Chen et al. (1984) and Collings et al. (1981), cooking the starch (which allows complete gelatinization) renders the starch rapidly digested.

Glycemic index (relative glycemic response)

The glycemic index has been proposed as a method of ranking foods on the basis of the positive incremental blood glucose responses they produce for a given amount of CHO (Jenkins et al., 1981). Glycemic index is calculated by dividing the blood glucose incremental (i.e., baseline-adjusted) area under the curve (AUC) of the test food by the blood glucose AUC of the reference food and multiplying by 100 (Jenkins et al. 1981). The CHO content of the test and

reference food is the same (50 g available CHO). The reference food is typically glucose or white bread, which represents the standard glycemic index value of 100. The glycemic response of some CHO foods standardized against white bread was summarized by Jenkins et al. (1984). The glycemic index of some high-CHO foods and ingredients is presented in Table 2.1. The use of the glycemic index may have practical application in the evaluation of ingredients and product concepts that would be used in the formulation of nutritional products to normalize blood glucose levels in people with abnormal glucose tolerance.

Carbohydrate food or ingredient	Glycemic index
Sugars	
fructose	23
glucose	100
honey	58
lactose	46
maltose	105
sucrose	65
Starchy foods	
brown rice	55
white rice, short grain	72
white rice, long grain	56
spaghetti	41
white bread	70
whole wheat bread	69
stonegound whole wheat bread	53
Vegetables	
carrots	49
peas, green	48
potato, baked	93
sweet corn	55
Legumes	
kidney beans	27
lentils	30
soy beans	18
Fruits	
apple	38
banana	55
orange	44
pear	66

Table 2.1. Glycemic Index (Standardized Against a Glucose Control, Glucose = 100) of Some High-Carbohydrate Foods and Ingredients. Adapted from Brand-Miller et al. (1999).

Does the relative glycemic response or glycemic index, which is based on an acute meal glucose tolerance test, translate into improved long-term glycemic control? As previously discussed, epidemiological data suggest that the consumption of a low glycemic load diet reduces the risk for development of type 2 DM (Salmeron et al., 1997a, 1997b). Buyken et al. (2001) documented that a lower dietary glycemic index is related to lower glycosylated hemoglobin (Hb A_{1c}) concentrations (a marker of glycemic control) in 2,810 people with type 1 DM. The advantages of a low-glycemic load diet may extend beyond the glycemic effects, because traditional low-glycemic index foods (e.g., beans, lentils, and barley) contain other bioactive components (e.g., soluble fiber and vegetable protein), which are known to improve biomarkers of health (e.g., LDL cholesterol). Liu et al. (2000) suggest that a high dietary glycemic load from refined CHO increases the risk of coronary heart disease in women. This finding is supported by the recent reports of Liu et al. (2001) and Ford and Liu (2001). These epidemiological studies point to the question - can low-glycemic index diets improve the long-term metabolic control of diabetes?

Improved glycemic response to the subsequent meal following a low-glycemic index meal supports the theory that long-term glycemic control will improve with a low glycemic index diet. This concept was first demonstrated by Jenkins et al. (1980b) who found that the postprandial rise in blood glucose to an oral glucose tolerance test (OGTT) was lower 4 h after subjects had consumed a guar gum-containing OGTT. Jenkins et al. (1982) further showed that a breakfast containing lentils (a slow release dietary CHO) improved the

blood glucose response to a standard bread lunch that followed 4 h later. Wolever et al. (1988) found that the glycemic responses to a standard breakfast was lower on mornings when subjects consumed a low-glycemic index dinner than after a high-glycemic index dinner. Low-glycemic index diets (slowly digested CHO) may improve long-term metabolic control in people with glucose intolerance.

Wolever (2001, personal communication) summarized all 13 available controlled studies that have evaluated the glycemic effects of a low-glycemic index diet in subjects with diabetes. A total of 218 subjects with type 1 or 2 DM were fed for an average of 10 weeks low- or high-glycemic index diets containing, on average, 52% of kcal from CHO. The reduction in glycemic index was 21% (13 units) for the low-glycemic index diet. On average, subjects had a 7% reduction (95% CI –9.6 to –4.4) in glycated protein (fructosamine or glycated hemoglobin) during the low-glycemic index dietary period. While objections to the glycemic index concept and its clinical application have been raised (Hollenbeck et al., 1986), the above summary of the dietary trials of low-glycemic index diets provides justification for its use. Because asking questions is the first step in the research process, application of the glycemic index is generating new studies of the diabetic diet that will enable us to develop improved dietary recommendations. Long-term dietary intervention trials are still needed; however, one may theorize that these studies will be positive because acarbose, a compound that slows the rate of CHO digestion, has been proven to be clinically effective in the long-term management of glycemic

control in people with type 2 DM, regardless of concomitant antidiabetic medicine (Chiasson et al., 1994). As noted in its early research stages by Jenkins et al. (1988), the glycemic index may be more appropriate for the ranking of starchy foods. The glycemic index provides a practical method for comparing (and ranking) the glycemic response to CHO ingredients and high CHO foods.

Role of dietary fiber

The extent and duration of the glucose rise after a meal is dependent upon the rate of absorption, which in turn depends upon rate of gastric emptying and upon the rates of hydrolysis and diffusion of hydrolysis products in the small intestine. Purified viscous fibers have potential benefits for improving blood glucose control in diabetes (Anderson et al., 1987; Jenkins et al., 1978; Wolever and Jenkins, 1993). These effects may be moderated through delayed gastric emptying (Torsdottir et al., 1991) or slowed absorption from the small intestine (Jenkins et al., 1978; Leatherdale et al., 1982).

Although fiber has been increasingly recognized as an important dietary constituent, controversy and confusion still exist concerning the physiological effects of fiber. The American Diabetes Association (ADA) recommends a moderate increase in the intake of dietary fiber because of the cholesterol-lowering effects of soluble fiber; however, the effects of dietary fiber on glycemic control were considered inconsequential (ADA, 2001). In a well-controlled study, Chandalia et al. (2000) compared the effects of a recommended ADA diet (24 g fiber per day) to a high fiber diet (50 g per day,

25 g soluble) on glycemic control and plasma lipid concentrations in patients with type 2 DM. They found that the high-fiber diet improved glycemic control and reduced plasma lipid concentrations compared to the ADA diet. These data support an increase in the recommended intake of dietary fiber, especially soluble fiber, by patients with type 2 DM.

Nutrition should be used as adjunctive therapy in the treatment of people with DM. In order for basic research to have a practical benefit, it must have application to-and acceptability in-the clinical setting. Liquid medical nutritional products serve as a convenient means to provide nutrition (e.g., a meal replacement) to individuals who are on the go. The availability of fiber-fortified liquid nutritional products may have practical applications in the nutritional management of people with DM, especially when adherence to dietary recommendations regarding the addition of vegetables, fruits, and whole cereal products is poor.

There is evidence that the viscosity of purified dietary fiber is directly related to its effect on blood glucose control (Wolever and Jenkins, 1993). One type of purified dietary fiber, guar gum, is a viscous, water-soluble dietary fiber composed of a β-1,4 mannose backbone with galactose side chains joined together by α-1,6 linkages. This galactomannan is obtained from the endosperm of the seeds of the leguminous vegetable, the Indian cluster bean (*Cyamposis tetragonolobus*). It is widely used in the food industry as a stabilizer and as a thickening and film-forming agent. A second type of purified dietary fiber, alginate, is the sodium salt of alginic acid and is isolated from

brown seaweed (family Phaeophyceae; Whistler and BeMiller, 1997). It is composed of mannuronic (pK_a ~ 3.38) and guluronic acids (pK_a ~ 3.65). Alginate, in the absence of free polyvalent cations, is a relatively nonviscous soluble fiber. Alginate solutions gel upon addition of free calcium ions, which fill the cavities formed between parallel guluronic acid chains (G-block regions). These cavities contain two carboxylate and two hydroxyl groups, one from each chain. The result is a junction zone that has been called an "egg box" arrangement, with the calcium ions being likened to eggs in the pockets of an egg carton (Anderson et al., 1991). Torsdottir et al. (1991) have shown that sodium alginate supplementation (3.75 g mannuronic and guluronic acids) to a meal diminishes the postprandial rise in blood glucose and insulin. We (Murray et al., 1999) evaluated the effect of a novel alginate:insoluble calcium system incorporated into an enteral formula on nutrient digestion (including calcium) and showed that the alginate system does not negatively affect apparent nutrient digestion and absorption in dogs cannulated in the ileum. In addition, the alginate system reduced the rise in postprandial serum glucose in dogs (Murray et al., 1999).

Numerous clinical studies have evaluated the acute and long-term effects of supplemental guar gum on glycemic control. Although a dose response trial has not been completed, guar gum doses in the range of 1.8 to 15 g have been found to improve the postprandial glycemic response to oral glucose tolerance tests and meal glucose tolerance tests in nondiabetic (Gabbe et al., 1982; Gatti et al., 1984; Heijnen et al., 1995; Jarjis et al., 1984;

Krotkiewski 1984; Wolever et al., 1979) and diabetic (Ebeling et al. 1988; Fuessl et al., 1986; Gatti et al., 1984; Jenkins et al., 1980c, 1980d; Leatherdale et al., 1982) subjects, although Williams et al. (1980) reported no effect. In people with diabetes, medium- to long-term (range 4 weeks to 1 year) guar gum supplementation (9 to 60 g/d) has also shown metabolic improvements in blood glucose control (Aro et al., 1981; Atkins et al., 1987; Ebeling et al., 1988; Fuessl et al., 1987; Gatti et al., 1984; Groop et al., 1993; Jenkins et al., 1977, 1978b, 1980b; Kirsten et al., 1992; Lalor et al., 1990; Lim et al., 1990; Paganus et al., 1987; Peterson et al., 1987; Ray et al., 1983; Smith et al., 1982; Tagliaferro et al., 1985; Vuorinen-Markkola et al., 1992). However, many studies have reported no effect on glycemic control (Beattie et al., 1988; Botha et al., 1981; Bruttomesso et al., 1989, 1991; Carroll et al., 1981; Cohen et al., 1980; Niemi et al., 1988; Uusitupa et al., 1989; Wilson et al., 1989); the dose of guar gum or the method of guar gum preparation and timing of delivery may be the reason for the discrepancy between studies on glycemic control.

In the study of Williams et al. (1980), fiber supplements (guar, pectin, agar, locust bean gum) failed to improve postprandial glycemic control of subjects with type 2 DM. The powder fiber supplements (10 g dose) were mixed with a breakfast test meal or the pre-hydrated fiber was consumed before the test meal. It was noted by these investigators that the powder fiber supplements formed lumps when mixed with the test meal. This lack of thorough mixing with the meal may have been the reason as to why no clinical benefit was found. They suggest that the hydrated fiber supplement must be

fed with the CHO portion of the meal and also must be palatable in order for it to be of practical clinical utility.

A product that innately allows an intimate mixing of the meal with guar gum, which is essential for its effect on CHO metabolism (Wolever et al., 1978; Fuessl et al., 1986), should enhance clinical efficacy. In fact, Wolever et al. (1979) suggest that guar gum is most effective when added to the liquid phase of the meal, because the guar gum is fully hydrated and thus at its highest viscosity, which is an important factor in its clinical efficacy (Jenkins et al., 1978a; Ellis et al., 1986). Furthermore, when consumed as a pill, guar gum has been associated with esophageal obstruction (Seidner et al., 1990).

Even though guar gum has been shown to be clinically effective in the improvement of postprandial blood glucose and insulin responses, the lack of its incorporation into a palatable product has limited its use. Unfortunately, foodstuffs containing guar gum usually exhibit slimy mouth-feel (Williams et al., 1980), tooth packing (Tredger and Ransley, 1978), and poor palatability (Ellis et al., 1991). The overall hedonic quality of guar-containing foods can be improved by reducing the average molecular weight (i.e., through hydrolysis) of the galactomannan in guar gum (Ellis et al., 1991); however, this results in a concurrent loss of clinical efficacy (Jenkins et al., 1978a). Cohen et al. (1980) noted that the poor palatability of guar limits its use and adds uncertainty pertaining to the degree of patient acceptance and compliance. Therefore, they concluded that it is unlikely that guar would have a useful clinical role in the treatment of diabetes.

In addition to the challenge of making a palatable product, dietary supplementation with guar gum is also associated with gastrointestinal side effects (e.g., flatulence and diarrhea) due to its rapid fermentation in the colon (Flickinger et al., 2000). Several experiments have noted an increased incidence of gastrointestinal side effects with guar gum supplementation (Christiansen et al., 1980; Kirsten et al., 1992; Lim et al., 1990; Peterson and Mann, 1985; Smith et al., 1982; Todd et al., 1990; Tuomilehto et al., 1988; Uusitupa et al., 1989; Wilson et al., 1989) even resulting in subject drop out (Aro et al., 1981; Cohen et al., 1980). However, several studies have documented that these side effects subside after approximately 2 weeks of supplementation (Ebeling et al., 1988; Tuomilehto et al., 1980). Todd et al. (1990) suggested a gradual increase in the guar gum dose to reduce side effects and to restrict the dose at 15 g/d, which will reduce the incidence of flatulence. Other research groups found that subjects tolerate guar gum when given at lower daily doses (< 20 g/d) (Carroll et al., 1981; Cohen et al., 1980; Tuomilehto et al., 1988). In order for this technology to have clinical application, a palatable, efficacious, low guar gum dose product must be developed.

A few studies have evaluated the effect of supplemental guar gum on satiety and weight loss. Bruttomesso et al. (1989) found that the feeling of satiety was highest when subjects with type 1 DM consumed 9 g guar gum (3 g three times per day) for 4 weeks. In 9 obese females, Krotkiewski (1984) fed 20 g granulated guar gum (10 g two times per day) for 8 weeks and found a

significant reduction in body weight and sensations of hunger compared to control (20 g wheat bran). Likewise, Tuomilehto et al. (1980) found that 33 hypercholesterolemic females supplemented with 15 g guar gum (5 g three times day) for 4 months had significant weight loss compared to control. Wilmshurst and Crawley (1980) found a significant correlation between mean gastric emptying time and a subjective measure of satiety. Supplementation of 2 g guar gum to a low energy (207 kcal) test meal increased gastric transit time in 12 overweight patients (Wilmshurst and Crawley, 1980). Groop et al. (1993) found a significant reduction in body weight of 15 subjects with type 2 DM given 15 g supplemental guar gum (5 g three times per day) for 48 weeks.

Fructose metabolism and absorption

The nutritive sweetener, fructose, has a low glycemic index (Crapo et al., 1980a; Jenkins et al., 1981; Nuttall et al., 1992). Because of its reduced glycemic response, fructose is an ideal CHO for use in the dietary control of postprandial glycemia in people with diabetes mellitus. However, dietary recommendations for the diabetic have been made to avoid high intakes of simple CHO, especially fructose (Franz et al., 1994). It has been implied that high fructose consumption is associated with an increased risk for hyperlipidemia (increased serum cholesterol, LDL cholesterol, triacylglycerol; Henry et al., 1991; Hollenbeck, 1994). Data from rat studies show that dietary fructose stimulates lipogenesis, resulting in an increase in blood lipids (Zavaroni et al., 1982). Data from human studies as reviewed by Henry et al. (1991) and Hollenbeck (1993) are less conclusive. Part of this problem may be due to the

lack of rigorous control in nutrient intake or an inappropriate CHO source in the control diet such as sucrose, which in itself is half fructose.

A few well-controlled human studies in this area have been conducted. Bantle et al. (1992) evaluated the metabolic effects of isocaloric diets containing <3% or 20% of energy from fructose in subjects with type 1 and type 2 DM. They found a tendency toward reduced glycemia when subjects were consuming the high fructose diet over a 4-week period. However, they also found an increase in the fasting serum total and LDL cholesterol levels when subjects consumed the high fructose diet. No effect was found on fasting or peak postprandial serum triacylglycerol. More recently, Bantle et al. (2000) conducted a similar study in healthy male and female subjects. In a randomized, balanced, crossover design, 2 isocaloric diets (<3% and 17% total kcal as fructose) were fed for 6 weeks. They found that men had a higher fasting and postprandial plasma triacylglycerol concentrations during the high fructose diet period. The high fructose diet had no effect on these criteria in women and had no persistent effect on fasting plasma cholesterol or LDL cholesterol in either men or women. Hallfrisch et al. (1983) fed hyperinsulinemic and control males diets containing 0, 7.5, or 15% (of total kcal) fructose for 5 weeks each in a 3 period, crossover design. Plasma total cholesterol and LDL cholesterol was increased when they consumed 7.5 and 15% fructose. Plasma triacylglycerol levels increased as the level of fructose increased in hyperinsulinemic men. The adverse effects of fructose on plasma

lipids are associated with high, but clinically relevant, dietary levels, and subjects who are susceptible to hyperlipidemia.

Many dietary factors (e.g., macronutrient profile, CHO dose and type) affect the postprandial blood glucose excursion (Crapo et al., 1980b; Falko et al., 1980). Another factor, which is many times overlooked, that affects the postprandial blood glucose level is hepatic glucose uptake and production. The effects of dietary intake on postprandial CHO metabolism in hepatic and extrahepatic tissues are not well understood because the regulation of postprandial CHO metabolism is complex, and because clinical experiments in this area are intensive and can be complicated to conduct. Furthermore, the hepatic role in blood glucose control is abnormal in persons with type 2 DM. For example, the postprandial hyperglycemia in this population is a result of excessive hepatic glucose release coupled with a lack of an appropriate increase in hepatic glucose uptake (Dinneen et al., 1992).

One factor that affects postprandial hepatic glucose uptake is the liver's ability to phosphorylate glucose. Glucokinase, an isoenzyme of hexokinase, is specific to liver parenchymal cells. Unlike other hexokinases, glucokinase has a high K_m (~ 5 m*M* or 90 mg/dl compared with < 0.1 m*M*), and thus only utilizes glucose at a significant rate when blood glucose levels are elevated (e.g., postprandial). Furthermore, glucokinase is not inhibited by its end product, glucose-6-phosphate, as are other hexokinases (Van Shaftingen et al., 1994). Glucokinase is an inducible enzyme, meaning that the amount of enzyme present is influenced by various physiological conditions. Unlike other tissues

(e.g., skeletal muscle and adipocytes), the liver does not require insulin for glucose uptake; however, insulin does affect the level of glucokinase in the parenchymal cells. Insulin increases the amount of glucokinase by promoting the transcription of the glucokinase gene. The response is relatively slow, usually requiring several hours (see Harris, 1992 for review). Thus, a person who consumes large meals rich in CHO, which would elicit an insulin response, will have greater amounts of glucokinase in the liver than a person who does not. In theory, a liver in which glucokinase has been induced can make a greater contribution to the lowering of elevated postprandial blood glucose.

Consumption of a high-CHO diet has been shown to improve the postprandial glycemic response in humans (Jenkins et al., 1980a; Howard et al., 1991; Swinburn et al., 1991). I hypothesize that individuals who typically consume a high-CHO diet (and thus have higher postprandial insulin responses resulting in an increase in glucokinase) would be more likely to respond to fructose supplementation because they should also have a higher level of glucokinase. This presents a topic for future research.

Glucokinase is acutely regulated by fructose-6-phosphate and fructose-1-phosphate, two metabolites whose effects are dependent upon an inhibitory protein that tightly binds to glucokinase (Van Schaftingen et al., 1994). Fructose-6-phosphate promotes, but fructose-1-phosphate inhibits binding of the inhibitory protein to glucokinase. Thus, dietary fructose may promote hepatic glucose utilization by an indirect mechanism. Fructose is converted in the liver directly to fructose-1-phosphate (via fructokinase, an enzyme present

only in the liver), which competes with fructose-6-phosphate on the glucokinase regulatory protein, and activates glucokinase by promoting dissociation of its inhibitory protein (see Figure 2.1). In isolated rat hepatocytes, fructose at low concentrations stimulated the glycolytic flux (Fillat et al., 1993). Supplemental dietary fructose may enhance glucose flux through glucokinase in people with type 2 DM, who have an impaired ability to suppress endogenous glucose production during hyperglycemia, due in part to decreased glucose-induced flux through glucokinase (Mevorach et al., 1998). With an improvement in postprandial hepatic glucose uptake, the blood glucose level may be reduced after a meal containing supplemental fructose.

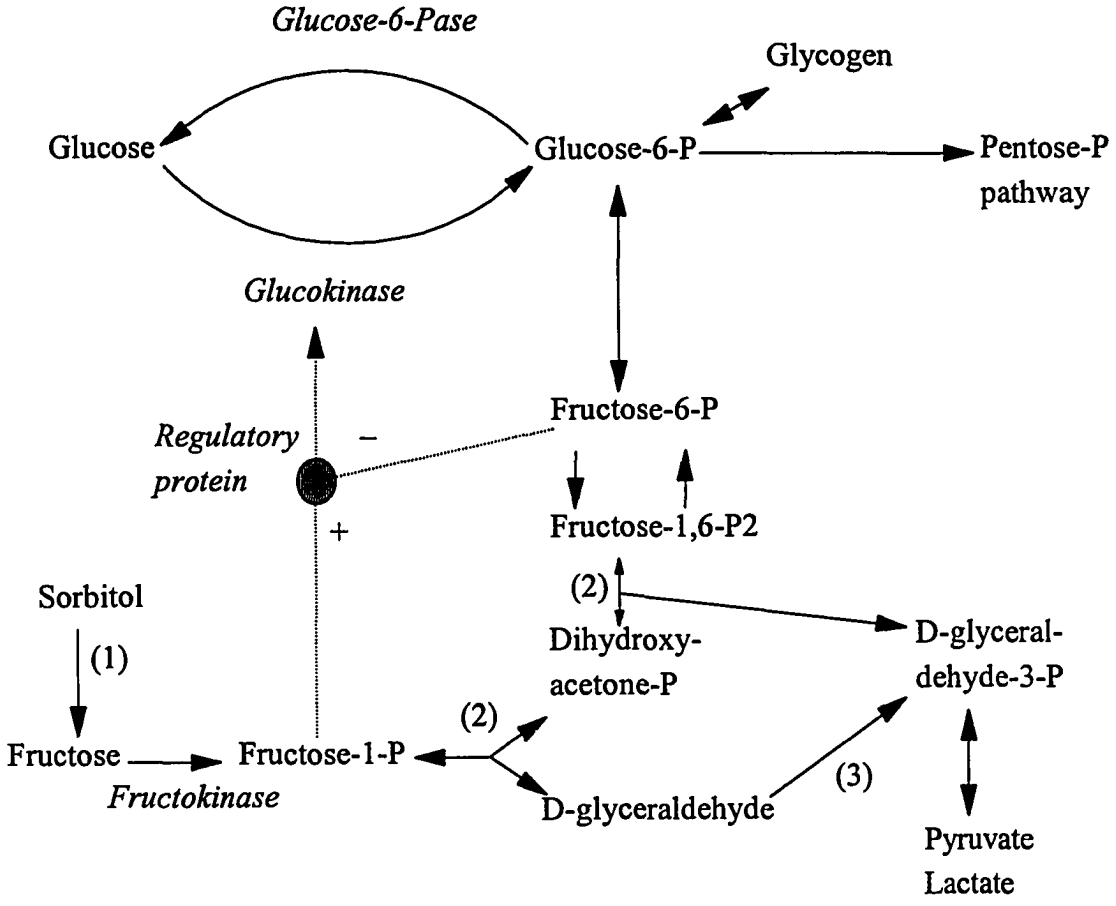

Figure 2.1. Metabolism of glucose, fructose, D-glyceraldehyde, and sorbitol in the liver. For the sake of clarity, cosubstrates are not shown. Sorbitol dehydrogenase (1); aldolase (2); triokinase (3). Adapted from Van Schaftingen et al., 1994.

Shiota et al. (1998) found that intraportal infusion of small amounts of fructose augment net hepatic glucose uptake during hyperglycemic hyperinsulinemia in dogs. Recently, we (Hadley et al., 2000) and several groups have demonstrated that supplemental fructose improves the postprandial glycemic response to an OGTT in rats (Atkinson et al., 2000), nondiabetic healthy volunteers (Moore et al., 1999) and people with type 2 DM (Moore et al., 2000). Furthermore, Hawkins et al. (1999) showed that intravenous fructose decreases hepatic glucose production during hyperglycemia in subjects with type 2 DM. During hyperglycemia and hyperinsulinemia in dogs, intraportal infusion of small amounts of fructose resulted in a 3-fold increase in net hepatic glucose uptake, a majority (69%) of which was stored as glycogen. Lower amounts were released as lactate (17%) or oxidized (8%). However, Moore et al. (2000) found a significant increase in blood lactate and concluded that it was more than adequate to account for the difference in plasma glucose concentrations.

Rats have a higher capacity for absorbing free fructose compared to the human. Fujisawa et al. (1991) determined that the amount of fructose that was completely absorbed by rats was 1.4 to 1.6 g/kg body weight, a 10-fold higher dose than that required to reduce the postprandial glycemic excursion in Zucker fatty *fa/fa* rats (Hadley et al., 2000).

