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IMPLICATIONS OF BREAKFAST COMPOSITION ON MID-DAY
MEAL METABOLIC RESPONSES IN ADULTS
WITH TYPE 2 DIABETES MELLITUS

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IMPLICATIONS OF BREAKFAST COMPOSITION ON MID-DAY MEAL METABOLIC RESPONSES IN ADULTS WITH TYPE 2 DIABETES MELLITUS

By

Clesteen A. Clark

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ABSTRACT

IMPLICATIONS OF BREAKFAST COMPOSITION ON MID-DAY MEAL METABOLIC RESPONSES IN ADULTS WITH TYPE 2 DIABETES MELLITUS

By

Clesteen A. Clark

Type 2 Diabetes Mellitus (DM) is a growing public health problem, comprising 90-95% of all cases of people who have diabetes in the U.S. Dietary treatment is an integral component of the medical management of this condition. Research suggests that breakfast meal composition affects glycemic, insulinemic and free fatty acid (FFA) responses. The objective of this study was to determine the relative importance of soluble fiber versus carbohydrate load in the breakfast meal on postprandial glucose, insulin and FFA after a standardized midday meal. In a randomized, cross-over design, 45 male and female subjects with Type 2 DM consumed 3 different breakfast meals:
(A) a high glycemic load consisting of farina plus a placebo drink; (B) a high glycemic load consisting of farina with a psyllium drink administered 20 minutes post-breakfast, and (C) a low glycemic load consisting of a psyllium loop cereal plus placebo drink. A standardized lunch was consumed 3.5 hours after the breakfast meal. Blood concentrations and area under the curve (AUC) values were measured for the morning and afternoon periods. These data indicated that a high glycemic load (Breakfasts A & B) resulted in significantly greater glucose and insulin AUC values ($p<0.05$) post-breakfast as compared to a low glycemic load (Breakfast C). FFA AUC values were significantly lower when subjects consumed Breakfasts A & B versus Breakfast C ($p<0.05$). After the

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midday lunch meal, glucose AUC values were similar for all three breakfast types. Insulin AUC values were similar with Breakfasts A and B, as were Breakfasts B and C, but only Breakfasts A and C were significantly different ($p<0.05$). FFA AUC values were unaffected by breakfast type. These data suggest that reducing the glycemic load at breakfast, independent of the fiber component improves the breakfast postprandial glycemic and insulinemic responses in individuals with Type 2 DM. This influence however, did not result in a second meal effect after the standardized mid-day lunch meal. This study provides support for the American Diabetes Association's guidelines that the amount of carbohydrate, or glycemic load, is more important than the source of carbohydrate in the management of Type 2 DM.

To the pursuit of dreams
While weathering storms
To my family and friends
Who created rainbows and clouds with silver linings
To my late parents who instilled in me the importance of an education
To cancer survivors
With hope, courage and perseverance in the struggle
To Leon, Cecily and Chris
For your love, sacrifices and support
But most of all...
...To our Savior who sustains me.

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INTRODUCTION

It is estimated that 18 million people, or about 7 % of the United States (U.S.) population have DM. There are 13 million cases diagnostically confirmed, and approximately 700,000 new cases diagnosed per annum (NIDDK, 2002). The more prevalent form of diabetes, Type 2, comprises 90-95 % of all people with diabetes, and is a growing public health concern. It is characterized by hyperglycemia resulting from insulin resistance and/or insulin deficiency. If not detected, or if blood glucose levels are poorly controlled, the condition is often associated with macro- and microvascular complications including coronary heart disease, neuropathy, nephropathy, and retinopathy (Chen et al, 1993; Orchard, 1994). These medical complications substantially reduce the overall quality of life and increase the morbidity and mortality associated with the disease (Eastman et al, 1997a,b).

Results of the largest and longest study on individuals with Type 2 DM, the United Kingdom Prospective Diabetes Study (UKPDS), demonstrate that an improvement in glycemic control reduces the incidence and severity of medical complications (Turner et al, 1996). Further, it has been shown that postprandial blood glucose levels might be a better indicator of glucose control than fasting glucose levels. Treatment strategies for diabetes specifically target controlling both fasting and postprandial glucose to lower glycosylated hemoglobin (referred to as glycated hemoglobin or HbA1C levels) (a measure of longer-term blood glucose control over approximately a 2-3 month period) and reduce glucose toxicity (Smith, 1994). Hence, dietary intervention is an integral component in the management and treatment of this

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illness (Franz et al, 1995). Consensus among medical organizations emphasizes dietary modification that is high in complex carbohydrate (CHO) and low in fat to improve glycemic control, lower LDL-cholesterol concentrations, and reduce insulin requirements (Brunzell et al, 1971; Anderson, 1977; Riccardi et al, 1984).

Recent research has criticized a high-CHO diet as contributing to elevated triglyceride levels, which can accentuate the risk of coronary heart disease in individuals with Type 2 DM (Coulston et al, 1989; Chen et al, 1995). As a result, alternative treatment options are emerging including the use of low-carbohydrate diets with high monounsaturated fat, or high-carbohydrate diets with specific modifications such as the inclusion of low glycemic index foods, and/or high dietary fiber foods.

Additionally, studies have shown that soluble dietary fiber can moderate postprandial glucose and insulin concentrations in adults with Type 2 DM (Florholmen et al, 1982; Karlstrom et al, 1988). In addition to reducing acute rises in serum glucose and insulin concentrations when administered with a meal, it has been suggested that soluble fibers may have second-meal effects that blunt the postprandial glucose rise after meals eaten several hours after the fiber ingestion (Jenkins et al, 1980). However, limited evidence of the second-meal effects of soluble fiber exists to date in adults with Type 2 DM.

This study addresses the relative importance of glycemic response after breakfast or the persistence of soluble fiber effects within the intestine (from the first meal) on postprandial glycemic and fatty acid biomarkers after a standardized lunch meal, (i.e., second meal effect) in adults with Type 2 DM. As such, the aim of this study was to

provide insights into the effects of dietary components beyond the immediate postprandial period.

LITERATURE REVIEW

DM is defined as "a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both" [ADA (American Diabetes Association), 2002a]. It is a chronic condition with disturbances in the intermediary metabolism of glucose, coupled with alterations in the metabolism of fat, protein and other substances (Anderson, 1999; Davis and Granner, 1995).

Background Physiology

Insulin secretion is stimulated from the β-cells of the pancreas when increased blood concentrations of glucose and amino acids are present after a meal, with glucose being the primary stimulus for secretion (Anderson, 1999; Davis and Granner, 1995). The hormone travels via the circulation throughout the body and is responsible for "anabolic" processes within the cell, including the uptake, utilization, and storage of glucose, amino acids, and fatty acids (Davis and Granner, 1995). Insulin lowers the level of glucose in the blood by permitting glucose to enter the cells, where glucose is used as fuel for cellular functions (Saudek et al, 1997). A significant portion of the glucose released after a meal is received by muscle, adipose, and other tissues (Anderson, 1999). In muscle cells, insulin also facilitates the storage of glucose in the form of glycogen and the conversion of amino acids into protein (Anderson, 1999). In addition, it aids in converting glucose into fatty acids for storage as triglyceride in adipose tissue cells (Anderson, 1999).

Serum insulin levels gradually fall during the transition from the fed to the fasted state (Anderson, 1999). Low insulin levels promote cellular "catabolic" processes such as

the breakdown and mobilization of storage depots of glycogen, triglyceride, and protein (Anderson, 1999; Davis and Granner, 1995). The fasting state resembles the diabetic state, particularly in the adaptive responses of the liver, muscle, and adipose tissue due to the absolute or relative deficiency of insulin. Liver cells revert from utilizing glucose to producing glucose. When liver glycogen reserves are depleted, the liver utilizes amino acids released by muscles and other tissues to continue producing glucose for the brain, other nervous tissue, and the renal medulla, which have ongoing requirements for glucose. Adipose tissue is stimulated to release fatty acids. The liver utilizes fatty acids to meet its energy needs and to fuel the production of glucose. Muscle and other tissues meet their energy needs through fatty acids and fatty acid oxidation products known as ketones (Anderson, 1999).

In healthy individuals, insulin secretion by pancreatic β-cells is a tightly regulated process, designed to maintain stable blood glucose concentrations during both the fed and fasting state (Anderson, 1999; Davis and Granner, 1995). The extent of the rise in blood glucose after a meal, and the rate at which it returns to baseline is largely dependent on the composition of the meal (Saudek et al, 1997).

Blood glucose levels also fluctuate in healthy individuals, usually rising after meals, but stays within a range of 70 to 140 mg/dl (Saudek et al, 1997). However, in individuals with diabetes, the β-cells of the pancreas do not secrete adequate amounts of insulin and/or the target tissues are resistant to insulin action. Therefore, blood glucose levels are higher than in healthy individuals, because a significant portion of the glucose is unable to enter tissue cells (Saudek et al, 1997). Some individuals with diabetes have persistently high blood glucose that does not vary widely during the course of the day,

whereas others have wide daily blood glucose fluctuations that on average are high, or greater than 140 mg/dl (Saudek et al, 1997).

Classification and Diagnosis of Diabetes Mellitus

New recommendations for the classification and diagnosis of DM were developed in 1997 through an International Expert Committee under the sponsorship of the American Diabetes Association (ADA, 2002a). This new classification system identifies four types of DM: Type 1, Type 2, Gestational Diabetes, and Secondary and Other Types [American Diabetes Association, 2002a; Centers for Disease Control (CDC), 1998]. This work will specifically address Type 2 DM, formerly called adult-onset DM. Sometimes the research literature may refer to non-insulin dependent DM, or NIDDM. For consistency, however, this review and study will reference the newer, more commonly used term, Type 2 DM.

Type 2 DM, is characterized by relative (rather than absolute) insulin deficiency and insulin resistance in peripheral tissues (American Diabetes Association, 2002a; Mayfield, 1998). Risk factors for Type 2 DM include a family history of diabetes, older age, obesity, history of gestational diabetes, impaired glucose tolerance, physical inactivity, and race or ethnicity (CDC, 1998). Unlike the acute onset observed in Type 1 DM, Type 2 DM is often asymptomatic in its early stages and may be undiagnosed for many years, because hyperglycemia develops gradually (American Diabetes Association, 2002a). Type 2 DM, as previously mentioned, accounts for the majority of all diagnosed cases of diabetes (CDC, 1998), and will be further investigated in this research study design.

In 1979, the National Diabetes Data Group (NDDG) developed criteria for the classification and diagnosis of diabetes in the U.S. [American Diabetes Association, 2002a; National Diabetes Data Group (NDDG), 1979]. The World Health Organization (WHO) endorsed these diagnostic criteria in 1985 (WHO, 1985; American Diabetes Association 2002a). According to the NDDG/WHO recommendations, diabetes was diagnosed if fasting plasma glucose was 140 mg/dl or higher or if an oral glucose tolerance test produced a 2-hour post load plasma glucose value of 200 mg/dl or greater.

An International Expert Committee was appointed by the American Diabetes Association in 1995 to review the scientific literature from 1979 and to determine whether new classification and diagnosis criteria were warranted. Following the review of considerable data, the Expert Committee chose to modify the NDDG/WHO recommendations (American Diabetes Association, 2002a). The revised criteria outlines three methods of diagnosing diabetes that require each method to be confirmed, on a subsequent day, either by repeating the same test or one of the two other tests (American Diabetes Association, 2002a):

> Fasting plasma glucose (FPG) level of 126 mg/dl or higher. Fasting is defined as no caloric intake for 6 hours.
>
> or
>
> Non-fasting, random plasma glucose level of 200 mg/dl or higher with classic symptoms of diabetes. Testing may be conducted any time of day without regard to time of last meal.
>
> or

Plasma glucose level of 200 mg/dl or higher, two hours after administering 75 grams of glucose dissolved in water (2-h PG following oral glucose tolerance test)

In their report, the Expert Committee recognized an intermediate group of subjects whose blood glucose levels were not high enough for a diagnosis of diabetes, but had pre-diabetic conditions, or blood glucose levels that were significantly higher than normal. This intermediate group is considered to have impaired glucose tolerance or impaired fasting glucose, with fasting plasma glucose levels between 110 mg/dl and 126 mg/dl (American Diabetes Association, 2002a; CDC, 1998).

Epidemiology of Type 2 Diabetes Mellitus

U.S. Prevalence and Incidence of Type 2 Diabetes Mellitus

Data from the Third National Health and Nutrition Examination Survey, 1999 (NHANES III) has been used to determine the current prevalence of diabetes in the adult U.S. population (Harris et al, 1998). NHANES III consisted of a probability sample that included 18,825 adults ≥20 years of age who were interviewed to determine whether they had been diagnosed with diabetes. A subsample of 6,587 adults who had not been diagnosed with diabetes was subjected to a fasting plasma glucose test to determine the prevalence of undiagnosed diabetes and impaired fasting glucose.

According to NHANES III, the prevalence of diagnosed diabetes in the U.S. is estimated to be 5.1 % for adults 20 years of age or older (Harris et al, 1998). Using the American Diabetes Association's criteria of fasting plasma glucose ≥ 126 mg/dl for diagnosing diabetes, an additional 2.7 % of the population have undiagnosed diabetes and 6.9 % have impaired fasting glucose, based on fasting glucose levels of 110 to 126 mg/dl

(Harris et al, 1998). Extrapolation from the 1997 census of the U.S. population, 10.2 million Americans have diabetes, 5.4 million have undiagnosed diabetes, and 13.4 million have impaired fasting glucose levels (Harris et al, 1998). Approximately 15.6 million U.S. adults, 20 years or older have diabetes (Harris et al, 1998). Although the data does not differentiate among the different types of diabetes, it is safe to assume that the vast majority of cases (approximately 90-95 %) have Type 2 DM.

The National Institutes of Health and the CDC indicate there has been a six-fold increase in diabetes in the U.S. over the last four decades (CDC, 1999). In the 1950s, less than 2 million persons had diabetes compared to estimates of over 10 million in recent years. The increase is particularly relevant among older age groups. For example, among people who were 40-74 years, prevalence increased from 8.9 % in the period 1976-1980 to 12.3 % in the period 1988-1994 (Harris, 1998). (On the basis of WHO criteria, the same age group experienced an increase in the prevalence of diabetes from 11.4 % to 14.3 % from 1976-1980 to 1988-1994. Percentages are different because WHO uses different diagnostic criteria than those currently endorsed by the American Diabetes Association and other U.S. agencies). The American Diabetes Association estimates that 1 person in 14 in the U.S. either has diabetes or will develop diabetes in their lifetime (Berdanier, 1999).

It is estimated that 7 % or 13.4 million of the U.S. population have impaired fasting glucose (CDC, 1998). At present, it is not clear how many of these individuals will develop diabetes over the course of their lifetime. The WHO estimates a 19-61 % progression rate to Type 2 DM within 5-10 years of detecting impaired glucose tolerance (Bennett, 1997).

U.S. Prevalence by Age, Gender, and Ethnicity

Diabetes (diagnosed and undiagnosed) prevalence rates increase with age (Harris et al, 1998). Prevalence rose from 1-2 % at ages 20-39 years to 18-20 % at ages 60-74 years, and reached a plateau at 75 years or older (Harris, 1998). There were minor gender differences in prevalence among adults 60 years or older, but overall the age-standardized rates were similar with 8.4 % and 7.7 % for men and women, respectively (Harris et al, 1998). Individuals with Type 1 and Type 2 DM were not separated in this analysis, but due to the age criteria of the sample (i.e. ≥ 20 years of age), it is likely the majority (about 90 %) experienced Type 2 DM. In general, there is little evidence that the risk for Type 2 DM differs between males and females (Rewers, 1995). Similarly, the National Institute of Diabetes, Digestive, and Kidney Diseases (NIDDKD) reports that Type 1 DM occurs in equal frequency among males and females (NIDDKD, 1998).

Differences in prevalence rates were apparent among ethnic groups. Prevalence rates for diagnosed diabetes were 5.0 % for non-Hispanic whites, 6.9 % for non-Hispanic blacks, and 5.6 % for Mexican-Americans (Harris, 1998). On the basis of 1997 population projections, the number of people 20 years of older who have diagnosed diabetes was estimated to be 7.5 million for non-Hispanic whites, 1.5 million for non-Hispanic black, and 0.8 million for Mexican-Americans (Harris, 1998). Rates were similar for non-Hispanic white men and women, but were higher for non-Hispanic black and Mexican-American women (Harris et al, 1998). Undiagnosed diabetes prevalence rates were highest among Mexican-Americans (4.5 %), followed by non-Hispanic blacks (3.6 %), and non-Hispanic whites (2.5 %) (Harris et al, 1998).

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Prevalence of impaired fasting glucose was higher for men than for women in each ethnic group (Harris et al, 1998). Among males, Mexican-Americans had higher rates of prevalence (8.9 %) than non-Hispanic whites (6.8 %) or non-Hispanic blacks (7.0 %) (Harris et al, 1998). Translation of these rates to the projected 1997 population indicates impaired fasting glucose levels for 10.3 million non-Hispanic whites, 1.3 million for non-Hispanic blacks, and 0.9 million for Mexican-Americans (Harris et al, 1998).

Total prevalence of diabetes and impaired fasting glucose combined, is estimated to be 21.6 million for non-Hispanic whites, 3.6 million for non-Hispanic blacks, and 2.1 million for Mexican-Americans (Harris et al, 1998). In addition to these groups, the CDC reports that 9 % of American Indians and Alaska Natives have diagnosed diabetes (CDC, 1998). These two groups are 2.8 times more likely to have diagnosed diabetes than non-Hispanic whites of similar age (CDC, 1998). National diabetes prevalence data for Asian Americans and Pacific Islanders are currently limited, although they have been identified as high-risk groups (CDC, 1998).

Global Prevalence and Incidence

Worldwide, WHO projects a 122 % increase in diabetes between 1995 and 2025 (WHO, 14 September 1998). The largest proportion of this increase will be from developing countries, with India expected to experience the largest increase during this period (WHO, 1998). Listed in Table 1 are the top ten countries that had the largest number of persons with diabetes according to WHO diagnostic criteria and statistics in 1995, with projections of the top ten countries in 2025 if current demographic projections hold (WHO, 1998).