The absorption of fructose in the small intestine of man is poorly understood. Burant et al. (1992) found that small intestinal absorption of fructose occurs through a specific fructose transporter, GLUT5. However,

malabsorption of fructose (as measured by breath hydrogen) is common when given alone as an acute challenge (Riby et al., 1993). This effect is eliminated when equimolar amounts of glucose (Riby et al., 1993; Rumessen and Gudmand-Hoyer, 1986; Truswell et al., 1988), galactose (Kneepkens et al., 1984), or certain amino acids (Hoekstra and van den Aker, 1996) are given with the fructose challenge. Evidence in rats supports the hypothesis that fructose, when fed with glucose, might be absorbed by the disaccharidase-related transport system as if it was a product of the enzymatic hydrolysis of sucrose. Fujisawa et al. (1991) found that sucrose competes with the mixture of glucose and fructose for the saturation of the absorptive mechanism and that Acarbazone (an inhibitor of disaccharidases, e.g., sucrase) also inhibits glucose facilitation of fructose absorption. However, in humans, it was found that fructose absorption is not by a disaccharide-related transport system (Hoekstra and van den Aker, 1996; Shi et al. 1997). Shi et al. (1997) suggested that fructose is transported transcellularly by facilitated diffusion and paracellularly (based on lactulose transport) together with glucose-activated solution drag [i.e., water and solute (e.g., fructose) pass the mucosa through the same pathway]. Hoekstra and van den Aker (1996) came to similar conclusions based on their observation that fructose absorption was enhanced from a solution containing certain amino acids (e.g., L-alanine). They also suggested that the increase in intraluminal fructose concentration, as a result of extracted water, might lead to an increase in fructose transport.

Further studies are needed to elucidate the mechanism of fructose absorption in humans, which may improve the dietary use of fructose for the control of postprandial blood glucose. Eight of ten healthy adult subjects given 50 g of free fructose showed malabsorption, as measured by breath hydrogen, compared to 20 g of lactulose (Rumessen & Gudmand-Hoyer, 1986). It seems likely that the lower glycemic index of fructose may be partly due to the malabsorption of free fructose. Because fructose is not malabsorbed when consumed as sucrose in humans (Kneepkens et al., 1984; Rumessen and Gudmand-Hoyer, 1986; Shi et al., 1997), the addition of sucrose to a medical nutritional product may reduce the postprandial glycemic response to a high CHO (starch) meal. This hypothesis should be tested in a human experiment.

Summary

Tight glycemic control is of paramount importance for the medical treatment of people with DM. Because intensive treatment with insulin is associated with an increased risk of hypoglycemia and weight gain, nutrition should serve as adjunctive therapy for this population. To improve the postprandial glycemic response to foods, it is essential to gain more knowledge about the factors responsible for the metabolic features of CHO foods. Of the macronutrients, dietary CHO has the most significant effect on postprandial glycemia. Both the amount and type of dietary CHO are important determinants of postprandial glucose response to a meal. Sugars typically have a flatter postprandial glycemic response compared with starch; however, nutritional recommendations have been made to avoid excessive sugar intake. Starch

constitutes the largest proportion of energy intake, and the rate of its digestion is directly related to the postprandial glycemic response. Several factors affect the rate of starch digestion and should, therefore, be considered in the formulation of nutritional products for people with DM.

Purified viscous forms of dietary fiber have potential benefits for improving blood glucose control. While high viscosity appears to be a necessary property of fiber to maximize its benefits on blood glucose, high viscosity reduces the palatability of foods and is a major impediment to the practical use of viscous fiber. Novel methods of incorporating viscous fiber into palatable products may enable the practical use of dietary fiber in the diabetic diet and improve glycemic control.

Dietary fructose may promote hepatic glucose utilization via its indirect role in the regulation of glucokinase. If small amounts of oral fructose can increase hepatic glucose uptake, adding supplemental fructose to the diet of individuals with DM may be useful in reducing the postprandial glycemic response.

Identification of ingredients that slow the rate of digestion or enhance hepatic glucose uptake should improve the glycemic control of people with DM. In addition, the identification of ingredients that slow the rate of digestion and(or) absorption should reduce the risk of hypoglycemia in DM patients placed on an intensive blood glucose control program. The glycemic index provides a practical method for comparing (and ranking) the glycemic response to CHO ingredients and high CHO foods. Each of these concepts may have

practical utility in the development of a CHO system for the formulation of products for people with DM. But first, physiological efficacy must be demonstrated.

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CHAPTER 3

EFFECTS OF CHEMICAL MODIFICATION ON IN VITRO FOOD STARCH HYDROLYSIS - AN ATTEMPT TO DISCOVER A SLOWLY DIGESTED STARCH

INTRODUCTION

As part of a healthy diet, it is generally recommended that the intake of fat (as a percentage of calories) should be decreased and the intake of complex carbohydrate (i.e., starch) and dietary fiber concomitantly increased to meet caloric requirements. For individuals with diabetes mellitus, however, the carbohydrate must not exacerbate postprandial hyperglycemia and must prevent hypoglycemia. Differences in glycemic and insulinemic responses to dietary starch are directly related to rate of starch digestion (O'Dea et al., 1981; Jenkins et al., 1984).

Chemical modification of starch may ultimately affect its rate and extent of digestion in the small intestine. Flickinger et al. (2000) found that little digestion of a dextrinized cornstarch hydrolysate [Fibersol 2(E)] occurred in the small intestine of dogs cannulated in the ileum. Furthermore, they documented that rate and extent of *in vitro* fermentation of Fibersol 2(E) were lower compared with other low molecular weight carbohydrates. These data are

supported by Tsuji and Gordon (1998), who estimated that Fibersol 2, a dextrinized cornstarch hydrolysate, had a caloric value of 2.2 kJ/g in rats.

Numerous chemically modified food starches are available as ingredients for processed foods. Chemical treatments currently allowed and used to produce modified starches for food use in the U.S. include: esterification, etherification, acid modification, bleaching, and oxidation (Whistler and BeMiller, 1997). Multiple modifications of starch are a common occurrence for designing starches with specific applications in the food industry. I postulated that the use of these modifications might allow for the production of a slowly digested starch that could be used for the treatment of certain medical modalities (e.g., glycogen storage disease and diabetes mellitus).

Given the high cost associated with the conduct of *in vivo* studies, a number of groups have developed *in vitro* methods to predict the digestion of starch in the small intestine (Englyst et al., 1992; Muir and O'Dea, 1992, 1993; Brighenti et al., 1995). Methods have been adapted to predict the amounts of starch that would be rapidly digested, slowly digested, or not digested (resistant) in the small intestine. These methods provide an inexpensive and rapid means to screen large numbers of starch substrates for their *in vivo* glycemic response (glycemic index). The purpose of this research was to screen chemically modified starch ingredients that may serve as a source of slowly digested starch in liquid enteral formulas.

MATERIALS AND METHODS

Starch Sources. Extent of *in vitro* starch hydrolysis was determined on chemically modified starch and their unmodified controls. Chemical modifications evaluated were substitution with propylene oxide (etherification), crosslinking with phosphorous oxychloride (creating phosphate intermolecular bridges between starch molecules), acid modification with heat (dextrinization), and oxidation with sodium hypochlorite. The extent of chemical modification varied among starch ingredients and was within the legal limits allowed by the U.S. Food and Drug Administration. In addition, different genetic varieties were evaluated: common starch (~ 27% amylose), waxy starch and dull waxy starch (0% amylose), and a high amylose variety (50% amylose). Several experiments were conducted to ascertain the effects of chemical modification, variety of starch, and combinations of the above on *in vitro* starch hydrolysis.

Chemically modified starch ingredients and their unmodified controls were obtained from commercial starch suppliers. Modified starch descriptions, including degree of substitution, are presented in Table 3.1. Details regarding starch processing are proprietary industry procedures. Two lab reference materials were used in our experiments: raw potato starch and raw cornstarch (Sigma Chemical Co., St. Louis, MO). Potato buds were obtained from a local grocery. Dry matter was determined according to Association of Official Analytical Chemists methods (AOAC, 1984). In general, starch ingredients contained approximately 0.35% protein, 0.55% fat, 0.2% ash, and 98.9% carbohydrate (dry matter basis) as determined by the ingredient supplier.

In Vitro Hydrolysis. A modification of the Muir and O'Dea (1992, 1993) *in vitro* starch digestion technique was used. Approximately 0.1 g carbohydrate was suspended in 1 mL pepsin (Sigma) solution (1 g/L; pH was adjusted to 2.0 with HCl) and incubated for 30 min at 37 °C. The solution then was neutralized with 0.5*M* NaOH (0.5 mL). Five milliliters of 0.2*M* sodium acetate buffer (pH was adjusted to 5.0 with glacial acetic acid) and 1 mL enzyme solution containing 10 mg α-amylase (Sigma) and 28 U amyloglucosidase (Sigma) dissolved in the sodium acetate buffer (pH 5.0) were added, and samples were incubated at 37 °C in a shaking water bath for various time points, as described below. After the appropriate incubation time, samples were centrifuged at 3000 × *g* for 10 min, and the supernate was removed. The precipitate was washed three times by resuspension with 1.5 mL sodium acetate buffer (pH 5.0) and centrifugation (10 min, 3000 × *g*). All supernates from washings were pooled with the original supernate. This fraction allowed for the prediction of digestion that would occur in the small intestine (i.e., digestible starch, DS).

The washed precipitate was lyophilized; this material was considered the resistant starch (RS) component of the sample. The precipitate was resuspended in 5 mL dimethyl sulfoxide (DMSO) and incubated in a boiling water bath for 5 min. Then 20 mL of 0.15*N* sodium acetate buffer (pH adjusted to 4.5 with glacial acetic acid) was added and incubated in a boiling water bath for 20 min. Samples then were autoclaved for 1 h at 1.055 kg/cm^2 and 121 °C. Samples were allowed to cool to room temperature before addition of 10 mL amyloglucosidase solution containing 580 U amyloglucosidase dissolved in

water. Samples were incubated for 24 h at 55 °C (with occasional vortexing) and then centrifuged for 10 min at 10,000 to 15,000 × *g*. The supernate was removed for glucose analysis. After starch digestion (hydrolysis), the released glucose was measured by a glucose oxidase method (Glucose Test Kit 510-A, Sigma). The final glucose reading was multiplied by 0.9 to convert free glucose back to polysaccharide. All values are reported as means of duplicate analyses, expressed as a percentage of ingredient dry matter. Duplicate samples were reanalyzed if duplicates differed by > 5%.

Experiment 1. Raw starch ingredients and cooked starch ingredients were evaluated for their extent of *in vitro* hydrolysis. A 15-h *in vitro* incubation was used to determine the amount of digestible starch because this timeframe has been shown to correlate with the amount of starch escaping digestion in the small intestine using this *in vitro* assay (Muir and O'Dea, 1993). An attempt to quantify resistant starch also was conducted as described above. In addition, raw starch ingredients were evaluated for total starch content by the method of Thivend et al. (1972). For cooking, raw starch ingredients were made into 5 and 10% (wt/vol) starch solutions with water. The use of a hot plate (to warm the starch solution) was required to suspend some starch ingredients. Samples then were autoclaved for 10 min at 1.055 kg/cm^2 and 121 °C. The starch solutions were frozen (-20 °C) and lyophilized in a Tri-Philizer MP microprocessor-controlled lyophilizer. Lyophilized samples were ground with a mortar and pestle in preparation for analysis.

Experiment 2. The rate of starch digestion was estimated by analysis of *in vitro* starch hydrolysis over time. Samples were analyzed at 0, 2.5, 5, 10, and 15 h. Separate tubes containing ~0.1 g carbohydrate were used for each time point. Free glucose (0 h time point) was analyzed for each substrate after pepsin digestion. Starches were suspended in 10% (wt/vol water) solutions and sterilized in order to mimic their use in a liquid nutritional product. Starch samples that had a negative impact on the solution's viscosity were predigested with α-amylase (Dexlo-S, Genencor International, Inc., Rochester, NY). Starches that did not have a negative impact on the solution's viscosity were cooked into 10% starch solutions at 91 °C for 30 min. Starches that required pre-digestion were cooked as 10% solutions at 71 to 77 °C until the starch was hydrated and the solution was translucent in appearance at which time the temperature was raised to 93 to 97 °C for 5 to 10 min in order to inactivate the α-amylase. An aliquot of 1,000 g of the cooked 10% starch solutions was filled in 1-L ready-to-hang bottles (Ross Products Division, Altavista, VA). The bottles were sealed, sterilized by heating to 122 °C, and held at this temperature for 12 min before being allowed to cool to room temperature. The starch solutions were frozen (-20 °C) and lyophilized in a Tri-Philizer MP microprocessor-controlled lyophilizer. Lyophilized samples were ground with a mortar and pestle in preparation for analysis.

Experiment 3. The initial rate of starch digestion for a few chemically modified starch ingredients was estimated by analysis of *in vitro* starch hydrolysis over time. Samples were analyzed at 0, 0.5, 1, 1.5, 2, and 2.5 h.

Separate tubes containing ~0.1 g carbohydrate were used for each time point. Starches were prepared as described in Experiment 2.

RESULTS

Experiment 1. The starch concentration and extent of *in vitro* hydrolysis of chemically modified starches are presented in Table 3.2. The total starch content of raw starch ingredients (DS + RS) as measured by the method of Muir and O'Dea (1992, 1993) was similar to the values obtained by the method of Thivend et al. (1972). The unmodified waxy and dull waxy starch ingredients (WS and dWS, respectively) contained high levels of DS (> 90%), whereas the unmodified 50% high amylose starch ingredient (hAS) contained a significant proportion of RS. Total starch was lower in propylene oxide-substituted starches (hPOlXL-WS, mPOmXL-dWS, hPO-hAS) compared with their unmodified controls (WS, dWS, hAS, respectively), suggesting a decrease in their digestibility. A similar phenomenon was noted for the dextrinized starches. As the degree of dextrinization increased, the total amount of digestible starch decreased (lD-CS > mD-CS > hD-CS). Starch modification by crosslinking did not appear to affect starch digestibility (compare WS to hXL-WS), whereas the highly oxidized waxy starch (hO-WS) was less digestible than its unmodified control (WS).

Overall, the amount of digestible starch and RS were similar between 5 and 10% cooked solutions. Of those raw ingredients that contained a significant amount of RS, this portion became digestible upon cooking. For example, raw propylene oxide-substituted starch ingredients (hPOlXL-WS,

mPOmXL-dWS, and hPO-hAS) contained more RS compared with their cooked counterparts. In addition, hAS, the raw 50% amylose hybrid (unmodified) had a high concentration of RS (52%) compared with its cooked version (~16%). Digestible starch values were similar for cooked and raw dextrinized starches (ID-CS, mD-CS, hD-CS). The lab reference materials, raw cornstarch and raw potato starch, contained a high level of total starch; however, a majority of the raw potato starch was quantified as RS.

Figure 3.1 shows the *in vitro* hydrolysis of raw cornstarch, corn syrup solids (DE =20), and potato buds over time. This figure shows that a standard carbohydrate ingredient used in liquid food products, corn syrup solids, is rapidly and completely digested. The digestible portion of potato buds was digested very rapidly, with the remaining portion being resistant to digestion. Raw cornstarch was digested more slowly but was also extensively digested over time in this assay (i.e., a slowly digested starch).

Experiment 2. Because a 15-h incubation has been shown to correlate with the amount of starch escaping digestion in the small intestine (Muir and O'Dea, 1993), we evaluated the extent of *in vitro* digestion over this period of time. The *in vitro* hydrolysis of chemically modified starches is presented in Table 3.3. In general, these results show that the portion of starch that was digestible was digested within 2.5 h, and the remaining starch was resistant to digestion (i.e., RS). The only starch that demonstrated a "slow" rate of digestion was the lab reference material, raw cornstarch. Sixty-seven percent of raw cornstarch was digested at 2.5 h, and its digestibility rose to 86% by 15 h.

The extent of starch digestion (i.e., 15-h time point) of individual starch ingredients was similar to the values obtained in experiment (Exp.) 1. Thus, the effect of chemical modification on extent of *in vitro* hydrolysis was similar between Exp. 1 and Exp. 2. The extent of *in vitro* hydrolysis at 15 h for the lab reference controls raw cornstarch and raw potato starch were 83.9 and 38.3% in Exp. 1 and 86.0 and 37.6% in Exp. 2, respectively.

Experiment 3. Because no slowly digested modified starch ingredients were identified over the 15-h *in vitro* period, we evaluated the extent of starch digestion over the initial 2.5 h (Table 3-4). No starch ingredients appeared to be slowly digested compared with our lab reference material, raw cornstarch. Thirty-five percent of raw cornstarch was digested at 0.5 h, and its digestibility rose to 64% by 2.5 h. Using this *in vitro* procedure, most modified cornstarch ingredients chosen for these experiments contained only a small proportion of slowly digested starch when processed into a liquid solution.

In vitro digestion values for the 2.5-h time point tended to be higher for the same chemically modified starch ingredients in Exp. 3 compared with Exp. 2. Potential sources of experiment-to-experiment variation include: water bath temperature (± 1 °C), water bath shaker speed (i.e., sample suspension in the buffer), enzyme concentrations, number of samples (i.e., speed at which samples could be processed upon reaching desired incubation time), and the large number of steps for this assay. Thus, appropriate control samples were run with each experiment.

DISCUSSION

Worldwide, starch and its products constitute most of the digestible carbohydrate in the human diet. The rate and extent of starch digestion in the small intestine are dependent upon several intrinsic and extrinsic factors (reviewed by Englyst et al., 1992). All starch was once assumed to be hydrolyzed and absorbed in the small intestine because pancreatic α-amylase can be produced in ample amounts (Fogel and Gray, 1973). However, a substantial amount of starch is now known to escape digestion in the small intestine and enter the colon (Englyst and Cummings, 1985; Berry, 1986). In an attempt to nutritionally categorize starch, Englyst et al. (1992) developed an *in vitro* method to classify starch into three categories: rapidly digested starch (RDS), slowly digested starch (SDS), and resistant starch (RS).

For the purposes of this research, we have defined SDS as starch that is likely to be completely digested in the small intestine but at a slower rate, as described by Englyst et al. (1992). Considering this, there are several conditions for which the nutritional use of a SDS would be of physiological benefit. For example, individuals with type 2 diabetes mellitus may benefit from a foodstuff that contains a SDS in order to improve the postprandial glycemic response (i.e., prevention of hyperglycemia and hypoglycemia). In addition, a SDS may prolong satiety and could be incorporated into foodstuffs marketed for weight loss programs. Finally, SDS may be beneficial in products that are utilized by athletes, because SDS would provide a longer, more consistent source of systemic glucose.

Type I glycogen storage disease is associated with the absence or deficiency of glucose-6-phosphatase (glucose-6-phosphate →glucose), which results in hypoglycemia during fasting. Raw (uncooked) cornstarch has been shown to serve as an effective oral therapy for the prevention of nighttime hypoglycemic episodes in these patients (Wolfsdorf and Crigler, 1997). Chen et al. (1984) made similar observations; however, ingestion of cooked cornstarch resulted in a sharp rise in blood glucose levels followed by a rapid fall to hypoglycemic levels. These results suggest that raw cornstarch is a SDS, and that cooking disrupts the cornstarch granules, making them more rapidly digestible. Considering these data, we chose to include raw cornstarch in our *in vitro* experiments as a positive SDS control.

In the present study, total starch content of raw starch ingredients, as measured by the method of Muir and O'Dea (DS + RS; 1992, 1993), was similar to the values obtained by the method of Thivend et al. (1972). Compared with their unmodified controls, propylene oxide-substituted and dextrinized starches had lower total starch values (Table 3.2). This may be due to incomplete hydrolysis of the modified starch ingredients by the enzymes used in these *in vitro* methods. Both of these *in vitro* methods are based upon starch hydrolysis by α-amylase and(or) amyloglucosidase followed by glucose determination (via glucose oxidase) to quantify starch concentration. Alternate sources of enzymes (e.g., Megazyme International Ireland Ltd.; Wicklow, Ireland) were evaluated to determine if improved quantification could be obtained. No differences were found in the ability of alternate enzyme sources to improve

starch quantification (data not shown). A substituted starch polymer, although completely hydrolyzed, probably would not be quantified because glucose oxidase would not react with a substituted glucose molecule. However, the decrease in total starch quantification found in the present study is much greater than the absolute level of substitution. For example, the theoretical reduction in the extent of digestion for hPOlXL-WS (degree of substitution = 0.12) would be 12%; however, an approximately 40% reduction in *in vitro* hydrolysis was found (Table 3.2). Thivend et al. (1972) noted that some glucose polymers obtained from certain starch treatments (e.g., dextrinization) were not hydrolyzed by glucoamylase (i.e., amyloglucosidase). They suggested that total hexose content be determined in conjunction with a hydrolysis method (e.g., anthrone method) for these types of ingredients. We attempted to combine the anthrone method with our *in vitro* procedure but were unable to obtain meaningful results (data not shown).

In the present study, the RS pellet was resuspended in DMSO in order to gelatinize this material. The limited digestibility of high amylose starch is related to its gelatinization. This is clearly demonstrated in the hAS ingredient (compare raw sample to 5 and 10% cooked solutions, Table 3.2). However, solvent (i.e., DMSO) treatment of chemically modified starches did not improve the quantification of RS in these ingredients. We postulated that some of the RS was not quantified because the enzymes used in our *in vitro* system could not hydrolyze it. As with total starch quantification, the use of alternate enzyme sources did not improve RS quantification (data not shown). We also

postulated that the polymer size of the RS was small enough that it was not pelleted during the centrifugation and, therefore, was not quantified in the RS separation. An attempt was made to improve the Muir and O'Dea (1992, 1993) procedure by increasing the force of centrifugation from 1,200 × *g* to 3,000 × *g*; however, it was not successful. This method for measuring RS only works on insoluble forms of RS, and we conclude that this procedure is not appropriate for the quantification of RS in modified starch ingredients because much of the RS was soluble (especially for dextrinized starches). In addition, we attempted to utilize the *in vitro* method described by Englyst et al. (1992); however, we were unable to replicate the method and obtain meaningful results. More recently, Englyst et al. (1999) have modified the procedure.

Gelatinization refers to the disruption of molecular order within starch granules that are heated in the presence of excess water. The incorporation of chemically modified starch ingredients into 5 and 10% solutions with heating significantly increased the digestible component of the starch (Table 3.2, compare to raw starch values), especially for those ingredients made from high amylose starch (hAS). Thus, our data support the analysis of digestible starch in the final product as prepared for consumption, because cooking may alter the proportion of digestible starch in the foodstuff.

The present study shows that the level of digestible starch is decreased by several chemical modifications. Propylene oxide substitution (etherification) significantly reduced the *in vitro* digestibility of starch. I proposed that the propylene oxide substitution interferes with the binding of α-amylase and/or

amyloglucosidase, thus decreasing starch digestion. Likewise, a high degree of oxidation decreased the extent of starch digestion; however, crosslinking with phosphorus oxychloride did not alter the *in vitro* digestibility of starch in our experiment. The legal limit of starch crosslinking may be too low to significantly alter starch digestion.

Partial hydrolysis of starch using acid and heat (i.e., dextrinization) results in molecular rearrangement of the starch molecule such that α and β-(1,2) and -(1,3) linkages are formed in addition to reconfiguration of existing α-(1,4) and -(1,6) bonds into β bonds (Bryce and Greenwood, 1963). Our data document that dextrinization decreases the *in vitro* digestibility of starch. The data generated by this *in vitro* assay are supported by the *in vivo* work of Flickinger et al. (2000) and Tsuji and Gordon (1998). Data presented in Table 3.2 show that, as the degree of dextrinization increases, *in vitro* digestibility decreases.

Most modified starches contained only RDS and RS. The only ingredient from the present study that contained a significant amount of SDS was raw cornstarch. The lack of SDS in other starches could be due to the nature of the ingredients tested (i.e., isolated in pure form), and because they were "cooked". The use of raw cornstarch as a method to prevent nighttime hypoglycemic episodes is fairly common. It appears that the structural nature of raw cornstarch retards the ability of α-amylase to hydrolyze the starch molecule, thus slowing its rate of digestion and absorption. In the case of the modified starches used in this experiment, that component of the starch that was

digestible was hydrolyzed rapidly, with the remaining component being completely resistant to α-amylase hydrolysis.