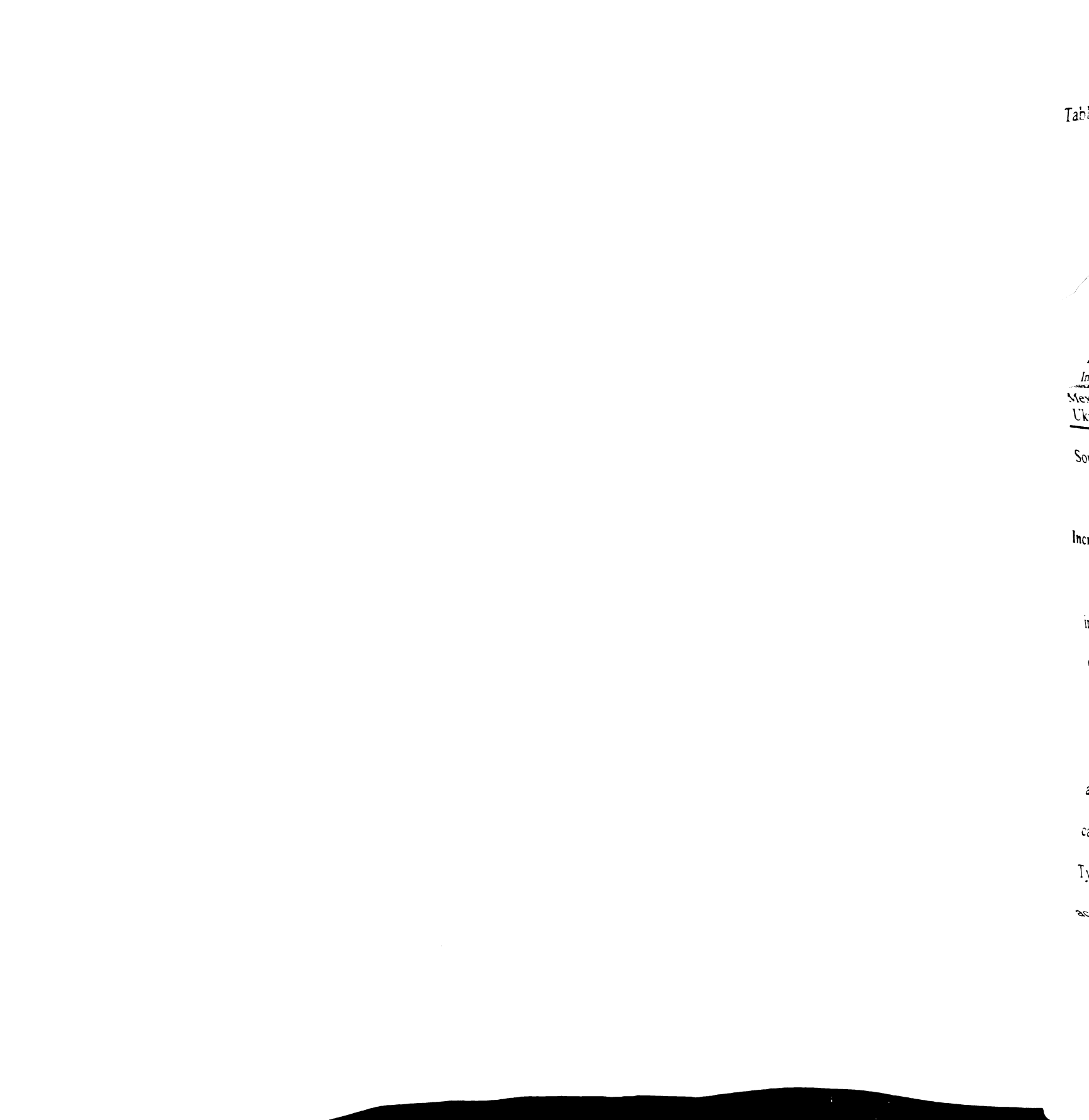

Table 1. Current and Projected Prevalence of Diabetes Mellitus

1995	Million	2025	Million
India	19	India	57
China	16	China	38
USA	14	USA	22
Russian Federation	9	Pakistan	15
Japan	6	Indonesia	12
Brazil	5	Russian Federation	12
Indonesia	5	Mexico	12
Pakistan	4	Brazil	11
Mexico	4	Egypt	9
Ukraine	4	Japan	9

Source: World Health Organization, 1998

Increasing Prevalence in Youth

Incidence of Type 2 DM, commonly considered an adult disease, is significantly increasing among children and adolescents, 10-19 years of age (Rosenbloom et al, 1999; CDC, 1999). This trend has been found among Pima Indians in Arizona, Native Indians in Manitoba, Canada, Native Indians in Ontario, Canada, African-Americans in Ohio, Mexican-Americans in California, Arabs in Libya, and Japanese in Japan (Rosenbloom et al, 1999). Although the overall prevalence rate is less than 1 % of those in this age category, it is disturbing to note the increase in incidence in recent years. Before 1992, Type 2 DM comprised 2-4 % of all childhood diabetes, but by 1994, Type 2 DM accounted for 16 % of all new cases (Rosenbloom et al, 1999). Among Native North American Indian youth, 30 % of new cases of diabetes have Type 2 DM. Similar findings are reported among Mexican American children in California (Rosenbloom et al, 1999).

There has been more than a 30-fold increase in the incidence of Type 2 DM among Japanese school children, concomitant with changing food consumption patterns and the rising rate of obesity (Rosenbloom et al, 1999).

Etiology of Type 2 Diabetes Mellitus: Genetics and Environment

Study of the causes of Type 2 DM indicates it is a condition with multifactorial components that involve the complex interaction of genetic and environmental factors. Increasing age is well recognized as a major factor in the development of this disease (Bennett, 1997). Type 2 DM is "most likely to appear when genetic susceptibility and/or age interact with additional environmental factors" (Bennett, 1997).

Genetic Component

Although environment has a strong role in Type 2 DM susceptibility, there are several lines of evidence, which suggest a genetic link in the etiology of this disease. Analysis of prevalence data clearly demonstrates that specific ethnic subgroups have a much higher prevalence of the disease than the population as a whole. In the U.S. for example, African-Americans, Mexican-Americans, Native Americans, Asian-Americans, and Pacific Islanders have higher prevalence rates than the rest of the population (American Diabetes Association, 2002b). The fact that prevalence varies widely among diverse ethnic groups sharing a common environment suggests a genetic basis for the disease (Lebovitz, 1999).

The Native American subgroup in Arizona—the Pima Indians, have the highest reported prevalence of Type 2 DM of any population group in the world (Lebovitz, 1999; Bennett, 1999). Prevalence is 10-fold higher in this group compared to the general U.S. population, despite results of recent surveys, which indicate similarities in dietary intake

between the two groups (Lebovitz, 1999). By age 60, diabetes is prevalent in 50-70 % of the Pima population (Bloomgarden, 1995). Higher rates of obesity in this group do not entirely explain the higher diabetes rates, because even lean Pimas have a higher incidence of diabetes (Lebovitz, 1999). As in other populations, diabetes is strongly familial, and if diabetes develops before age 45 years, Pima offspring have a 2-4-fold increase risk of developing diabetes (Lebovitz, 1999). Furthermore, factors that predict the development of the disease, such as obesity, higher fasting glucose levels, abnormal glucose tolerance tests, insulin resistance, and impaired insulin secretion are also heritable traits in this community (Lebovitz, 1999). In one well-documented study, it has been shown that the prevalence of Type 2 DM in the Pima Indian community is inversely related to the extent of interbreeding with the white population (Knowler et al, 1988).

Twin studies provide further evidence for the genetic basis of Type 2 DM (Ghosh and Schork, 1996). Concordance rates of Type 2 DM in monozygotic twins have been estimated to be between 20 % and 90 % (Ghosh and Schork, 1996). The wide disparity in concordance has been partly attributed to methodological differences among studies. If twin pairs are followed a long time, concordance rates increase due to the increased prevalence of Type 2 DM with age (Ghosh and Schork, 1996). Monozygotic twins have higher concordance than dizygotic twins, further indicating the hereditability of Type 2 DM (Ghosh and Schork, 1996).

Molecular geneticists are currently attempting to identify specific genotypes of Type 2 DM. One approach has been to develop detailed genetic maps of families with clearly inherited forms of diabetes to identify which area of the genome has the disease over generations (O'Rahilly and Savill, 1997). Another approach has been to identify all

the substances involved in normal insulin secretion and action and then examine whether patients with inherited forms of diabetes have defects in the genes that control the synthesis and action of these substances (O'Rahilly and Savill, 1997).

One of the genotypes that have been well studied is the maturity-onset diabetes of the young (MODY), characterized by early onset, usually before 25 years of age, and inherited as a simple dominant trait (Ghosh and Schork, 1996; Vionnet et al, 1992; Neel, 1999). The clinical features of MODY, however, are similar to late onset Type 2 DM (Vionnet et al, 1992). Studies indicate a defect in glucose-stimulated insulin secretion, suggesting a dysfunction of pancreatic β-cells, rather than a condition due to insulin resistance (Yamagata et al, 1996). Since its recognition, MODY has been divided into five subtypes, each associated with a specific aspect of glucose metabolism and each linked to a mutated gene (Neel, 1999; Yamagata et al, 1996). For example, one of the MODY subtypes has a mutation in the gene encoding for glucokinase, an enzyme involved in the regulation of insulin secretion and integration of liver intermediary metabolism (Vionnet et al, 1992; Yamagata et al, 1996).

Genome scans have been used to assess a number of chromosome loci carrying diabetes predisposing genes in hundreds of different ethnic and racial families (Permutt and Hattersley, 2000). One such study carried out in Mexican Americans, identified a susceptibility locus, designated as Type 1 DM, on chromosome 2 (Cox et al, 1999). The Type 1 DM susceptibility gene may also contribute to the development of diabetes in other populations such as various European groups, Japanese, and Pima Indians, but to a lesser degree than in Mexican Americans (Horikawa et al, 2000). Researchers working within the Type 1 DM area have recently described a calpain-10 gene, which encodes for

the cysteine protease, calpain-10 (Yamagata et al, 1996). The contribution of calpain-10 gene to the development of Type 2 DM is estimated to be 14 % in Mexican Americans and 4 % in Europeans (Finnish and German) (Yamagata et al, 1996). Preliminary studies indicate that genetic variation in calpain-10 also affects other population groups as well.

Discovery of this gene suggests the potential of a new biochemical pathway that may contribute to the development of diabetes. Calpains are proteases that cleave specific substrates, causing its activation or inactivation (Saido et al, 1994). They are implicated in variety of cellular functions, including the down regulation of insulin receptor substrate-1, an important mediator of insulin action (Smith et al, 1996). Calpain-10 mRNA has been found in pancreatic, liver and muscle cells, key tissues involved in glucose homeostasis (Yamagata et al, 1996). Due to the diversity of proteases and the numerous functions they perform, it is speculated that additional proteases and other calpain genes may also be implicated in Type 2 DM susceptibility (Yamagata et al, 1996).

Environmental Factors

Genetic variation alone cannot explain the dramatic increase in the incidence of Type 2 DM in the U.S. and worldwide. For example, there is an even more pronounced increase in the incidence of diabetes among Asian Indians who migrate to Europe from the Indian subcontinent, as they become exposed to western lifestyles (Ghosh and Schork, 1996). Similar patterns are emerging from studies of migrants from rural to urban areas within less developed countries (O'Rahilly and Savill, 1997). Populations undergoing westernization in the absence of migration, such as the Pima Indians also experience higher prevalence, rates of obesity and Type 2 DM (Gohdes et al, 1993).

According to one hypothesis, it is speculated that polygenic forms of Type 2 DM are a consequence of a "thrifty genotype" (a theory involving the adaptation of genes to the environment) that has evolved to accommodate an abundant food supply and a sedentary lifestyle from ancient times when food was scarce and physical activity was high (Neel, 1999). The theory suggests our tribal, hunter-gatherer ancestors had a higher muscle mass than modern man, whereas modern man has muscle cells that are well padded with fat. Fat and skeletal muscle cells have markedly different insulin sensitivities (Venkatesan et al, 2001), and therefore the overall sensitivity of insulin to skeletal muscle cells was profoundly altered in the transition to a westernized lifestyle, leading to obesity, hypertension, and diabetes. These diseases of civilization are rarely encountered in tribal populations whose sustenance is based on "hunting, foraging, and limited agricultural practices" (Neel, 1999).

A wealth of information into the etiology of Type 2 DM has been obtained from studying the Pima Indians of Arizona. Study of this population has clearly demonstrated how the environmental factors in addition to genetic factors contribute to the development of Type 2 DM. Historical and archaeological evidence indicate the Pima Indians have lived in the deserts of central Arizona for more than 2000 years (Lebovitz, 1999; Bennett, 1999). Diabetes was not found in this community until the second half of the 20th century (Lebovitz, 1999; Bennett, 1999). Unexpectedly in 1963, it was revealed that several individuals in the community had diabetes. Later studies also indicated Pima Indians were hyperinsulinemic and insulin resistant compared to other ethnic groups, and that even among those with an early onset, β-cell antibodies were not a part of the disease process (Lebovitz, 1999). In the last 50 years, prevalence of Type 2 DM among the Pimas

has progressively increased, and virtually all of the diabetes is of the Type 2 category (Lebovitz, 1999; Bennett, 1999). Several lifestyle changes coincide with this increase and appear to promote the development of this condition.

An illustration of how environmental factors influence the clinical expression of diabetes can be further explained by comparing the diabetes prevalence rates and lifestyle of the Pima Indians of Arizona with the Pima Indians in northern Mexico. The Pimas of northern Mexico were separated from the Pimas of Arizona 700-1000 years ago and have lived a secluded life from Western influences. Their lifestyle involves high physical activity and a traditional diet low in saturated fat and high in complex carbohydrates. Obesity is an infrequent occurrence and the prevalence of diabetes is considerably lower than that of the Pima Indians in Arizona (Lebovitz, 1999). Thus, further discussion is warranted of these primary influencers: obesity, physical inactivity, and diet.

Obesity

Obesity is well recognized as a major risk factor for Type 2 DM (Rewers and Hamman, 1995; Pi-Sunyer, 1996). Numerous cross-sectional, retrospective and prospective studies consistently demonstrate the association between obesity and the prevalence of Type 2 DM (Rewers and Hamman, 1995). One of the groups most studied is the Pima Indians.

Since the 1960s, there has been a dramatic increase in mean body mass index (BMI) and obesity among Pima Indians, particularly among those under the age of 50 years (Bennett, 1999). Incidence of diabetes is strongly linked to BMI in this population. Estimates indicate the risk of diabetes is 10-fold higher in those with a BMI above 40 compared to those with a BMI of less than 20 (Lebovitz, 1999). The higher prevalence of

obesity is associated with increased insulin resistance and reduced glucose tolerance (Lebovitz, 1999).

Increased risk of diabetes with increasing BMI has also been demonstrated in several other populations, including British men (Wannamethee and Shaper, 1999), U.S. nurses (Colditz et al, 1990), Swedish men (Ohlson et al, 1988) Israeli men (Medalie et al, 1974), and Norwegians (Midthjell et al, 1999). Data from NHANES III indicate a significantly higher prevalence of Type 2 DM among obese men and women younger than 55 years than normal weight individuals of the same age (Must et al, 1999). Most children affected with Type 2 DM are likely to be obese (Rosenbloom et al, 1999; CDC, 1999). In addition to the level of obesity, duration of obesity is also an important Type 2 DM risk factor (Saido et al, 1994; Wannamethee and Shaper, 1999). Studies assessing risk factors of diabetes consider obesity the largest environmental influence on the development of diabetes in a population (Maggio and Pi-Sunyer, 1997).

Physical Inactivity

A sedentary lifestyle is also associated with the increased risk of Type 2 DM. Low physical activity promotes obesity and is also an independent risk factor for diabetes (Lebovitz, 1999). Prospective epidemiological studies consistently demonstrate an inverse relationship between physical activity and the development of Type 2 DM (Wing et al, 2001; Rewers and Hamman, 1995; Perry et al, 1995; Helmrich et al, 1991). The evidence exists that among the Pima Indians with Type 2 DM, there is lower lifetime physical activity than those without diabetes (Kriska et al, 1993; Lebovitz, 1999). It is postulated that in most individuals, the protective effect of physical activity is its ability to lower insulin resistance (Rewers and Hamman, 1995).

Diet

Diet also plays a role in the development of this disease. This is clearly evident among the Pima Indians. In recent years, traditional farming has declined, resulting in the increased reliance on government surplus commodities, typically high in fat (Lebovitz, 1999). The composition of the Pima diet changed from one that provided 70% calories from carbohydrate, 15% calories from fat, and 15% calories from protein to one that provides 50% calories from fat, 30% calories from carbohydrates, and 20% calories from protein (Lebovitz, 1999). A high-fat, low-complex carbohydrate diet is associated with reduced glucose tolerance, higher fasting glucose levels, and impaired β-cell function (Lebovitz, 1999; Gannon et al, 1998; Frape et al, 1997; Frape et al, 1998).

However, epidemiological studies of diet and the development of Type 2 DM have produced mixed results in part due to the difficulties of accurately evaluating dietary intake (Rewers and hamman, 1995). The Nurses' Health Study (Colditz et al, 1992), the Zutphen Study (Feskens and Kromhout, 1989), and the Israeli Heart Study (Medalie et al, 1978) found no relationships with dietary factors (carbohydrate and fiber intake) and Type 2 DM, but a four-year, follow-up study of elderly subjects did observe an association between carbohydrate intake and glucose intolerance, after adjusting for obesity and other interfering variables (Feskens et al, 1991). A retrospective study of Hispanic and non-Hispanic whites found higher fat intake was linked to undiagnosed Type 2 DM and impaired glucose tolerance in only those who were sedentary (Marshall et al, 1991). A two-year, follow up of Hispanic and non-Hispanic whites with impaired glucose tolerance indicated that a

40 g higher fat intake increased the risk of Type 2 DM by seven-fold, after adjusting for age, obesity, and other confounding factors (Marshall and Hamman, 1988. Additional discussion on dietary composition will ensue in the section on dietary intervention of Type 2 DM.

Pathogenesis of Type 2 Diabetes Mellitus

As suggested earlier, Type 2 DM is most likely to manifest itself when genetic susceptibility and/or age interact with additional environmental factors (Bennett, 1997). It is a progressive condition that develops over the course of several years. Current theories envision two separate arms in the pathogenesis of Type 2 DM. Initiators of the process include "genotype, age, obesity, physical inactivity, and diet," which contribute to insulin resistance and impaired glucose (Bloomgarden, 1995). Promoters of the disease include "glucose toxicity, genotype, age and decreased β-cell mass," which contribute via insulin deficiency, leading to the progression from impaired glucose tolerance to overt Type 2 DM (Bloomgarden, 1995).

In the early stages, individuals are not usually aware of the disease process. Both insulin resistance and deficient insulin secretion are important early determinants of abnormal glucose tolerance, and are necessary for the development of overt diabetes. Table 2 outlines the three phases acknowledged in the development of Type 2 DM.

Table 2. Development of Type 2 Diabetes Mellitus

Phase I: Insulin Resistance	Phase II: Impaired Glucose Tolerance	Phase III: Insulin Secretory Failure
• Normal β-cell function • Normal glucose tolerance • Increased postprandial hyperglycemia and hyperinsulinemia • Insulin resistance influenced by genetic susceptibility and environmental factors, e.g. obesity	• Evidence of declining β-cell function • Rising glucose levels	• Reduced β-cell function • Fasting hyperglycemia • Insulin deficiency • Overt Symptoms of Diabetes

urce: Data summarized from DeFronzo et al, 1992; Bennett, 1997

ase I: Insulin Resistance

Epidemiological studies suggest insulin resistance and hyperinsulinemia as some he earliest metabolic aberrations detected among individuals susceptible to Type 2 I (DeFronzo et al, 1992; Bennett, 1997; Kekalainen et al, 1999). Studies utilizing the lycemic insulin-clamp technique demonstrate that development of insulin resistance is ociated with the progression of normal to impaired glucose tolerance (DeFronzo et al, 2). During this phase, the pancreas is able to increase insulin secretion to offset the ılin resistance, and glucose tolerance remains normal (DeFronzo et al, 1992). Mild ns of insulin resistance may be difficult to detect because of the increased onsiveness of β-cells during this phase.

Although insulin resistance often precedes impaired glucose tolerance, it should be noted that it might not be a prerequisite for all individuals. For example, in maturity-onset diabetes of the young (MODY), diabetes develops due to a mutation of the glucokinase gene, which results in impaired β-cell function (Krentz, 1996).

Phase II: Impaired Glucose Tolerance

Persistent insulin resistance eventually leads to rising glucose levels and impaired glucose tolerance because the continued demand for increased insulin production cannot be met by pancreatic β-cells (Reusch, 1998; Bennett, 1997; DeFronzo et al, 1992). Impaired glucose tolerance is currently the best predictor of developing Type 2 DM. Its presence suggests that insulin resistance and compensatory insulin secretion are in a state of precarious balance (Bennett, 1997). An increase in insulin resistance or a reduction in insulin secretion will result in progressive hyperglycemia and the development of Type 2 DM (Bennett, 1997). The progression from normal glucose tolerance to impaired glucose tolerance is marked by increases in both fasting and glucose-stimulated plasma insulin levels (Bennett, 1997).

Phase III: Insulin Secretory Failure

Insulin secretory inadequacy is the final stage in the development of Type 2 DM (Bennett, 1997; DeFronzo et al, 1992). Deficiency in insulin secretion is believed to result from an impairment in β-cell function and/or the inability to progressively secrete sufficient insulin to compensate for the increase in insulin resistance (Bennett, 1997). Fasting hyperglycemia and overt symptoms of diabetes are eventual outcomes. The precise cause of pancreatic exhaustion is unknown, but may be related to glucose toxicity

(i.e. deleterious effects of hyperglycemia) in a genetically predisposed β-cell (Bennett, 1997; Flatt et al, 1997; Lebovitz, 1999).

Insulin Resistance and Obesity

It has been well documented that obesity is a major factor in the development of insulin resistance. Common indices of obesity, such as BMI, are only moderately associated to insulin resistance (Chisholm et al, 1997). Central or android obesity on the other hand, characterized by upper body (i.e. abdominal) fat distribution, is linked to the risk of Type 2 DM, in contrast to gynoid obesity, characterized by fat distribution in the lower (i.e. hip) region of the body (Kissebah et al, 1982). In fact, there is a strong relationship between waist circumference, which is more highly predictive on intra-abdominal fat than waist:hip ratio, and Type 2 DM development (Chan et al, 1994; Carey et al, 1997; Han et al, 1998). Populations with a high prevalence of Type 2 DM, such as the Pima Indians, Mexican Americans, and South Asians are predisposed to abdominal obesity or visceral fat deposition, indicated by a high waist:hip ratio (Krentz, 1996). Computerized tomography and magnetic resonance imaging have demonstrated a strong relationship between central intra-abdominal fat and insulin resistance (Chisholm et al, 1997; Yamashita et al, 1996; Han et al, 1997). This technique indicates central obesity consists primarily of intra-abdominal fat accumulation rather than subcutaneous fat accumulation (Brunzell and Hokanson, 1999). The amount of intra-abdominal fat is correlated with insulin resistance even among men with normal BMI and among women with typical fat accumulation in the hips and thighs (Brunzell and Hokanson, 1999). Twin studies indicate the presence of specific genetic determinants of central abdominal obesity, independent of overall obesity (Chisholm et al, 1997). Caloric restriction and/ or

exercise reduces intra-abdominal fat and insulin resistance (Brunzell and Hokanson, 1999).

Mechanisms through which central abdominal fat increases insulin resistance are not well understood. Studies conducted *in vitro* indicate that intra-abdominal fat is more responsive to lipolytic hormones than fat from the glutofemoral region C. This finding has been confirmed *in vivo* studies, which suggest increased free fatty acid (FFA) turnover in abdominal fat depots and an increased flow of fatty acids to the liver via the portal vein (Jensen et al, 1989). The direct release of FFA into the portal vein (Lebovitz, 1999; Chisholm et al, 1997), increases gluconeogenesis and glucose output from the liver. Elevated plasma FFA enhance cellular FFA uptake and stimulate lipid oxidation (DeFronzo et al, 1992). In muscle, the accelerated rate of fat oxidation reduces insulin-mediated glucose disposal by inhibiting glucose oxidation and impairing glycogen synthesis (DeFronzo et al, 1992). Animal studies demonstrate a close relationship between muscle insulin resistance and fat stores in muscle, suggesting both circulating FFA and intramuscular fat stores are likely to contribute to muscle insulin resistance (Chisholm et al, 1997). It has been shown that high physiological concentrations of FFA significantly inhibit insulin binding, degradation, and action in isolated rat hepatocytes (Svedberg et al, 1990). Other processes may also contribute to the obesity and insulin resistance relationship. It has been shown that hormones such as tumor necrosis factor (TNFα) secreted from fat cells may impair insulin action (Reusch, 1998; Chisholm et al, 1997).

Individuals with central adiposity are more likely to develop the metabolic insulin resistance syndrome known as syndrome X (Reusch, 1998; Brunzell and Hokanson,

1999). This syndrome is associated with insulin resistance, hyperinsulinemia, hypertriglyceridemia, obesity, impaired glucose tolerance, diabetes, hypertension, and cardiovascular disease. It is postulated that central obesity leads to insulin resistance and elevated FFA, which promotes gluconeogenesis and hypertriglyceridemia (Yang et al, 2003). Elevated plasma triglycerides and insulin resistance are associated with decreased high-density lipoprotein (HDL) cholesterol levels and increased production of small, dense low-density lipoprotein (LDL) particles (Brunzell and Hokanson, 1999). In 75-85 % of patients studied, Type 2 DM is preceded by insulin resistance, hyperinsulinemia, obesity, and the unique dyslipidemia described (Lebovitz, 1999). The clustering of hypertriglyceridemia, low HDL-cholesterol, and small dense LDL-cholesterol particles is also associated with cardiovascular disease risk, and has been termed the "atherogenic lipoprotein phenotype" (Brunzell and Hokanson, 1999).

The recent identification of the obesity (ob) gene and its product, leptin, are providing new insights into the relationship between obesity and insulin resistance (Krentz, 1996). Rodent studies indicate alterations in the production of leptin can induce obesity and diabetes (Krentz, 1996). In humans, raised leptin concentrations and insulin resistance are linked, independent of body fat mass (Krentz, 1996). A causal relationship between leptin and reduced insulin sensitivity is currently being investigated.

Morbidity and Mortality in Type 2 Diabetes Mellitus

Epidemiological studies have shown that hyperinsulinemia, a hallmark of insulin resistance is a risk factor for morbidity and mortality in common diseases (Reaven, 1988). A consequence of this condition (insulin resistance) is an increased risk of macro- and micro-vascular complications (Svedberg, 1990).

Macrovascular Complications

Atherosclerosis is the most frequently observed complication of diabetes (Anderson, 1999). Compared to healthy individuals, patients with diabetes have a two-to four fold higher risk for coronary heart disease (American Diabetes Association, 2002c). They also have a two to four times higher relative risk of stroke, and it is estimated 60 to 65 % of individuals with diabetes have high blood pressure (CDC, 1998).

Cardiovascular disease is the primary cause of death in 60-70 % of patients with Type 2 DM in industrialized countries (Savage and Narayan, 1999). Furthermore, NHANES 1971-93 data indicate that the decline in heart disease mortality observed in the general U.S. population is smaller for diabetes patients (Gu et al, 1999). Deaths from heart disease dropped 36 % in men without diabetes compared to only 13 % in men with diabetes. Among women with diabetes, death from heart disease rose 23 % compared to a 27 % decrease in women with no diabetes. Typically, cardiovascular disease is less common in pre-menopausal women than in their male counterparts, due to the protection offered by female sex hormones (Kaseta et al, 1999). The presence of diabetes removes the gender-specific protection observed in premenopausal women because of the strong link between diabetes and cardiovascular disease (Kaseta et al, 1999). With the increasing prevalence of diabetes and the smaller decline in heart disease mortality, it is anticipated that diabetes may become an increasingly important factor for heart disease mortality in the U.S. population (Gu et al, 1999).

The mechanisms through which diabetes accelerates atherosclerosis have not been well defined. It is possible that hyperglycemia, hyperinsulinemia, and insulin resistance may each influence the onset and development of atherosclerotic disease, by causing lipid

abnormalities, platelet disturbances, altered coagulability, and arterial wall thickening and stiffness (Massi-Benedetti and Federici, 1999). Dyslipidemia commonly found in Type 2 DM patients--elevated triglyceride levels, decreased HDL-cholesterol levels, and the preponderance of small, dense LDL-cholesterol particles are associated with increased macrovascular disease (American Diabetes Association, 2002c). LDL- cholesterol levels may be normal or only modestly elevated (Nuttall and Chasuk, 1998). Hypertension, frequently found among patients with Type 2 DM, is yet another risk factor.

Microvascular Complications

In addition to cardiovascular complications, diabetes is characterized by microvascular complications that significantly increase morbidity and mortality. Approximately 20-30 % of individuals with Type 2 DM develop nephropathy or kidney disease (American Diabetes Association, 2002d). Diabetes is the leading cause of kidney failure or end-stage-renal disease, occurring more frequently with Type 1 DM and to a lesser extent with Type 2 DM (Nuttall and Chasuk, 1998). Diabetes injures the small blood vessels in the kidneys, impairing their ability to filter impurities from the blood for excretion in the urine (NIDDK, 1994). The first sign of nephropathy is the presence of albumin in the urine, referred to as microalbuminuria (Nuttall and Chasuk, 1998). About 20-40 % of individuals with Type 2 DM with microalbuminuria will develop overt nephropathy without intervention, and about 20 % will progress to end-stage-renal-disease (Nuttall and Chasuk, 1998). Presence of albumin in the urine is also a biomarker for increased cardiovascular morbidity and mortality (Nuttall and Chasuk, 1998). Preliminary evidence suggests that lowering blood cholesterol may also reduce

albuminuria (Nuttall and Chasuk, 1998). Hypertension, frequently found in Type 2 DM significantly accelerates the progression of nephropathy (Nuttall and Chasuk, 1998).

Another microvascular complication of Type 2 DM is neuropathy or nerve disorder. Three types of neuropathy can occur with diabetes (Joslin Diabetes Center, 1999a). Sensory neuropathy affects the nerves that carry information from the peripheral parts of the body to the brain. It is the most common form of neuropathy found in diabetics and can lead to numbness, tingling, pain, and inability to feel heat and cold in the hands and feet. Autonomic neuropathy affects the nerves that control the involuntary actions of the body, including the heart, stomach, intestine, and bladder. This type of neuropathy can lead to diarrhea, bloated stomach, impotence, and the inability to empty the bladder completely. Motor neuropathy affects the nerves that carry signals to muscles, which can lead to muscle weakness. About 60 % of patients with diabetes have neuropathy, but 30-40 % have no symptoms (NIDDK, 1995).

Diabetic retinopathy is reported to be the most common cause of blindness in adults in the 20-74 age category (American Diabetes Association, 2002e). It is a vascular complication of the retina related to the duration of diabetes (American Diabetes Association, 2002e). Over 60 % of patients have some degree of retinopathy after 20 years of the disease (American Diabetes Association, 2002e).

Mortality

The death rate attributed to diabetes has risen by 30 % since 1980, while it has fallen for other diseases such as cardiovascular disease and stroke (Joslin Diabetes Center, 1999b). Life expectancy among individuals with Type 2 DM averages 10-15 years less than that of the general population (Joslin Diabetes Center, 1999b).

Cardiovascular disease, diabetes, malignant neoplasms, and cerebrovascular disease are the four leading causes of death in individuals with Type 2 DM (Geiss et al, 1995). Some of the risk factors for mortality in persons with diabetes include age, age at onset, gender, duration of diabetes, cardiovascular risk factors—including smoking, hypertension, abnormal lipids, and physical inactivity (Geiss et al, 1995). Risk factors may also include central obesity, insulin use, and erratic glycemic control (Geiss et al, 1995). In addition, individuals with Type 2 DM who have clinically apparent microvascular complications are also at higher risk for mortality than individuals without diabetes who do not show such complications (Geiss et al, 1995). Therefore, efforts to prevent Type 2 DM may be of significant economic, clinical, and public health importance.

Prevention of Type 2 Diabetes Mellitus

Diabetes researchers now consider the primary prevention of Type 2 DM a practical reality (Bennett, 1997). Although genetic predisposition is part of the disease equation, as a result of the increasing understanding of the etiology and pathogenesis of Type 2 DM, several target groups and key environmental associative factors—obesity and inactivity, have been identified that are amenable to lifestyle interventions (Bennett, 1997). A greater knowledge of the role of diet and pharmacological therapies in reducing risk are also important factors in promoting prevention. Furthermore, Type 2 DM develops progressively, with increasing insulin resistance, and increasing impaired glucose tolerance (IGT) being apparent before the clinical manifestation of diabetes (Assal, 1997). Thus, individuals may be identified on the basis of disturbed glucose/insulin metabolism, prior to the overt development of diabetes (Assal, 1997).

As a means of more definitively determining whether Type 2 DM can be prevented, the National Institutes of Health announced in 1996 their intention to begin a randomized clinical trial called the Diabetes Prevention Program (DPP) (Diabetes Prevention Program, 1999). The goal of this nationwide research study was to determine whether Type 2 DM can be prevented or delayed in high-risk individuals with elevated fasting plasma glucose concentrations and impaired glucose tolerance (Diabetes Prevention Program, 1999). At least 3,000 non-diabetic participants were expected to comprise the study group. At least half of this group consisted of ethnic minorities who have disproportionately high rates of Type 2 DM, namely African-Americans, Hispanics, American Indians, Asian Americans, and Pacific Islanders (Diabetes Prevention Program, 1999). Approximately 20 % of the volunteers were age 65 or older, and 20 % were women who had gestational diabetes during their pregnancies. Recruitment of subjects began in June 1996 and continued through the first half of 1999 (Diabetes Prevention Program, 1999). Treatment and follow-up were so successful that the study concluded ahead of the completion date of mid-2002.

Study participants were randomized into one of the following treatment groups:

a) An intensive lifestyle intervention focusing on healthy diet and exercise;

b) Hypoglycemic drug supplementation e.g. metformin, with standard diet and exercise recommendations;

c) Placebo with standard diet and exercise recommendations.

Regardless of treatment assignment, all subjects received standard healthy lifestyle recommendations. They were encouraged to follow the USDA Food Pyramid Guidelines and the National Cholesterol Education Program Step 1 Diet; to lose 5-10 %

of their initial weight through diet and exercise; and to increase their physical activity to meet a goal of at least 30 minutes, five times a week; and to avoid excessive alcohol intake (Diabetes Prevention Program, 1999). The goals of the intensive lifestyle intervention were the same as the standard recommendations, but more intensive, --i.e. achieve and maintain a weight reduction of at least 7 % and at least 150 minutes/week of moderate intensity physical activity, such as walking and bicycling (Diabetes Prevention Program, 1999).

The results of these subjects indicated that waist circumference, fasting plasma glucose concentration, serum insulin concentration two hours after oral glucose challenge, triglyceride concentration and blood pressure decreased significantly more among subjects in the intervention group than among those in the control group. The cumulative incidence of Type 2 DM was lower in the intervention group than in the control group as a result of lifestyle changes in high-risk subjects (Tuomilehto et al, 2001). The results of this study are likely to influence the management of diabetes now and well into the future.

Treatment Goals for Type 2 Diabetes Mellitus

The primary goal of diabetes treatment is to lower blood glucose to or near normal levels (American Diabetes Association, 2002h). Blood glucose levels before meals should be approximately 80-120 mg/dl and 100-140 mg/dl at bedtime (Table 3). The glycosylated A1C fraction of hemoglobin, or HbA1C, level is another marker used in monitoring diabetes control. Table 3 summarizes the goals for whole blood and plasma glucose and HbA1C levels. Laboratory methods measure plasma glucose, which is typically 10-15 % higher than whole blood glucose values (American Diabetes

Association, 2002h). Some home blood glucose monitors and test strips are calibrated for whole blood readings, whereas others calibrate blood glucose readings to plasma values for consistency with laboratory methods. Glucose combines with different fractions of hemoglobin, providing an integrated measure of blood glucose concentration over time (Lipkin, 1999). HbA1C is also a marker for products associated with end-organ pathology (Lipkin, 1999). The goal of therapy is to achieve an HbA1C level below 7 %.

Table 3. Glycemic Control for People with Diabetes Mellitus[1,2]

Biochemical Index	Normal	Goal	Additional Action Suggested
Whole blood values			
Avg. preprandial glucose (mg/dl)[1]	< 100	80-120	< 80/> 140
Avg. bedtime glucose (mg/dl)[1]	< 110	100-140	< 100/> 160
Plasma values			
Avg. preprandial glucose (mg/dl)[1]	<110	90-130	< 90/> 150
Avg. bedtime glucose (mg/dl)[1]	< 120	110-150	< 100/> 180
HbA1C (%)	< 6	< 7	> 8

[1]Values in this table are calibrated to plasma glucose. They are generalized to the entire population of individuals with diabetes. Patients with co-morbid diseases, the very young and older adults, and others with unusual circumstances may warrant different treatment goals. These values are for nonpregnant adults. "Additional action suggested" depends on individual patient circumstances. Such actions may include enhanced diabetes self-management education, co-management with a diabetes education team, referral to an endocrinologist, change in pharmacological therapy, initiation of or increase in self-monitoring of blood glucose, or more frequent contact with patient. HbA1C is referenced to a non-diabetic range of 4.0-6.0 % (mean 5.0 %, SD 0.5 %).

[2]Source: American Diabetes Association, 2002h.

Daily self-monitoring of blood glucose in important for patients taking insulin or sulfonylureas to prevent hypoglycemia (American Diabetes Association, 2002h). The

optimal frequency of self-monitoring of blood glucose is not known in patients with Type 2 DM, especially in patients that are stable with Type 2 DM treated with diet alone (American Diabetes Association, 2002h). Overall treatment approaches for Type 2 DM include medical nutrition therapy, exercise, weight reduction if warranted, and use of oral glucose-lowering agents and/or insulin when necessary (American Diabetes Association, 2002h). Management should also include an assessment of cardiovascular risk factors including hypertension, dyslipidemia, smoking, and family history (American Diabetes Association, 2002h).

A large-scale, well known intervention study, the United Kingdom Prospective Study (UKPDS) conducted among adults with Type 2 DM, strongly linked the degree of hyperglycemia to risk of microvascular complications (American Diabetes Association, 2002g). However, the relationship between glycemia and cardiovascular risk was less certain (American Diabetes Association, 2002c). Therefore, it is unknown whether an intensive effort to maintain normal glucose concentrations will significantly reduce the rate of cardiovascular events (Nuttall and Chasuk, 1998). Also, cardiovascular risk factors may already be present before the onset of Type 2 DM. Because CHD is the leading cause of death among individuals with Type 2 DM, aggressive screening for diabetes with an emphasis on glycemic control and a multi-faceted approach to reducing CHD are recommended (American Diabetes Association, 2002c). An effort to minimize diabetic dyslipidemia is one approach. Diabetes with elevated LDL-cholesterol levels should lower LDL-cholesterol concentration to ≤ 100 mg/dl, levels typically recommended for patients with pre-existing CHD (American Diabetes Association, 2002c). Behavioral (i.e. diet) interventions are initiated for LDL-cholesterol levels >100

mg/dl. Initiation of pharmacological intervention (e.g. statins) is set at LDL-cholesterol levels ≥130 mg/dl, although in patients with multiple risk factors, some recommend initiation of drug therapy when LDL-cholesterol levels are between 100 and 130 mg/dl (American Diabetes Association, 2002c). The first priority in the treatment of dyslipidemia is lowering the LDL-cholesterol because it is associated with reduced CHD and possible over-all mortality.

Optimal HDL-cholesterol levels are >45 mg/dl and desirable triglyceride levels are <200 mg/dl (American Diabetes Association, 2002c). Weight loss, increased physical activity, and abstaining from smoking are behavioral interventions that may increase HDL-cholesterol. Pharmacological interventions may also be employed to raise HDL-cholesterol levels. In the case of hypertriglyceridemia, behavioral interventions such as weight loss, increased physical activity, and moderation of alcohol consumption are the first course of treatment. Improved glycemic control also reduces triglyceride levels. Use of glucose-lowering agents may also aid in lowering triglyceride concentrations (American Diabetes Association, 2002c).

Hypertension contributes to the development and progression of diabetic complications. Lifestyle modifications such as weight loss, exercise, reduction of dietary sodium, and moderation of alcohol consumption are initially employed to reduce elevated blood pressure (American Diabetes Association, 2002h). The goal for hypertensive control in diabetes is a systolic blood pressure <130 mm Hg and/or diastolic blood pressure <85 mg Hg (American Diabetes Association, 2002h). Risk for end-organ damage appears to be lowest when systolic blood pressure is <120 mm Hg and diastolic blood pressure is <80 mm Hg (American Diabetes Association, 2002h). While the goal is

to optimize blood glucose and lipid concentrations, monitoring blood pressure is an important area to address in the overall therapeutic treatment plan.

Dietary Management of Type 2 Diabetes Mellitus

The beneficial effects of dietary composition on insulin sensitivity are well known and important (Nuttall and Gannon, 1991; Jones et al, 1984; Gannon et al, 1998). Because of the risk of cardiovascular disease mentioned previously, dietary recommendations have focused primarily on low-energy diets that are high in complex CHO (>55 % of total energy) and low in fat (<30 % of total energy) content (American Diabetes Association, 2002i). This approach is embodied in the Medical Nutrition Therapy (MNT) used to maintain near-normal blood glucose levels (American Diabetes Association, 2002i).

Medical Nutrition Therapy

Medical nutrition therapy (MNT) is an integral part of diabetes management (American Diabetes Association, 2002i). As far as possible, MNT should be individualized and appropriate for the lifestyle and treatment goals of the individual with diabetes (American Diabetes Association, 2002i). To ensure diabetes management goals are being met, it is necessary to monitor glucose and HbA1C, lipids, blood pressure, and renal status (American Diabetes Association, 2002i). If an individual's blood glucose levels are still elevated after making diet and exercise changes, an oral glucose-lowering drug and/or insulin may need to be included in the therapeutic plan (American Diabetes Association, 2002i). The overall goal of MNT is:

a) maintenance of near-normal blood glucose levels by balancing food intake with insulin or oral glucose-lowering medications, and physical activity;

b) achievement of optimal serum lipid levels;

c) provision of adequate calories for maintaining or attaining reasonable weight for adults; normal growth and development for children and adolescents; increased metabolic needs during pregnancy and lactation, or recovery from catabolic illness. Reasonable weight may not be ideal body weight but the weight the individual and health care provider have agreed upon;

d) prevention and treatment of acute complications of insulin-treated diabetes such as hypoglycemia, short-term illnesses, and exercise-related problems; and long-term complications such as renal disease, neuropathy, hypertension, and cardiovascular disease;

e) improvement of overall health through optimal nutrition. Dietary Guidelines for Americans and the Food Guide Pyramid provide recommendations for all healthy Americans. The Diabetes Food Guide Pyramid is more specific for individuals with diabetes. All three guidelines may be used for diabetes management to make healthy choices (American Diabetes Association, 2002i).