According to the U.S. food regulations, "[n]on-digestible dietary fiber will be determined by the method 'Total Dietary Fiber in Foods, Enzymatic Gravimetric Method, First Action, in the Journal of the Association of Official Analytical Chemists (JAOAC) 68: 399, 1985, as amended in JAOAC 69: 370, 1986.' [21 CFR 101.9(c)(3)]". The dietary fiber that is recovered with this gravimetric procedure includes celluloses, hemicelluloses, pectins, other non-starch polysaccharides, lignins, and a portion of RS. Not detected by this method are some nondigestible polysaccharides, such as inulin and polydextrose, which are soluble in 78 to 80 percent ethanol. In addition, this method would not quantify all of the RS fractions of the chemically modified starches evaluated in this study because this RS would remain soluble in 78 to 80 percent ethanol. Thus, this material would be removed during the filtration process when analyzing for dietary fiber. Because a significant amount of chemically modified starch is not digested or quantified as RS, alternative methods of its determination/quantification must be sought to improve and allow meaningful nutritional labeling (energy values) of food products that contain a significant proportion of chemically modified starch. The use of these ingredients (modified food starch, dextrin) may result in a higher intake of 'dietary fiber' by the U.S. population.

Our results verify the slowly digestible properties of raw cornstarch. The level of SDS in cooked, chemically modified starches was small; however,

these modifications resulted in an increase in the levels of RS. For most modified starches, as the degree of modification increased, the level of digestible starch decreased, suggesting an increase in the amount of RS. The present *in vitro* procedure was not appropriate for the quantification of RS in chemically modified starch ingredients. The use of chemically modified starches allows for the fortification of many foods with a dietary fiber-like material. However, alternative methods of RS quantification should be sought in order to allow appropriate nutritional labeling for foods containing these ingredients. Because of their increased RS composition, the use of chemically modified starch ingredients should attenuate the glycemic response and decrease the caloric density of foods containing them. Caution must be used when interpreting starch values for foodstuffs because most enzymatic-based methods are probably underestimating the content of RS, and some may be overestimating the availability of starch digestion in the small intestine. Appropriate clinical studies are necessary to validate these hypotheses. This study highlights the need for methodologies to accurately and reproducibly quantify chemically modified starches, which are being continually used in the food supply.

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Table 3.1. Identification and Description of Starch Ingredients[a]

ingredient	description[b]	degree of substitution[c]	crosslinking[d]
waxy starch			
WS	unmodified waxy starch (0% amylose)	0	0
hXL-WS	highly crosslinked, waxy starch	0	0.00093
hPOlXL-WS	highly propylene oxide-substituted, lightly crosslinked, waxy starch	0.12	0.000085
hO-WS	highly oxidized, waxy starch	0	0
mD-WS	moderately converted dextrin, waxy starch	0	0
hD-WS	highly converted dextrin, waxy starch	0	0
dull waxy starch			
dWS	unmodified dull waxy starch (0% amylose)	0	0
mPOmXL-dWS	moderately propylene oxide-substituted, moderately crosslinked, dull waxy starch	0.07	0.00037
common starch			
CS	unmodified common starch (27% amylose)	0	0
lD-CS	lightly converted dextrin, common starch	0	0
mD-CS	moderately converted dextrin, common starch	0	0
hD-CS	highly converted dextrin, common starch	0	0
high amylose starch			
hAS	unmodified high amylose starch (50% amylose)	0	0
hPO-hAS	highly propylene oxide-substituted, high amylose starch	0.15	0

(continued)

Table 3.1 (continued)

[a] All chemical modifications were within the legal limits allowed by the U.S. Food and Drug Administration. [b] Degree of dextrinization and oxidation as determined by ingredient manufacturer. [c] Degree of substitution is the average number of hydroxyl groups per glycosyl unit that have been derivatized by etherification. [d] Crosslinking values represent the amount (g/100g) of phosphorous oxychloride ($POCl_3$) added to the starch during processing.

Table 3.2. Starch Concentration and In Vitro Hydrolysis of Chemically Modified Corn Starch Ingredients (Exp. 1)

	"raw" starch			5% starch solution[a]		10% starch solution[a]	
	Thivend[b]	Muir & O'Dea[c]		Muir & O'Dea[c]		Muir & O'Dea[c]	
ingredient[c]	starch, %	DS, %	RS, %	DS, %	RS, %	DS, %	RS, %
waxy starch							
WS	96.8	98.0	0.0	101.9	0.9	96.1	0.0
hXL-WS	97.8	97.6	1.5	94.2	1.7	99.1	0.0
hPOlXL-WS	58.9	34.4	27.1	62.0	0.8	56.7	0.2
hO-WS	99.4	92.5	1.3	91.3	1.2	84.2	0.0
hD-WS	49.1	45.6	0.3	45.7	0.9	44.9	0.0
dull waxy starch							
dWS	96.3	90.6	0.9	90.6	1.6	95.2	0.0
mPOmXL-dWS	72.5	68.7	4.4	69.4	1.6	83.9	0.0
common starch							
lD-CS	90.1	87.6	1.0	85.4	3.0	86.2	0.3
mD-CS	84.2	77.8	0.2	75.1	3.2	77.2	0.0
hD-CS	63.8	63.4	0.6	64.9	1.0	62.3	0.1
high amylose starch							
hAS	92.5	47.1	51.9	72.8	16.6	77.2	15.4
hPO-hAS	58.9	53.8	7.6	59.7	2.3	58.1	0.2
lab reference controls							
raw cornstarch	97.9	83.9	9.0	N/A[d]	N/A	N/A	N/A
raw potato starch	95.0	38.3	65.7	N/A	N/A	N/A	N/A

(continued)

Table 3.2 (continued)

[a] For cooking, raw starch ingredients were made into 5 and 10% (wt/vol) starch solutions with water, then cooked by autoclaving 10 min at 1.055 kg/cm^2 and 121°C. [b] Total starch, expressed as a percentage of ingredient dry matter, determined by the method of Thivend et al. (1972). [c] DS = digestible starch, and RS = resistant starch, expressed as a percentage of ingredient dry matter, determined by the method of Muir and O'Dea (1992, 1993). All values are means of duplicate samples, and samples were re-analyzed if duplicates differed by > 5%. [c] Identification and description of starch ingredients are presented in Table 3.1. [d] N/A, not applicable.

Table 3.3. In Vitro Hydrolysis Over Time of Chemically Modified Cornstarch Ingredients (Exp. 2)[a]

ingredient [b]	incubation time, hours				
	0	2.5	5	10	15
waxy starch					
WS	1.1	88.5	87.6	86.3	90.6
hXL-WS	1.0	87.5	88.0	89.1	96.3
hPOlXL-WS	1.0	54.5	56.0	52.9	60.2
hO-WS	1.5	84.0	82.9	86.9	89.1
mD-WS	0.7	81.8	81.6	79.2	83.0
hD-WS	0.9	46.1	47.5	42.2	49.6
dull waxy starch					
dWS	0.7	89.7	87.1	88.5	91.2
mPOmXL-dWS	1.1	67.8	64.0	68.1	66.2
common starch					
lD-CS	0.2	86.1	86.3	82.6	90.1
mD-CS	0.9	73.9	74.6	71.0	68.9
hD-CS	0.1	58.0	59.2	54.1	60.6
high amylose starch					
hAS	0.9	77.2	77.3	76.2	83.7
hPO-hAS	0.3	59.3	60.0	54.3	57.5
lab reference controls					
raw cornstarch	0.0	67.3	69.8	78.3	86.0
raw potato starch	0.0	32.6	29.7	38.0	37.6

(continued)

Table 3.3 (continued)

[a] Starch ingredients were made into 10% solutions (wt/vol) and processed into 1-L ready-to-hang bottles. Hydrolyzed starch, expressed as a percentage of ingredient dry matter, determined by the method of Muir and O'Dea (1992, 1993; α-amylase and amyloglucosidase enzyme system); a 15-h *in vitro* incubation has been shown to correlate with the amount of starch escaping digestion in the small intestine (Muir and O'Dea, 1993). Time 0 values represent free glucose in samples. All values are means of duplicate samples, and samples were re-analyzed if duplicates differed by > 5%. [b] Identification and description of starch ingredients are presented in Table 3.1.

Table 3.4. In Vitro Hydrolysis Over Time of Chemically Modified Cornstarch (Exp. 3)[a]

ingredient [b]	time, hours					
	0	0.5	1	1.5	2	2.5
waxy starch						
hXL-WS	0.8	90.9	92.8	97.9	97.9	99.9
hPOlXL-WS	0.9	56.1	52.5	58.1	57.0	57.6
hO-WS	1.6	87.0	87.7	94.0	94.6	96.1
mD-WS	0.7	82.3	81.5	89.5	90.2	90.4
common starch						
ID-CS	0.2	83.0	92.7	95.3	94.7	95.8
mD-CS	0.9	76.7	76.3	79.3	77.7	78.4
hD-CS	0.1	60.5	60.4	59.9	61.2	62.4
high amylose starch						
hPO-hAS	0.3	62.4	63.4	64.1	63.6	63.8
lab reference controls						
raw cornstarch	0.0	35.4	46.1	50.9	62.2	63.5
raw potato starch	0.0	29.5	27.4	28.9	22.6	25.3

[a] Starch ingredients were made into 10% solutions (wt/vol) and processed into 1-L ready-to-hang bottles. Hydrolyzed starch, expressed as a percentage of ingredient dry matter, determined by the method of Muir and O'Dea

(continued)

Table 3.4 (continued)

(1992, 1993; α-amylase and amyloglucosidase enzyme system); a 15-h *in vitro* incubation has been shown to correlate with the amount of starch escaping digestion in the small intestine (Muir and O'Dea, 1993). Time 0 values represent percent free glucose in samples. All values are means of duplicate samples, and samples were re-analyzed if duplicates differed by > 5%. [b] Identification and description of starch ingredients are presented in Table 3.1.

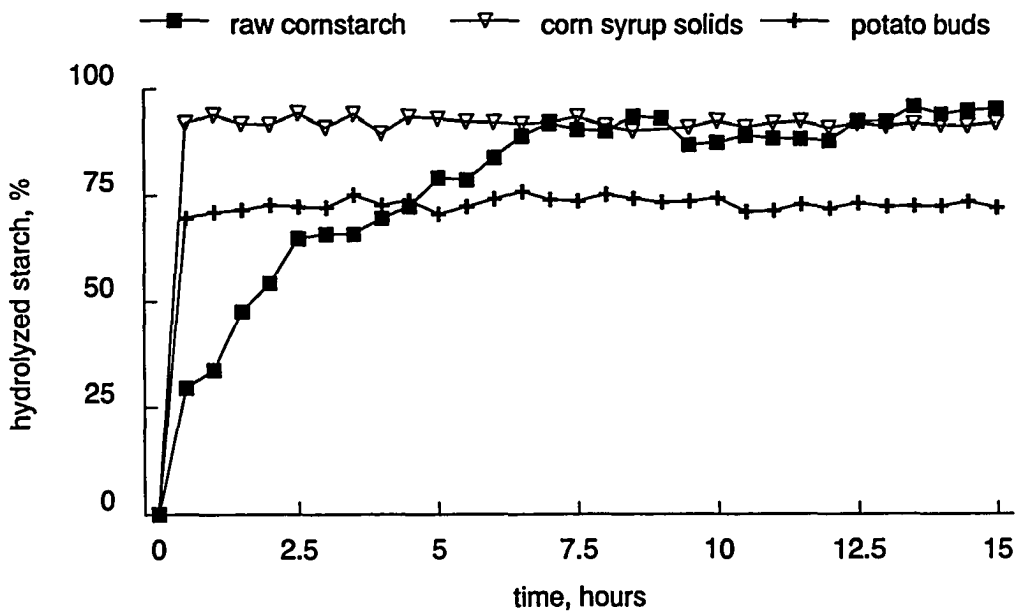

Figure 3.1. *In vitro* hydrolysis of raw starches

CHAPTER 4

COMPARISON OF THE GLYCEMIC EXCURSION OF RAW CORNSTARCH WITH AN ORAL GLUCOSE TOLERANCE TEST IN NONDIABETIC HEALTHY ADULTS

INTRODUCTION

Carbohydrates typically constitute the largest percentage of daily caloric intake; thus, knowing their effects upon glycemic response is of primary importance, especially for the nutritional management of people with glucose intolerance. Because of the discrepancy in the glycemic response of different carbohydrates and high carbohydrate foods, Jenkins et al. (1981) proposed a method of ranking foods on the basis of the incremental blood glucose responses they produce for a given amount of carbohydrate (i.e., the glycemic index). The use of the glycemic index may have a practical application in the evaluation of carbohydrate ingredients and product prototypes that would be used in the formulation of nutritional products to normalize blood glucose concentrations in people with glucose intolerance.

Although the methodology and criteria for measuring glycemic response (Klimt et al., 1969) and glycemic index (Wolever et al., 1991) are standard,

slight differences in methodology exist. For example, blood glucose concentration may be measured in whole blood, plasma, or serum. In addition, blood samples may be obtained via venipuncture (venous blood) or finger-prick (capillary blood). Differences between venous and capillary blood glucose concentrations are expected as evidenced by their different diagnostic values for the OGTT (WHO, 1985). Capillary blood glucose values are higher because venous samples represent blood returning from the capillary beds where glucose has been taken up by peripheral tissues. Thus, capillary blood samples may be more sensitive to the analysis of glycemic response to dietary carbohydrates. Wolever and Bolognesi (1996) found that measuring blood glucose on finger-prick capillary blood samples reduced the variability in the glycemic index compared to blood samples obtained via venipuncture.

The objective of this dissertation was to evaluate and formulate a carbohydrate system (package) for use in liquid medical nutritional products that will not exacerbate the postprandial glycemic response. Before initiating *in vivo* experiments in human subjects, an *in vivo* MGTT utilizing finger-prick as the means to obtain blood samples for glucose analysis needed to be validated. The present study was conducted to validate finger-pricking methodology by evaluating the glycemic response of healthy nondiabetic adult subjects fed two carbohydrates that differ greatly in their glycemic response. The primary objective of this study was to compare the glycemic response of 75 g of RCS with a 75-g OGTT. A secondary objective was to evaluate the effects of an acute challenge of 75 g of RCS on subjective gastrointestinal tolerance.

MATERIALS AND METHODS

In Vitro Starch Hydrolysis. Because differences in the glycemic response to dietary starch are directly related to rate of starch digestion (O'Dea et al., 1981), an *in vitro* starch hydrolysis method was used to predict the extent of starch digestion over time in the small intestine. The percentage of digestible starch was determined as described by Wolf et al. (1999), who used a modification of the method of Muir and O'Dea (1992, 1993; α-amylase and amyloglucosidase enzyme system). A 15-h *in vitro* incubation has been shown to correlate with the amount of starch escaping digestion in the small intestine (Muir and O'Dea, 1993). Raw cornstarch (Agro, CPC International, Englewood Cliffs, NJ) was obtained from a local grocery.

Subjects. A total of 15 healthy nondiabetic (fasting plasma glucose value of < 110 mg/dL (6.1 mmol/L); American Diabetes Association, 1997) volunteers (11 men and 4 women) were recruited. Subjects had a mean (± SE) age of 40 ± 2 y, weight of 79.3 ± 3.8 kg, and body mass index of 26 ± 1 kg/m^2. Fourteen were self-described as Caucasian; and one, as Asian. Subjects did not have active gastrointestinal or metabolic diseases, a first-degree family history of diabetes mellitus or glucose intolerance, recent infection, surgery or corticosteriod treatment. No subjects were receiving oral contraceptives. All subjects gave written informed consent to the protocol, which was approved by the Ross Products Division Review Board (Columbus, OH).

Dietary Treatments. Two dietary treatments were evaluated in the study: (1) RCS: raw cornstarch (Agro) supplemented to provide 75 g of glucose after digestion (due to hydration of glucose after hydrolysis of the starch polymer) per 296 mL serving and 2) OGTT: 75 g of glucose (Glucose Tolerance Drink, Criterion Sciences, Riverdale, NJ) per 296 mL serving. Raw cornstarch was mixed immediately before consumption with a hand held blender (Rival Ultra Blend Model 950, Rival Mfg. Co. Kansas City, MO).

Experimental Design. The study was a double-blind crossover design in which subjects participated in two 4-h MGTT on separate occasions. Subjects were randomly assigned to one of two treatment sequences. After an overnight fast, subjects consumed either the RCS or OGTT product. To ensure that subjects had similar glycogen stores on the two test days, they were instructed to consume a high-carbohydrate diet (goal 300 g/day, minimum 150 g/day) for 3 d before each MGTT and also were asked to avoid exercise for 24 h before the experiment. On the evening before each MGTT, all subjects consumed a low-residue dinner consisting of one 8 fl oz (237 mL) can chocolate Ensure Plus® with additional Honey Graham Crunch Ensure® Bars to provide one-third of each subject's individual daily caloric requirement as estimated by the Harris-Benedict equation multiplied by an activity factor of 1.3 (Harris and Benedict, 1919). After their low-residue evening meal, subjects were instructed to fast overnight, during which they were allowed to consume only water. Smoking was prohibited. Subjects returned within 14 d for repeat analysis with the appropriate crossover treatment. Subjects were allowed ice chips (300 g)

during each 4-h test. All subjects were recruited and enrolled from one study site.

Blood Glucose Analysis. After an overnight fast (mean of 13 h, range: 11 to 15 h), a finger-prick capillary blood sample was obtained after 30 min of rest and immediately analyzed for plasma glucose. Subjects then consumed the appropriate test meal within 10 min. Finger-prick capillary blood was obtained at 15, 30, 60, 90, 120, 150, 180, 210, and 240 min postprandial. Capillary blood was measured for plasma glucose by the glucose oxidase method utilizing a Precision·G Blood Glucose Testing System (Medisense, Inc., Bedford, MA). The precision of this testing system for the range of values obtained is 3.4 to 3.7% (coefficient of variation), as reported by the manufacturer.

Gastrointestinal Tolerance. Using a questionnaire, subjects were asked to report the frequency and intensity of the following symptoms: nausea, cramping, distention, and flatulence for the 24-h period immediately following consumption of the test material. Intensity was set to a 100-mm line scale (0 representing "absent" and 100 "severe") as was frequency (50 representing "usual", 0 "less than usual", and 100 "more than usual"). Subjects placed a single perpendicular slash mark across the 100-mm horizontal line to indicate their scores for each of these variables of frequency and intensity. A score of 5 or less was considered to be not physiologically meaningful.

Breath Hydrogen and Methane. A subset of seven subjects was evaluated for carbohydrate malabsorption by measuring their end-alveolar hydrogen and methane concentrations hourly for 4 h after they ingested their

test meal. The concentration of carbon dioxide, hydrogen and methane in breath samples were analyzed by gas chromatography (Microlyzer Gas Analyzer, model SC; Quintron Instruments, Milwaukee, WI). The observed hydrogen and methane values were corrected for atmospheric contamination of alveolar air by normalizing the concentrations of observed carbon dioxide to 5.5%.

Study Variables. The primary variable for this study was incremental (i.e., baseline adjusted) peak plasma glucose response. Secondary variables for this study were: net incremental AUC for plasma glucose, relative glycemic response, and subjective gastrointestinal tolerance factors. Exploratory variables for this study were mean incremental change from baseline in plasma glucose at 15, 30, 60, 90, 120, 150, 180, 210, and 240 min postprandial.

Calculations. Net incremental AUC (Gannon et al., 1989; Wolever, 1989) for plasma glucose was calculated as (AUC 0 to 120 min postprandial) – (120 × baseline glucose concentration at 0 min). The areas after the challenge were calculated with the trapezoid rule. Relative glycemic response was calculated as (net incremental AUC for RCS / net incremental AUC for OGTT) × 100 (also known as relative glucose area; Gannon and Nuttall, 1987).

Statistical Methods. Data obtained during the two testing days for the glucose parameters and symptoms of gastrointestinal tolerance were fit to a two-period crossover model. The residuals obtained from fitting the two-period crossover model were examined for evidence of a normal distribution with the Shapiro-Wilk test. If the assumption of normality was rejected ($P < 0.05$ for the Shapiro-Wilk test), a nonparametric model was used. The effects of sequence, period, and treatment were examined by two-sided t-test or two-sided Wilcoxon rank sum test, as appropriate (SAS version 8.0, SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Our *in vitro* hydrolysis data support the hypothesis that RCS is a SDS that will attenuate the postprandial glycemic response (Table 4.1). Raw cornstarch was slowly hydrolyzed in this *in vitro* assay, indicating that it would be a SDS *in vivo*. Wolf et al. (1999) showed that RCS has a slow rate of digestion *in vitro*, which agrees with its use as a SDS *in vivo* (Chen et al., 1984; Kaufman et al., 1995; Wolfsdorf and Crigler, 1997).

The mean fasting plasma glucose concentration was not different between treatments (93.5 ± 1.6 and 93.2 ± 2.5 mg/dL for OGTT and RCS, respectively). Table 4.2 presents data for mean peak incremental change from baseline in plasma glucose and net incremental AUC for plasma glucose. Mean peak incremental change from baseline in plasma glucose was reduced 58% ($P < 0.01$) for the RCS treatment. Over the 4-h experiment, the net incremental AUC for plasma glucose was not different between treatments, although from 0 to 180 min, the net incremental AUC was reduced 53% ($P <$

0.05) by RCS compared to OGTT. The relative glycemic response at 180 min was calculated to be 70 ± 19. The glycemic excursion is presented in Figure 4.1. Raw cornstarch reduced ($P < 0.01$) the postprandial incremental change from baseline in plasma glucose at 15, 30, and 60 min compared to the OGTT. In addition, RCS maintained plasma glucose levels near basal values at 180, 210, and 240 min, whereas the OGTT values fell below baseline (treatment difference $P < 0.01$), reaching moderate hypoglycemia when subjects consumed OGTT.

The clear difference in the postprandial glycemic response to an OGTT and RCS (Figure 4.1) support the finger-pricking methodology as a means to evaluate glycemic response. Castro et al. (1970) tested the postprandial glycemic response of several glucose loads in healthy subjects. They reported an incremental peak plasma glucose concentration of 64.4 mg/dL when subjects were given a 75-g OGTT. This observation closely matches our observed incremental peak plasma glucose concentration of 62.9 mg/dL. Elliott et al. (1993) found an incremental peak plasma glucose concentration of ~68 mg/dL when healthy volunteers were challenged with 75 g of glucose. Falko et al. (1980) evaluated the serum glucose response to a 75-g OGTT fed to healthy volunteers. They found an incremental peak serum glucose concentration of ~50 mg/dL. Castro et al. (1970) and Falko et al. (1980) reported that the blood glucose concentration fell below fasting (baseline) levels near 150 min postprandial, which is similar to the observation of the present experiment (Figure 4.1), whereas Elliott et al. (1993) observed that the plasma glucose

concentration fell below baseline at 120 min. These similarities in the postprandial glycemic response to a 75-g OGTT in healthy subjects support the use of our finger-pricking methodology.

Optimally, a SDS should be completely digested in the small intestine so that it would have minimal effects on gastrointestinal tolerance (e.g., flatulence). The extent of *in vitro* hydrolysis by α-amylase and amyloglucosidase at 15 h was high (~80%). Minimal effects on gastrointestinal symptoms (intensity and frequency of nausea, cramping, distention, and flatulence) were noted for both products, with no clinically significant differences between products (data not shown). No adverse events were documented for subjects consuming either product. Breath hydrogen and methane data are presented in Table 4.3. No clinically significant differences were found between treatments. These data point toward a high degree of digestion in the small intestine or a lack of fermentation by the colonic microflora (Olesen et al., 1992). Furthermore, these data document the excellent tolerance of healthy nondiabetic adult subjects given an acute challenge of 75 g of RCS.

In conclusion, RCS provides a means of maintaining plasma glucose levels by reducing the early phase excursion and by appropriately maintaining the later phase excursion, thus avoiding hypoglycemia in healthy adult subjects. This *in vivo* study supports the hypothesis that RCS is a SDS. In addition, it corroborates the use of RCS as nutritional therapy for the prevention of nighttime hypoglycemia (Chen et al., 1984; Kaufman et al., 1995; Wolfsdorf and Crigler, 1997). Furthermore, this study validates the use of finger-prick capillary

blood samples as a means to evaluate glycemic response, confirming the results of Wolever and Bolognesi (1996). Raw cornstarch is an excellent SDS positive control for which to compare other carbohydrates or carbohydrate blends.

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Table 4.1. In Vitro Hydrolysis of Raw Cornstarch (RCS) Over Time of Incubation[a]

	time of incubation (hours)					
ingredient	0	0.5	1	2.5	5	15
	% starch hydrolyzed (dry matter basis)					
RCS	0.1 ± 0.1	35.3 ± 2.2	36.2 ± 2.7	43.7 ± 1.5	58.3 ± 2.7	79.5 ± 1.9

[a] Values are means ± SEM, n = 4. Hydrolyzed starch, expressed as a percentage of ingredient dry matter; a 15-h *in vitro* incubation has been shown to correlate with the amount of starch escaping digestion in the small intestine (Muir and O'Dea, 1993). Time 0 values represent free glucose in samples.