Total Calories, Protein, Fat and Carbohydrates

It is recommended that individuals with Type 2 DM consume a diet moderately restricted in calories (i.e. 250-500 calories less than the caloric intake suggested by diet history), and a nutritionally adequate plan with a reduction in total fat and saturated fat, accompanied by an increase in physical activity (American Diabetes Association, 2002i). A hypocaloric diet and weight loss each independently increases sensitivity to insulin and improves blood glucose control (Heibronn et al, 1999). Irrespective of starting weight, moderate weight loss in the range of 10-20 pounds, reduced hyperglycemia,

dyslipidemia, and hypertension (American Diabetes Association, 2002i). Very-low-calorie diets are not recommended because they have not been effective in achieving long-term weight loss (American Diabetes Association, 2002i).

Since obesity has been implicated in the expression of diabetes, reducing visceral fat mass is an important factor in preventing and treating Type 2 DM. Unfortunately, the tools currently available for reducing body fat mass in obese individuals are limited (Nuttall and Chasuk, 1998). Nutrition and exercise intervention may be temporarily effective, but rarely result in significant long-term weight loss or maintenance, particularly among individuals with refractory obesity (Nuttall and Chasuk, 1998; American Diabetes Association, 2002i). Development of new pharmacological agents to treat obesity may be effective in reducing obesity in the future.

The most recent American Diabetes Association position is that the distribution of calories from fat, protein and carbohydrate can vary based on individual assessment and treatment goals (American Diabetes Association, 2002i). In the U.S., dietary intake of protein is reported to be similar across all ages from infancy to older age, and represents about 15-20% of caloric intake. This intake patterns appears to be similar in individuals with diabetes as well. The 2002 American Diabetes Association Nutrition Recommendations recommend increasing protein intake beyond the Recommended Dietary Allowance, but not greater than usual intake. This recommendation results from research conducted among individuals with Type 2 DM, which demonstrates that moderate hyperglycemia can contribute to an increased turnover of protein, which suggests an increased need for protein. Protein intake in the usual range is reported to be similar in patients with and without nephropathy. However, the long-term effects of a

protein diet containing greater than 20 % calories from protein on the development of nephropathy has not been determined and thus, it would be prudent to limit intake to less than this amount (American Diabetes Association: Clinical Practice Recommendations, 2002).

The recommended percentage of calories from fat is dependent on the individual's lipid profile and the treatment goals for glucose, lipids, and weight (American Diabetes Association, 2002i). Individuals with diabetes who have normal lipid levels and have normal weight are encouraged to follow the recommendations of the National Cholesterol Education Program (NCEP) (American Diabetes Association, 2002i). The NCEP recommends the following daily intakes: fat < 30 % calories, saturated fatty acids < 10% calories, polyunsaturated fatty acids < 10 % calories, monounsaturated fatty acids (MUFA) in the range of 10-15 % of calories, and $\leq$ 300 mg of cholesterol. If serum LDL-cholesterol is elevated, the NCEP Step II diet is recommended: reduction of saturated fat to 7 % of total calories and dietary cholesterol to < 200 mg/day.

Individuals with diabetes who have elevated serum triglycerides and very-low-density lipoprotein (VLDL)-cholesterol may attempt a moderate increase in MUFA at the expense of carbohydrate intake (American Diabetes Association, 2002i). However, one caveat is that care should be taken to ensure that increased fat intake does not perpetuate or aggravate obesity. In individuals with very high triglyceride levels (> 1000 mg/dl), it is recommended that total fat be limited to < 10 % of total energy (American Diabetes Association, 2002i). It is also essential to monitor glycemic control, lipid status, and body weight changes with any dietary fat modifications. This is of importance because a high-fat diet and elevated plasma triacylglycerol (TAG) may induce insulin resistance, and the

resulting blood glucose intolerance is a risk factor for cardiovascular disease (Ohlson et al, 1989; Sidery et al, 1990).

As in the case of total carbohydrate, the American Diabetes Association suggests that carbohydrate and MUFA together should provide 60-70 % of energy intake. In persons with Type 2 DM on weight maintenance diets, replacing carbohydrate with MUFA reduces postprandial glycemia and triglyceridemia (Manson and Spelsberg, 1994). However, there is concern that increasing fat intake from 30 % to 40 % of total energy may promote weight gain. Therefore, the contributions of carbohydrate and monounsaturated fat should be individually evaluated based on the metabolic profiles, nutritional assessments and treatment goals for weight loss when determining the MUFA content of the diet (American Diabetes Association, 2002i).

Effect of Fat and Carbohydrate on Insulin Sensitivity

It is well established that carbohydrate (CHO) and fat metabolism are interrelated (Randle et al, 1963; Whitley et al, 1997), and the distribution of fat and CHO can be adjusted to yield an improvement in insulin sensitivity. A reduced fat intake has beneficial effects on cholesterol concentrations while an increased carbohydrate intake leads to moderate increases in fasting triglyceride concentrations and low HDL-cholesterol (Smith, 1994). In the past several decades, a large number of studies have examined the relationship between the ratio of fat and carbohydrate in the diet and insulin sensitivity. There is strong and consistent experimental evidence from studies using animal models that high fat, low CHO diets are associated with insulin resistance (Lichtenstein and Schwab, 2000; Storlien et al, 2000). Several of these studies have reported the mechanisms by which high fat diets cause insulin resistance. There appears

to be no effect of a high fat diet on insulin receptor number or binding (Olefsky and Saekow, 1978) but there are reported reductions in insulin receptor phosphorylation (Watarai et al, 1988; Nagy et al, 1990), and increases in triglyceride content of skeletal muscle cells (Storlien et al, 1986).

In contrast with studies in animals that show clear and consistent effects of high fat diets on reducing insulin action, studies in humans have been less clear. Several have shown that the consumption of a high-CHO diet is associated with improvements in insulin sensitivity (Brunzell et al, 1971; Collier et al, 1987; Swinburn et al, 1991). Other studies have reached the opposite conclusion suggesting insulin and glucose responses to oral glucose are lower or no different following the consumption of a high-fat diet as compared to a high-CHO diet (Sarkkinen et al, 1996). These studies, which have included healthy men, women, individuals with diabetes, and obese individuals, fail to show any easily identifiable reasons for the discrepant results. It could be related to the methodological limitations in techniques used to measure diet parameters, e.g. euglycemic clamp and frequently sampled blood glucose tolerance test to assess insulin action versus older techniques that examine fasting or mean insulin levels throughout the day, or inadequate control for obesity and other risk factors (Lichtenstein and Schwab, 2000).

The Importance of Postprandial Hyperglycemia and Its Dietary Determinants

The major contributor to both the acute and chronic complications of diabetes is hyperglycemia. While treatment strategies historically focused on the fasting plasma glucose to lower HbA1C levels, more recent studies have prompted attention on the role of postprandial (blood glucose 1-2 hours after eating a meal) plasma glucose in the

etiology and treatment of diabetes. The UKPDS showed that postprandial glucose is a better indicator of glucose control than fasting glucose levels (Harris et al, 1994). A persistent or sustained elevation of postprandial glucose is one of the first major defects to occur in the pre-diabetic phase, and this remains a predominant effect throughout the course of diabetes. Given that postprandial plasma hyperglycemia is primarily a result of markedly blunted insulin-stimulated muscle glucose uptake in the face of a nutrient challenge, there are several mechanistic approaches that can be used to control postprandial glucose. These include modification of nutrient intake, smaller feedings, weight loss and exercise, as well as various drug therapeutic regimens (Gavin, 1999). The treatment of postprandial hyperglycemia is critical to achieving optimal outcomes in Type 2 DM (DeVeciana et al, 1995).

The following discussion will focus on the key macronutrient dietary determinants, e.g. fat, carbohydrate, and fiber, of postprandial glycemic response, or the change in blood glucose over time after a meal has been consumed. Protein will not be specifically addressed in this review as there are only limited studies on non-glucose yielding foods in individuals with Type 2 DM, and because this study proposal does not include protein as a determinant.

Attention was given to the breakfast meal occasion for several reasons. First, most of the postprandial literature is focused at this time period since breakfast is the first meal after an overnight fasting period. Second, individuals with Type 2 DM exhibit a more pronounced insulin resistance in the morning as compared to in the afternoon (Perriello et al, 1988; Shapiro et al, 1991). This pertubation had a negative effect on both the fasting and morning postprandial blood glucose concentrations (Bolli, 1988; Ferrannini et al,

1988). Third, since consuming breakfast helps to achieve nutrition targets for fat, carbohydrate and dietary fiber intakes, it is an important contributor of macro- and micronutrient intake and nutritional status. Breakfast consumption, particularly if the meal contains breakfast cereals, has been associated with lower daily intakes of fat and higher intakes of carbohydrate, dietary fiber, and certain micronutrients (Ruxton and Kirk, 1997). Thus, an examination of the dietary and metabolic contributors to postprandial hyperglycemia is appropriate at the breakfast occasion, and was the focus of this research study.

Dietary Fat

Ingested fat does not independently stimulate insulin secretion, but when ingested with carbohydrate, it is generally considered to reduce postprandial elevations in plasma glucose and insulin concentrations because of reduced upper gastrointestinal motility (Welch et al, 1987). Fat also potentiates gastric inhibitory polypeptide (GIP) secretion, which may have an acute effect on increasing insulin secretion (Collier et al, 1988). Intestinal hormones, such as GIP and others, undoubtedly are playing a role in the insulin secretory response, and more data are required to fully understand the differences in responses to macronutrients in individuals with and without diabetes. The predominance of the literature addresses varying levels and types of fat fed in combination with carbohydrate and this is addressed below in more detail.

Extensive research examining various amounts of fat ingested at breakfast has been conducted by Dr. Frape (N.S. Research, Suffolk, UK) in healthy subjects. Specifically, he investigated the acute postprandial responses to meals consumed at the

reakfast and lunch meals with varying levels of fat content, and this work will be ummarized.

In experiments conducted by Frape et al (1997), healthy volunteers were fed over 4-day period (Tuesday-Friday) meals of two compositions, providing similar amounts metabolizable energy (2.1 MJ, or 502 Kcal): moderately high-fat, low-carbohydrate nelette meal breakfasts (33 g fat, or 57 % Kcal from fat, 21 % Kcal from carbohydrate CHO), or low-fat, high-carbohydrate cereal breakfasts (5.5 g fat, or 11 % Kcal from fat, nd 75 % Kcal from CHO). An alternative treatment was no breakfast and a moderately gh-fat lunch equivalent to the moderately high fat breakfast. A standard evening dinner omposition undisclosed) was provided to all subjects. Blood samples were taken at riodically pre-determined times throughout the day, and plasma glucose, insulin and C-ptide were measured.

The results indicated significantly higher area under the curve (AUC) plasma ucose, insulin and C-peptide responses following the low-fat, high carbohydrate reakfast meal than the high-fat, low-carbohydrate meal. The values were also larger for sulin and C-peptide responses following breakfast than lunch for both low-fat, high HO meals and moderately high-fat meals. This suggests that subjects were more sistant to insulin at breakfast than at lunch, leading to higher circulating levels of ulin after breakfast. Further it was shown that the plasma glucose response to a fatty nch was increased by a fatty breakfast, and this was associated with a considerable vation in plasma non-esterified fatty acids in the afternoon (Frape et al, 1994). High culating levels of fatty acids have been shown to contribute to the insulin-resistant te (Frayn et al, 1997; Prins, 1997).

Subsequent work by Frape et al (1998) explored the relationship between breakfast fat consumption and carbohydrate intakes on glucose tolerance and the relationship to risk factors of atherosclerosis. Twenty-four healthy adult men were given low-fat, high-carbohydrate cereal meals (L) (5.5 g fat), or high-fat, low-carbohydrate breakfast meals (25.7 g fat) (M) of similar energy content for 28 days. The low-fat, high carbohydrate meal consisted of cornflakes with skim milk and orange juice, while the high-fat, low-carbohydrate meal was a lean-meat vegetable pastry. Fasting blood characteristics were measured on day 1, and an OGTT was given at 09.00 hours. On Day 29, each subject received a breakfast of either L or M at 09.00 hours, followed by an OGTT at 13.00 hours. Blood samples were analyzed for glucose, insulin, C-peptide, triacylglcerol (TAG) and non-esterified fatty acid (NEFA) responses.

The results showed there were no significant differences between treatments for fasting blood characteristics on either Day 1 or Day 29, or for the AUC values of glucose, insulin, TAG, or NEFA on Day 1. However, after consuming the diet for 29 days, the postprandial results indicate that the moderately high-fat breakfast meal led to significantly higher OGTT C-peptide responses and higher AUC of OGTT serum glucose and insulin responses compared with the OGTT responses to the low-fat, high-carbohydrate breakfast meal treatment. Before the OGTT, serum NEFA concentrations were greater for the M group than the L group. After breakfast in the morning, serum NEFA AUC concentrations were 59 % lower with the low-fat, high carbohydrate breakfast treatment than the high-fat, low-carbohydrate treatment, while serum TAG were similar with both treatments. This suggests that even at lower levels of fat consumption (5.5 g versus 25.7 g total fat) from isoenergetic breakfasts (in contrast to Dr.

Frape's work discussed previously with high fat (75 g) intake) has a potentially large influence on elevated circulating plasma NEFA, and this effect was observed for up to six hours after the morning meal. An inability to suppress postprandial plasma NEFA concentrations is positively associated with reduced glucose tolerance, and an elevated insulin response.

Frape et al (2000) further explored this hypothesis by focusing on the fatty acid subtypes, or the composition of NEFA. In a study of twenty-four males, he fed isoenergetic breakfast meals of similar fat composition, but of low (L) (Cornflakes, low-fat milk and orange juice) and moderate fat (M) (lean-meat vegetable pastry) levels of fat content as previously described (5.5 g Fat, 113 g CHO versus 26 g Fat, 56 g CHO, respectively). Subjects were asked to fast until 09.00 hours on Days 1 and 29, and were provided low-fat meals on the evening of Days 0 and 28. On Day 1 fasting blood characteristics were measured and all subjects were given an OGTT at 09.00 hours. On Day 29 each subject received one of the two breakfasts at 09.00 hours, followed by an OGTT at 13.30 hours.

The results from the previously described study (Frape et al, 2000) indicated that there were no significant differences in fasting NEFA composition. The total NEFA AUC with treatment L was only 59 % of that of Treatment M on Day 29 three hours following the breakfast meal. Treatment differences were also observed between 1 and 3 hours following breakfast in total saturated and total monounsaturated fatty acids, where the proportions of 16:0 and 17:0 chain length fatty acids were greater ($p=0.026$ and 0.005, respectively) and that of 18:1 chain length fatty acids were lower ($p=0.003$) in treatment (L) relative to treatment (M). Saturated and monounsaturated fatty acids constituted about

90 % of the total measured fatty acids of plasma NEFA during the OGTT and the postprandial period following breakfast. Serum insulin averaged 35 and 65 mU/L in treatments (M) and (L) respectively, during this period. It was concluded that a substantial rise in postprandial insulin concentration was associated with a rise in the proportion of saturated fatty acids and a decrease in the proportion of monounsaturated fatty acids in plasma NEFA. It was proposed that this change is the result of a suppression of fat mobilization, which may partly account for the difference in the postprandial plasma NEFA between high versus low fat meals.

The postprandial research previously discussed focused on normal subjects. The research conducted among individuals with Type 2 DM has examined the postprandial effect of substituting saturated fatty acids or carbohydrate with monounsaturated fatty acids to address the concern that the these dietary components when consumed in excess of the American Diabetes Association Nutrition Guidelines (2002) may adversely affect TG and HDL-cholesterol levels. Support for incorporating MUFA in the diets of individuals with diabetes is based on intervention studies that indicates that MUFA improves fasting plasma glucose (Garg, 1998), serum insulin, insulin sensitivity (Vessby et al, 2001) and serum lipids while having no adverse effect on HDL-cholesterol (Garg, 1998; Vessby et al, 2001).

Carbohydrates

The majority of research in the area of Medical Nutrition Therapy and diabetes management has been focused on carbohydrates and its various components: sugars, starch, and fiber. A number of factors influence the postprandial glycemic and insulin response of carbohydrate foods including the type of carbohydrate (glucose versus

fructose), the amount, nature of the starch present, its botanical structure and particle size, and the rate of digestion. Fructose produces much lower glucose and insulin responses than glucose because it is slowly converted to glucose in the liver, and only some of this glucose is released into the circulation (Wolever and Brand-Miller, 1995). However, fructose and sucrose may raise serum triglycerides (Frayn and Kingman, 1995) and LDL-cholesterol (Swanson et al, 1992). Therefore, the use of large amounts of fructose and sucrose as a way of reducing postprandial insulin is unlikely to be a recommended approach to the management of insulin resistance or glycemic control, and will not be further reviewed in this research study.

In the past decade, much research has been conducted examining the role that the mount and rate of absorption of dietary carbohydrate play in influencing the outcomes f diabetes (Garg et al, 1994; Chen et al, 1995; Jenkins et al, 1982; 1988). These studies ave indicated that postprandial insulin and glucose concentrations can be influenced by ducing the rate of CHO absorption using low-glycemic index (GI) foods. Alternatively, e can vary the amount of carbohydrate in the diet, or increase the monounsaturated fat ake combined with carbohydrate-rich foods as previously mentioned. A newer ncept, glycemic load (GL), which takes into account both the amount and type of bohydrate, has emerged. This review will discuss the application of the GI and GL, luding a discussion of dietary fiber.

cemic Index (GI)

Differences in glycemic responses to various carbohydrate foods are related to differences in how the carbohydrate is digested and absorbed. Foods eliciting a low emic response have been reported to facilitate blood glucose regulation and to

improve lipid metabolism in diabetes (Jenkins et al, 1981; 1982; 1988). The GI was introduced in the early eighties, as a means of ranking foods according to their glycemic effect. It expresses the rise in blood glucose elicited by a carbohydrate food as a percentage of the rise in blood glucose that would occur if the same individual ingested an equal amount of carbohydrate from white bread or glucose. The method of assessing the GI value involves calculating the area under the three-hour glucose response curve for a 50 g CHO portion of food and dividing by the area under the three-hour curve for the equivalent amount of carbohydrate as glucose or bread (Jenkins et al, 1981). Carbohydrate foods with a GI in the range of 30-50 relative to glucose or 40-70 relative to white bread are considered to be in low to moderate GI range, while foods in the range of 70-80 relative to glucose or 100-120 relative to white bread are considered to be high (Brand et al, 1991). Importantly, the GI does not always correlate with the fiber content of foods (Jenkins et al, 1983).

Today, the usefulness of the GI remains controversial. Proponents of the GI as a tool for dietary guidance argue that substituting foods with low glycemic indexes for those with higher indexes results in reduced serum insulin and glucose responses, urinary C-peptide excretion (a marker of insulin production) and HbA1C concentrations in both diabetic and non-diabetic subjects. Additionally, foods with high glycemic indexes are associated with increased insulin resistance (Brand-Miller, 1994), lower concentrations of HDL-cholesterol (Frost et al, 1999), and hypertriglyceridemia (Jenkins et al, 1987).

Conversely, Franz et al (1999) in the American Diabetes Association Position Statement indicated that the data available does not reveal a clear trend in outcome benefits of low GI diets on glycemia and lipemia, and any long-term effects appear to be

modest. As a result, the American Diabetes Association does not support the use of the GI for several reasons, including:

(1) nutrition education using the GI is too complex, requiring individuals to add another step of categorizing foods to meal planning;

(2) the use of the GI not only limits food choices, but promotes the concept of "good" and "bad" foods;

(3) the reproducibility of the glucose response in the same subjects has not been adequately studied, as current data suggest considerable variability;

(4) limited predictability, as mixed meal models using the GI did not necessarily result in the post-meal area under the curve glucose response that would be predicted for the individual foods included;

(5) the GI was determined using the first meal of the day, and it is now known that the first meal can affect the glucose response to an identical meal ingested 4 hours later;

(6) meal components can be manipulated to yield a favorable GI value that in fact can have deleterious effects in individuals with Type 2 DM (e.g. the use of fructose lowers the GI value, but can have an adverse impact on glucose control).