Table 4.2. Glycemic Response for Subjects Consuming Glucose or Raw Cornstarch [a]

Item	OGTT	RCS
incremental peak glucose (mg/dL)	62.9 ± 8.9	26.7 ± 2.9[b]
180 min net incremental AUC ($mg \cdot min \cdot dL^{-1}$)	4763 ± 822	2236 ± 423[c]
240 min net incremental AUC ($mg \cdot min \cdot dL^{-1}$)	3628 ± 739	2540 ± 601

[a] Mean ± SEM, n = 15. Fasting plasma glucose concentrations were 93.5 ± 1.6 and 93.2 ± 2.5 mg/dL for subjects consuming 75 g of glucose from an oral glucose tolerance test (OGTT) and raw cornstarch (RCS), respectively. To convert glucose mg/dL to mmol/L, multiply mg/dL by 0.0555. Glucose of 90 mg/dL = 5.0 mmol/L. [b] ($P < 0.01$). [c] ($P = 0.02$).

Table 4.3. Breath Hydrogen and Methane Responses Over Time after Subjects Consumed a 75-g OGTT or 75 g of RCS[a]

	time (hours)				
treatment	0	1	2	3	4
	hydrogen (ppm)				
OGTT	2.3 ± 0.2	2.4 ± 0.3	2.1 ± 0.3	1.9 ± 0.3	2.0 ± 0.5
RCS	6.6 ± 2.2	3.4 ± 1.0	2.9 ± 0.6	2.6 ± 0.4	2.9 ± 0.5
	methane (ppm)				
OGTT	12.3 ± 4.4	6.0 ± 3.0	10.3 ± 2.2	13.9 ± 2.6	15.3 ± 3.2
RCS	7.7 ± 3.7	5.6 ± 3.4	8.1 ± 2.8	10.6 ± 3.5	9.6 ± 3.4

[a] Values are means ± SEM, n = 7.

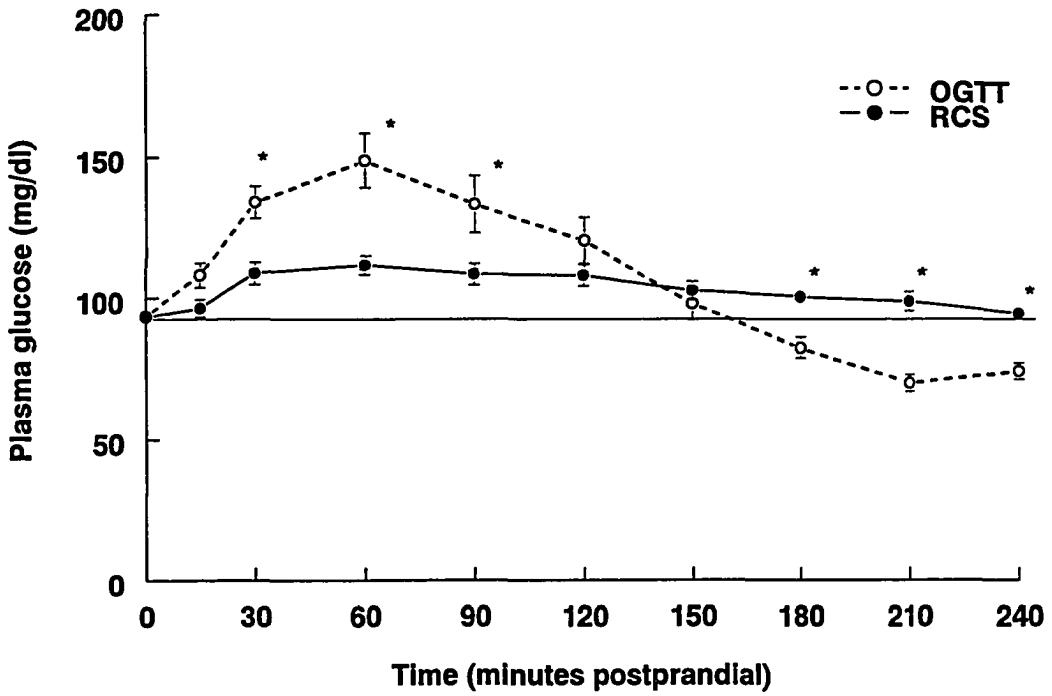

Figure 4.1. Plasma glucose response after 15 subjects consumed 75-g glucose (OGTT) or 75 g of raw cornstarch (RCS). Values are means ± SEM. Fasting plasma glucose concentrations were 93.5 ± 1.6 and 93.2 ± 2.5 mg/dL for subjects consuming OGTT and RCS, respectively. Horizontal line at 93 mg/dL represents fasting plasma glucose concentration. To convert glucose mg/dL to mmol/L, multiply mg/dL by 0.0555. Glucose of 90 mg/dL = 5.0 mmol/L. * $P <$ 0.01.

CHAPTER 5

GLYCEMIC RESPONSE TO A FOOD STARCH ESTERIFIED BY 1-OCTENYL SUCCINIC ANHYDRIDE IN HUMANS

INTRODUCTION

The prevalence of physician-diagnosed diabetes in U.S. adults is estimated to be 5.1% (Harris et al., 1998), and it continues to be a major health problem because of the increasing frequency of obesity and sedentary lifestyles. Many medical experts believe that there is potential for the long-term management of the complications associated with diabetes through tight glycemic control (American Diabetes Association, 2000). Intensive therapy to control blood glucose has been shown to improve quality of life in people with diabetes (DCCT Research Group, 1993; UKPDS Group, 1998), and dietary choices can have a profound impact upon glycemic control (Brand et al., 1991). Dietary recommendations have been made to replace saturated fat with complex carbohydrates (e.g., starch); however, the glycemic responses to different dietary carbohydrate sources are not the same (Crapo et al., 1977; Jenkins et al., 1980).

Numerous chemically modified food starches are available as ingredients for processed foods and are used to enhance physical and nutritional stability of

the product. Chemical treatments that are currently allowed and used to produce modified starches for food use in the United States include esterification, etherification, acid modification, bleaching, and oxidation (Whistler and BeMiller, 1997). Multiple modifications of starch are a common occurrence for making starches with specific applications in the food industry. Wolf et al. (1999) postulated that the use of these modifications might allow for the production of a slowly digested starch that could be used for the treatment of certain medical modalities (e.g., glycogen storage disease and diabetes mellitus) by improving the postprandial glycemic excursion (i.e., prevention of hyperglycemia and hypoglycemia).

Wolf et al. (1999) found that the etherification of waxy and high-amylose cornstarch with propylene oxide decreased the extent of starch digestion *in vitro.* In the case of dextrinization, as the degree of modification increased, the level of digestible starch decreased, suggesting an increase in the amount of resistant starch. They postulated that the use of chemically modified starch should attenuate the glycemic response. In healthy young men, Raben et al. (1997) evaluated the glycemic response to isocaloric meals containing 50 g of modified potato starch. The glycemic and insulinemic responses were similar between a 1 to 2% acetylated potato starch and an unmodified potato starch. In contrast, β-cyclodextrin supplementation at the level of 2% flattened the glucose curve and lowered the insulin response. On the basis of these two studies, the glycemic response to modified starch appears to be dependent upon both the type and level of modification.

Starch esterified by octenyl succinic anhydride (OSA) has been used by the food industry for over 30 years. Esterification of starch with OSA provides hydrophobic domains that enhance the emulsifying ability of starch. As a result, OSA-modified starch improves the mixing characteristics and stability of elemental or protein-hydrolysate formulas in which protein is absent or the natural emulsifying properties of milk or vegetable proteins have been destroyed by partial protein hydrolysis (Mahmoud, 1987). Toxicology studies have shown that OSA-modified starch is safe when fed up to 15 g/kg of body weight/d in rats (Buttolph and Newberne, 1980). Even though OSA-modified starch has been in the food supply for many years, limited clinical data on this ingredient are available (Kelley, 1991), and the glycemic response of this modified starch is unknown.

We postulated that OSA substitution should interfere with the binding of α-amylase, thus decreasing the rate and/or extent of starch digestion. The formulation of novel products with carbohydrates of low glycemic index should, therefore, enhance the use of nutrition as adjunctive therapy for people with diabetes mellitus. The primary objective of this study was to determine the glycemic response to a food starch esterified by OSA in healthy nondiabetic adult subjects. A secondary objective was to evaluate the effects of an acute challenge of 25 g of OSA-substituted starch on subjective gastrointestinal tolerance.

MATERIALS AND METHODS

In Vitro Starch Digestion. Because differences in the glycemic response to dietary starch are directly related to rate of starch digestion (O'Dea et al., 1981), an *in vitro* starch digestion method was used to predict the extent of starch digestion over time in the small intestine. The percentage of digestible starch was determined as described by Wolf et al. (1999), who used a modification of the method of Muir and O'Dea (1992, 1993; α-amylase and amyloglucosidase enzyme system). A 15-h *in vitro* incubation has been shown to correlate with the amount of starch escaping digestion in the small intestine (Muir and O'Dea, 1993).

The extent of OSA-substituted starch (Capsul, dextrose equivalency ≅ 3, degree of substitution ≅ 0.07; National Starch and Chemical Co., Bridgewater, NJ) hydrolysis over time was compared with the extent of digestion of common cornstarch (Agro, CPC International, Englewood Cliffs, NJ). Both starches were tested in a raw and cooked state. For cooking, 0.1 g of carbohydrate was suspended in 1 mL of water and autoclaved for 30 min at 2.1 kg/cm^2 and 121 °C. Immediately after autoclave treatment, starch samples were cooled in a cold water bath for 10 min and then used in the *in vitro* procedure as described by Wolf et al. (1999).

Subjects. A total of 30 healthy nondiabetic (fasting plasma glucose value of < 6.1 mmol/L; American Diabetes Association, 1997) volunteers (12 men and 18 women) were recruited. Subjects had a mean (± SE) age of 43 ± 3 y (range: 20 to 74 y), weight of 68 ± 2 kg (range: 50 to 93 kg), and body mass index of

24.1 ± 0.6 kg/m^2 (range: 19.4 to 32.2 kg/m^2). Twenty-five were self-described as Caucasian, four as Asian or Pacific Islander, and one as other. Subjects did not have active gastrointestinal or metabolic diseases, a first-degree family history of diabetes mellitus or glucose intolerance, recent infection, surgery or corticosteriod treatment. No subjects were receiving oral contraceptives. During subject screening, a fasting blood draw was obtained for determination of routine serum chemistry values (St. Michael's Hospital, Toronto, ON, Canada). All subjects gave written informed consent to the protocol, which was approved by the Western Institutional Review Board (Olympia, WA).

Dietary Treatments. Two dietary treatments were evaluated in the study (Table 5.1): (1) Glucose: 25 g of dextrose (Corn Products International Inc., Bedford Park, IL) per 238 g serving and (2) OSA: 25 g of 1-octenyl succinic anhydride-substituted starch (Capsul; National Starch and Chemical Company) per 241 g serving. Details regarding the starch processing parameters are proprietary industry trade secrets. Ingredients were made into a 10.4% solution with water, filled into 250-mL glass bottles, and terminally sterilized (Ross Products Division of Abbott Laboratories, Columbus, OH). Sodium citrate and citric acid were added as buffers to both products in order to prevent the isomerization of glucose in the Glucose product. The products were incorporated into pourable solutions that were consumed as a beverage (viscosity < 5 mPa·sec).

Experimental Design. The study was a double-blind crossover design in which subjects participated in two 2-h meal glucose tolerance tests on separate

occasions. Subjects were randomly assigned to one of two treatment sequences. After an overnight fast, subjects consumed either the Glucose or OSA product. To ensure that subjects had similar glycogen stores on the two test days, subjects were instructed to consume a high-carbohydrate diet (goal 300 g/day, minimum 150 g/day) for 3 d before each meal glucose tolerance test and also were asked to avoid exercise for 24 h before the experiment. On the evening before each meal glucose tolerance test, all subjects consumed a low-residue dinner consisting of one 8 fl oz (237 mL) can of chocolate Ensure Plus® with additional Honey Graham Crunch Ensure® Bars to provide one-third of each subject's individual daily caloric requirement as estimated by the Harris-Benedict equation multiplied by an activity factor of 1.3 (Harris and Benedict, 1919). After their low-residue evening meal, subjects were instructed to fast overnight, during which they were allowed to consume only water. Smoking was prohibited. Subjects returned within 14 d (range 5 to 14 d) for repeat analysis with the appropriate crossover treatment. Subjects were allowed water (250 mL) during each 2-h test. All subjects were recruited and enrolled from one study site.

Blood Glucose Analysis. A fasting (mean of 13 h, range 10 to 14.5 h) finger-prick capillary blood sample was obtained and collected into a fluoro-oxalate tube after 30 min of rest. Subjects then consumed the appropriate test meal within 10 min. Finger-prick capillary blood was obtained at 15, 30, 45, 60, 90, and 120 min postprandial. Samples were stored at -20° C for a maximum of 3 d until analysis of whole blood glucose. Capillary blood glucose was

measured by the glucose oxidase method using a YSI analyzer (model YSI 2300 STAT PLUS, Yellow Springs Instruments, Yellow Springs, OH).

Gastrointestinal Tolerance. Using a questionnaire, subjects were asked to report the frequency and intensity of the following symptoms: nausea, cramping, distention, and flatulence for the 24-h period immediately following consumption of the test material. Intensity and frequency were set to a 100-mm line scale (0 representing "absent" and 100 "severe" and 0 representing "usual" and 100 "more than usual," respectively). Subjects placed a single perpendicular slash mark across the 100-mm horizontal line to indicate their scores for each of these variables of frequency and intensity. A score of 5 or less was considered to be not physiologically meaningful.

Study Variables. The primary variable for this study was incremental (i.e., baseline-adjusted) peak blood glucose response. Secondary variables for this study were net incremental area under the curve (AUC) for blood glucose, relative glycemic response, and subjective gastrointestinal tolerance factors. Exploratory variables for this study were mean incremental change from baseline in blood glucose at 15, 30, 45, 60, 90, and 120 min postprandial.

Calculations. Net incremental AUC (Gannon et al., 1989; Wolever, 1989) for glucose was calculated as (AUC 0 to 120 min) – (120 × baseline blood glucose concentration at 0 min). The areas after the challenge were calculated with the trapezoid rule. Relative glycemic response was calculated as (net incremental AUC for OSA / net incremental AUC for Glucose) x 100 (also known as relative glucose area; Gannon and Nuttall, 1987).

Statistical Methods. Prior to conducting this experiment, a power analysis was prepared utilizing the data generated in a similar study with healthy subjects (Goetz et al., 1987; unpublished observations). Peak glucose response was used as the variable to calculate power. A conservative estimated difference of 0.75 standard deviation (~20% decrease in peak glucose response) between treatments was used for the power calculation. We determined that a sample size of 30 would give 80% power (significance level of 0.05) to detect differences between treatments.

Data obtained during the two testing days for the glucose parameters and symptoms of gastrointestinal tolerance were fit to a two-period crossover model. The residuals obtained from fitting the two-period crossover model were examined for evidence of a normal distribution with the Shapiro-Wilk test. If the assumption of normality was rejected ($P < 0.05$ for the Shapiro-Wilk test), a nonparametric model was used. The effects of sequence, period, and treatment were examined by a two-sided *t* test or a two-sided Wilcoxon rank sum test, as appropriate (SAS version 8, SAS Institute, Cary, NC).

RESULTS

The extent of *in vitro* starch hydrolysis over time is presented in Table 5.2. Compared with cooked cornstarch, OSA substitution decreased the extent of starch hydrolysis by approximately 30 percentage units, indicating an increase in the amount of resistant starch. A majority of the digestible component of OSA was hydrolyzed quickly, in contrast to the extent of hydrolysis for raw cornstarch over time. Cooking OSA had minimal effects on

its extent of hydrolysis over time. On the other hand, cooking cornstarch dramatically increased its in vitro extent of hydrolysis over time by α-amylase and amyloglucosidase.

Clinical chemistry values of the subjects evaluated in this experiment are presented in Table 5.3. The mean fasting blood glucose concentrations were not different ($P > 0.20$) between treatments (4.38 ± 0.06 and 4.31 ± 0.07 mmol/L for Glucose and OSA, respectively). Table 5.4 presents data for mean peak incremental change from baseline in blood glucose and net incremental AUC for blood glucose. Mean peak incremental change from baseline and net incremental AUC for blood glucose were lower ($P < 0.01$ and $P < 0.05$, respectively) for the OSA treatment. In addition, the relative glycemic response was calculated to be 93.8 ± 11.6, indicating that OSA-substituted starch has a blunted glycemic response compared with that from Glucose. The postprandial incremental change from baseline in blood glucose was reduced ($P < 0.01$) for the OSA treatment at 15 and 30 min and tended ($P < 0.08$) to be lower at 45 min (Figure 5.1). The postprandial incremental change from baseline in blood glucose did not differ ($P > 0.20$) between treatments at 60 and 90 min, but was higher ($P < 0.01$) for the OSA treatment at 120 min.

Minimal effects on gastrointestinal symptoms (intensity and frequency of nausea, cramping, distention, and flatulence) were noted for both products, with no clinically significant differences between products (data not shown). No adverse events were documented for subjects consuming either product.

These data document the excellent tolerance of healthy nondiabetic adult subjects given an acute challenge of 25 g of OSA.

DISCUSSION

Starch digestion primarily occurs within the lumen of the small intestine. Pancreatic amylase is present in the small intestinal lumen in large amounts such that substrate rather than activity limits digestion (Fogel and Gray, 1973). It was once assumed that all starch was hydrolyzed and absorbed within the small intestine. However, it is now known that a substantial amount of starch escapes digestion in the small intestine (Englyst and Cummings, 1985; Berry, 1986). Starch that escapes digestion in the small intestine enters the colon, where, through fermentation by the colonic microflora, it may influence large bowel physiology. The rate and extent of starch digestion in the small intestine are dependent upon several intrinsic and extrinsic factors (reviewed by Englyst et al., 1992).

Processing treatments, storage conditions, chemical modification, and genetic breeding influence the digestibility of starch (Dreher et al., 1984; Wolf et al., 1999). For example, as the amount of amylose increases, the extent of *in vitro* hydrolysis decreases (Wolf et al., 1999) and the glycemic response is improved (Behall et al., 1989; Larsen et al., 1996). Limited data are available on the effects of these criteria on the rate of starch digestion. This topic is of nutritional importance because the rate of starch digestion may have therapeutic application. For example, individuals with type 2 diabetes mellitus could benefit from a foodstuff that contains slowly digested starch in order to

improve the postprandial glycemic response (i.e., prevention of hyperglycemia and hypoglycemia).

Wootton and Chaudhry (1979) found that starch substitution with hydroxypropyl or acetate (degree of substitution = 0.06 and 0.07, respectively) reduces the hydrolysis of gelatinized modified wheat starch by pancreatic amylase. We hypothesized that OSA substitution would decrease the extent and/or rate of starch digestion and thus attenuate the postprandial glycemic response. Our *in vitro* digestion data support the hypothesis that OSA will attenuate the postprandial glycemic response, but appropriate clinical studies are necessary to validate this hypothesis. The present clinical study was initiated to test this hypothesis with a food starch esterified with OSA in comparison to glucose in an oral meal glucose tolerance test in nondiabetic healthy adult subjects. The present experiment found that the postprandial glycemic excursion following a challenge with OSA-substituted starch is lower than that from glucose. Time to peak glucose concentration was similar between treatments; however, baseline-adjusted peak glucose response was reduced 19% ($P < 0.01$) by OSA. The lack of change in the time to peak glucose concentration may suggest that the difference in glycemic response is due to an overall decrease in the extent of digestion. Our *in vitro* data show that OSA substitution has a minimal effect on the extent of starch hydrolysis over time compared with raw cornstarch [a clinically effective slowly digested starch (Chen et al., 1984; Kaufman et al., 1995; Wolfsdorf and Crigler, 1997)]. These data may suggest that OSA substitution does not decrease the rate of

starch digestion, but it decreases the extent of starch digestion. Our *in vitro* digestion data that show that extent (15 h) of OSA digestion is decreased compared with cooked cornstarch support this hypothesis. Using our *in vitro* data, we would predict that 7.5 g of the 25 g OSA challenge would be resistant to digestion in the small intestine and would become available for fermentation in the large bowel. Because fermentation of carbohydrate contributes fewer calories to the host (Roberfroid et al., 1993), foodstuffs containing OSA would have a reduced caloric density. Starch substituted with OSA may enable the formulation of a product that could be used in weight management as well as improving glycemic control in people with diabetes mellitus. On the other hand, no difference in symptoms of gastrointestinal intolerance (a subjective measure of malabsorption) was noted in this double-blind study. In addition, the postprandial blood glucose response at 120 min was higher ($P < 0.05$) for OSA compared with Glucose. This difference may indicate a slow, prolonged absorption rather than malabsorption of the OSA starch. Kelley (1991) has shown that infants fed formula containing OSA-substituted starch excrete OSA and its metabolites in the urine, which suggests that the substituted units of the starch are at least partially hydrolyzed and absorbed in the small intestine. Jenkins et al. (1978) found that the addition of 14.5 g of guar gum (a soluble, viscous dietary fiber) to a 50-g glucose tolerance test improved the postprandial glycemic excursion. This effect was attributed to a delayed mouth-to-cecum transit (measured by breath hydrogen concentration) and delayed absorption (measured by urine xylose excretion). They documented a higher blood

glucose concentration at 120 min postprandial, which may be another marker of slower, prolonged absorption.

Furthermore, Jenkins et al. (1990) conducted a clinical study in healthy volunteers to specifically evaluate the rate of glucose absorption on postprandial metabolic effects. Nine subjects consumed a 50-g bolus of glucose or sipped 50 g of glucose (3.57 g/0.25 h over 3.5 h) in a crossover design. The blood glucose concentration was higher at 120 and 180 min postprandial when subjects sipped the glucose meal (simulating a slow rate of glucose absorption). Lower doses of a carbohydrate challenge, which would be the same situation as malabsorption, correspond to a quicker decrease in blood glucose, as shown in healthy volunteers (Castro et al., 1970; Sisk et al., 1970) and subjects with slightly impaired glucose tolerance (de Noble and van't Laar, 1978). These data support the hypothesis that at least part of the OSA is slowly digested. Perhaps some of the OSA is malabsorbed, but a concurrent breath hydrogen test should be conducted to test this hypothesis.

In conclusion, starch substitution with OSA attenuated the postprandial glycemic excursion compared with an equivalent glucose challenge. Because of its lower relative glycemic response, OSA-substituted starch may serve as a carbohydrate source in a medical nutritional product developed for people with diabetes. An acute challenge of 25 g of OSA was well tolerated (i.e., no clinically significant gastrointestinal discomfort) by fasting healthy adult subjects. The nutritional use of OSA-substituted starch should attenuate the

postprandial glycemic response and may decrease the caloric density of food containing it.

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Table 5.1. Ingredient Composition of Test Products[a]

ingredient	treatment	
	Glucose	OSA
	g/100 g	
dextrose	10.40	0.00
OSA	0.00	10.40
sodium citrate	0.15	0.15
citric acid	0.10	0.10
water	89.35	89.35

[a] Product fill weights were 238 ± 3 g and 241 ± 3 g for glucose and OSA, respectively.

Table 5.2. In Vitro Hydrolysis of Cornstarch and OSA-Modified Starch Ingredients[a]

ingredient	time (hours)					
	0	0.5	1	2.5	5	15
	% starch hydrolyzed (dry matter basis)					
OSA, raw	1.1	51.7	55.1	58.2	61.0	67.8
OSA, cooked	1.2	53.4	56.9	61.7	66.5	70.0
cornstarch, raw	2.0	8.5	15.5	26.5	40.3	68.6
cornstarch, cooked	2.5	70.0	81.4	91.0	98.7	99.9

[a] Values are means of triplicate samples. Hydrolyzed starch, expressed as a percentage of ingredient dry matter, determined by the method of Muir and O'Dea (1992, 1993; α-amylase and amyloglucosidase enzyme system); a 15-h *in vitro* incubation has been shown to correlate with the amount of starch escaping digestion in the small intestine (Muir and O'Dea, 1993). Time 0 values represent percent free glucose in samples.

Table 5.3. Clinical Chemistry Values of Subjects at Time of Screening

	value[a]
Hb A_{1c} (% of total hemoglobin)	5.0 ± 0.1
aspartate aminotransferase (U/L)	25.4 ± 1.2
LDL cholesterol (mmol/L)	2.94 ± 0.14
total cholesterol (mmol/L)	4.86 ± 0.15
chloride (mmol/L)	103 ± 0.3
total CO_2 (mmol/L)	29.3 ± 0.3
creatinine (µmol/L)	80.9 ± 2.5
HDL cholesterol (mmol/L)	1.37 ± 0.07
potassium (mmol/L)	4.3 ± 0.06
sodium (mmol/L)	144 ± 3.3
triglycerides (mmol/L)	1.23 ± 0.10
urea (mmol/L)	5.16 ± 0.28

[a] Mean ± SEM; n = 30 except for Hb A_{1c} for which n = 28. Hb A_{1c} normal range = 3.5 to 6.5%.

Table 5.4. Incremental Change from Baseline in Peak Blood Glucose Concentration and Net Incremental Area under the Blood Glucose Curve (AUC) for Subjects Consuming 25 g of Glucose or 25 g of OSA-Substituted Starch[a]

Item	Glucose	OSA
incremental peak glucose (mmol/L)	3.30 ± 0.19	2.66 ± 0.16[b]
net incremental AUC (mmol · min/L)	127 ± 14	107 ± 14[c]

[a] Mean ± SEM, n = 30. Fasting blood glucose concentrations were 4.38 ± 0.06 and 4.31 ± 0.07 mmol/L for subjects consuming Glucose and OSA, respectively. To convert glucose mmol/L to mg/dL, multiply mmol/L by 18.01. Glucose of 5.0 mmol/L = 90 mg/dL. [b] Different from Glucose, $P < 0.01$. [c] Different from Glucose, $P < 0.05$.

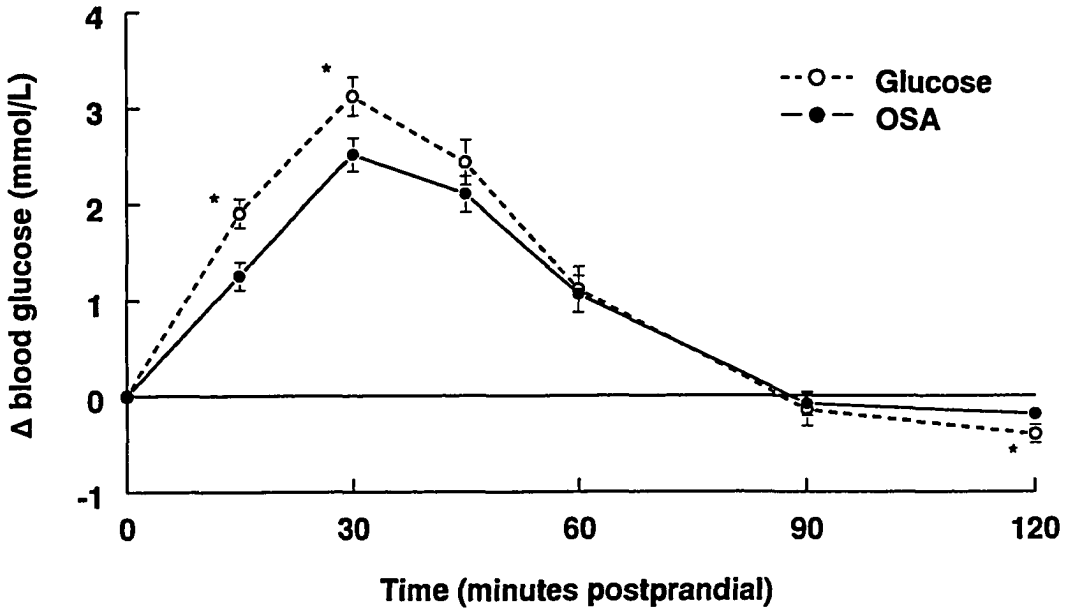

Figure 5.1. Incremental change from baseline in capillary blood glucose response for 30 subjects consuming 25 g of glucose or 25 g of 1-octenyl succinic anhydride-substituted starch (OSA). Values are mean ± SEM. Fasting blood glucose concentrations were 4.38 ± 0.06 and 4.31 ± 0.07 mmol/L for subjects consuming Glucose and OSA, respectively. To convert glucose mmol/L to mg/dL, multiply mmol/L by 18.01. Glucose of 5.0 mmol/L = 90 mg/dL. * $P < 0.05$.

CHAPTER 6

GLYCEMIC AND INSULINEMIC RESPONSES OF NONDIABETIC HEALTHY ADULT SUBJECTS TO AN EXPERIMENTAL ACID-INDUCED-VISCOSITY COMPLEX INCORPORATED INTO A GLUCOSE BEVERAGE

INTRODUCTION

The concept that dietary fiber may aid in the treatment of hyperglycemia has been suggested since the 1970's (Jenkins et al., 1978). Viscous soluble fiber (e.g., guar gum, psyllium, oat β-glucan) supplementation to test meals has been shown to effectively blunt postprandial glycemia (Braaten et al., 1991; Jenkins et al., 1978; Pastors et al., 1991). Despite the existence of some *in vivo* evidence, however, there is still considerable doubt about the efficacy of dietary fiber in the treatment of hyperglycemia (Franz et al., 1994). This doubt may exist because different types of dietary fibers have different physiological effects. For example, soluble viscous fibers generally have a greater effect on carbohydrate metabolism in the small intestine by slowing the rate of absorption (Jenkins et al., 1978), although delayed gastric emptying also may play a role (Torsdottir et al., 1991). These phenomena should decrease the rate at which glucose enters the systemic circulation and delay the postprandial rise in blood glucose. While the applicability of this concept is evident, its clinical application

is limited. Unfortunately, foodstuffs containing viscous fibers (e.g., guar gum) usually exhibit slimy mouth-feel, tooth packing, and poor palatability (Ellis et al., 1991). The overall hedonic quality of guar-containing foods can be improved by reducing the average molecular weight (e.g., through chemical hydrolysis) of the galactomannan in guar gum (Ellis et al., 1991); however, this results in a concurrent loss in clinical efficacy (Jenkins et al., 1978).

We developed an acid-induced-viscosity (acid I-V) complex that is based on the following concepts. The dietary fiber, alginate, which does not form highly viscous solutions at relatively modest concentrations, contains high levels of uronic acid groups. These groups can react with multivalent cations like calcium to cross link individual alginate molecules, producing a highly viscous solution (or gel at high alginate concentrations). We hypothesized that we could formulate a liquid product that is not viscous at neutral pH but becomes viscous upon acidification (i.e., acid-induced-viscosity). If the hydrochloric acid in the gastric chyme reacts with insoluble calcium salts like tricalcium phosphate to produce ionized calcium, the resultant free calcium ion can potentially cross-link with uronic acid groups within the alginate polymer and increase the viscosity of this chyme. Theoretically, a product formulated with the proper type of fiber and an acid-soluble calcium source should have a low viscosity at a neutral pH, be drinkable, and become highly viscous following ingestion.

Before the recommendation of a product concept, safety and physiological efficacy must be demonstrated. Murray et al. (1999) evaluated

the effect of the acid I-V complex incorporated into an enteral formula on nutrient digestion (including calcium). They showed that the acid I-V complex does not negatively affect apparent nutrient digestion and absorption in dogs cannulated in the ileum. In addition, the acid I-V complex reduced the rise in postprandial serum glucose in dogs (Murray et al., 1999). The present experiment evaluated the clinical efficacy of an acid I-V complex in a glucose-based product matrix. The primary objective of this study was to assess the ability of the acid I-V complex to attenuate the postprandial serum glucose and insulin responses in nondiabetic healthy adults.

MATERIALS AND METHODS

Experimental treatments. The acid I-V complex (alginate:citrate:calcium) was incorporated into a glucose-based beverage and compared to a control glucose-based beverage that had a similar total dietary fiber (TDF) level and initial viscosity (acid I-V and Control, respectively; Table 6.1). Ingredients were made into 31% solutions with water, filled into 250-mL glass bottles, and terminally sterilized (Ross Products Division of Abbott Laboratories, Columbus, OH). Experimental products were supplied as a liquid 250 g single serving.

Chemical composition of experimental treatments was determined in order to verify level of ingredient addition. Total solids were determined according to Association of Official Analytical Chemists methods (AOAC, 1984). Mineral composition was determined by an Inductively Coupled Plasma spectrometry method (Ross Products Division). Monosaccharides were

quantified by an ion chromatographic method utilizing a Dionex DX-300 chromatograph fitted with a PA1 CarboPac column (Dionex, Sunnyvale, CA) as described by Hogarth et al. (2000). Total dietary fiber was determined using the method of Prosky et al. (1984). Product viscosity was measured utilizing a rotational-type viscometer (Brookfield model DV-II+, #3 spindle at 60 rpm; Brookfield Instruments, Stoughton, MA) at 23 °C.

Subjects. A total of 30 healthy nondiabetic adult subjects (19 females and 11 males) were recruited. Subjects had a mean (± SE) age of 36 ± 2 y, weight of 66 ± 2 kg and body mass index between 21 and 28 (24.2 ± 0.4 kg/m^2). Seventeen were self-described as Caucasian, ten as Hispanic, and three as other. None of them were taking medication, had a family history of diabetes (first degree relatives), or suffered from a gastrointestinal disorder. Utilizing standard procedures, a complete medical exam (i.e., serum chemistry, hematology, urinalysis, physical exam, and medical history) was performed to document the subject's health prior to enrollment into the study (Diabetes and Glandular Disease Clinic, San Antonio, TX). The protocol and informed consent were reviewed and approved by the Western Institutional Review Board (Olympia, WA).

Experimental design. The experiment was conducted as a double-masked crossover study, and experimental products were tested as an oral glucose tolerance test. To ensure that subjects had similar glycogen stores on the two test days, subjects were instructed to consume a high-carbohydrate diet (minimum 200 g/day) for 3 d before each meal tolerance test and also were

asked to avoid exercise 24 h before the experiment. Subjects completed dietary histories for the 3 d before each test in order to estimate carbohydrate intake. Subjects fasted overnight (at least 12 h) prior to each test, during which they were allowed to consume only water. Smoking was prohibited. Subjects returned within 12 d (mean 7 ± 0.3 d, range 4 to 12 d) for repeat analysis with the appropriate crossover treatment. Subjects were allowed water (250 mL) during each 3-h test. All subjects were recruited and enrolled from one study site.

Blood sampling. After the overnight fast, an indwelling catheter was placed in a peripheral vein. Subjects were allowed to relax for 30 min, and a baseline blood sample was taken. Subjects then consumed the appropriate product within 10 min, and additional blood samples were taken at 15, 30, 60, 90, 120, 150, and 180 min postprandial. Serum glucose was measured by the glucose oxidase method using the Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA), and serum immunoreactive insulin was analyzed by Corning Clinical Laboratories (Irving, TX).

Gastrointestinal tolerance. Using a questionnaire, subjects were asked to report the severity of the following symptoms: nausea, cramping, distention, vomiting, burping, and reflux following each oral glucose tolerance test. Severity was set to the following scale: 0 = absent, 1 = mild, 2 = moderate, 3 = severe.

Study variables. The primary variable for this study was incremental (i.e., baseline-adjusted) peak serum glucose response. Secondary variables for

this study were: incremental peak serum insulin response, net incremental area under the curve (AUC) for serum glucose and insulin, relative glycemic response, mean incremental change from baseline in serum glucose and insulin at 15, 30, 60, 90, 120, 150, and 180 min postprandial, and subjective gastrointestinal tolerance factors.

Calculations. Net incremental AUC (Gannon et al., 1989; Wolever, 1989) for serum glucose and insulin was calculated as (AUC 0 to 180 min postprandial) – (180 × baseline serum concentration at 0 min). The areas after the challenge were calculated with the trapezoid rule. Relative glycemic response was calculated as (net incremental AUC for acid I-V / net incremental AUC for Control) ×100 (also known as relative glucose area; Gannon and Nuttall, 1987). Glycemic index was calculated as (incremental AUC for acid I-V / incremental AUC for Control) ×100 (Jenkins et al., 1981). Incremental AUC was calculated, ignoring the area below the fasting serum glucose concentration (Wolever and Jenkins, 1986).

Statistical methods. Prior to conducting this experiment, a power analysis was prepared utilizing the data generated in a similar study with healthy subjects (Goetz et al., 1987; unpublished observations). Peak glucose response was used as the variable to calculate power. A conservative estimated difference of 0.75 standard deviation (~20% decrease in peak glucose response) between treatments was used for the power calculation. We determined that a sample size of 30 would give 80% power (significance level of 0.05) to detect differences between treatments.

Data obtained during the two testing days for the glucose parameters, insulin parameters, and symptoms of gastrointestinal tolerance were fit to a two-period crossover model. The residuals obtained from fitting data to the two-period crossover model were examined for evidence of a normal distribution with the Shapiro-Wilk test. If the assumption of normality was rejected ($P < 0.05$ for the Shapiro-Wilk test), a nonparametric model was used. For the glucose and insulin data obtained at individual time points, if the assumption of normality was rejected at one or more individual time points, all time points were analyzed with nonparametric methods. The effects of sequence, period, and treatment were examined by two-sided *t* test or two-sided Wilcoxon rank sum test, as appropriate (SAS version 8.0, SAS Institute, Cary, NC).

RESULTS

Chemical composition of dietary treatments is presented in Table 6.2. Dietary treatments were formulated to deliver 75 g glucose for the oral glucose tolerance test. However, isomerization of glucose occurred during the heat processing (sterilization) of both products. Based upon product analysis, ~61 g glucose (12 g fructose and 0.5 g galactose) was present in each 250 g serving. It was decided that a 61 g glucose challenge was acceptable because 50-g carbohydrate loads are typically used in studies of glycemic response (Jenkins et al., 1981; Wolever et al., 1991). Total dietary fiber composition was similar for the Control and acid I-V treatments (4.7 and 4.4 g TDF per serving, respectively). In addition, initial product viscosity was similar for the Control and acid I-V treatments (289 and 249 mPa·sec, respectively).

Clinical characteristics of the subjects at screening are presented in Table 6.3. The baseline serum glucose value did not differ ($P > 0.10$) between groups (82.6 ± 1.3 and 84.6 ± 1.5 mg/dL for Control and acid I-V, respectively). Table 6.4 presents data for mean peak incremental change from baseline and net incremental AUC for serum glucose and insulin. There was a trend ($P < 0.06$) toward a reduced mean peak incremental change in serum glucose for the acid I-V product. In addition, the net incremental AUC was lower ($P < 0.01$) for the acid I-V product. The relative glycemic response over the 180-min test was calculated to be 23.3 ± 84.9, whereas the glycemic index over the 180-min test was calculated to be 91.2 ± 14.7. Postprandial glycemic response to the glucose challenge is graphically depicted in Figure 6.1. Data are presented as the incremental change from baseline. The postprandial change in serum glucose was not different ($P > 0.20$) between the two products at 15 and 30 min. However, there was a trend toward the blunting of glycemia at 60, 90, and 150 min postprandial for the acid I-V product ($P < 0.05$, $P = 0.06$, and $P < 0.05$, respectively). Serum glucose fell below baseline values at about 120 min postprandial for both products. Time to peak glucose did not differ between products.

The mean peak incremental change from baseline in serum insulin was higher ($P < 0.05$) for the acid I-V product (Table 6.4). Net incremental AUC for serum insulin over the 180-min test did not differ ($P > 0.20$) between products. Postprandial serum insulin responses are depicted in Figure 6.2. The baseline serum insulin value did not differ ($P > 0.20$) between groups (5.2 ± 0.8 and 5.4 ±

0.8 mIU/L for Control and acid I-V, respectively). Incremental change from baseline for insulin did not differ ($P > 0.10$) across all time points except at 90 min postprandial in which the Control product had a lower ($P < 0.01$) insulin response. Time to peak for insulin did not differ between products.

Gastrointestinal symptoms were minimally affected by the products fed in this glucose tolerance test (data not presented). No differences were noted between products. The most frequently reported symptoms were burping and nausea, most of which were mild in nature. No adverse events were documented for subjects consuming either product.

DISCUSSION

Alginate is the sodium salt of alginic acid and is isolated from brown seaweed, family Phaeophyceae (Whistler and BeMiller, 1997). It is composed of mannuronic (pKa ~ 3.38) and guluronic acid (pKa ~ 3.65). Alginate, in the absence of free polyvalent cations, is a relatively nonviscous soluble fiber. Alginate solutions gel upon addition of free calcium ions, which fill the cavities formed between parallel guluronic acid chains (G-block regions). These cavities contain two carboxylate and two hydroxyl groups, one from each chain. The result is a junction zone that has been called an "egg box" arrangement, with the calcium ions being likened to eggs in the pockets of an egg carton (Anderson et al., 1991). The induction of viscosity depends upon the release of free calcium ions. Tricalcium phosphate was used in the present formula as the source of insoluble calcium. Upon acidification within the stomach, free calcium ions would be released and allowed to react with alginate, inducing viscosity.

Potassium citrate was added to our products because citrate optimizes the viscous properties of alginate (Torsdottir et al., 1991). Citrate competes with alginate for the free calcium ion. Through this competition, citrate prevents the formation of a rigid gel that could be broken down under the shear of the stomach, resulting in a loss of viscosity.

The primary objective of the present study was to determine the ability of an acid I-V complex to attenuate the postprandial blood glucose and insulin responses in healthy nondiabetic adults. The products were formulated to mimic an oral glucose tolerance beverage, which ultimately challenged the subjects with 61 g of glucose. We found that the acid I-V formula reduced serum glucose at 60, 90, and 150 min postprandial. However, no differences were found at the earlier time points (15 and 30 min). The relative glycemic response over the 180-min test was calculated to be 23.3 ± 84.9, whereas the glycemic index over the 180-min test was calculated to be 91.2 ± 14.7. The calculation of relative glycemic response differs from glycemic index in the estimation of incremental AUC. In the calculation of net incremental AUC (for relative glycemic response), the negative area (i.e., the area below baseline if postprandial glucose values fall below basal fasting glucose) is subtracted from the net area, whereas the calculation of incremental AUC (for glycemic index) ignores this area below baseline (also called positive incremental area, Allison et al., 1995). In the case of our data, large discrepancies are seen between the relative glycemic response and glycemic index because serum glucose concentrations fell below the basal fasting concentration near 120 min.

Discrepancies such as this have generated much debate about which method is most appropriate (Gannon and Nuttall, 1987; Wolever, 1989; Allison et al., 1995). For this reason, Wolever et al. (1991) suggest that the postprandial glycemic response should not be measured past 2 h for calculating incremental AUC in normal subjects, thus avoiding the large discrepancies between values. Their position is justified because, in normal subjects, the postprandial blood glucose values typically return to baseline within 2 h following the consumption of a rapidly digested carbohydrate (Jenkins et al., 1990). In order to provide a more clinically meaningful value (e.g., effects on insulin and counter-regulatory responses) in the ranking of carbohydrate foods, the early part of the blood glucose response curve should be emphasized.

Subjects had a higher incremental peak insulin response after consuming the acid I-V product compared to the Control. The acid I-V complex improves the glycemic response without decreasing insulin response. These data suggest that the acid I-V complex increases insulin secretion relative to the glycemic stimulus, and this is deserving of further study, especially in subjects with type 2 diabetes mellitus.

Torsdottir et al. (1991) have shown that sodium alginate supplementation (3.75 g mannuronic and guluronic acids) diminishes the postprandial rise of blood glucose and insulin. The subjects (7 men with type 2 diabetes) were fed a liquid test meal (340 kcal) with or without sodium alginate. Torsdottir et al. (1991) also documented a slower gastric emptying rate due to alginate supplementation in their subject population and suggested that these data

support the theory that viscous dietary fiber attenuates the glycemic excursion after a meal primarily by slowing the rate of gastric emptying.

Because the present data did not completely corroborate that of Torsdottir et al. (1991), we evaluated the buffering capacity of the experimental treatments *in vitro*. Incremental levels of simulated gastric fluid were added to 100 g of product and evaluated for changes in pH and viscosity. We found that the viscosity of the acid I-V product did not increase until the pH dropped below 5.0, upon which a sharp rise in viscosity was noted until the pH reached approximately 4 (data not shown). As the pH dropped below 4.0, a large reduction in viscosity was noted. This corroborates well with the pKa of guluronic acid (~3.65). As the pH drops below 4.0, the calcium bridges are broken due to the protonation of the uronic acid groups with hydrogen. Knowing this, we can conclude that there is a small pH range upon which the acid I-V complex would be efficacious; however, this pH range is achievable under physiological conditions in the stomach. Future research should consider this finding, because buffering the physiological pH in this range should enhance product efficacy. Furthermore, this *in vitro* study documented the very strong buffering capacity of these products, which have a similar buffering capacity to a standard tube-fed product, Jevity® Plus (Ross Products Division, Columbus, OH). It took approximately 50 mL of simulated gastric fluid to bring about the induced-viscosity of 100 g of product. This suggests that it would require approximately 125 mL of gastric fluid (with 250 g product) to produce an induced-viscosity complex *in vivo*. It has been estimated that the healthy adult

produces around 100-170 mL of gastric fluid over the first hour postprandial (Davenport, 1977). Thus, not enough acid might have been produced by the subjects in our study to induce viscosity at the early time points (15 and 30 min postprandial), and this may explain why differences were only noted at later time points (60, 90, and 150 min postprandial).

Based upon these *in vitro* results, it appears that the induced-viscosity complex would have its largest impact upon gastric emptying and would probably have only a small direct effect upon glucose absorption within the small intestine. It is well established that duodenal pH is buffered to ~ 6.0 postprandial due to pancreatic and bile secretions, which contain a considerable amount of bicarbonate (Solomon, 1987). Thus, once the intestinal chyme pH is above 5, the induced viscosity would be lost. This effect is also dependent upon a dilution effect of added intestinal secretions. These effects would require a fair amount of shear stress to break-up the induced-viscosity complex.

Upon a more thorough review of the postprandial glucose responses of our subject population, we noted that the peak serum glucose response (123 ± 3.5 and 117 ± 4.2 mg/dL for Control and acid I-V, respectively) was significantly lower than glucose responses found in the literature. For example, Crapo et al. (1980) found a peak postprandial serum glucose response of approximately 165 mg/dL in normal (nondiabetic) adult subjects given a 50-g load of dextrose. Similar postprandial glucose responses to 50-g glucose loads in nondiabetic adult subjects have been found by others (Castro et al., 1970; Jenkins et al.,

1978, 1990). The reduced glucose response in the present study may have limited our ability to detect a benefit of the acid I-V complex.

I hypothesized that the reduced peak glucose response in the present study may be partly attributed to the presence of fructose in the test meal. Therefore, an animal experiment in fatty Zucker *fa/fa* rats (a model of type 2 diabetes mellitus) was undertaken. Fructose addition (0.16 g/kg body wt) to a glucose challenge (1.0 g/kg body wt) reduced ($P < 0.05$) the incremental AUC for plasma glucose by 34% over the 3-h experiment (Hadley et al., 2000). In addition, postprandial incremental change from baseline in plasma glucose at 30, 60, and 90 min was lower ($P < 0.05$) for rats fed supplemental fructose. These data support the hypothesis that small amounts of oral fructose may be useful in lowering the postprandial glucose response. This concept deserves further evaluation, as it may be useful for the dietary treatment of people with diabetes.

No differences in subjective gastrointestinal tolerance symptoms were noted between products in this double-masked experiment. Overall, those symptoms that were reported were scored as mild by the subjects. The primary fiber sources utilized in the formulation of these products, gum arabic and alginate, have been shown to be slowly and poorly fermented (Chow et al., 1998). These fermentation properties translate into a well-tolerated fiber source, unlike rapidly fermented fibers (e.g., pectin), which can be associated with bloating and intestinal cramping.

In conclusion, the acid I-V complex may help attenuate the postprandial glycemic response. Additional studies evaluating the acid I-V complex in a complete nutritional product *in vitro* and then *in vivo* in subjects with diabetes should be undertaken to more appropriately evaluate this concept's clinical application.

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Table 6.1. Ingredient Composition of Experimental Treatments [a]

ingredient	treatment	
	Control	acid I-V
	g/100 g	
water	66.6	66.6
glucose	30	30
sodium alginate	0.0	1.5
gum arabic	1.2	0.0
guar gum	0.3	0.0
potassium citrate	1.2	1.2
microcrystalline cellulose	0.2	0.2
tricalcium phosphate	0.15	0.15
flavor, lemon	0.2	0.2

[a] Product fill weights were 250 g; acid I-V = acid-induced-viscosity.

Table 6.2. Chemical Composition of Experimental Treatments [a]

item	treatment	
	Control	acid I-V
	(units/100 g product)	
total solids, g	31.5	30.8
potassium, mg	466	462
calcium, mg	67.4	60.8
phosphorus, mg	27.6	27.7
total dietary fiber, g	1.88	1.77
glucose, g	24.5	24.7
fructose, g [b]	4.8	4.7
galactose, g [b]	0.2	0.2
viscosity, mPa·sec	289	249

[a] Treatments were delivered as a liquid 250-g single serving; acid I-V = acid-induced-viscosity. [b] Fructose and galactose were formed during heat processing from the isomerization of glucose.

Table 6.3. Clinical Characteristics of Subjects at Time of Screening

	value [a]
age (y)	36 ± 2
body weight (kg)	66.4 ± 1.6
height (cm)	165 ± 1.4
body mass index (kg/m^2)	24.2 ± 0.4
glucose (mg/dL)	84.6 ± 1.2
triglycerides (mg/dL)	79.4 ± 12.2
total cholesterol (mg/dL)	177 ± 7
HDL cholesterol (mg/dL)	57.4 ± 2.7

[a] Mean ± SEM; n = 30.