Yet, there is momentum building for the use of the GI as a practical tool in diabetes management and education. Previous long-term studies have indicated that consuming a low-GI diet improves overall blood glucose and lipid control in individuals with Type 2 DM (Jenkins et al, 1985; 1987; 1988; Wolever et al, 1992). In a more recent study, Jarvi et al (1999) examined the effects of two diets with pronounced differences in GI, while the macronutrient content and type and amount of fiber were identical.

Differences in GI were achieved by altering the botanical structure or the chemical starch structure in an effort to eliminate or minimize the potential variations in dietary fiber of nutrient composition. In a randomized crossover trial, twenty patients with Type 2 DM were given two diets with either a low or high GI during two consecutive 24-day periods, consisting of breakfast, lunch and dinner and an evening snack (Jarvi et al, 1999). The energy derived from the diets for protein, fat and carbohydrate was 16 %, 28 %, and 55 %, respectively. The average GIs of the low- and high-GI diets, as expressed in relation to that of white bread, were 57 (range of 53 – 61) and 83 (77 – 85). Blood samples were drawn following an overnight fast, and at fixed time intervals during the day for determination of plasma glucose, serum fructosamine, plasma insulin, C-peptide, serum ipoproteins, NEFA, fatty acid composition, and plasminogen activator inhibitor-1 (PAI-).

The results showed that the area under the glucose response curve during the 9-our day was 31 % lower ($p< 0.05$) after the period with the low-GI diet than the after the gh-GI diet. Similarly, plasma insulin was 27 % lower ($p< 0.01$) after the low-GI diet. ne C-peptide levels were significantly higher after the high-GI diet, compared with the w-GI diet at 120 minutes ($p< 0.01$) and 300 minutes ($p< 0.01$) after breakfast. The um cholesterol concentration was lower in subjects on the low-GI diet as compared h those on the high-GI diet (-5 %, $p< 0.01$). There were no changes in the fasting ues of NEFA, but there were significant differences between the dietary periods ing the day, with NEFA levels being about 40 % higher at 120 and 180 minutes and % lower at 300 minutes on the low-GI diet compared with the high-GI diet. The fatty composition was similar after the two dietary periods. The PAI-1 decreased

substantially on the low-GI diet by 58 % ($p < 0.01$), but remained unchanged on the high-GI diet. When comparing the two periods, the PAI-1 was 53 % lower on the low-GI diet than after the high GI-diet. These data are consistent with the long-term studies and extends the results showing postprandial benefits of GI reduction.

These results indicated that a strictly controlled, low-GI diet consisting of starchy foods resulted in a considerably improved metabolic profile of glucose, insulin and lipid parameters when compared with a corresponding high-GI diet (Jarvi et al, 1999). It should be noted that the differences in GI were obtained by manipulating the structure of the starchy foods, yielding larger amounts of resistant starch in the low-GI diets. Resistant starch, like dietary fiber, reduces the rate of absorption and is fermented by the colonic microflora, thereby producing short-chain fatty acids (Jenkins et al, 1987). Increased colonic fermentation is associated with improved glucose tolerance (Thorburn et al (1993). It is interesting, however, that the results from this study were reportedly due to the calculated difference in GI (31 %) between the two diets, and not the dietary fiber content (dietary fiber was corrected for resistant starch, 38 g versus 34 g for the low- and high-GI diets, respectively). Thus, it is not clear what role, if any, that the fiber content of foods with a low GI plays in eliciting the low glycemic responses of these foods. In fact, research shows that although foods with a high fiber content typically have a low glycemic index, the two concepts are independent. Foods with a low glycemic index and high fiber content typically raise postprandial blood glucose concentrations less than foods that have the same fiber content but higher values on the glycemic index (Jarvi et al, 1999). The following discussion will specifically examine the relationship between dietary fiber and glycemic control.

Dietary Fiber and Glycemic Control

Viscous, soluble fibers such as guar, psyllium, pectin, ß- glucan, barley, xanthan, locust bean gum, and beet fiber have low glycemic index (Jenkins et al, 1983; Wolever et al, 1987; 1988). They act to lower postprandial glycemia and improve glucose tolerance (Jenkins et al, 1978; Frape and Jones, 1995; Wursch and Pi-Sunyer, 1997). These fibers lower glucose and insulin peaks by increasing the viscosity of the contents of the stomach and small intestine, reducing the rate at which nutrients are absorbed, and lengthening the rate of digestion (Lavin and Reed, 1995; Edwards et al, 1988). The factors influencing fiber's therapeutic effect include the amount of fiber used in the study (Nuttal, 1993; Chandalia et al, 2000), the method of administration (Wolever et al, 1991), the source of dietary fiber, the composition of the diet, and both within- and between-individual variability in response (Wolever, 1990; Chuang et al, 1992).

The majority of the research literature has examined the effects of guar gum (Jenkins et al, 1978; Holt et al, 1979; Blackburn et al, 1984; Jarjis et al, 1984). Guar gum administered at levels of 12 g and higher, mixed in a 50 g glucose solution, has been shown to form a very viscous gel when added to water and also impair glucose absorption (Jenkins et al, 1976; 1977; 1978). The more viscous the gel formed, the greater the effect on glucose rise when fiber was mixed in a glucose solution. Guar is the most viscous fiber studied, and it has been demonstrated that it attenuates the rise in glucose concentration and extended the time required for the glucose to return to a fasting value (Jenkins et al, 1978; Jarjis et al, 1984).

Psyllium soluble fiber also increases viscosity, or forms gels, although it is known to be considerably less viscous (Edwards et al, 1987). The focus of this research will be on psyllium, and the reasons are two-fold:

(1) psyllium supplementation and Type 2 DM. Most of the research that exists focuses on the cholesterol-lowering effect of psyllium and hence, the basis for the U.S. Food and Drug Administration's approval of the health claim on psyllium and heart health in 1996.

(2) Kellogg Company had invested considerable resources in developing psyllium products (a line of functional foods containing psyllium was launched in 1997), and was keenly interested in extending the value of these products to other metabolic areas.

Therefore, clinical data investigating the use of psyllium among individuals with Type 2 DM would be beneficial in addressing these limitations, and was further explored.

The literature contains a limited number of studies showing the efficacy of psyllium soluble fibers in lowering glucose and insulin concentrations. Early research showed that a psyllium supplemental drink, regardless of meal composition (administered in the dosage of ~ 6 g of psyllium) was beneficial in reducing postprandial blood glucose and insulin responses in individuals with Type 2 DM (Florholmen et al, 1982; Sartor et al, 1981). Subsequent research suggested psyllium potentially had advantages over guar gum because it is less readily fermented and therefore, would likely cause less flatulence and abdominal bloating (McBurney and Thompson, 1989). Psyllium was also of benefit because it has fecal bulking action (Fagerberg, 1982; Tomlin and Read, 1988), an effect usually evident with insoluble fibers that do not show metabolic effects (Jenkins et al,

1978; 1979). Thus, there was greater interest to determine if psyllium would be beneficial in reducing postprandial glucose and insulin concentrations in patients with Type 2 DM.

In the recent investigations on psyllium, Anderson et al (1999) conducted an investigation of the chronic safety and efficiency of psyllium soluble fiber used adjunctively to a traditional diet for diabetes. Thirty-four men with Type 2 DM and mild to moderate hypercholesterolemia were randomly assigned to receive 5.1 g psyllium soluble fiber, or cellulose placebo twice daily for 8 weeks. The dosage was administered as orange-flavored powders, and was packaged in identical foil packets. Subjects were instructed to mix each packet in 240 ml liquid and drink immediately (20-30 min) before the morning and evening meals. The study consisted of a two-week dietary stabilization phase during which subjects followed a diet for diabetes of $\leq$ 30 % of total energy as fat, $\leq$ 10 % energy as saturated fat, and $\geq$ 55 % of energy as carbohydrate. Diet was not the major focus of the intervention, and the goal of dietary instruction was to encourage subjects to maintain their dietary patterns throughout the study. Serum lipid and glycemic responses were measured bi-weekly on an outpatient basis, and at 0 and 8 weeks in a metabolic ward.

Significant differences in changes from baseline between placebo control and treatment groups were seen in both glycemic and lipid responses evaluated in the metabolic ward, with the psyllium group showing improved metabolic control and consistently below baseline values as compared to the placebo group at week 8 (Anderson et al, 1999). Although most changes observed during the outpatient evaluations were not significantly different between treatment groups, directional changes also suggested metabolic control. These data support the supplementation of

diets with psyllium soluble fiber as well tolerated in the dosage provided (5.1 g x 2/d) and improved glycemic and lipid responses over the duration of eight weeks.

The results from Anderson et al (1999) are in contrast with earlier research by Wolever et al (1991). Wolever et al (1991) conducted a series of five acute experiments using a flaked bran breakfast cereal, bran flakes enriched with psyllium at four levels, and psyllium alone. Ten healthy subjects (4 males, 6 females, 28 years, 107 % ± 3 % ideal body weight) were studied after a 10-12 hour overnight fast on seven occasions in random order in the morning. They consumed 50 g available carbohydrates portions of bran flakes cereal, bran flakes cereal enriched with psyllium at four levels: 5 %, 10 %, 15 %, or 20 % (with the composition in grams of psyllium and a small amount of wheat fiber of 17.1 g, 21.3 g, 23.4 g and 24.3 g per 100 g, respectively), or bran flakes cereal plus psyllium (20 g), with the psyllium either sprinkled onto the cereal in the bowl just before eating, or taken with 125 ml of water just before consuming the cereal. Additionally, on three occasions, the test meal consisted of white bread. The bread and cereal test meals were eaten with 250 ml of 2 % butterfat milk and a standard beverage of hot tea or coffee with or without milk.

Six patients with Type 2 DM (2 females, 4 males, 71 years, 120 % ± 10 % ideal body weight) were also studied using the same bran flakes and 20 % psyllium-enriched bran flakes test meals as the normal subjects in random order in the morning following overnight fasts and 5-10 minutes after taking their normal dose of insulin or glyburide. Blood samples were collected at 15, 30, 45, 60 and 90 minutes, and the area under curve values for glucose were calculated geometrically (Wolever and Jenkins, 1986).

The mean glycemic responses in normal subjects indicated that the response of bran flakes was similar to (specific level not provided) white bread, but greater than the 20 % psyllium-enriched bran flakes and bran flakes with psyllium sprinkled on top. Taking psyllium (20 g) in water before bran flakes had no significant effect on the GI, 104 +/- 15 compared to bran flakes alone (108 ± 8) or white bread (100). The GI values of 20 % psyllium-enriched bran flakes (58 ± 10) and psyllium sprinkled onto bran flakes (48 ± 11) were not significantly different from each other, but were lower than the other meals. (The GI was calculated by expressing the glycemic response area for the cereals as a percent of the mean response are of the white bread test meals taken by the same subject). The mean area for blood glucose in subjects with diabetes after 20 % psyllium-enriched bran flakes (559 ± 115 mmol min/L) was significantly less than after white bread, and bran flakes alone (1099 ± 136 mmol min/L). The GI values for bran flakes (124 ± 6) and 20 % psyllium-enriched bran flakes (61 ± 10) in subjects with diabetes were not significantly different from those obtained in normal subjects for the same foods.

These results demonstrated a dose-dependent blood glucose lowering effect of psyllium, which is similar in normal and diabetic subjects and is only evident, when the fiber is mixed with the food, not when consumed in water before the meal. Although sprinkling psyllium onto the cereal just before consumption was as effective in reducing the glycemic response as the psyllium enriched cereal, it was significantly less palatable. However, taking psyllium as a drink before the meal had no significant effect on blood glucose, and this potentially resulted in a lack of a therapeutic effect. A follow-up *in vitro* digestibility study supported this suggestion that the food must be surrounded by the gel

in order for the effect to be observed. This was confirmed by the observation that the rate of digestion was significantly reduced only when psyllium was mixed with the cereal prior to digestion.

The chronic assessment among subjects with Type 2 DM by Anderson et al (1999) however, is contradictory to the research by Wolever et al (1991), where Anderson et al (1999) observed efficacy using a supplemental psyllium drink. Although not likely, the difference in observations could be due to preparation method, as proper dispersion of the psyllium in water is required (Fuessl, et al 1987). Additionally, Wolever et al (1991) included subjects with Type 2 DM who used hypoglycemic agents. Subjects using these hypoglycemic agents were instructed to take their medication prior to treatment (four were treated with insulin and two with glyburide), and thus the use of these agents, insulin in particular, may have been a confounding variable in this study. Anderson et al (1999) excluded subjects using insulin from his research study. It is interesting to note that the fiber effect was observed by Anderson et al (1999), despite the levels of fiber used being lower (10.2 g/day of psyllium soluble fiber versus psyllium administered at the highest treatment level of 20 g). Thus, the Wolever et al (1991) study that suggested that the psyllium must be incorporated into the food in order to demonstrate efficacy may not be reproducible.

Indeed, subsequent research confirmed the findings by Anderson et al (1999) that psyllium supplementation as a drink, was acutely beneficial in reducing postprandial glucose concentrations, and especially insulin requirements in both healthy subjects (Sierra et al, 2001), and later in subjects with Type 2 DM. Sierra et al (2002) evaluated the acute and chronic effects of psyllium in twenty patients with Type 2 DM. The study

included three phases: Phase 1 (1 week), Phase 2 (treatment, 14 g of psyllium soluble fiber/day; 6 weeks) and Phase 3 (4 weeks, following a 2-week washout period). At the end of each phase, a clinical evaluation was performed after the ingestion of a standard breakfast (436 Kcal, 53 % CHO, 26 % Protein, and 21 % Fat), which consisted of 80 g low-fat boiled ham, two slices (60 g) white bread and 200 ml low-fat milk with non-sweetened black coffee. The psyllium dose was administered four times a day: before breakfast, lunch, afternoon snack and dinner (14 g of psyllium/day). During Phases 1 and 3, the patients received the same volume of water (300 ml) as in Phase 2 without psyllium before meals. Blood samples were drawn at –15, 0, 10, 20, 30, 45, 60, 75, 90, and 120 minutes after breakfast ingestion and following an overnight fast (with –15 and 0 averaged to obtain glucose and insulin fasting values).

The results for this study showed that the area under the serum glucose concentration curve was 12.2 % lower in the presence of psyllium fiber than that obtained at the end of Phase 1, and 11.9 % lower than that obtained at the end of Phase 3 (significant differences, Wilcoxon's test, at $p < 0.05$) (Sierra et al, 2002). Serum insulin AUC decreased 5% in Phase 2 in comparison with the value obtained in Phase 1, and it was 15 % lower than in Phase 3 (no significant differences, Friedman's test, at $p < 0.05$). The mean postprandial glucose concentrations corresponding to breakfast, lunch, and dinner were always lower during Phase 2 than during Phases 1 and 3. The decreases in these values were 13.8 % in breakfast, 7.8 % in lunch, and 8.2 % in dinner (Phase 2 versus Phase 1), and 4.0 % in breakfast, 6.7 % in lunch, and 4.4 % in dinner (Phase 2 versus Phase 3). Significant differences were found between postprandial glycemia after breakfast between Phases 1 and 2. These data confirm that glucose absorption decreased

in the presence of psyllium postprandially among subjects with Type 2 DM. There were however, important inter-individual variations found in both glucose and insulin responses (scarcely modified to 30 % decreases), and as a result, consideration should be given to individualized treatment regimes and meal patterns for greater efficiency.

In contrast to the previous discussion, however, there is the suggestion that soluble fiber plays only a minor role in glycemic control and diabetes management (Nuttal, 1993). Indeed, the research by Frape and Jones (1995) failed to show efficacy using low levels of psyllium of 3 g or less. They investigated the acute postprandial responses of plasma, insulin, glucose and lipids in healthy middle-aged adults of both sexes. Subjects were given high-fat breakfasts and lunches with four treatments administered in tablet form in a randomized order, consistent with a 4 x 4 Latin squares design over a four-day period: 1) Control with no supplement, 2) Treatment A: purified 1.1 g psyllium, 3) Treatment B: 1.1 g water-soluble psyllium and 1.1 g of purified citrus pectin, and 4) Treatment C: Repeat of Treatment A. The tablets were chewed over a period of 5 minutes immediately before breakfast and lunch.

The psyllium and psyllium-citrus pectin mixture had no significant effects on the postprandial measurements of plasma glucose, insulin:glucose ratio, total-cholesterol, LDL- and HDL-cholesterol, and triacylgycerol. The absence of a glycemic effect may indicate that quantities greater than 2-3 g fiber per meal are required (Braaten et al, 1991). However, the efficiency of soluble fiber in the amounts used in this study previously demonstrated a cholesterol-lowering effect with a meal, or given prior to the meal (Anderson et al, 1991; 1992; Zhang et al, 1992; Landin et al, 1992). Previous research using guar gum at levels of 12 g and higher have been shown to reduce

postprandial hyperglycemia in normal subjects, and in persons with Type 2 DM (Jenkins et al, 1976; 1777; 1978). Additionally, research among individuals with Type 2 Diabetes using the American Diabetes Association Diet with a moderate dietary fiber intake (24 g/day, of which there was16 g of insoluble fiber and 8 g of soluble fiber) indicated that it was not as efficacious as a diet with higher amounts of fiber at 50 g/day (25 g/day each of soluble and insoluble fiber) (Chandalia et al, 2000). Furthermore, research conducted recently among hyperinsulinemic adults indicated that a whole-grain diet versus a more refined diet (dietary fiber 17 g/day versus 28 g/day reported) was more beneficial in improving insulin sensitivity (Periera et al, 2002). Thus, the amount of dietary fiber consumed can have a significant impact on determining glycemic response outcomes.

It is must be acknowledged however, that it is unknown how the glycemic response and insulin sensitivity to psyllium soluble fiber compare to the serum cholesterol response to pysllium. There were about 57 human studies conducted between 1965 and 1996 which, taken as a whole, demonstrate that the consumption of psyllium (typically 7-15 g daily) led to decreased levels of serum total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) and a concomitant reduction in the risk of coronary heart disease. These studies demonstrated that psyllium has been efficacious in lowering cholesterol levels in more studies, and more consistently than fiber from oat bran or oatmeal in lowering cholesterol levels in mild to moderate hypercholesterolemics (Anderson et al, 1988; 1991; 1992). Every one percent reduction in average serum cholesterol within a population represents a two to four percent reduction in coronary heart disease risk (Lipids Research Clinics Program, 1984). Thus, psyllium consumption has can lead to heart health benefits. It is unclear from the data reported to date however,

whether or not cholesterol is more sensitive to psyllium than glucose, and if a similar amount of psyllium fiber is required to be efficacious with hyperglycemia.

In summary, the findings on psyllium soluble fiber and its relationship to glycemic control in Type 2 DM are inconsistent and inconclusive. Further work is need to better understand both the acute and chronic implications of diets supplemented with psyllium soluble fiber, low GI foods as a modifiable risk factor for Type 2 DM. Additional research would also be valuable in understanding both postprandial effects immediately following the first meal eaten, and also the residual effects that blunt postprandial glucose rise after meals eaten several hours after the fiber ingestion (Jenkins et al, 1980; 1982). The capacity of soluble dietary fiber, specifically guar gum, to influence the next meal, or the "second-meal" effect, has been shown in healthy subjects, and will be discussed as it pertains to psyllium and Type 2 DM.

Psyllium Soluble Fiber and Second-Meal Effect

It is well established that the addition of guar gum at levels of 12 g or higher to carbohydrate-rich test meals or oral glucose loads lowers postprandial hyperglycemia in normal subjects and individuals with diabetes (Jenkins et al, 1976; 1977; 1978). Guar gum not only improved first meal tolerance to glucose, but also resulted in a more flattened postprandial glycemia after the subsequent meal even though guar was not ingested with the second meal (Jenkins et al, 1980; Trinick et al, 1986). As referenced earlier, it is hypothesized that guar and other viscous fibers act by delaying gastric emptying (Holt et al, 1979; 1981), or alternatively reducing the rate at which glucose is absorbed from the small intestine (Blackburn et al, 1981). Another hypothesis proposed is that the residual effect may be attributable to an increased glucose utilization rate, which

would be secondary to a decreased free fatty acids concentration that in turn would result in an increased glucose oxidation rate (Jenkins et al, 1980). The exact mechanism responsible for this residual effect remains unknown. Nevertheless, a consistent post-meal effect has been seen only with guar gum administered in large amounts (at levels of 12 g and higher) (Jenkins et al, 1980).

Subsequent research suggested that gum tragacanth and oat gum might also produce similar second meal results (Nuttal, 1993). The postmeal effect is not a property of soluble fiber in general, and thus, there are limited data examining the second meal effect of various types of soluble fibers, especially in individuals with Type 2 DM. Other polysaccharide substances including legumes, bran, and resistant starch either alone or in combination with low- and high-GI foods have been studied, and have shown mixed results on second meal effect on glucose and insulin concentrations. The following discussion will address the limited data reported on the effect of psyllium on glucose response at the second meal in individuals with Type 2 DM.

Pastors et al (1991) examined psyllium fiber postprandially and its second meal effects. In a placebo-controlled 15-hour, crossover trial, 18 patients with Type 2 DM were randomly assigned to receive either placebo or a 6.8 g psyllium pre-meal dose (mixed into a 240 ml glass of water) immediately before breakfast and dinner (13.6 g/day) using standard test meals that patients with diabetes might reasonably be expected to consume outside the clinic. These meals provided an average of 53 % of calories as carbohydrates, 27 % as fat and 20 % as protein, with a daily total of 14 g of dietary fiber without psyllium supplementation. (Chandalia et al (2000) has shown that a basal dietary fiber intake of 50 g per day from non-fortified foods with one half each soluble and

insoluble fiber, improved glycemic control, decreased hyperinsulinemia, and lowered plasma lipid concentrations in patients with Type 2 DM). Postprandial effects were evaluated in patients controlled by diet alone, and in patients controlled by hypoglycemic agents.

Postprandial glucose elevation was reduced by 14 % at breakfast and 20 % at dinner relative to placebo. Postprandial insulin concentrations measured after breakfast were reduced by 12% as compared to placebo. Second-meal effects after lunch showed a 31% reduction in postprandial elevation relative to placebos. There were no significant differences observed between patients whose diabetes were controlled by diet alone and patients using oral hypoglycemic drugs. These data indicated that psyllium as a meal supplement reduced acute proximate and second-meal postprandial glucose and insulin concentrations in patients with Type 2 DM. These findings were in contrast however, to the results previously described by Wolever et al (1991), as are other studies noted earlier. The Wolever et al (1991) study that suggested that the psyllium must be incorporated into the food in order to demonstrate efficacy may not be reproducible.

In the aforementioned research by Anderson et al (1999), he similarly demonstrated the second meal effect of psyllium. Two doses of psyllium (5.1 g) were taken immediately (20-30 minutes) before breakfast and dinner as a supplemental drink. The results showed significantly lower metabolic measurements of all-day postprandial glucose and postlunch serum glucose concentrations in the psyllium than in the placebo group. All-day and postlunch postprandial glucose concentrations were 11.0 % and 19.2 % lower, respectively, than in the placebo group.

Additional research is needed to fully understand psyllium soluble fiber's second meal effects and its benefits in patients with Type 2 DM. Despite the very valid concerns for the utility of the GI, and the questions raised on the feasibility of achieving high dietary fiber intake levels, the fact remains that these emerging interventions show promising results, and warrant further investigation.

Parallel with these advances, the concept of glycemic load (GL) was introduced by researchers at Harvard University in 1997 to quantify the overall effect of a food portion, or the amount, of carbohydrate. The following discussion will address GL and its utility in assessing diabetes risk.

Glycemic Load (GL)

By definition, the glycemic index (GI) compares equal quantities of carbohydrate and provides a measure of carbohydrate quality but not quantity. The GL recognizes that both the quantity and quality (i.e., nature or source) of carbohydrate influence the glycemic response, and may be interpreted as a measure of dietary insulin demand (Salmeron et al, 1997a). Therefore, the higher the GL, the greater the expected elevation in blood glucose, and the insulinogenic effect of the food (Foster-Powell et al, 2002).

There are only three studies that have been reported in the literature on the GL concept relative to carbohydrate intake and the risk of Type 2 DM and coronary heart disease. All are prospective studies, and were conducted by researchers from Harvard University. The first two examined the relationship between dietary patterns and risk of Type 2 DM in men (Salmeron et al, 1997a) and in women (Salmeron et al, 1997b). Both sets of data were taken from the Nurses Health Study and the Health Professionals Follow-up Study, which are longitudinal studies of diet and lifestyle factors. To assess

participants' diets, they used a validated semi-quantitative food frequency questionnaire, and derived for each participant an average GL value by summing the products of the carbohydrate content per serving for each food and multiplying it by the average number of servings for that food per day. To calculate the GL, the sum of the carbohydrate for each food was then multiplied times its GI. The researchers used published data for the GI, and the carbohydrate content in each serving was reported by the U.S. Department of Agriculture. In 1986, participants provided information on height, weight, age and smoking status. In 1987, participants provided information on history of Type 2 DM in first-degree relatives. On follow-up questionnaires mailed every two years (1988, 1990, and 1992), participants indicated whether diabetes had been newly diagnosed. For those participants who affirmatively indicated diagnosis, a supplementary questionnaire was provided to ascertain the date and procedure of diagnosis, as well as clinical data and treatment. The criteria used corresponded with those proposed by the National Diabetes Data Group (National Diabetes Data Group, 1979) and the World Health Organization (WHO Expert Committee on DM, 1985). Relative risks were estimated as odds ratios using a logistic regression analysis.

There were 915 incident cases documented of Type 2 DM during the six- year, follow-up in women and 523 men among a baseline population of 42,759 men and 65,173 women. In these cases, the results showed a significant inverse association between total dietary fiber intake and the risk of Type 2 DM. Among the different sources of fiber, cereal fiber was inversely associated with Type 2 DM, whereas fruit and vegetable fiber were not clearly related to risk. Although total carbohydrate intake was not related to risk of Type 2 DM, both the GI as well as the GL score were positively

associated with risk. A high GL score (>165) in combination with a low cereal intake (<2.5 g/d) had a relative risk of 2.50, more than two-fold greater relative to consumption of a diet high in cereal fiber (>5.8 g/d) and low in GL (<143). A similar pattern was observed in the parallel cohort of men (Salmeron et al, 1997a). It is interesting to note that the GL score only became significant after adjustment for cereal fiber intake (cereal fiber intake was added to the model, and included bran and whole-grain cereal varieties).

Observations from these prospective data among both women and men indicated that diets with a high GL and low cereal fiber content (<2.5 and <3.2 in women and men, respectively) were positively associated with the risk of Type 2 DM, independent of other dietary factors and currently known risk factors. These data suggest that the fiber intake is closely linked with the low GL score, and that it is this positive association that contributes to their beneficial effect in reducing the risk of Type 2 DM. There is extensive literature as previously discussed, addressing the link between viscous, soluble fiber and its role in glycemic control. However, there was no distinction made in this study in the type of cereal fiber, soluble versus insoluble fiber, as the data reported were inclusive of both types.

Subsequent research by Liu et al (2000) using the cohort of women previously described examined the types of carbohydrate foods contributing to the GL, and the load did not appear to be determined by any particular food. However, the two most important contributors identified as influencing the dietary GL in this study were mashed or baked potatoes, and cold breakfast cereals. Other carbohydrate-containing foods contributed smaller amounts. It was concluded that a high glycemic load from refined carbohydrates increases the risk of Type 2 DM and coronary heart disease in U.S. women. It was

suggested that grains should be consumed in a minimally refined form to reduce the incidence of these diseases.

The GL concept is in its infancy, and additional research would be beneficial to better understand the role of dietary fiber in influencing the GL score, or whether or not there is an independent effect of dietary fiber influencing the relative risk of Type 2 DM. While the researchers indicated that prospective design of these studies eliminated many potential sources of bias, especially recall bias, it would also be helpful to determine if the results from these prospective studies would be reproducible in clinical trials. These large-scale, observational studies have proven very useful in permitting new insights into the relation between the relative risk of carbohydrate-rich food portions and Type 2 DM. The first international table of glycemic load values was published in early 2003, and thus, this concept is likely to increase in its utility in assessing the effects of different carbohydrates and health (Foster-Powell et al, 2002). However, as previously noted, the ADA Position Statement does not currently support the application of the GI, nor the GL, in the dietary modification and management of diabetes (ADA, 2002).

In summary, many reasons exist for considering a high-carbohydrate, low-fat diet emphasizing high fiber foods for individuals with and without Type 2 DM. These include the reduction in postprandial hyperglycemia as well as lower blood lipids. The existing data however, are not consistent that psyllium soluble fiber has a well-defined role in achieving these benefits. Additional clinical research would be useful in providing further evidence of its effect on glycemic response, and whether or not a change in glycemic response is due to the existence of a fiber effect, or alternatively, the carbohydrate load administered in a test meal, or a combination. A deeper understanding of research in this

area can potentially lead to dietary formulations and educational campaigns that may yield significant health benefits in patients with Type 2 DM.

RATIONALE AND OBJECTIVES

The management and treatment of Type 2 DM and its co-morbidities encompass specific dietary considerations, and an exercise regime (Chisholm et al, 1997; Zinker, 1999; Wing et al, 2001). Dietary modification has focused on the macronutrient intake including carbohydrates, fat, and protein due to their influence on glycemic control and insulin response. Additionally, the inclusion of soluble dietary fiber in the diet reportedly improves glucose tolerance of that meal (first meal tolerance), and this effect potentially extends to subsequent meals (second meal tolerance) (Jenkins et al, 1982; Trinick et al, 1986; Wursch and Pi-Sunyer, 1997). The mode of action hypothesized is that fiber alters hormonal and metabolic responses to food by reducing glucose absorption via reductions in gastric emptying, and diffusion rates of glucose from the lumen to the enterocyte brush border transporters (Taylor et al, 1980; Blackburn et al, 1984).

More recent data however, suggest that soluble dietary fiber may not result in lower glycemic responses unless it is consumed in large amounts (Chandalia et al, 2000); Nuttall, 1993). Although the debate on the beneficial effects of dietary fiber continues, the American Diabetes Association (ADA) has recommended that individuals with Type 2 DM should increase their fiber intake consistent with dietary advice for healthy persons, as fiber helps improves carbohydrate metabolism (ADA Position Statement, 1987; Nuttall, 1993). Additional research in this area is required to help further understand the benefits of dietary fiber in the management of Type 2 DM. My research goal is to provide evidence for the role of soluble dietary fiber consumed at breakfast in

control of plasma glucose, insulin and free fatty acids, and to determine if these effects extend to the next meal.

While there have been a few studies examining metabolic effects of lowering postprandial hyperglycemia at dinner on the attenuating second-meal effects after breakfast, the overwhelming majority of the literature has reported on the breakfast occasion. This trial similarly focuses on breakfast for several reasons. Individuals with Type 2 DM are more insulin resistant in the morning than afternoon (Perriello et al, 1988; Shapiro et al, 1991). Additionally, the use of breakfast cereals is an excellent way to deliver dietary fiber and achieve a targeted level of intake. Importantly, Kellogg Company was very interested in extending the benefits of psyllium beyond its cholesterol-lowering properties to other metabolic areas.

It has been well established that psyllium soluble fiber has benefits in lowering cholesterol, and thus, contributes to decreased risk of coronary heart disease (Anderson et al, 1991; 1994). The U.S. FDA has authorized a health claim on the use of soluble fiber from psyllium in reducing hypercholesterolemia, a risk factor for coronary heart disease (Federal Register, 1998). There are limited research data however, investigating the use of psyllium soluble fiber in lowering glucose and insulin concentrations. Most of the research reported has been done with guar gum, and even when psyllium supplementation has been used, it was incorporated in the study as a drink with a 50 g glucose load (Jarjis et al, 1984).

To date, only Wolever et al (1991) and Frape et al (1997) have conducted research on glycemic control where psyllium soluble fiber has been incorporated into breakfast cereals in both Canada and the United Kingdom, respectively. In the United

States, Kellogg is the only manufacturer marketing a psyllium-containing product, and was interested in expanding the benefits and use of these products beyond lowering cholesterol. Thus, this research study would be very beneficial in helping Kellogg to determine future strategies for existing products and potential new innovations using psyllium soluble fiber in other metabolic areas. This is particularly applicable given the growing incidence and prevalence of Type 2 DM (Harris et al, 1998).

Scientific investigations examining the benefits of high carbohydrate meals including soluble fiber on glycemic control are ongoing. The more recent variation of this research includes the utility of the glycemic index and glycemic load of carbohydrate meals. It has been shown that carbohydrate meals with high glycemic index foods appear to increase insulin demand and accentuate hyperinsulinemia (Jenkins et al, 1980; Trinick et al, 1986; Wolever et al, 1992). Conversely, a prolonged digestive phase, which typically occurs after the consumption of carbohydrate meals with a low glycemic index, results in a lower production of free fatty acids (Jenkins et al, 1980; Trinick et al, 1986). Fatty acids promote insulin resistance, and lower resistance after a meal results in lower blood glucose concentrations. Thus, dietary carbohydrates may mediate their effects on insulin sensitivity, at least in part, by altering plasma free fatty acid concentrations (Wolever, 2000).

Additionally, recent prospective research suggests that a high glycemic load (an indicator of carbohydrate's ability to raise blood glucose, and is calculated as glycemic index of a food times the amount of total carbohydrate) is associated with increased risk of developing Type 2 DM in both men (Salmeron et al, 1997a) and women (Salmeron et al, 1997b). Conversely, a low glycemic load, emphasizing cereal fiber, resulted in a lower

risk of developing Type 2 DM. It was suggested that this positive association was due more to the glycemic index portion of the equation, and was independent of the amount of total carbohydrate consumed. Similar results have been observed with low-glycemic index foods in relation to heart disease in the Nurses' Study (Liu et al, 1999) and in the Iowa cohort (Meyer et al, 2000).

This research trial will examine breakfast meal composition with the addition of psyllium soluble fiber and its effect on glycemic response, or the change in blood glucose over time after a meal. Importantly, we will also examine the residual or second meal effects of fiber in individuals with Type 2 DM. This research approach is significant as there is limited evidence that exists on the relationship of psyllium soluble fiber to glycemic response, especially in subjects with Type 2 DM, and conflicting results on second meal effects of soluble fibers.

The consequence of the addition of dietary fiber to the breakfast meals is a change in the amount of total carbohydrate. Thus, it will be possible to also determine if glucose, insulin and free fatty responses to a high-carbohydrate breakfast meal differs from a moderate or low-carbohydrate breakfast meal, with and without psyllium soluble fiber. The prospective data suggest that a high glycemic load, particularly when consumed in combination with a low cereal fiber intake increases the risk of diabetes (Salmeron et al, 1997a, b). The aim of my clinical trial is to provide insights on whether or not there is an independent effect of psyllium soluble fiber, or varying the amount of total carbohydrate is more beneficial in influencing glycemic response. This metabolic trial was designed to provide normal mixed meals versus the 50 g glucose challenge that has been widely used in previously published literature. Thus, these data should provide evidence of the

influence resulting from two different meal types on glycemic response, and aid our understanding on the utility of varying amounts of carbohydrate and the addition of psyllium soluble fiber on plasma glucose, insulin and free fatty acid concentrations.

The hypothesis of this research is that a low carbohydrate, high psyllium soluble fiber meal will have a more favorable influence on glucose, insulin and free fatty acid concentrations than a high carbohydrate meal with no psyllium soluble fiber. Further, the breakfast meal containing psyllium will also show a sustained effect or influence beyond the mid-day standardized lunch. The specific objectives of this study are:

1. To determine if the presence of psyllium soluble fiber at breakfast influences second meal effects after a standardized, mid-day lunch has been consumed
2. To determine if varying levels of carbohydrate foods with and without psyllium soluble fiber and different GI values will significantly influence Type 2 DM biomarkers

The results from this research should be useful in determining the effects of psyllium soluble fiber independently, or in combination with the glycemic load in modulating postprandial glucose control, insulin release and free fatty acid responses in individuals with Type 2 DM. As a result, these data may be meaningful in influencing dietary recommendations for individuals with Type 2 DM, and ultimately help reduce the complications of the illness through diet modification.

MATERIALS AND METHODS

Subjects

The study protocol and consent form were approved by the Michigan State University Committee on Research Involving Human Subjects and Sparrow Hospital (Appendix A). Subjects were recruited through hospital clinics. This proved disappointing and therefore, we placed advertisements in the university journal and local newspaper (Appendix B), and we were subsequently successful in our recruitment. Prospective subjects were invited to attend a lecture explaining the purpose and procedures of the study. Time was allotted for questions and answers, and interested subjects were then asked to read and sign the consent form.

A total of 75 adult men and women between the ages of 38 and 82 were recruited initially. Individuals were eligible for the study if they were over 18 years of age; were medically diagnosed with Type 2 DM (Fasting Plasma Glucose level of 126 mg/dl or higher) a minimum of six months; their condition was controlled with diet only or diet plus oral hypoglycemic agents; had no other chronic disease diagnosis, were regular breakfast eaters (four out of seven days), and had no known allergy to psyllium seed husk (Table 4). Forty-five subjects qualified and were selected to participate in the study as a result of screening, and a statistical power analysis previously conducted to discern differences between the treatments at 80 % power with 15 persons in each group (the power calculation is based on a 12 % reduction between the treatments as noted in Appendix C). Individuals were excluded from the study if they had a history of myocardial infarction, other chronic medical conditions, or major surgical procedures

within the previous six months, as were individuals who were unable to participate for a three-week consecutive time period.

Table 4. Inclusion Criteria for Subjects

Adults over 18 years of age
Medically diagnosed with Type 2 DM for a minimum of 6 months
Good to excellent glucose control with HbA1C ~7-8
No use of insulin, but oral hypoglycemic agents acceptable
No known allergy to psyllium seed husk
No evidence of chronic disease
Does not routinely skip breakfast

All participating subjects who gave written, informed consent were allowed to continue in the study. They were remunerated $50 per day at the end of the test cycle, which was scheduled either Tuesday or Thursday for a three-week period. If subjects were unable to participate for three consecutive times due to illness, family emergencies or other conflicts, they were allowed to come the following test day within the cycle. A confirmation letter, or phone call, and a one-day parking permit were provided to each subject. Subjects were instructed to arrive at staggered times from 6:30 A.M., fifteen minutes apart and were advised they would be required to stay approximately all day. They were free-living and visited the G. Malcolm Trout Building at Michigan State University (MSU) in Room 1 where a clinical setting was approximated.

Subjects using medication were instructed to bring it with them, and they were allowed to take as prescribed following the baseline blood draw and just prior to the breakfast meals. They were also encouraged to drink plenty of fluids, and counseled on

the possibility of experiencing slight discomfort or flatulence. However, the level of fiber consumed did not pose a problem, and the subjects tolerated the meals well.

Study Design

The study had a randomized, cross-over design with three treatment arms as shown in Table 5:

1) High glycemic load containing Malt-O-Meal farina and a sugar-free (sucralose) placebo beverage without psyllium soluble fiber (Breakfast A)
2) High glycemic load containing Malt-O-Meal farina and a sugar-free (sucralose) beverage containing 6.6 g of psyllium soluble fiber administered 20 minutes post the breakfast meal (Breakfast B)
3) Low glycemic load containing a loop ready-to-eat cereal with 6.6 g of psyllium soluble fiber incorporated into the cereal and a sugar-free (sucralose) placebo (Breakfast C)

Table 5. Food Composition of Breakfast Meals[1,2]

Food Items	Breakfast A	Breakfast B	Breakfast C
Breakfast cereal (2 servings)	Farina	Farina	Psyllium Loop
W/Milk (1 cup)	Skim	Skim	Skim
Bread (1 slice)	Toast	Toast	Toast
Spread (1 Tsp)	Margarine	Margarine	Margarine
Beverage	Coffee or Tea	Coffee or Tea	Coffee or Tea
Post-meal Beverage (2 servings)	Placebo Drink	Psyllium Drink	Placebo Drink

[1]Goals for meals were 30% of total daily energy intake, based on an 1800-Kilocalorie Diet.
[2]A serving of farina is 1 cup (3 tbsp/1 cup cooked); a serving of the psyllium loop is 2/3 cup. A serving of post meal beverage, psyllium drink or placebo, is 1 prepared packet dissolved in an 8-oz glass of water.

The level of psyllium soluble fiber of 6.6 g was chosen due to several considerations:

(1) research published at the time this study was proposed examining the relationship between psyllium and glycemic control had used a range of 3.3-6.6 g per serving in a beverage drink. There was no effect seen at the lowest range, while efficacy has been demonstrated using 5 g or higher levels of psyllium (Frape and Jones, 1995). Dosage at 6.6 g produced mixed effects when the first meal effect was examined (Florholmen et al, 1982; Sartor et al, 1981; Jarjis et al, 1984; Frape and Jones, 1995; Frost et al, 2003). My aim was to determine if efficacy would be seen at the 6.6 g level, and if this effect extended beyond lunch, or the second meal.