Table 6.4. Glycemic and Insulinemic Responses of Healthy Nondiabetic Subjects to an Experimental Acid Induced-Viscosity (I-V) Complex [a]

	glucose (mg/dL)		insulin (mIU/L)	
item	Control	acid I-V [b]	Control	acid I-V [b]
peak value [c]	40.4 ± 3.3	32.8 ± 3.4 [e]	32.6 ± 3.7	59.8 ± 11.9 [g]
AUC (unit·min) [d]	1717 ± 433	429 ± 276 [f]	2723 ± 326	3366 ± 560

[a] Mean ± SEM; n = 30. Baseline values for serum glucose were 82.6 ± 1.3 and 84.6 ± 1.5 mg/dL for Control and acid I-V, respectively. Baseline values for serum insulin were 5.2 ± 0.8 and 5.4 ± 0.8 mIU/L for Control and acid I-V, respectively. To convert glucose mg/dL to mmol/L, multiply mg/dL by 0.0555. Glucose of 90 mg/dL = 5.0 mmol/L. [b] acid-induced-viscosity. [c] Mean peak serum insulin values represent period one data only. [d] Net incremental area under the curve 180 min postprandial. [e] acid I-V different from Control ($P < 0.06$). [f] acid I-V different from Control ($P < 0.01$). [g] acid I-V different from Control ($P < 0.05$).

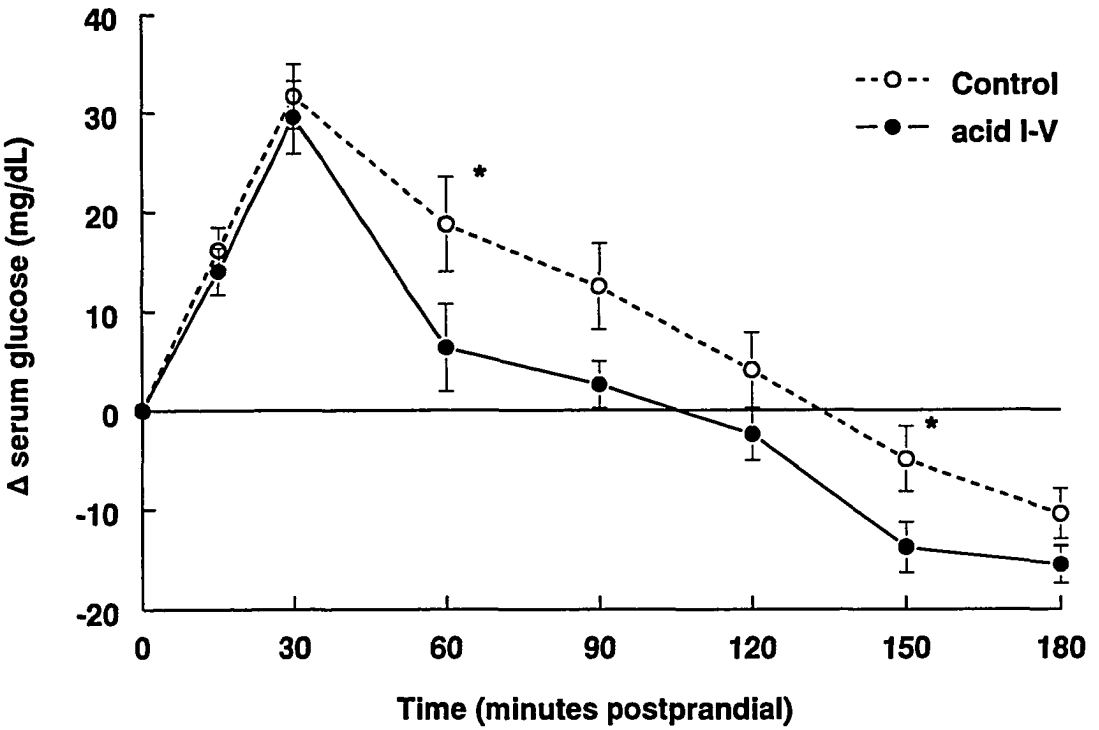

Figure 6.1. Incremental change from baseline in serum glucose after ingestion of a glucose beverage (Control) and a glucose beverage containing an experimental acid-induced-viscosity complex (acid I-V) by healthy nondiabetic adult subjects. Baseline serum glucose values did not differ between groups (82.6 ± 1.3 and 84.6 ± 1.5 mg/dL for Control and acid I-V, respectively). To convert glucose mg/dL to mmol/L, multiply mg/dL by 0.0555. Glucose of 90 mg/dL = 5.0 mmol/L. Values represent mean incremental serum glucose (mg/dL) ± SEM, n = 30. *, $P < 0.05$.

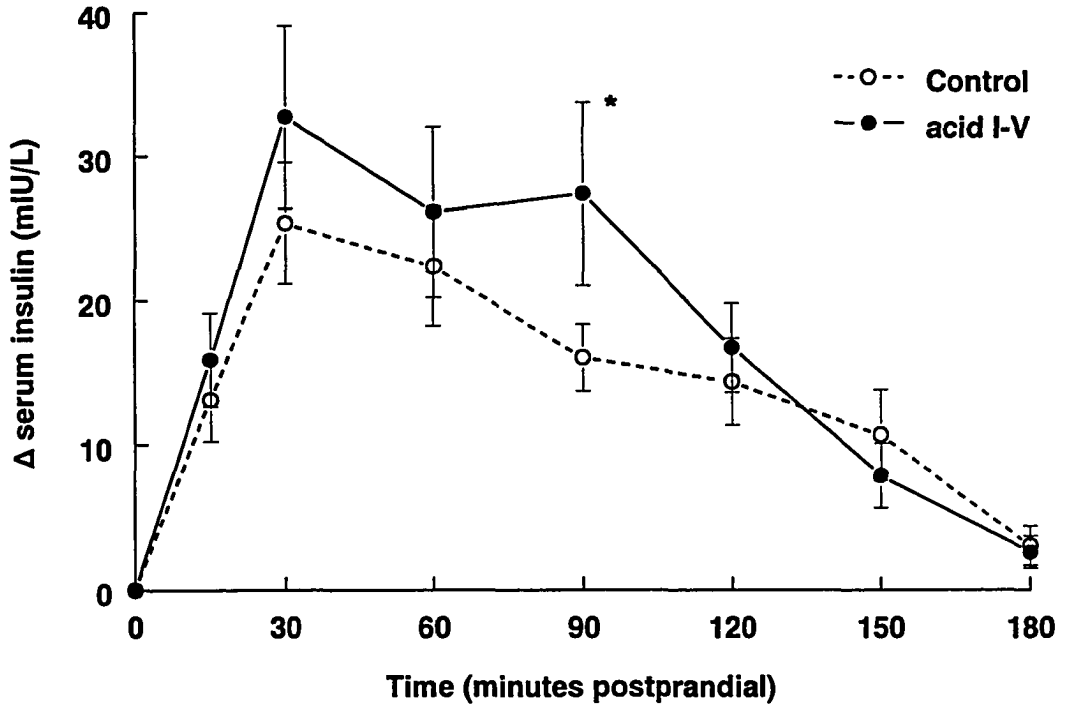

Figure 6.2. Incremental change from baseline in serum insulin after ingestion of a glucose beverage (Control) and a glucose beverage containing an experimental acid-induced-viscosity complex (acid I-V) by healthy nondiabetic adult subjects. Baseline insulin values did not differ between groups (5.2 ± 0.8 and 5.4 ± 0.8 mIU/L for Control and acid I-V, respectively). Values represent mean incremental serum insulin (mIU/L) ± SEM, n = 30. *, $P < .01$.

CHAPTER 7

SUPPLEMENTAL FRUCTOSE ATTENUATES POSTPRANDIAL GLYCEMIA IN ZUCKER FATTY *FA/FA* RATS

INTRODUCTION

In the evaluation of a novel acid-induced-viscosity concept on postprandial glycemia (Chapter 6), which delivered ~61 g of glucose, the peak serum glucose response was lower (123 ± 3.5 and 117 ± 4.2 mg/dL for control and acid-induced-viscosity test meals, respectively) than predicted from published literature (Castro et al., 1970; Crapo et al., 1980; Jenkins et al., 1978, 1990). For example, Crapo et al. (1980) found a peak postprandial serum glucose response of approximately 165 mg/dL in normal (nondiabetic) adult subjects given a 50-g load of dextrose. This discrepancy led to my hypothesis that supplemental fructose, which was present in the glucose-based experimental beverages at ~12 g, attenuates postprandial glycemia.

Fructose alone increases the postprandial blood glucose concentrations less than isocaloric amounts of glucose (Crapo et al., 1980; Jenkins et al., 1981; Nuttall et al., 1992). Shiota et al. (1998) found that intraportal infusion of small amounts of fructose augment net hepatic glucose uptake during

hyperglycemic hyperinsulinemia in dogs. In isolated rat hepatocytes, Fillat et al. (1993) demonstrated that fructose at low concentrations stimulated the glycolytic flux. This effect may be mediated through the control of hepatic glucose phosphorylation. Glucokinase is acutely regulated by fructose-6-phosphate and fructose-1-phosphate, two metabolites whose effects are dependent upon an inhibitory protein that tightly binds to glucokinase (Van Schaftingen et al., 1994). Fructose-6-phosphate promotes, but fructose-1-phosphate inhibits binding of the inhibitory protein to glucokinase. Thus, dietary fructose may promote hepatic glucose utilization by an indirect mechanism. Fructose is converted in the liver directly to fructose-1-phosphate (via fructokinase an enzyme present only in the liver), which competes with fructose-6-phosphate on the glucokinase regulatory protein and activates glucokinase by promoting dissociation of its inhibitory protein. Supplemental dietary fructose may enhance glucose flux through glucokinase in people with type 2 diabetes mellitus; they have an impaired ability to suppress endogenous glucose production during hyperglycemia, due in part to decreased glucose-induced flux through glucokinase (Mevorach et al., 1998). With an improvement in postprandial hepatic glucose uptake, the blood glucose level may be reduced after a meal containing supplemental fructose. In Chapter 6, I postulated that I serendipitously found that fructose supplementation to a glucose challenge attenuates the glycemic response in healthy nondiabetic adult subjects.

In this chapter, I will describe a series of experiments that were conducted to evaluate the effects of supplemental fructose on postprandial glycemia. In Zucker fatty *fa/fa* rats (a model of type 2 diabetes mellitus), we found that supplemental fructose attenuates postprandial glycemia.

MATERIALS AND METHDOS

Animals: Experiments were performed on 400- to 450-g Zucker fatty *fa/fa* rats (Harlan Sprague Dawley, Inc.; Indianapolis, IN). Rats were individually housed in microisolator cages on dry bedding and were given ad libitum access to water and rat chow (pelletted; 8640 Harlan Teklad 22/5 Rodent Diet; Harlan Teklad, Madison, WI). The housing facility was maintained at 19 to 23 °C, 30 to 70 % relative humidity, and 12-h light-dark cycle. Rats were handled 4 to 5 times per week and were trained to orally consume test meals for the meal glucose tolerance test (MGTT). The animal use protocol was reviewed and approved by The Ohio State University Animal Care Committee (Columbus, OH).

Experimental design: In a series of experiments, dietary treatments were evaluated in a randomized crossover design with a 7-d (range 6 to 9 d) washout period between each MGTT. Within each experiment, every rat received each treatment. After overnight food deprivation, rats were orally fed test meals as a solution. Rats consumed the test meal within a 1-min time period. Blood samples were collected from the tail vein and immediately analyzed for plasma glucose by the glucose oxidase method utilizing a Precision·G Blood Glucose

Testing System (Medisense, Inc., Bedford, MA) before (0 min) and 30, 60, 90, 120, and 180 min postprandial. Rats had free access to water throughout the MGTT.

Test carbohydrates: Raw cornstarch (RCS; Argo, CPC International, Englewood Cliffs, NJ) was obtained from a local grocery. Purified glucose, fructose, sucrose, and maltose were obtained from Sigma (St. Louis, MO). Lodex 15 (maltodextrin) was obtained from Cerestar USA, Inc. (Hammond, IN) and had a dextrose equivalence of 15.

Experiment 1: The objective of this experiment was to compare a high- and low-glycemic carbohydrate in the Zucker fatty *fa/fa* model of type 2 diabetes mellitus. The postprandial glycemic response to glucose was compared with a slowly digested starch, RCS (Collings et al., 1981; Chen et al., 1984; Wolf et al., 1999) in 20 male rats. Two dietary treatments were evaluated: (1) glucose and (2) RCS. Carbohydrates were made into 50 % (wt/vol) solutions with water prior to challenge. Test meal volume was approximately 1 mL and was adjusted such that each rat was delivered an equivalent carbohydrate challenge (1.0 g/kg body wt).

Experiment 2: The objective of this experiment was to evaluate the effects of supplemental fructose on the postprandial glycemic response to glucose in 10 female Zucker fatty *fa/fa* rats. Two dietary treatments were evaluated: (1) glucose (1.0 g/kg body wt) and (2) as 1 plus supplemental fructose (0.16 g/kg body wt). This fructose dose approximates that fed in Chapter 6.

Experiment 3: The objective of this experiment was to evaluate the effects of supplemental fructose on the postprandial glycemic response to a rapidly digested starch. Maltodextrin (partially hydrolyzed cornstarch) was chosen as the rapidly digested starch (Philipson et al., 1992). Two dietary treatments were evaluated: (1) maltodextrin (1.0 g/kg body wt) and (2) as 1 plus supplemental fructose (0.16 g/kg body wt). Maltodextrin was made into a 50 % (wt/vol) solution with water and fructose was added to the appropriate treatment. Each solution was heated in a microwave for 30 sec to completely solubilize the carbohydrate solutions 1 h before testing in 10 male Zucker fatty *fa/fa* rats.

Experiment 4: The objective of this experiment was to evaluate if supplemental sucrose (as an indirect source of fructose) would respond the same as purified fructose on the postprandial glycemic response to a rapidly digested starch. Two dietary treatments were evaluated: (1) maltodextrin (1.0 g/kg body wt) plus maltose (0.16 g/kg body wt) and (2) maltodextrin (1.0 g/kg body wt) plus sucrose (0.32 g/kg body wt, which is 0.16 g/kg body wt fructose equivalent). Treatments were prepared as described in experiment 3 and fed to 10 male Zucker fatty *fa/fa* rats. One rat did not complete the sucrose treatment because feed had not been withheld overnight.

Experiment 5: The objective of this experiment was to evaluate the effects of supplemental fructose on second meal glucose tolerance. The two test meals were: (1) maltodextrin (1.0 g/kg body wt) and (2) as 1 plus supplemental fructose (0.16 g/kg body wt). A 3-h MGTT was conducted in the a.m. followed ~1.5 h later with a second 3-h MGTT. The four treatments were: (1) meal 1

followed by meal 1 (M/M); (2) meal 1 followed by meal 2 (M/F); (3) meal 2 followed by meal 1(F/M); and (4) meal 2 followed by meal 2 (F/F). Treatments were prepared as described in experiment 3 and fed to 20 male Zucker fatty *fa/fa* rats. Many data points were missing at the 180-min time point; therefore, this time point was dropped from the analysis.

Experiment 6: The objective of this experiment was to evaluate the dose response of supplemental fructose. Four dietary treatments were evaluated: (1) maltodextrin (1.0 g/kg body wt); (2) as 1 plus supplemental fructose (0.10 g/kg body wt); (3) as 1 plus supplemental fructose (0.20 g/kg body wt); and (4) as 1 plus supplemental fructose (0.50 g/kg body wt). Treatments were prepared as described in experiment 3 and fed to 20 male Zucker fatty *fa/fa* rats.

Experiment 7: The objective of this experiment was to evaluate a low dose of supplemental fructose. Two dietary treatments were evaluated: (1) maltodextrin (1.0 g/kg body wt) and (2) as 1 plus supplemental fructose (0.075 g/kg body wt). Treatments were prepared as described in experiment 3 and fed to 19 male Zucker fatty *fa/fa* rats.

STATISTICS

Positive incremental area under the glucose curve (AUC) over the 180-min postprandial period was calculated according to Wolever et al. (1991). If a rat had one or more glucose measurements missing between 0 and 180 min (both inclusive) during a MGTT, its data for that MGTT were not included in the analyses of peak incremental change from baseline (i.e., peak rise) and positive incremental AUC. Data were analyzed using a mixed model for crossover

trials, with treatment and period effects as fixed, and subject effect as random. Baseline blood glucose concentration was used as a covariate for blood glucose concentrations at individual postprandial time points. Different covariance structures were tested according to Brown and Prescott (1999). Model fit was checked by comparing Akaike's Information Criterion (AIC). Compound symmetry variance pattern was found to be the most adequate for all experiments. Residuals from these models were plotted against the predicted values in order to check the appropriateness of the model. In experiment 5, treatments were arranged as a 2 × 2 factorial by presence or absence of fructose at the first or second meal. The first meal fructose × second meal fructose interaction was not significant. In experiment 6, the linear and quadratic effects of fructose dose were examined. All results were considered to be statistically significant if the significance level was less than 0.05 (SAS version 8.0, SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

In the Zucker fatty *fa/fa* rat model of type 2 diabetes mellitus, we were able to differentiate a low- and high-glycemic test meal. The postprandial glycemic response to RCS was delayed and the early phase excursion reduced ($P < 0.01$) compared with a glucose challenge (Figure 7.1). Peak rise in plasma glucose was lower ($P < 0.01$) for RCS (5.03 ± 0.38 compared with 6.32 ± 0.38 mmol/L). These *in vivo* rat data are consistent with the starch hydrolysis data of Wolf et al. (1999), who found that RCS had a slow extent of *in vitro* hydrolysis over time and the *in vivo* human findings of Chapter 4. Furthermore, it

corroborates the use of RCS as nutritional therapy for the prevention of nighttime hypoglycemia (Chen et al., 1984; Kaufman et al., 1995; Wolfsdorf and Crigler, 1997). The Zucker fatty *fa/fa* rat may be an effective model for evaluating the postprandial glycemic response to dietary carbohydrates.

Shiota et al. (1998) found that intraportal infusion of small amounts of fructose augment net hepatic glucose uptake during hyperglycemic hyperinsulinemia in dogs. In the present study, orally supplemented fructose was found to attenuate the postprandial glycemic response to glucose (Figure 7.2) and a rapidly digested starch (Figure 7.3). At a dose of 0.16 g/kg body wt, fructose reduced ($P < 0.05$) the incremental AUC by 34 % when supplemented to a glucose challenge and by 32 % when supplemented to a maltodextrin challenge. In addition, peak rise in plasma glucose was lower ($P < 0.05$) when fructose was supplemented to a glucose (2.45 ± 0.35 compared with 3.80 ± 0.35 mmol/L) or maltodextrin (4.00 ± 0.53 compared with 5.87 ± 0.53 mmol/L) challenge. The present study supports our hypothesis that orally administered supplemental fructose attenuates postprandial glycemia.

Sucrose (glucose α-1,2 fructose) has a reduced glycemic index (Jenkins et al., 1981). Because sucrose is well absorbed in the small intestine (Rumessen and Gudmand-Hoyer, 1986; Truswell et al., 1988; Riby et al., 1993), I postulated that sucrose, as an indirect source of fructose, would reduce postprandial glycemia. Similar to our results with fructose supplementation, sucrose reduced ($P = 0.0575$) the incremental AUC for plasma glucose (Figure 7.4). Peak rise in plasma glucose was lower ($P < 0.01$) when rats were fed

supplemental sucrose (5.43 ± 0.58 compared with 7.12 ± 0.56 mmol/L). Small amounts of oral sucrose may be useful for the prevention of postprandial hyperglycemia in people with diabetes.

As previously noted, Shiota et al. (1998) found that intraportal infusion of small amounts of fructose augment net hepatic glucose uptake. In addition, they determined that 69 % of this glucose was stored as hepatic glycogen. Thus, we asked the question, can liver glycogen stores be maximized and worsen postprandial glycemia at the second meal? Second meal glycemic response was not affected by fructose supplementation during the first meal, and fructose supplementation during the second meal reduced ($P < 0.05$) postprandial glycemia after fructose supplementation during the first meal (Figure 7.5). These results may suggest that glycogen stores are not filled or that fructose enhances glycolysis. The latter case is supported by the study of Atkinson et al. (2000), who found that fructose feeding raised plasma lactate concentration. Further research is needed in this area to understand the metabolic implications of fructose supplementation.

The dose-related effect of supplemental fructose on an oral glucose tolerance test in AP rats was recently reported in abstract form (Atkinson et al., 2000). These investigators found that high levels of fructose supplementation (1, 2, and 4 g/kg body wt) reduced the early (10 min) postprandial plasma glucose concentrations following a glucose challenge. In the present study, we evaluated the dose response of fructose supplementation at lower levels (0.1, 0.2, and 0.5 g/kg body wt). Similar to the findings of Atkinson et al. (2000), all

three doses were found to reduce ($P < 0.05$) the early rise in plasma glucose; however, incremental AUC for plasma glucose was unchanged (Figure 7.6). Supplemental fructose reduced ($P < 0.01$) the peak rise in plasma glucose (linear and quadratic effect). A final experiment was conducted to test an even lower supplemental fructose dose (0.075 g/kg body wt). Again, fructose reduced ($P < 0.05$) the postprandial glycemic response (Figure 7.7). Incremental AUC for plasma glucose was reduced ($P < 0.05$) 18 %. Peak rise in plasma glucose was lower ($P < 0.01$) when rats were fed supplemental fructose (4.99 ± 0.27 compared with 6.34 ± 0.27 mmol/L). These findings have great clinical potential for the prevention of postprandial hyperglycemia in people with diabetes. Because fructose and sucrose intake have been shown to cause hypertriacylglycerolemia and hypercholesterolemia in rats (Mayes, 1993) and humans (Hollenbeck, 1993; Bantle et al., 2000; Parks and Hellerstein, 2000), nutritional recommendations have been made to avoid supplementation of simple sugars to the diabetic diet (Franz et al., 1994; Uusitupa, 1994). However, these negative attributes of fructose feeding are only well documented at high (<17 % total kcal) dietary intakes (Bantle et al., 1992; Bantle et al., 2000). The findings of the present study support a low level of dietary supplementation of fructose for the attenuation of postprandial glycemia.

CONCLUSIONS

These data support the hypothesis that small amounts of oral fructose or sucrose may be useful in lowering the postprandial blood fatty glucose

response. These findings provide further support for the addition of fruit and honey, which naturally contain fructose and sucrose, to the diabetic diet. This concept deserves further clinical evaluation, as it may be useful for the dietary treatment of people with diabetes.

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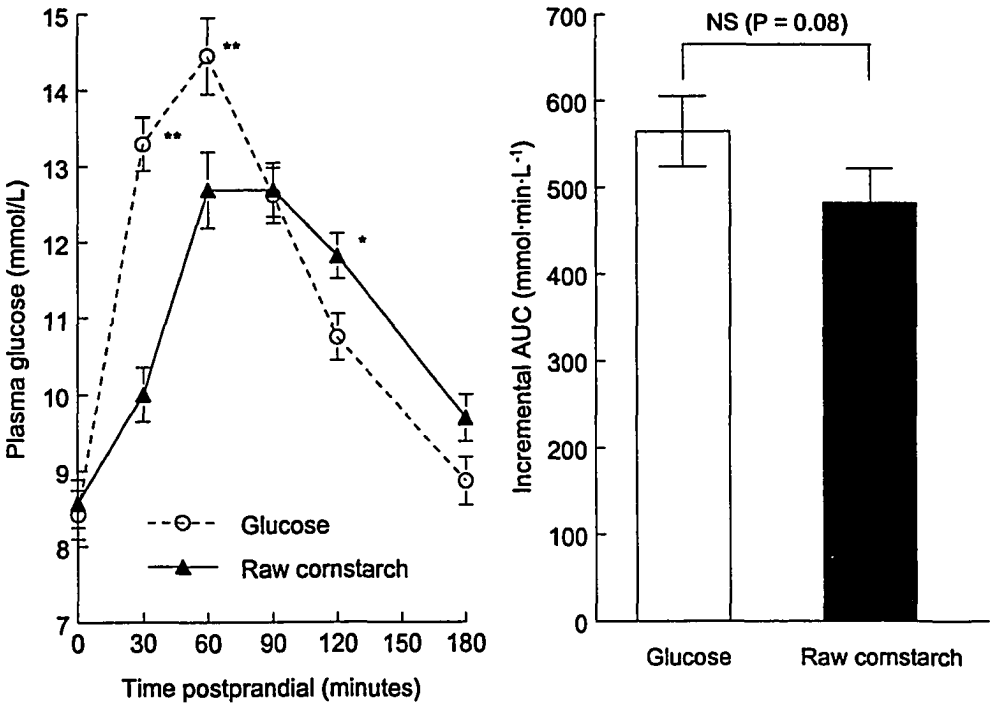

Figure 7.1. Postprandial glycemic response and incremental area under the curve (AUC) after a 1.0 g/kg body wt oral glucose or raw cornstarch (RCS) challenge in male fatty Zucker *fa/fa* rats (Experiment 1). Basal fasting plasma glucose concentrations were not different (8.42 ± 0.32 vs. 8.57 ± 0.32 mmol/L; Glucose vs. RCS, respectively). Points at the same time interval differ, $^{*}P < 0.05$, $^{**}P < 0.01$. Data are least squares means ± SEM, n = 20.

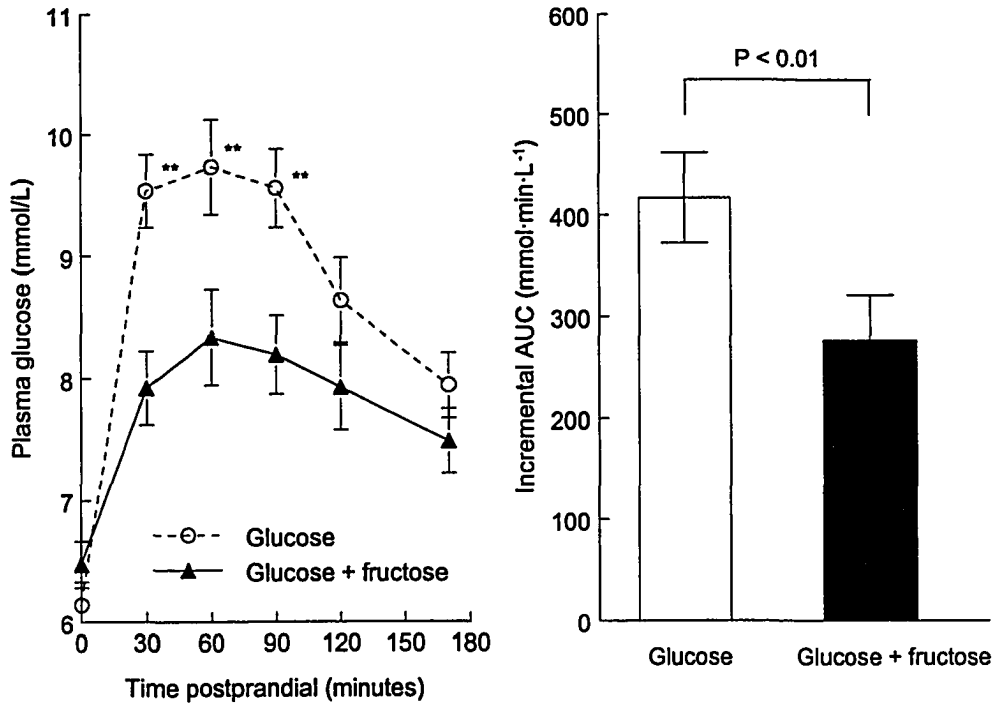

Figure 7.2. Postprandial glycemic response and incremental area under the curve (AUC) after a 1.0 g/kg body wt oral glucose (Glucose) or the same plus 0.16 g/kg body wt supplemental fructose (Glucose + fructose) challenge in female fatty Zucker *fa/fa* rats (Experiment 2). Basal fasting plasma glucose concentrations were not different (6.14 ± 0.19 vs. 6.47 ± 0.19 mmol/L; Glucose vs. Glucose + fructose, respectively). Points at the same time interval differ, $^{**}P < 0.01$. Data are least squares means ± SEM, n = 10.

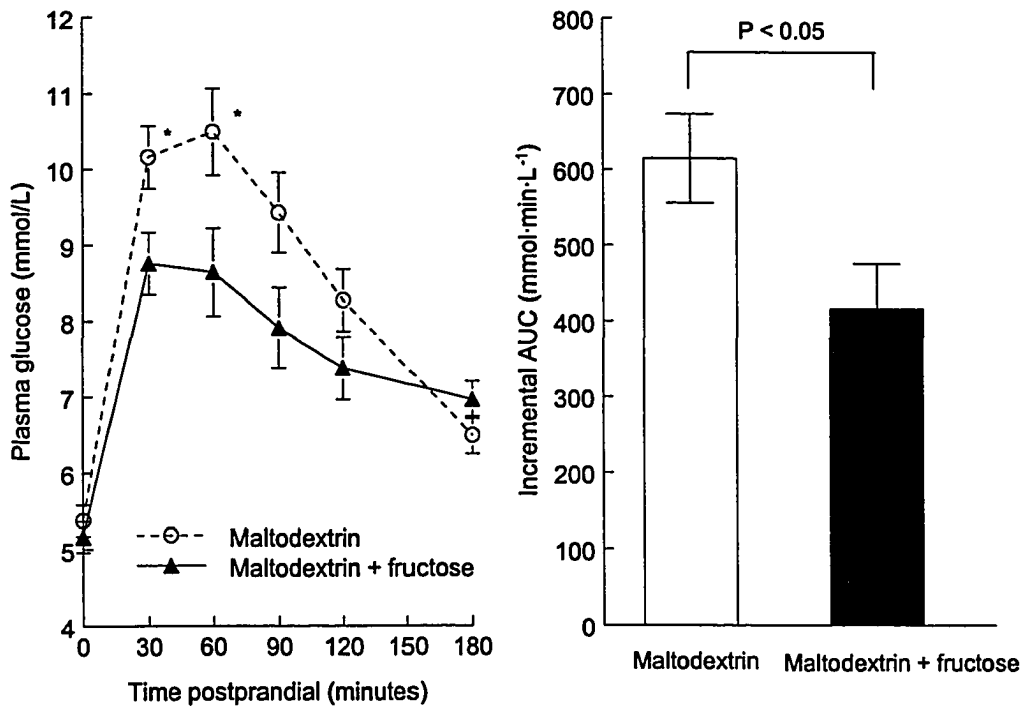

Figure 7.3. Postprandial glycemic response and incremental area under the curve (AUC) after a 1.0 g/kg body wt oral maltodextrin (Maltodextrin) or the same plus 0.16 g/kg body wt supplemental fructose (Maltodextrin + fructose) challenge in male fatty Zucker *fa/fa* rats (Experiment 3). Basal fasting plasma glucose concentrations were not different (5.38 ± 0.20 vs. 5.16 ± 0.20 mmol/L; Maltodextrin vs. Maltodextrin + fructose, respectively). Points at the same time interval differ, $^{*}P < 0.05$. Data are least squares means ± SEM, n = 10.

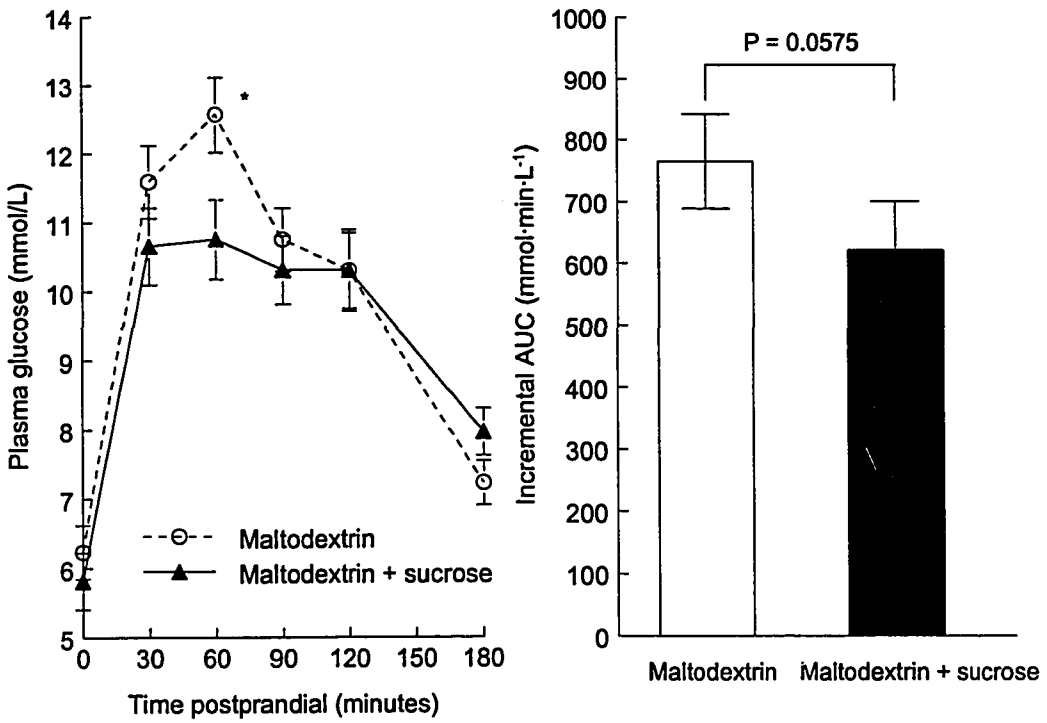

Figure 7.4. Postprandial glycemic response and incremental area under the curve (AUC) after a 1.0 g/kg body wt oral maltodextrin plus 0.16 g/kg body wt maltose (Maltodextrin) or 1.0 g/kg body weight oral maltodextrin plus 0.32 g/kg body wt sucrose (Maltodextrin + sucrose) challenge in male fatty Zucker *fa/fa* rats (Experiment 4). Basal fasting plasma glucose concentrations were not different (6.23 ± 0.39 vs. 5.82 ± 0.41 mmol/L; Maltodextrin vs. Maltodextrin + sucrose, respectively). Points at the same time interval differ, $^*P < 0.05$. Data are least squares means ± SEM, n = 10, except for Maltodextrin + sucrose n = 9.

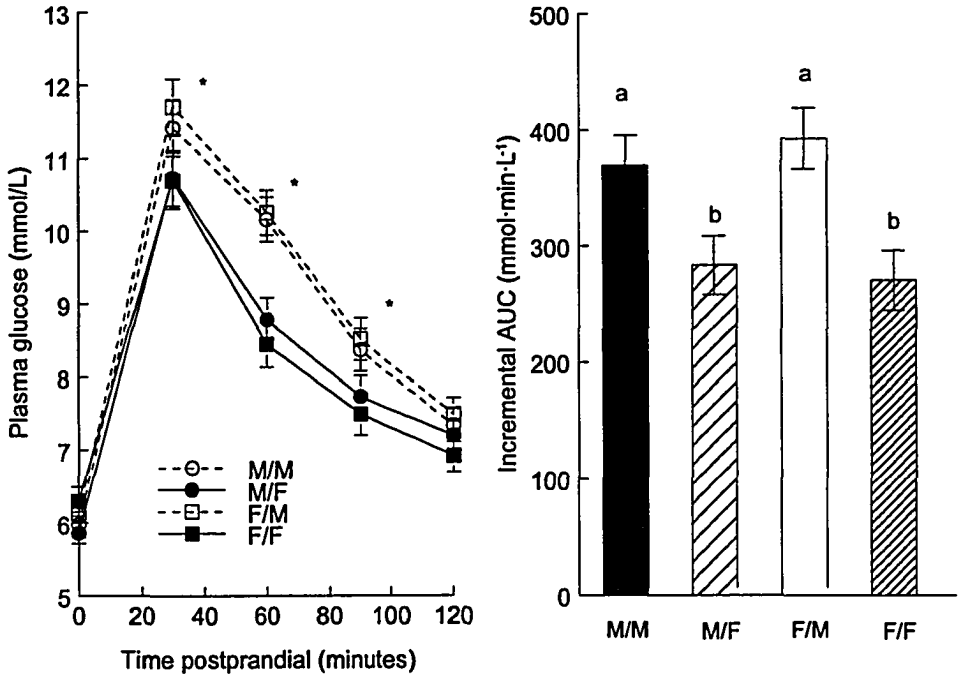

Figure 7.5. Second meal postprandial glycemic response and incremental area under the curve (AUC) after a 1.0 g/kg body wt oral maltodextrin (meal 1 = M) or the same plus 0.16 g/kg body wt supplemental fructose (meal 2 = F) challenge in male fatty Zucker *fa/fa* rats (Experiment 5). The four treatments were: (1) M followed by M (M/M); (2) M followed by F (M/F); (3) F followed by M (F/M); and (4) F followed by F (F/F). At the second meal, basal plasma glucose concentrations were 5.98 ± 0.17, 5.86 ± 0.14, 6.13 ± 0.25, and 6.30 ± 0.20 mmol/L for M/M, M/F, F/M, and F/F, respectively. Main effect of fructose at the second meal *$P < 0.01$, points at the same time interval differ. Main effect of fructose at the second meal $P < 0.01$, bars with unlike letters differ. Data are least squares means ± SEM, n = 19, except for M/F n = 20.

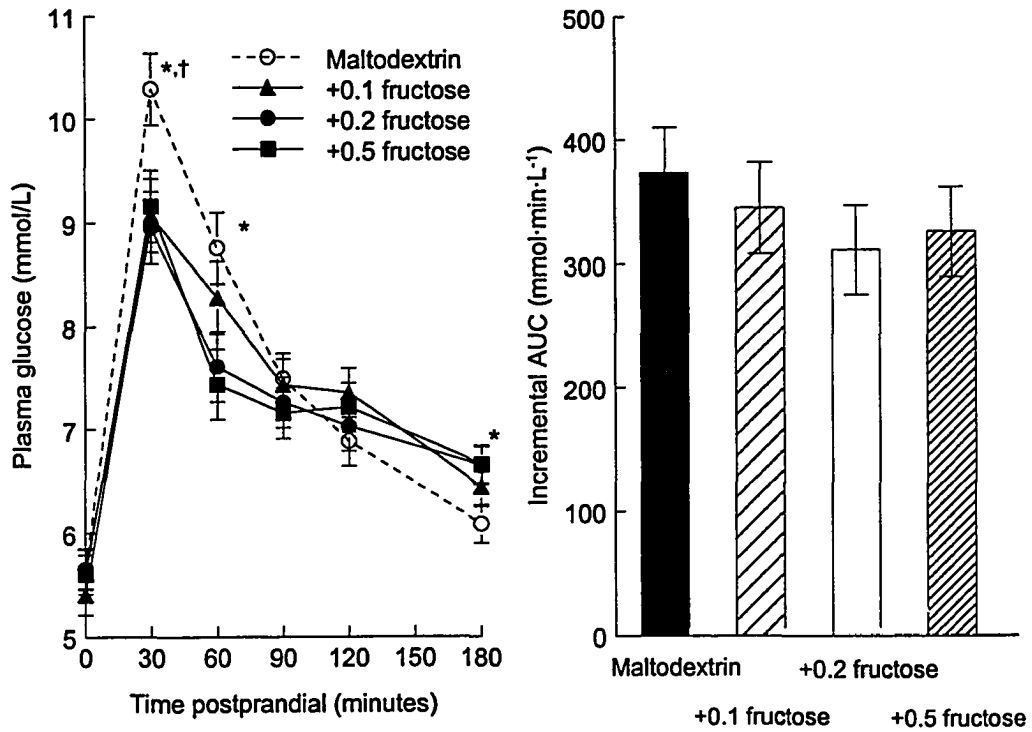

Figure 7.6. Postprandial glycemic response and incremental area under the glucose curve (AUC) after a 1.0 g/kg body wt oral maltodextrin (Maltodextrin) or the same plus 0.1, 0.2, or 0.5 g/kg body wt supplemental fructose (+0.1 fructose, +0.2 fructose, and +0.5 fructose, respectively) challenge in male fatty Zucker *fa/fa* rats (Experiment 6). Basal fasting plasma glucose concentrations were not different (5.65 ± 0.20, 5.60 ± 0.19, 5.65 ± 0.19, and 5.40 ± 0.19 mmol/L for Maltodextrin, +0.1 fructose, +0.2 fructose, and +0.5 fructose, respectively). Linear effect of fructose, $^{*}P < 0.05$. Quadratic effect of fructose, $^{\dagger}P < 0.05$. Data are least squares means ± SEM, n = 20, except for +0.1 fructose n = 19.

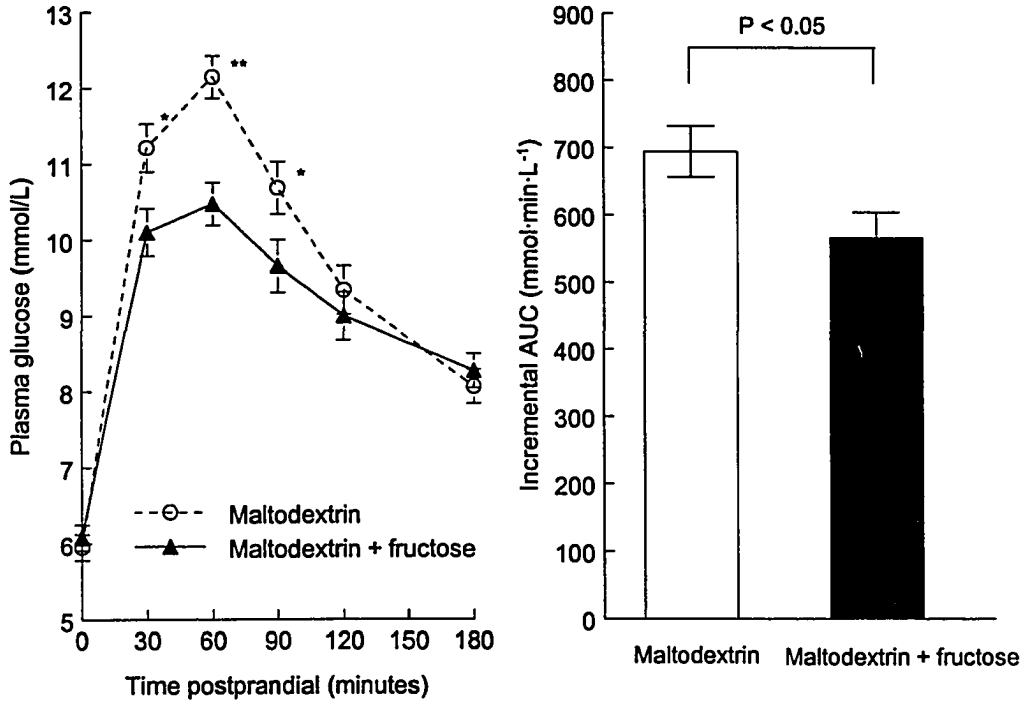

Figure 7.7. Postprandial glycemic response and incremental area under the glucose curve (AUC) after a 1.0 g/kg body wt oral maltodextrin (Maltodextrin) or the same plus 0.075 g/kg body wt supplemental fructose (Maltodextrin + fructose) challenge in male fatty Zucker *fa/fa* rats (Experiment 7). Basal fasting plasma glucose concentrations were not different (5.95 ± 0.17 vs. 6.08 ± 0.17 mmol/L; Maltodextrin vs. Maltodextrin + fructose, respectively). Points at the same time interval differ, $^{*}P < 0.05$, $^{**}P < 0.01$. Data are least squares means ± SEM, n = 19.

CHAPTER 8

POSTPRANDIAL GLYCEMIC RESPONSE OF HEALTHY NONDIABETIC ADULT SUBJECTS TO A GUAR GUM-BASED AMYLASE-INDUCED-VISCOSITY SYSTEM AND(OR) FRUCTOSE INCORPORATED INTO A LOW DEXTROSE EQUIVALENT MALTODEXTRIN-BASED BEVERAGE

INTRODUCTION

Purified viscous forms of dietary fiber have potential benefits for improving blood glucose control in diabetics (Jenkins et al., 1978; Anderson et al., 1987; Wolever and Jenkins, 1993). Although high viscosity appears to be a necessary property of fiber to maximize its benefits on blood glucose (Jenkins et al., 1978), high viscosity reduces the palatability of foods (Ellis et al., 1991), which is a major impediment to the practical use of viscous fiber. Recently, low dextrose equivalent (DE) maltodextrins were reported to prevent the dissolution of konjac flour (glucomannan) (Crosby, 2000). We have found that certain protein sources or low DE maltodextrins can prevent the dissolution of neutral polymers like guar gum (galactomannan; unpublished information). A product formulated with the proper type of fiber and an appropriate starch source could have low viscosity in the absence of α-amylase but, upon ingestion, salivary

amylase would hydrolyze the maltodextrin, freeing water and allowing the fiber to solubilize and form a viscous digesta. This novel concept will be referred to as amylase induced-viscosity (I-V). I hypothesized that the amylase I-V system would attenuate postprandial glycemia.

Fructose has a low glycemic index (Crapo et al., 1980; Jenkins et al., 1981). In Zucker fatty *fa/fa* rats (a model of type 2 diabetes mellitus), Hadley et al. (2000) found that fructose addition (0.16 g/kg body wt) to an oral glucose challenge (1.0 g/kg body wt) reduced ($P < 0.05$) the incremental area under the curve (AUC) for plasma glucose by 34% over the 3-h experiment. Additional experiments (Wolf et al., 1999; unpublished observations) in Zucker fatty *fa/fa* rats demonstrated that supplemental fructose attenuates the postprandial glycemic response to rapidly digested starch (e.g., maltodextrin). I hypothesized that small amounts of oral fructose may be useful in lowering the postprandial glucose response to a carbohydrate (maltodextrin) challenge. Furthermore, I hypothesized that there may be a synergistic effect of amylase I-V and fructose.

Before the recommendation of a product concept, physiological efficacy must be demonstrated. The objective of this experiment was to evaluate the effects of an amylase I-V system and(or) fructose on postprandial glycemia, gastrointestinal tolerance, and satiety in healthy nondiabetic individuals.

MATERIALS AND METHODS

Subjects. A total of 30 healthy nondiabetic (fasting plasma glucose value of < 6.1 mmol/L; American Diabetes Association, 1997) volunteers (13

men and 17 women) were recruited. Subjects had a mean (± SE) age of 51 ± 3 y (range: 18 to 75 y), weight of 68.4 ± 1.8 kg (range: 55.4 to 84.0 kg), and body mass index of 24.2 ± 0.4 kg/m^2 (range: 20.2 to 27.9 kg/m^2). Twenty-two were self-described as Caucasian, four as African American, and four as Asian or Pacific Islander. Subjects did not have active gastrointestinal or metabolic diseases, a first-degree family history of diabetes mellitus or glucose intolerance, recent infection, surgery or corticosteroid treatment. No subjects were receiving oral contraceptives. During subject screening, a fasting blood draw was obtained for determination of routine serum chemistry values (St. Michael's Hospital, Toronto, ON, Canada). All subjects gave written informed consent to the protocol, which was approved by the Western Institutional Review Board (Olympia, WA). Informed consent was obtained from all participants.

Dietary Treatments. Subjects consumed 50 g of available carbohydrate from DE 1 maltodextrin (25 g, Star D, A.E. Staley Manufacturing Co., Decatur, IL) and white bread (25 g) at each meal glucose tolerance test (MGTT). Four DE 1 maltodextrin-based beverages were formulated to test the effects of the amylase I-V system, fructose, and their combination (Table 8.1). Maltodextrin and the carbohydrate from white bread are rapidly digested starches (Wolever et al., 1991; Philipson et al., 1992). The inclusion of these carbohydrate sources enabled the evaluation of the exploratory concepts during a MGTT. For the beverages, ingredients were made into a solution with water,

filled into 250-mL metal cans, and terminally sterilized (Ross Products Division of Abbott Laboratories, Columbus, OH).

White bread was made from the following recipe (Wolever and Bolognesi, 1996): 250 mL of warm water, 334 g of all purpose flour, 7 g of sugar (sucrose), 4 g of salt, and 6.5 g of dry instant yeast. The bread maker was set for a 2 h bake, and turned on. After the bread was made, it was removed from the container, set for 1 h, and weighed. Each loaf contained 250 g of carbohydrate, giving ten 25-g carbohydrate portions. The end crusts were discarded; thus, eight portions were available for the MGTT.

A 50-g available carbohydrate load for a MGTT is a standard practice (Jenkins et al., 1981; Wolever et al., 1991). A 5-g guar gum (3.6 g galactomannan) level was chosen because it was at the upper limit of addition to the 240-g test formula (approximately 2% solution) and is a level known to be effective in lowering the postprandial glycemic response when incorporated into a snack bar (Johnson et al., 1998). In addition, Wolever and Jenkins (1993) document that guar gum levels as low as 2.5 g are effective in reducing postprandial blood glucose and insulin concentrations. A 5-g fructose level was chosen because this dose (approximately 0.075 g/kg body weight) has been shown to effectively blunt the postprandial glycemic response to a rapidly digested starch in Zucker fatty *fa/fa* rats (Wolf et al., 1999; unpublished information). In addition, 5 g of fructose is at an appropriate level to sweeten the product.