(2) This was the maximum dosage allowable in the test foods without adversely affecting palatability.

(3) Fiber consumption among U.S. consumers is on average about 16 g per day (National Health and Examination Survey, III, 1999). Thus, we wanted to minimize any potential side effects that can occur when increasing your fiber intake e.g. bloating, flatulence, and constipation.

(4) While this study is not a dose response trial, using a lower level of psyllium was advantageous because at the time of the research design, there was no published literature on whether or not psyllium would be tolerated at high levels in patients with Type 2 DM [Subsequent to the start of this study, research was published showing that psyllium was well

tolerated by subjects with Type 2 DM at levels of 11g per day, fed *in two* separate doses of 5.5 g each (Anderson et al, 1999)].

The crossover design used six breakfast meal sequences: ABC, BCA, *CAB, BAC,* CBA and ACB. It was uniform with respect to sequence (each meal appears *the same* number of times within the six sequences) and uniform with *respect to week (each meal* appears the same number of times (twice) *within each week). It was also balanced with respect* to the first order carryover effects *(each meal precedes* each of the other two breakfast meals the same number of times). Any carryover effect was assumed to be the same for the three *breakfast* treatments. Each subject served as his or her own control, and were randomized according to a fixed diet sequence for Breakfast A, B or C using a random order table. For example, the combination, CAB means Breakfast C in Week 1, Breakfast A in week 2, and Breakfast B in week 3.

Calculation of Glycemic Index and Glycemic Load

The glycemic load is a concept that takes into account both the quantity, which refers to the amount of carbohydrate, multiplied by the quality, which is the glycemic index, of the carbohydrate food consumed (Salmeron et al, 1997a,b). The load value for each treatment meal is similarly derived by summing the products of the carbohydrate content per serving for each food times the average number of servings of that food during the breakfast meal and multiplying the total carbohydrate content of the food times the glycemic index (GI) for that food (Table 6).

Concentration was on the difference in the test cereal products were consumed as part of the breakfast meal occasion, since the other foods consumed were the same for each treatment. *The* GI values for the test cereals were analyzed using Glycaemic Index

Testing, Inc. (Ontario, Canada) while the other meal components were obtained from published data Wolever, 1990; Wolever et al, 1994). The GI values of the psyllium loop and Malt-O-Meal farina cereals were analyzed using white bread as a reference (GI ranges between 100-120) and the results were 56 and 64 respectively (See Appendix D for methodology and test results of GI testing conducted by Glycaemic Index Testing Inc., Ontario, Canada).

Table 6. Glycemic Load of 1800-Calorie Breakfast Meal Plan [1,2]

	Total CHO Breakfasts A/B	Total CHO Breakfasts C	GI (Bread Value)	Glycemic Load Breakfasts A/B	Glycemic Load Breakfasts C
1 slice wheat bread	12	12	83	996	996
1 Cup (C) skim milk (12 g/C)	12	12	46	552	552
2 svg Dry Cereal (17 g/C)	--	34	56	--	1904
2svg Cooked Cereal (25 g/C)	50	--	64	3200	--
2svg Psyllium Drink 7.4 g w/ 240ml H_2O (6 g/serving)	12	--	0	0	--
2svg Placebo Drink 2.5 g w/ 240 ml H_2O (2 g/svg)	4	4	0	--	0
			Total	4748	3452

[1] Table references reported in the International table of glycemic index and glycemic load values: 2002.
[2] GI values for the psyllium drink and placebo, and margarine (0.04 g of total carbohydrate) were assumed to be zero, based on communication with Glycaemic Testing, Inc., Ontario, Canada.

This yielded a difference in GI of 13 %, which was substantially lower than expected. Published data for Nabisco Cream of Wheat cereal, which is similar in food type and form to the Malt-O-Meal farina, indicated a value closer to white bread, and thus the expectation was that a higher value would have been obtained for this test food. This difference may be potentially due to the preparation method in which milk and cereal were combined and heated in a microwave versus our method of preparation where the cereal was cooked conventionally and milk was added to the cereal when served. Additionally, skim milk was used in this trial versus the 2 % milk used in the GI testing. Another consideration may be related to the variability of glucose responses of the subjects studied, the number of subjects studied and the number of tests done (Wolever and Bolgonesi, 1996).

The calculated difference between the high glycemic load versus the low glycemic load meal was 38 % (Table 6). The glycemic test conducted in this trial confirmed a difference in subjects consuming a high glycemic load versus a low glycemic load breakfast meal.

Treatment Products

The farina was purchased commercially from Malt-O-Meal Company (Northfield, MN) (Appendix E). The psyllium soluble fiber cereal loop, sugar-free psyllium soluble fiber beverage and cellulose placebo beverage were developed and manufactured by Kellogg Company (Battle Creek, MI) (Appendix F,G, and H). The non-caloric sweetener used with the psyllium beverage was powdered sucralose provided courtesy of McNeil Specialty Products, Inc. (New Brunswick, NJ). It was added to both the psyllium soluble

fiber drink and the cellulose placebo in droplet form (5 droplets per 240 ml of cold water).

The sugar-free psyllium beverage was developed and prepared using sugar-free, orange-flavored Metamucil ® (Proctor and Gamble Company, Cincinnati, OH) as a prototype. The cellulose placebo was similarly prepared excluding psyllium soluble fiber. The psyllium soluble fiber beverage was initially developed in two flavor varieties, strawberry banana and tangerine. Based on a qualitative sensory evaluation conducted by the principal investigators for the study, the tangerine flavor variety was selected for use in the study.

Treatment Meals

The breakfast treatment meals were prescribed according to the energy requirements based on body weight using the 1999 American Diabetes Association Nutrition Guidelines. The treatment meals based on an 1800-Calorie diet were planned to deliver approximately 30% of energy intake at breakfast. The grocery shopping for the meals was done the day before each test to ensure freshness and good quality. All meals were prepared in the MSU Sensory Laboratory in the G. Malcolm Trout Building. The composition of both control and treatment breakfast meals are shown in Table 7. The nutrient content of these meals was analyzed using Nutritionist V ™Data Analysis Software, First Databank Inc., 1999-2000 (San Bruno, CA), and is shown in Table 8.

Table 7. Nutrient Composition of Breakfast Meals[1,2]

	Breakfast A	Breakfast B	Breakfast C
Energy (Kcal)	438	470	362
Protein (g)	19	19	15
Carbohydrate (g)	78	86	62
Fat (g)	5.5	5.5	6.0
Dietary Fiber (g)	3.4	13.4	12.4
Soluble Fiber (g)	1.0	7.6	6.6

[1]The nutrient content of the meals was analyzed using Nutritionist V Data Analysis Software, First Databank Inc., 1999-2000, and Kellogg Chemistry Laboratory.
[2]The difference in fiber content of Breakfasts A, versus B and C is due to the amount of fiber contained in the psyllium loop cereal and the psyllium drink. Both the loop cereal and the psyllium drink provided 6.6 g of soluble fiber and 4.4 g of insoluble fiber. The remaining fiber present came from the farina (1g each of soluble and insoluble fiber) and the cracked wheat bread (1.4 g of insoluble fiber). Similarly, there is a difference in the amount of Total Carbohydrate for Breakfast A (4 g) versus Breakfast B (12 g) where the placebo was used in Breakfast A versus the psyllium drink in Breakfast B.

Table 8. Nutrient Composition of the Standardized Lunch[1,2]

Energy (Kcal)	568
Protein (g)	24
Carbohydrate (g)	75
Fat (g)	19
Dietary Fiber (g)	2

[1]The nutrient content of the meals was analyzed using Nutritionist V Data Analysis Software, First Databank Inc., 1999-2000.
[2]Values shown are for serving sizes based on an 1800-Calorie Meal Plan.

The breakfast meal was provided and twenty minutes following the meal, either the cellulose placebo or the psyllium beverage was administered. Previous work by Wolever et al (1991) has suggested that a similar amount of psyllium soluble fiber incorporated into a breakfast cereal was more effective than when taken as a beverage twenty minutes prior to the meal. Thus, this design will help ascertain whether or not the

timing and method of administration influenced the degree of efficaciousness of the fiber, in addition to comparing the fiber versus the load effect.

The other breakfast foods included in the meals were skim milk, toast, margarine and sugar-free jam. Coffee, tea, water, artificial sweetener and non-fat creamer were offered freely. The lunch meals consisted of split-top, low-fiber wheat bread (Taystee), American cheese, 99% fat-free turkey, lettuce, a slice of tomato, fat-free mayonnaise, a mustard package and non-caloric beverages. Similarly, as for the breakfast, the energy content for lunch was based on energy requirements in accordance with the 1999 American Diabetes Association Nutrition Guidelines. The goal was to provide a low-fat meal that delivered approximately 30% of the daily kilocalorie intake.

The 1999 Block 98.2 Food Frequency Questionnaire was administered to help gauge usual or routine intake patterns. These data were used to provide a further assessment of nutrient intake and data interpretation, and an explanation of any confounding variables.

Blood Collection and Analyses

At each of the three visits, baseline, fasting glucometer readings were conducted to ensure that the subjects were not hypoglycemic. Subjects at or near normalcy were allowed to proceed. Only one subject was not near normalcy during the trial, and thus, was asked to return the next test date in the three-day period cycle. An angiocatheter was inserted into the hand or forearm vein for the blood draw procedures. After an 8-10 hour overnight fast, blood samples were drawn at fasting or time zero for determination of glucose, insulin, HbA1C, free fatty acids, triglycerides, HDL- and LDL-cholesterol and total cholesterol:HDL ratio. The angiocatheter was flushed with 6 ml of saline solution,

and each subject's blood draw was administered with 1 ml of heparinized saline (100 USP/ml) per draw (14 ml over the breakfast and lunch periods) to minimize the development of thrombi (McBurney et al, 1995). There were 2 ml of blood discarded from each draw in an effort to remove the heparin.

Postprandial measurements of serum glucose, insulin and free fatty acids were determined at various intervals: 30, 45, 60, 90, 120, 180 and 210 minutes after the breakfast. These tubes were labeled as 1-7. Lunch was fed at 260 minutes. Additional blood was drawn post-lunch at 285, 300, 330, 360, 390, 420, and 450 intervals, and these tubes were labeled 9–15. Data derived from these analyses were used to determine an acute assessment of the primary outcomes of glucose, insulin and free fatty acids based on the test meals consumed and also to assess whether or not there is any carryover, or second meal effect of breakfast. Interest in these specific metabolic measures is due to the association of Type 2 DM with glucose intolerance, hyperinsulinemia, and hyperlipidemia.

The primary data assessments were made using calculations of area under the curve (AUC) values. This measurement provides an estimate of how these metabolic outcomes of insulin and glucose vary when different test foods or meals are consumed. The unit of time for AUC calculations is expressed in hours, 0-210 minutes for the AM period, and 285-450 minutes for the PM period. The time period for the PM period AUC calculations began at 285 minutes due to the absence of a baseline blood draw at lunch. Of the total PM measurements for the AUC values, approximately 10 % is missing for the three treatments. However it is unlikely, in retrospect, that this would influence the results. The insulin AUC is expressed in mIU x hr, glucose is expressed as mg/dl x hr,

and free fatty acid concentration is expressed in mg/dl x hr. The AUC calculations were made post-breakfast (AM) and post-lunch (PM) periods.

The blood samples were processed for commercial analyses according to the instructions provided by the Sparrow Hospital Laboratory (SHL), Lansing, MI (The algorithm of the blood collection is shown in Appendix I). The samples were held at a temperature of 4 °C for 45 minutes and centrifuged at 2000 x g for 10 minutes using Hamilton Bell VanGuard V65000 model centrifuge. The serum was then harvested and stored in the refrigerator until they were retrieved on the same day of testing, and transported on dry ice by a SHL representative for analyses. The SHL performed the fasting blood glucose, HbA1C, insulin and lipoprotein analyses, and the treatment glucose and insulin tests. The Mayo Clinic (Rochester, MN) was contracted to perform the free fatty acid analyses. The results of the analyses were provided within seven days to the principal investigators. Biohazardous materials associated with blood collection were disposed of according to the current policies and procedures of the Office of Radiation, Chemical and Biological Safety.

Clinical Measurements

Serum glucose concentrations were determined using the hexokinase and ultraviolet methods with an Olympus AU640 Spectrophotometer (Olympus America, Inc., Melville, NY). Serum insulin concentrations were determined using immunoenzymatic and chemiluminescence methods with the Beckman Access Detector (Beckman Instruments, Brea, CA). Glycated hemoglobin was determined using HPLC with the Tosoh A1C 2.2 Detector (Tosoh Medics, Inc., South San Francisco, CA). Blood samples for free fatty acid analyses were sent to the Mayo Clinic (Rochester, MN). The

free fatty acid concentrations were determined with enzymatic and colorimetric methods using the Hitachi 912 Spectrophotometer (Roche Diagnostic Corp., Indianapolis, IN). Other lipid measurements were done locally. Serum cholesterol (HDL, with homogenous, liquid selective detergent) and triglyceride concentrations were determined using the enzymatic method with the Olympus AU640 Detector (Olympus America, Inc., Melville, NY). LDL-cholesterol was calculated with the Friedewald formula (Friedewald et al, 1972).

Statistical Analyses

The study design was a three-diet, three-period crossover. Assessments of insulin, glucose and fatty acids were obtained at pre-determined time points in the post-breakfast, and post-lunch periods and summarized by subject-specific area under the curve (AUC). The AUC was computed by the trapezoidal rule, with the baseline being zero. There are several methods of calculating the area under the curve response including: (1) the total area which includes all values, (2) incremental area above the baseline glucose, insulin or free fatty acid values, (3) incremental area above the minimum blood glucose, insulin, or free fatty acid value, and (4) net area under the curve (difference between total and incremental values) (Wolever, 2003, unpublished data). The method employed in this study includes the total area below the curve, including the area below the fasting concentration. This method was used because it measured average blood glucose, insulin and free fatty acid concentration values over time, and allowed the blood increments for all subjects to be utilized, irrespective of the value being negative or positive.

The total area under the curve is calculated by applying the trapezoid rule to all blood glucose, insulin and free fatty acid increments over a three and a half hour time

period. The values were then summed across the time period for each subject. The following formula was used for the trapezoidal area calculation:

Area under the curve = ½ (B + b) * height (as shown in Figure 1)

Where B represents the longest base and b is the shortest base of the trapezoid.

The analysis of each AUC measure was performed separately, and all analyses are expressed as the log-transformed AUC as a dependent variable in a mixed model with each patient as random effect, and diet and week as fixed effects. The log transformation mitigates the effect of skewness in AUC values. Graphical checks of normality were made by quantile-quantile (Q-Q) plots for each treatment, and for the entire test period (See Appendix J). The hypothesis of log AUC values for each of the diets was formally verified by the Shapiro-Wilk test, p-values exceeded 0.77 (Shapiro et al, 1968).

Analyses of the effects of diet on AUC values were based on a mixed model in which the log-transformed AUC was the dependent variable, with subject as random effect, and diet and period as fixed effects. Analyses of AUC values in the post-lunch period were controlled by including the post-breakfast AUC as a covariate in the model. Comparisons between diets (control vs. psyllium cereal, control vs. psyllium beverage, and by subtraction, psyllium beverage vs. psyllium cereal) were based on t-tests. Confidence intervals for differences used the Bonferroni adjustment for multiple comparisons. All statistical analyses were performed using SAS Software version 8.02 (SAS Institute, Cary, NC)

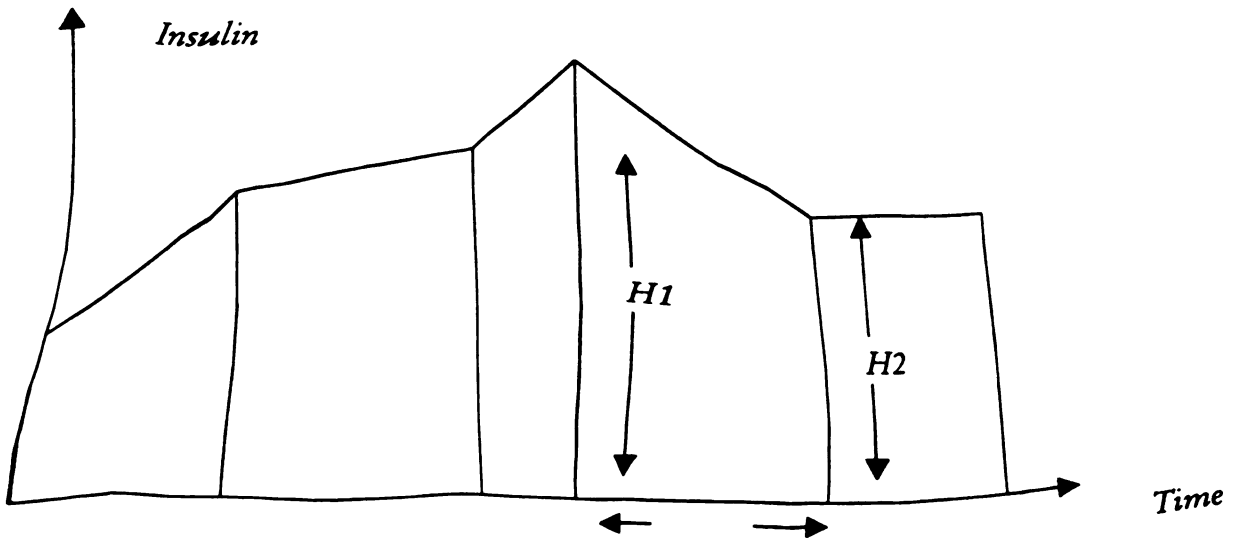

Figure 1. The total area under the curve response for blood glucose, insulin and free fatty acid concentrations represented by the area of the trapezium as $\frac{(H1+H2)}{2} \times B$.

RESULTS

Seventy-five adult subjects medically diagnosed with Type 2 DM were screened to participate in the study, and of this number, twenty-five either failed to meet the study inclusion criteria, or declined to participate in the study. The remaining fifty subjects were randomly assigned to the breakfast treatments, and forty-five subjects completed all three treatments. The other five subjects failed to fulfill the requirements for blood draws and their records were not evaluated. On the basis of subject interviews and observations, compliance to pre-experimental guidelines was determined to be excellent for both control (Breakfast A consisting of Malt-O-Meal cereal with no fiber) and treatments (Breakfast B consisting of Malt-O-Meal cereal followed twenty minutes later by a psyllium soluble fiber drink and Breakfast C consisting of a loop cereal with psyllium fiber incorporated).

Baseline Characteristics

Baseline physical characteristics of the study population are summarized in Table 9. There were thirty-two males and thirteen female participants. The mean BMI was 34.1 ± 1.8 kg/m^2 and 30.9 ± 0.9 kg/m^2 for males and females, respectively, indicating obesity, a common co-morbidity in Type 2 DM. Twenty-one subjects (thirteen males and eight females) managed their diabetes with diet alone whereas twenty-two subjects (eighteen males and four females) used oral hypoglycemic agents (OHA), one of whom used lipid-lowering medication. Additionally, two other subjects used antihypertensive medications alone, and one subject used lipid-lowering medication alone to help maintain control.

Table 9. Physical characteristics of study participants [1,2]

Characteristic	Males (n=32)	Females (n=13)
Age (yrs)	64 ± 2	59 ± 3
Height (m)	1.77 ± 0.01	1.65 ± 0.02
Weight (kg)	96.4 ± 2.8	92.1 ± 4.2
Body mass index (kg/m^2)	34.1 ± 1.8	30.9 ± 0.9
Diabetes management [2]		
Diet alone	13	8
Oral hypoglycemic agents		
Sulfonylurea	10	2
Metformin	4	2
Sulfonylurea and metformin	4	0
Antihypertensive medication	2	0
Lipid-lowering medication	0	1

[1] Mean ± SEM of values taken at week 0 prior to breakfast treatments.
[2] Totals are not additive as some subjects were using multiple prescribed medications.

Fasting baseline serum characteristics of the subjects were similar prior to the consumption of each of the three breakfast meals in this randomized, controlled, cross-over design study (Table 10). The clinical data were not analyzed by gender as the study was not designed, nor statistically powered, to detect gender differences.