Experimental Design. The study was a randomized, double-blind, four group, placebo-controlled, crossover design in which subjects participated in four 3-h MGTT on separate occasions. Subjects were randomly assigned to one of 24 treatment sequences. After an overnight fast, subjects consumed the appropriate test meal. To ensure that subjects had similar glycogen stores on the four test days, subjects were instructed to consume a high-carbohydrate diet (goal 300 g/day, minimum 150 g/day) for 3 d before each MGTT and also were asked to avoid exercise for 24 h before the experiment. On the evening before each MGTT, all subjects consumed a low-residue dinner consisting of one 237-mL can of chocolate Ensure Plus® with additional Honey Graham Crunch Ensure® Bars to provide one-third of each subject's individual daily caloric requirement as estimated by the Harris-Benedict equation multiplied by an activity factor of 1.3 (Harris and Benedict, 1919). After their low-residue evening meal, subjects were instructed to fast overnight, during which they were allowed to consume only water. Smoking was prohibited. Subjects returned on average within 9 d (range 5 to 42 d) for repeat analysis with the appropriate crossover treatment. Subjects were allowed water (250 mL) during each 3-h test. All subjects were recruited and enrolled from one study site.

Blood Glucose Analysis. A fasting (mean of 13 h, range 10 to 15 h) finger-prick capillary blood sample was obtained and collected into a fluoro-oxalate tube after 30 min of rest. Subjects then consumed the appropriate test meal within 10 min. Finger-prick capillary blood was obtained at 15, 30, 45, 60, 90, 120, and 180 min postprandial. Samples were stored at –20 °C for a

maximum of 3 d until analysis of whole blood glucose. Capillary blood glucose was measured by the glucose oxidase method using a YSI analyzer (model YSI 2300 STAT PLUS, Yellow Springs Instruments, Yellow Springs, OH).

Gastrointestinal Tolerance. Using a questionnaire, subjects were asked to report the frequency and intensity of symptoms of nausea, cramping, distention, and flatulence for the 24-h period immediately following consumption of the test material. Intensity and frequency were set to a 100-mm line scale (0 representing "absent" and 100 "severe" and 0 representing "usual" and 100 "more than usual", respectively). Subjects placed a single perpendicular slash mark across the 100-mm horizontal line to indicate their scores for each of these variables of frequency and intensity. A score of 5 or less was considered to be not physiologically meaningful.

Satiety. In order to assess the subjective feeling of hunger, subjects completed a satiety questionnaire immediately before the MGTT, at 1, 2, and 3 h postprandial, and immediately before and after their lunch meal after the MGTT. Subjects rated their feeling of hunger with the following scale: 1 = not at all hungry; 3 = slightly hungry; 5 = moderately hungry; 7 = very hungry; 9 = extremely hungry. In addition, subjects reported the amount of lunch consumed as: much less than usual, moderately less than usual, somewhat less than usual, slightly less than usual, about the same, slightly more than usual, somewhat more than usual, moderately more than usual, or much more than usual.

Breath Hydrogen and Methane. A subset of 10 subjects was evaluated for carbohydrate malabsorption by measuring their end-alveolar hydrogen and methane concentrations hourly for 8 h after they ingested their MGTT. Samples of end-alveolar air were collected into 10-mL glass vacuum tubes using an EasySampler® device (Quintron Instruments, Milwaukee, WI). At 5 h postprandial, subjects were given a standard lunch consisting of one or two 237-mL cans of chocolate Ensure Plus (the number of cans remained consistent for each subject). After the initial 3-h MGTT, these subjects were allowed free access to water.

The concentration of carbon dioxide, hydrogen and methane in breath samples were analyzed by gas chromatography (Microlyzer Gas Analyzer, model SC; Quintron Instruments). The observed hydrogen and methane values were corrected for atmospheric contamination of alveolar air by normalizing the concentrations of observed carbon dioxide to 5.26% (40 mm Hg, the partial pressure of carbon dioxide in alveolar air). Changes in hydrogen concentrations were calculated by subtracting the nadir hydrogen concentration at 0, 1, or 2 h. The nadir value was used as baseline because some subjects have residual hydrogen accumulation in the colon during sleep that may be excreted over the first few hours of the experiment, and therefore this does not reflect a true basal breath hydrogen level (Read et al., 1985; Hertzler et al., 1996). Subjects were classified as having CHO malabsorption (i.e., positive breath hydrogen test) if their breath hydrogen concentrations increased by more

than 10 parts per million (0.9×10^6 g of hydrogen per liter of air or 0.45 µmol per liter) from their basal nadir value.

Study Variables. The primary variable for this study was incremental (i.e., baseline-adjusted) peak blood glucose response. Secondary variables for this study were positive incremental AUC for blood glucose; relative glycemic response; mean incremental change from baseline in blood glucose at 15, 30, 45, 60, 90, 120, and 180 min postprandial; positive breath hydrogen test; subjective gastrointestinal tolerance factors; and subjective satiety factors.

Calculations. Positive incremental AUC for glucose was calculated according to Wolever et al. (1991). The areas after the challenge were calculated with the trapezoid rule, and areas below the baseline fasting blood glucose concentration were ignored. Relative glycemic response was calculated as (incremental AUC for treatment / incremental AUC for Control) x 100.

Statistical Methods. If a subject had one or more glucose measurements missing between 0 and 180 min (both inclusive) during a visit, their data for that visit were not included in the analyses of peak incremental change from baseline in blood glucose and positive incremental AUC. These data were analyzed using a mixed model approach for crossover trials, with treatment and period effects as fixed, and subject effect as random. Incremental change from baseline in blood glucose at individual time points was analyzed using a mixed model approach for crossover trials, with treatment effect as fixed, and subject effect as random. Different covariance structures

were tested according to Brown and Prescott (1999). Compound symmetry variance pattern was found to be the most adequate. Residuals from these models were plotted against the predicted values in order to perform model checking. Treatments were arranged as a 2 × 2 factorial by presence or absence of amylase I-V and fructose. The amylase I-V × fructose interaction was not significant; therefore, statistical results represent main effects of amylase I-V or fructose (SAS version 8.0, SAS Institute, Cary, NC). All results were considered to be statistically significant if the significance level was less than 0.05.

RESULTS

Clinical characteristics of subjects evaluated in this experiment are presented in Table 8.2. The mean fasting blood glucose concentration was not different among treatments. Table 8.3 presents data for incremental peak glucose concentration, positive incremental AUC, and relative glycemic response. Peak incremental blood glucose concentration was lower ($P < 0.01$) when subjects consumed either test meal containing amylase I-V compared to the other treatments, whereas fructose increased ($P < 0.05$) peak incremental blood glucose concentration. Incremental AUC was lower ($P < 0.01$) when subjects consumed the amylase I-V containing products, and fructose had no effect ($P > 0.05$). The relative glycemic response was 80 ± 5.8, 108 ± 4.2, and 91 ± 5.2 for Amylase I-V, Fructose, and I-V+Fructose, respectively.

The postprandial glycemic excursion is graphically depicted in Figure 8.1. When subjects consumed test meals containing amylase I-V, the postprandial rise in blood glucose was reduced ($P < 0.05$) at 15, 30, 45, and 60 min. In addition, there was a slower late postprandial decrease in blood glucose as shown by higher ($P < 0.05$) blood glucose concentrations at 120 and 180 min, indicating slower and prolonged carbohydrate absorption. Time to peak glucose concentration was delayed when subjects consumed products containing amylase I-V compared to the other treatments.

Subjects reported a higher intensity and frequency of cramping, distension, and flatulence when they consumed the amylase I-V containing products (Table 8.4). The frequency at which subjects tested positive for the breath hydrogen test was lower when subjects consumed Fructose compared to the other treatments (60, 50, 20, and 70% for Control, Amylase I-V, Fructose, and I-V+Fructose, respectively; data not analyzed). In contrast to the subjective gastrointestinal tolerance data, the frequency at which subjects tested positive for a breath hydrogen test was similar when subjects consumed Control and the amylase I-V containing products.

Two adverse events were reported following consumption of the guar gum-containing products (one following Amylase I-V and one following I-V+Fructose). These adverse events were associated with gastrointestinal intolerance, diarrhea, and cramping and were transient in nature (self-resolved within 24 hours). It is concluded that these adverse events are not of any safety concern. Certain individuals are more susceptible to gastrointestinal symptoms

following a dose of fermentable fiber (in this case, guar gum). No serious adverse events occurred throughout the trial.

Subjective ratings of hunger during the 3-h MGTT and immediately before and after their lunch meal were similar among treatment groups (data not shown). In addition, the estimated amount of food consumed during the lunch meal following the MGTT was similar among treatment groups.

DISCUSSION

Guar gum is a viscous, water-soluble dietary fiber composed of a β-1,4 mannose backbone with galactose side chains linked α-1,6. This galactomannan is obtained from the endosperm of the seeds of the Indian cluster bean (*Cyamposis tetragonolobus*), a leguminous vegetable. It is widely used in the food industry as a stabilizer and as a thickening and film-forming agent.

Numerous clinical studies have evaluated the acute and long-term effects of supplemental guar gum on glycemic control. Although a dose response trial has not been completed, guar gum doses in the range of 1.8 to 15 g have been found to improve the postprandial glycemic response to oral glucose tolerance tests and MGTT in nondiabetic (Gatti et al., 1984; Jarjis et al., 1984; Heijnen et al., 1995) and diabetic (Gatti et al., 1984; Fuessl et al., 1986; Ebeling et al., 1988) subjects, although Williams et al. (1980) reported no effect. In people with diabetes, medium- to long-term (range 4 weeks to 1 year) guar gum supplementation (9 to 60 g per day) also has shown metabolic improvements in blood glucose control (Aro et al., 1981; Gatti et al., 1984;

Kirsten et al., 1992; Groop et al., 1993). However, many studies have reported no effect on glycemic control (Cohen et al., 1980; Uusitupa et al., 1989; Wilson et al., 1989); the dose of guar gum or the method of guar gum preparation and timing of delivery may be the reason for the discrepancy between studies on glycemic control.

Our novel amylase I-V system allows the production of a palatable, liquid RTF, guar gum-containing product that innately allows an intimate mixing of the meal with guar gum, which is essential for its effect on carbohydrate metabolism (Wolever et al., 1978; Fuessl et al., 1986). We speculate that a meal replacement formulated with the amylase I-V system will have superior clinical efficacy than if it was given as a pre-meal drink or dietary supplement (e.g., capsule). In fact, Wolever et al. (1979) suggest that guar gum is most effective when added to the liquid phase of the meal because the guar gum is fully hydrated and thus at its highest viscosity, which is an important factor in its clinical efficacy (Jenkins et al., 1978; Ellis et al., 1986). Furthermore, when consumed as a pill, guar gum has been associated with esophageal obstruction (Seidner et al., 1990).

Even though guar gum has been shown to be clinically effective in the improvement of postprandial blood glucose and insulin responses, the lack of its incorporation into a palatable product has limited its use. Unfortunately, foodstuffs containing guar gum usually exhibit slimy mouth-feel (Williams et al., 1980), tooth packing (Tredger and Ransley, 1978), and poor palatability (Ellis et al., 1991). The overall hedonic quality of guar-containing foods can be

improved by reducing the average molecular weight (i.e., through hydrolysis) of the galactomannan in guar gum (Ellis et al., 1991); however, this results in a concurrent loss in clinical efficacy (Jenkins et al., 1978). Cohen et al. (1980) noted that the poor palatability of guar limits its use and adds uncertainty pertaining to the degree of patient acceptance and compliance. Therefore, they conclude that it is unlikely that guar will have a useful clinical role in the treatment of diabetes. Our amylase I-V products should be more palatable than other guar gum-containing products (e.g., guar bread), which is essential for long-term patient compliance.

In addition to the challenge of making a palatable product, dietary supplementation with guar gum is also associated with gastrointestinal side effects (e.g., flatulence and diarrhea) from its colonic fermentation, because guar gum is a rapidly fermented carbohydrate (Flickinger et al., 2000). Several experiments have noted an increased incidence of gastrointestinal side effects with guar gum supplementation (Uusitupa et al., 1989; Todd et al., 1990; Kirsten et al., 1992) even resulting in subject drop out (Cohen et al., 1980; Aro et al., 1981). However, several studies have documented that these side effects subside after approximately 2 weeks of supplementation (Tuomilehto et al., 1980; Ebeling et al., 1988). Todd et al. (1990) suggested a gradual increase in the guar gum dose to reduce side effects and to restrict the dose at 15 g/d, which will reduce the incidence of flatulence. Other research groups have found that subjects tolerate guar gum when given at lower daily doses (< 20 g per day) (Cohen et al., 1980; Carroll et al., 1981; Tuomilehto et al., 1988).

The present study was designed to test the efficacy of a low-dose guar gum-based amylase I-V system. We found that 3.6 g galactomannan supplemented to an amylase I-V system effectively blunted postprandial glycemia in healthy nondiabetic adult subjects.

We found that 5 g supplemental fructose when given with a MGTT did not improve the postprandial glycemic response. These results are similar to our recent results (Wolf et al., 2000, unpublished information) in which we found that 10 g supplemental fructose, when given with a MGTT (50 g carbohydrate from instant mashed potatoes), had no effect on the postprandial glycemic response. The lack of efficacy when fed with a meal in the present study, as compared to a free glucose beverage (Atkinson et al., 2000; Hadley et al., 2000; Moore et al., 1999, 2000), may be related to the effects of free glucose on the absorption of fructose (Hoekstra and van den Aker, 1996; Shi et al., 1997). Rats have a higher capacity for absorbing free fructose compared to the human. Fujisawa et al. (1991) determined that the amount of fructose that was completely absorbed by rats was 1.4 to 1.6 g/kg body weight, which is a 10-fold higher dose than that required to reduce the postprandial glycemic excursion in Zucker fatty *fa/fa* rats (Hadley et al., 2000). This may explain why our experiments in rats showed a positive response to supplemental fructose with a MGTT.

In summary, amylase I-V provided a means to maintain blood glucose levels by reducing the early phase excursion and by appropriately maintaining the later phase excursion in healthy nondiabetic humans. Supplementation of 5

g fructose to a 50-g starch meal did not improve the postprandial glycemic excursion in healthy nondiabetic humans. Healthy nondiabetic subjects reported a higher intensity and frequency of cramping, distension, and flatulence when they consumed the amylase I-V containing products. Amylase I-V as a component of a CHO beverage did not affect the subjective ratings of hunger in healthy nondiabetic humans undergoing a MGTT.

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Table 8.1. Composition of Novel Carbohydrate-Containing Test Products and Listing of Ingredients[a]

Ingredient composition	Control	Amylase I-V	Fructose	I-V+Fructose
	g/100 g product			
Water	89.39	87.31	87.31	85.23
DE 1 maltodextrin	10.42	10.42	10.42	10.42
Guar gum	0	2.08	0	2.08
Fructose	0	0	2.08	2.08
Orange flavor	0.12	0.12	0.12	0.12
Sucralose	0.07	0.07	0.07	0.07
Proximate analysis		g/100 g product		
Total solids	9.5	11.8	11.3	13.6
Carbohydrate	9.5	11.7	11.2	13.4
Fructose	0	0	1.95	2.00
TDF	0	1.80	0	1.75
Galactomannan	0	1.53	0	1.50
Nutrient		g/ 240 g serving[b]		
Fructose	0	0	4.68	4.80
TDF	0	4.32	0	4.20
Galactomannan	0	3.67	0	3.60
Maltodextrin	22.8	23.8	22.2	23.2
Viscosity, mPa·sec	8.0	156	8.5	142

[a]Amylase I-V = Amylase-Induced-Viscosity, I-V+Fructose = Amylase-Induced-Viscosity + Fructose, DE = dextrose equivalent, TDF = total dietary fiber.

[b]Products were filled to 240 ± 3 g per serving. Maltodextrin calculated as total solids – (ash + protein + fat + fructose + TDF).

Table 8.2. Clinical Chemistry Values of Subjects at Time of Screening

Characteristic	Value[a]
Glucose, mmol/L	4.5 ± 0.10 (< 6.1)
Triglycerides, mmol/L	1.28 ± 0.15 (< 2.20)
Cholesterol, mmol/L	5.02 ± 0.19 (< 5.20)
HDL cholesterol, mmol/L	1.42 ± 0.08 (> 0.90)
LDL cholesterol, mmol/L	2.98 ± 0.16 (< 3.90)
Hb A_{1c},% of total hemoglobin	5.3 ± 0.09 (3.5-6.5)
Sodium, mmol/L	142 ± 0.3 (135-147)
Potassium, mmol/L	4.2 ± 0.06 (3.5-5.0)
Chloride, mmol/L	103 ± 0.4 (96-106)
Total CO_2, mmol/L	28 ± 0.4 (22-30)
Urea, mmol/L	5.3 ± 0.3 (3.0-7.0)
Creatinine, µmol/L	78 ± 2.7 (< 110)
Aspartate amino transferase, U/L	26 ± 1.5 (8-39)

[a] Mean ± SEM; n = 30. Numbers in parentheses are normal range as defined by St. Michael's Hospital, Toronto, ON, Canada. To convert glucose mmol/L to mg/dL, multiply mmol/L by 18.01. Glucose of 5.0 mmol/L = 90 mg/dL.

Table 8.3. Glycemic Response of Healthy Nondiabetic Subjects Consuming Novel Carbohydrate-Containing Beverages in a Meal Glucose Tolerance Test*

	Treatment			
	Control	Amylase I-V	Fructose	I-V+Fructose
Incremental peak glucose (mmol/L) †,‡	4.2 ± 0.28	2.2 ± 0.16	4.3 ± 0.14	2.7 ± 0.14
Incremental AUC (mmol·min·L^{-1}) †	283 ± 22	215 ± 19	291 ± 20	248 ± 16
Relative response ƒ	100	80 ± 5.8	108 ± 4.2	91 ± 5.2

* Mean ± SEM, n = 30 except for I-V+Fructose, n = 29. Baseline fasting blood glucose concentrations were 4.37 ± 0.09, 4.48 ± 0.12, 4.46 ± 0.08, and 4.45 ± 0.10 mmol/L for subjects consuming Control, Amylase-Induced-Viscosity (Amylase I-V), Fructose, and Amylase-Induced-Viscosity + Fructose (I-V+Fructose), respectively. To convert glucose mmol/L to mg/dL, multiply mmol/L by 18.01 (glucose of 5.0 mmol/L = 90 mg/dL). AUC = area under the curve.

† Effect of amylase I-V, $P < 0.01$.

‡ Effect of fructose, $P < 0.05$.

ƒ Relative response = incremental AUC for treatment / incremental AUC for Control x 100.

Table 8.4. Subjective Gastrointestinal Tolerance Ratings of Subjects Consuming Novel Carbohydrate-Containing Beverages in a Meal Glucose Tolerance Test [a]

	Treatment			
	Control	Amylase I-V	Fructose	I-V+Fructose
Intensity of				
Nausea	1 ± 0.2	1 ± 0.3	0 ± 0.2	0 ± 0.2
Cramping	1 ± 0.5	5 ± 2.9	1 ± 0.2	3 ± 2.1
Distension	0 ± 0.2	4 ± 2.6	0 ± 0.2	5 ± 3.4
Flatulence	2 ± 1.7	5 ± 3.0	1 ± 0.8	9 ± 4.4
Frequency of				
Nausea	0 ± 0.2	1 ± 0.4	0 ± 0.2	0 ± 0.2
Cramping	0 ± 0.2	5 ± 3.0	0 ± 0.1	4 ± 2.6
Distension	1 ± 0.2	2 ± 1.5	0 ± 0.1	5 ± 3.1
Flatulence	2 ± 1.7	6 ± 3.2	2 ± 1.6	10 ± 4.6

[a] Amylase I-V = Amylase-Induced-Viscosity, I-V+Fructose = Amylase-Induced-Viscosity + Fructose. Mean ± SEM, n = 30. A score of 5 or less was considered not physiologically meaningful. Intensity and frequency were set to a 100-mm line scale (0 representing "absent" and 100 "severe" and 0 representing "usual" and 100 "more than usual," respectively).

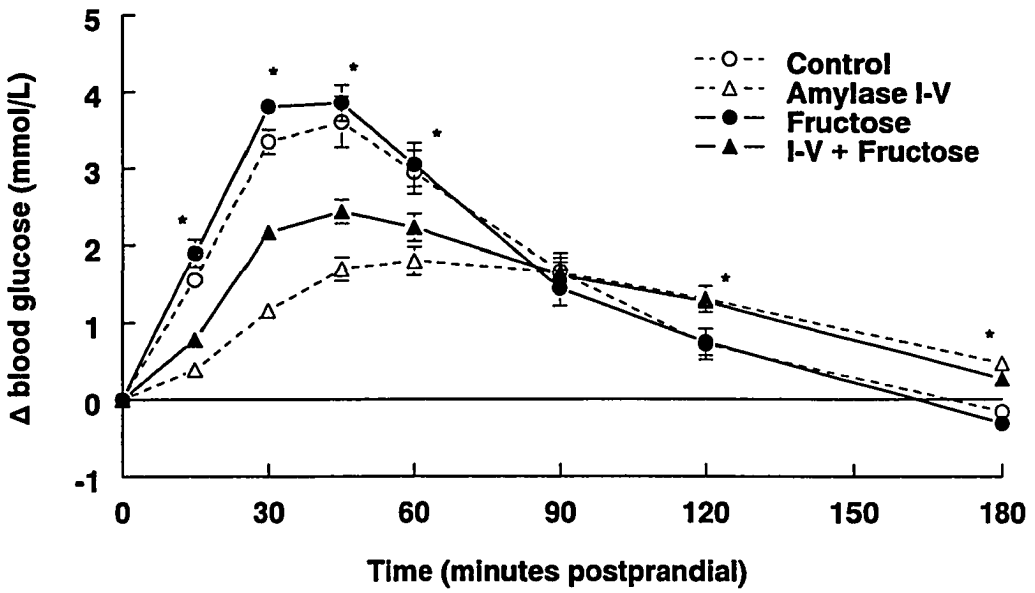

Figure 8.1. Incremental change from baseline in capillary blood glucose response for 30 volunteers consuming 50 g of available carbohydrate from maltodextrin and white bread. Values are mean ± SEM. Fasting blood glucose concentrations were 4.37 ± 0.09, 4.48 ± 0.12, 4.46 ± 0.08, and 4.45 ± 0.10 mmol/L for subjects consuming Control, Amylase-Induced-Viscosity (Amylase I-V), Fructose, and Amylase-Induced-Viscosity + Fructose (I-V + Fructose), respectively. To convert glucose mmol/L to mg/dL, multiply mmol/L by 18.01 (glucose of 5.0 mmol/L = 90 mg/dL). *$P < 0.05$ Control or Fructose compared with Amylase I-V or I-V+Fructose.

CHAPTER 9

CONCLUSIONS

Many of the chemically modified starches evaluated in this dissertation had a reduced extent of *in vitro* hydrolysis, suggesting an increase in the amount of resistant starch. Because of their increased resistant starch composition, the use of chemically modified starch ingredients should attenuate postprandial glycemia and decrease the caloric density of foods containing them. In the case of cornstarch esterified with 1-octenyl succinic anhydride, the postprandial glycemic response was reduced compared with an oral glucose tolerance test given at the same carbohydrate load. Future research should evaluate the caloric value of chemically modified starches and their effects on gut health.

Raw cornstarch was found to have a slow rate of *in vitro* hydrolysis. A study in nondiabetic individuals showed that raw cornstarch reduced the postprandial glycemic response, and it provided a means of normalizing glycemia by reducing the early phase excursion and by appropriately maintaining the later phase excursion, thus avoiding hypoglycemia. This study also provided justification for the use of finger-pricking methodology for the experimental evaluation of glycemic response. Because of its slow rate of

digestion, future product development should evaluate the possibilities of incorporating raw cornstarch into palatable matrices (e.g., snack bar) that will maintain the slow rate of digestion of the raw cornstarch granule.

Two novel approaches to incorporate viscous dietary fiber into liquid nutritional products are described in this dissertation. These examples (acid-induced-viscosity and amylase-induced-viscosity) offer advantages over other food forms (e.g., bread) that have been troubled with poor palatability. In addition, the clinical efficacy found with the low fiber dose should reduce gastrointestinal symptoms that have limited the clinical application of soluble fiber-fortified foods.

In the evaluation of the acid-induced-viscosity concept, the peak rise in blood glucose was lower than predicted from published literature. In a series of experiments using the Zucker fatty *fa/fa* rat model of type 2 diabetes, supplemental fructose (or sucrose) was found to reduce the postprandial glycemic response to glucose or rapidly digested starch. However, supplemental fructose did not improve the postprandial glycemic response to rapidly digested starch in healthy nondiabetic adult subjects. I postulate that this discrepancy may be attributed to the differences in fructose absorption between these two species. This concept deserves further evaluation as supplemental fructose may have clinical benefit in the normalization of postprandial glycemia in people with glucose intolerance.

Certain chemically modified starches and the induced-viscosity concepts provide a means for developing liquid, ready-to-feed nutritional formulas for

people with glucose intolerance. Appropriate intervention trials should be conducted to determine the effects of these concepts on long-term glycemic control. Because of their ability to attenuate postprandial glycemia, the concepts described in this dissertation may have application for products developed to prevent hypoglycemia or enhance satiety.

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