Table 10. Baseline fasting serum glycemic, insulin, and lipid parameters of subjects by breakfast treatment [1,2]

Variable	Breakfast A	Breakfast B	Breakfast C
Fasting serum glucose (mg/dl)	130.98 ± 4.93	131.72 ± 5.24	133.16 ± 4.37
Serum insulin (mIU/ml)	11.45 ± 1.27	11.73 ± 1.44	10.86 ± 1.14
HbA1C (%)	6.91 ± 0.18	6.88 ± 0.16	6.91 ± 0.16
Free fatty acids (mg/dl)	532.77 ± 43.22	515.57 ± 31.77	550.27 ± 33.68
Triglycerides (mg/dl)	188.33 ± 29.44	185.96 ± 27.52	185.56 ± 19.32
HDL cholesterol (mg/dl)	41.89 ± 1.43	41.76 ± 1.36	41.53 ± 1.28
LDL cholesterol (mg/dl)	111.09 ± 4.93	114.00 ± 5.46	113.67 ± 6.02
Total cholesterol (mg/dl)	186.02 ± 4.66	189.28 ± 5.79	189.91 ± 5.40
Total cholesterol: HDL Chol[3]	4.68 ± 0.23	4.74 ± 0.23	4.75 ± 0.19

[1] Mean ± SEM. Baseline values of key outcomes prior to each breakfast treatment. There were no significant differences between groups ($p>0.05$).
[2] Meal types consist of (1) Breakfast A with farina and no psyllium fiber, (2) Breakfast B with farina plus a psyllium drink 20 minutes postmeal, and (3) Breakfast C with a ready-to-eat psyllium loop cereal.
[3] Ratio of total cholesterol to HDL-cholesterol measure.

The dietary intakes recorded from each subject during the first visit using the Block Food Frequency Questionnaire are summarized in Table 11. The mean intake of energy for males was 1981 ± 96 kilocalories and 2121 ± 233 kilocalories for females. Similarly, the mean percent of calories from fat, protein, and carbohydrates were reported as 40 ± 1 %, 16 ± 0 %, and 44 ± 1 % for males and 38 ± 3 %, 17 ± 1 %, and 45 ± 3 % for females, respectively. Thus, their fat intake was higher and carbohydrate intake was correspondingly lower than the 1999 American Diabetes Association Nutrition Guidelines' recommendations of 30 % of calories of fat and 55-60 % of calories as carbohydrate. The percent of calories from saturated fat was 12 ± 1 % and 11 ± 1 % for males and females, respectively, which was slightly higher than the 1999 American Diabetes Association Nutrition Guidelines' recommendation of a maximum of 10 % of

caloric intake. The mean dietary cholesterol intake was reported to be 258 ± 21 mg for males and 272 ± 42 mg for females, which appears to be understated relative to the reported fat intake. Dietary fiber intake was similar for males and females at 23 ± 2 grams and 23 ± 3 grams, respectively, and consistent with the recommended guidelines.

Table 11. Nutrient intakes of subjects [1,2]

Variable	Males (n=32)	Females (n=13)
Calories (kcal)	1981 ± 96	2121 ± 233
Total fat (g)	90 ± 6	91 ± 12
Saturated fat (g)	27 ± 2	27 ± 4
Protein (g)	80 ± 5	90 ± 8
Carbohydrate (g)	220 ± 10	247 ± 34
% kcal from fat	40 ± 1	38 ± 3
% kcal from saturated fat	12 ± 0	11 ± 1
% kcal from protein	16 ± 0	17 ± 1
% kcal from carbohydrate	44 ± 1	45 ± 3
% kcal from sweets	7 ± 1	8 ± 1
% kcal from alcoholic beverages	1.8 ± 1	0.2 ± 1
Dietary cholesterol (mg)	258 ± 21	272 ± 42
Dietary fiber (g)	23 ± 2	23 ± 3

[1] Based on the 1999 Block 98.2 Food Frequency Questionnaire.
[2] Data are presented as mean ± SEM.

First Meal Responses

Glucose, Insulin and Free Fatty Acid Concentrations

Fasting and postprandial morning (AM) and afternoon (PM) glucose, insulin and free fatty acid concentrations are shown in Figures 2-4 (See Appendix K for absolute concentrations). Glucose concentrations peaked at 60-90 minutes for all three breakfast

types: Breakfasts A (230 ± 8 mg/dl), Breakfast B (228 ± 9 mg/dl), and Breakfast C (200 ± 8 mg/dl). The difference in peak concentrations for Breakfasts A and B were negligible, whereas Breakfast C was 12% lower than Breakfasts A and B. Concentrations of glucose steadily began to decline, and returned to near baseline levels by 210 minutes (Figure 2).

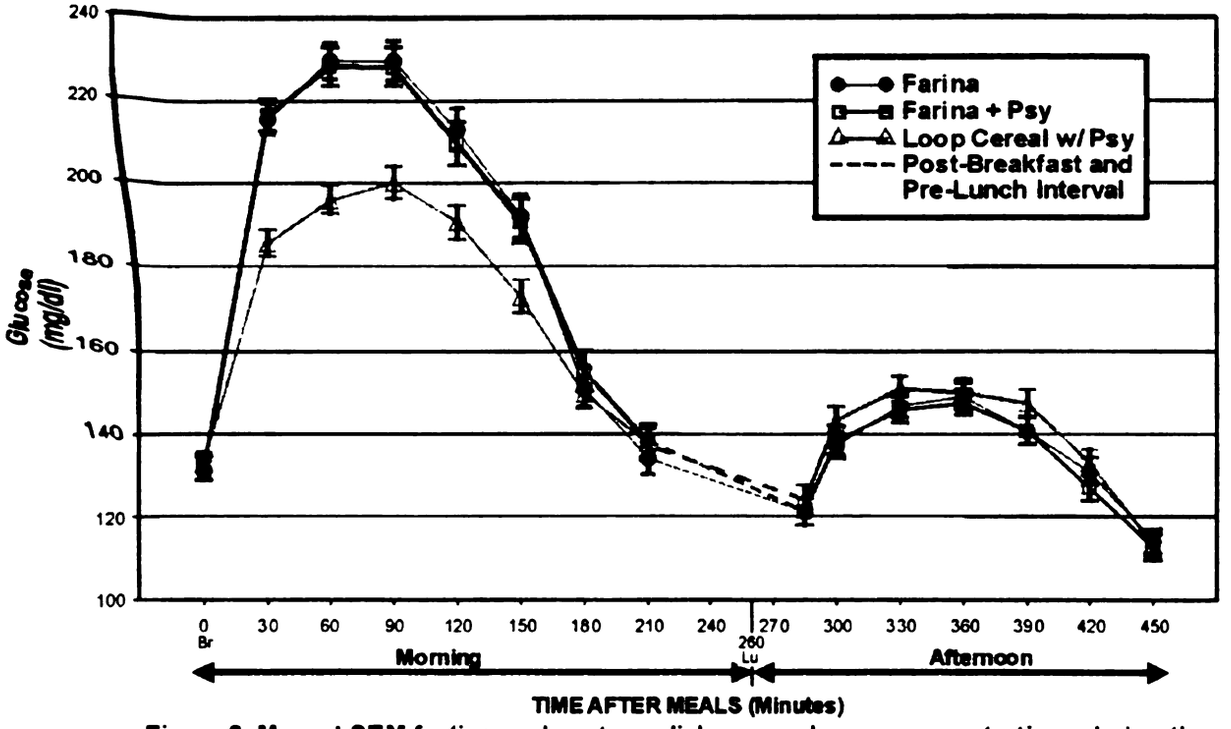

Figure 2. Mean ± SEM fasting and postprandial serum glucose concentrations during the AM and PM in subjects with Type 2 Diabetes Mellitus (n-45)

Insulin concentrations for both Breakfasts A and B peaked at 60 minutes, 74 ± 5 and 64 ± 8 uU/ml, while Breakfast C peaked at 90 minutes (45 ± 5 uU/ml) and remained significantly lower than Breakfasts A and B throughout the AM period (Figure 3).

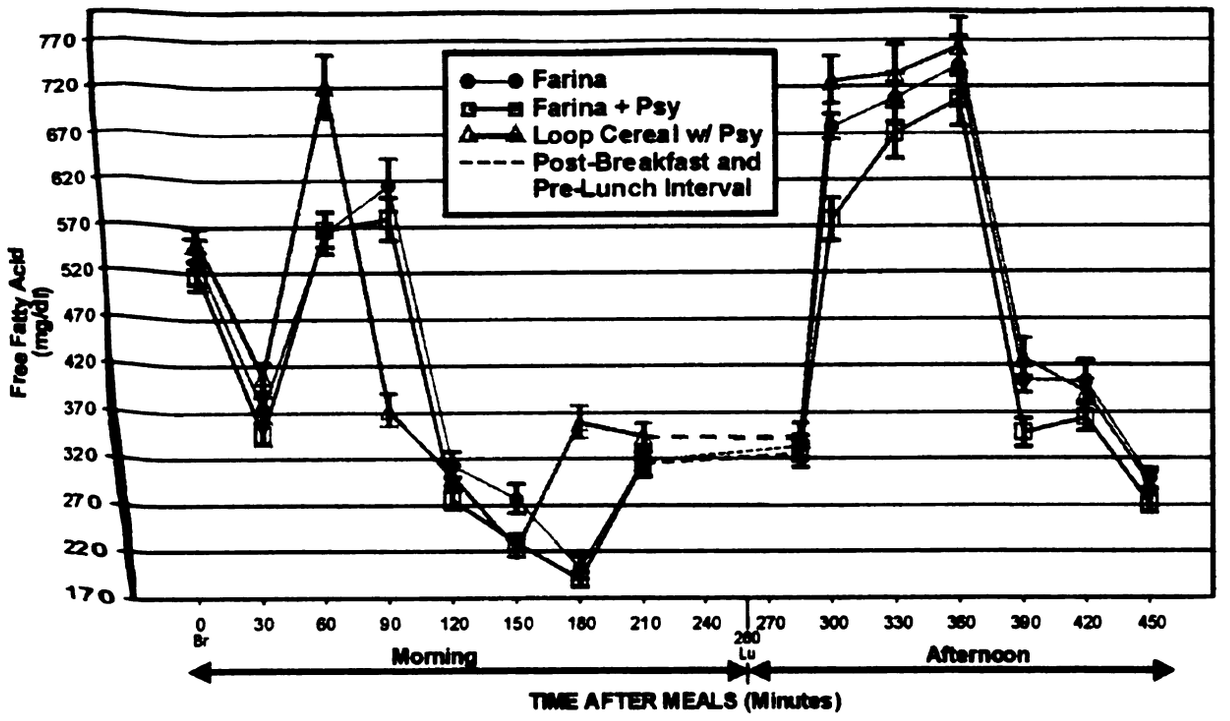

Figure 3. Mean ± SEM fasting and postprandial serum free fatty acid concentrations during the AM and PM in subjects with Type 2 Diabetes Mellitus (n-45)

The pattern of change for free fatty acid concentrations was markedly different from the glucose and insulin observations. Initially, morning FFA concentrations declined, and then increased at the 90-minute time period for Breakfasts A (615 ± 57) and B (579 ± 47), and at 60 minutes for Breakfast C (721 ± 69). The FFA concentrations then dropped to ~50% of these peak concentrations, and overall remained below baseline concentrations for all breakfast types for the duration of the AM morning period (Figure 4-).

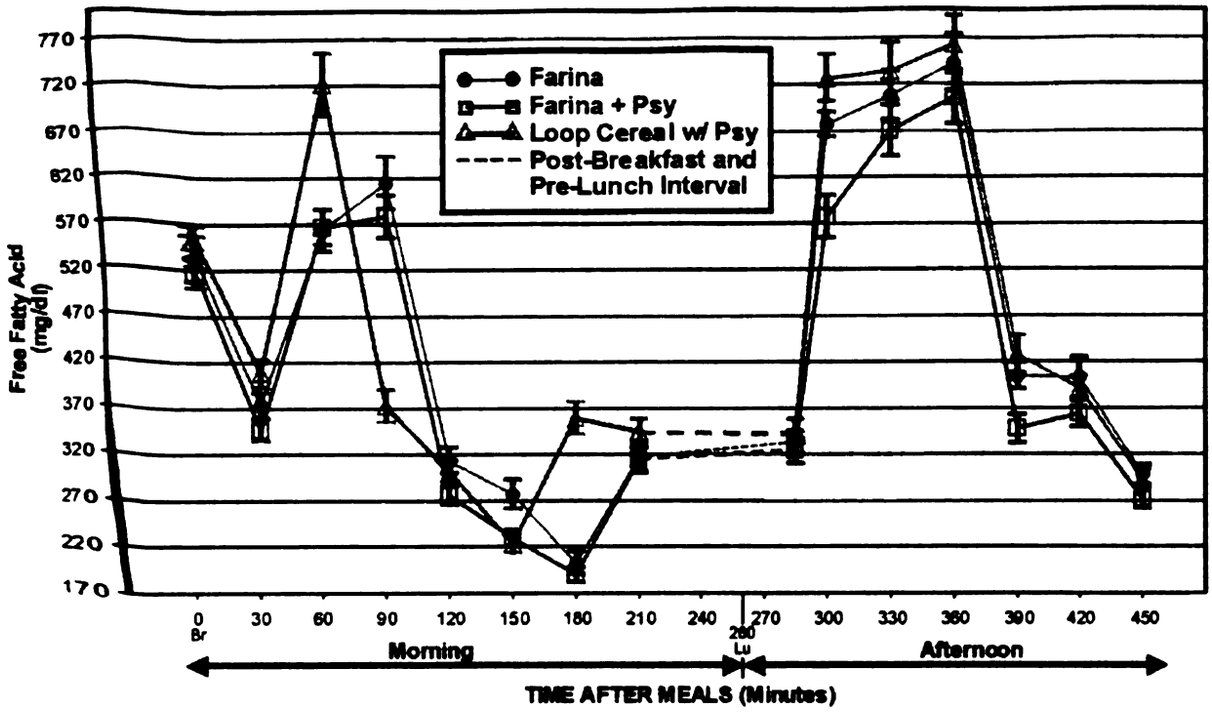

Figure 4. Mean ± SEM fasting and postprandial serum free fatty acid concentrations during the AM and PM in subjects with Type 2 Diabetes Mellitus (n-45)

Glucose, Insulin and Free Fatty Acids Area Under the Curve

The summary comparisons for AUC values for the subject responses were analyzed by diet and by week for the AM morning period and the PM post-lunch period (Appendix L). There was no effect of week for breakfast or lunch, and thus any carryover effect is presumed to be the same for the breakfast treatments.

Table 12 collapses the AM morning and PM afternoon data for all subjects (n=45) and presents the statistics by breakfast treatment with comparisons for glucose, insulin and free fatty acid responses using area under the concentrations-versus-time curves (using a log scale and expressed as log AUC).

Table 12.
Area under curve values for glucose, insulin, and free fatty acids by breakfast treatment in the AM and PM (n=45) [1,2,3]

Time of Day	Breakfast A	Breakfast B	Breakfast C
AM			
Glucose (mg/dl.hr)	6.45 ± 0.04[a]	6.44 ± 0.05[a]	6.36 ± 0.04[b]
Insulin (uU/ml.hr)	4.86 ± 0.11[a]	4.79 ± 0.10[a]	4.47 ± 0.10[b]
Free fatty acid (mg/dl.hr)	7.00 ± 0.07[a]	6.98 ± 0.05[a]	7.16 ± 0.07[b]
PM			
Glucose (mg/dl.hr)	5.86 ± 0.03[a]	5.85 ± 0.04[a]	5.92 ± 0.03[a]
Insulin (uU/ml.hr)	4.32 ± 0.06[a]	4.40 ± 0.05[ab]	4.58 ± 0.06[b]
Free fatty acid (mg/dl.hr)	7.11 ± 0.05[a]	7.03 ± 0.05[a]	7.05 ± 0.05[a]

[1] Values are expressed as mean log AUC values and standard errors of the mean.
[2] Values in the same row with different superscript letters are significantly different ($p \leq 0.05$).
[3] Meal types consist of (1) Breakfast A including farina with no psyllium soluble fiber, (2) Breakfast B including farina plus a psyllium drink 20 minutes postmeal, and (3) Breakfast C including a ready-to-eat psyllium loop cereal.

For the AUC morning analyses, the data assessment includes values at 0 minutes through 210 minutes as the AM period. As expected, a high glycemic load (Breakfasts A and B) resulted in significantly greater glucose AUC (6.45 +/- 0.04 mg/dl.hour and 6.44 +/- 0.05 mg/ml.hour) and insulin AUC values (4.86 +/- 0.11 uU/ml.hour and 4.79 +/- 0.10 uU/ml.hour) ($p<0.05$) than for subjects fed Breakfast C. Free fatty acid AUC were significantly lower on Breakfasts A (7.00 +/- 0.07 mg/dl.hour) and B (6.98+/- 0.05 mg/dl.hour) versus Breakfast C (7.16 +/- 0.07 mg/dl.hour) ($p<0.05$).

The dietary influence of the treatments with AUC calculations can be more clearly be seen by an unlogged scale using:

Exp(mean + 0.5*sigma^2).

The difference resulting from this calculation in the morning AUC values between Breakfasts A and B versus Breakfast C for glucose, insulin and FFA ranged from 8-9 %, 27-32 %, and 15-16 %, respectively.

Second Meal Responses

Glucose, Insulin and Free Fatty Acid Concentrations

The mid-day meal (all subjects were provided the same lunch) was consumed at 260 minutes. At 285 minutes, plasma glucose averaged 121 ± 6 mg/dl, 121 ± 6 mg/dl and 124 ± 6 mg/dl for Breakfasts A, B, and C respectively. These concentrations were below AM fasting baselines values (Table 10). Glucose values during the PM period were fairly flat for all breakfast types between 330 and 360 minutes, with Breakfasts A, B and C at 149 ± 7 mg/dl, 147 ± 6 mg/dl and 151 ± 6 mg/dl, respectively. At the end of the post-lunch period, values were below fasting baseline levels in the AM period (Figure 2).

Insulin concentrations moderated by the end of the AM period, but remained above the pre-breakfast concentrations. Plasma insulin concentrations of subjects fed Breakfast A abruptly peaked at 52 ±14 uU/ml by 300 minutes (40 minutes after consumption of the lunch). In contrast, insulin concentrations for subjects fed Breakfasts B and C slowly increased after lunch. Insulin concentrations for subjects fed Breakfast B peaked at time 360 minutes (45 ± 4 uU/ml), while insulin concentrations for subjects fed Breakfast C peaked at 390 minutes after lunch (47 ± 9 uU/ml). At the end of the post-lunch period, insulin concentrations were lowest for Breakfast A (24 ± 3 uU/ml) and highest for Breakfast C (31 ± 7 uU/ml), with insulin concentrations 22% higher in Breakfast C at the end of the PM period (Figure 3).

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Free fatty acid concentrations continued to moderate immediately post-lunch for all three breakfast types, but spiked at 300 minutes. Serum FFA concentrations for subjects fed all breakfast types similarly peaked at 360 minutes, ranging from 705 ± 58 mg/dl to 762 ± 64 mg/dl. At the end of the PM period, free fatty acid concentrations declined below baseline fasting values both in the AM and PM periods. Breakfast C resulted in the highest free fatty acid concentrations of all three breakfast types for both the AM and PM periods. However, by the end of the PM period (450 minutes) free fatty acid concentrations were similar for all breakfast types: 299 ± 21 mg/dl, 272 ± 20 mg/dl, and 293 ± 23 mg/dl for Breakfast A, B and C, respectively (Figure 4).

Glucose, Insulin and Free Fatty Acids Area Under the Curve

The post-lunch analyses for AUC calculations began at 285 minutes (this reflects missing blood draw values at baseline for all three treatments which accounted for approximately 10% of the area; given that the values for the three treatments were declining in a similar pattern and 90% of the area for the PM period was calculated, the impact on the results were determined to be inconsequential) (Table 12). The glucose AUC values were lower with both Breakfasts A (5.86 +/- 0.03 mg/dl.hour) and B (5.85 +/- 0.04 mg/dl.hour) versus Breakfast C (5.92 +/-0.03 mg/dl.hour), but none of the three treatments differed statistically. Insulin AUC values were lower with Breakfast A (4.32 +/- 0.06 uU/ml.hour) and Breakfast B (4.40 +/- 0.05 uU/ml.hour), but only Breakfast A (4.32 +/- 0.06 uU/ml.hour) differed statistically from Breakfast C (4.58 +/- 0.06 uU/ml.hour) ($p< 0.05$). In the post-lunch (PM) analyses, the free fatty acid AUC values were unaffected by breakfast type.

The dietary influence of the treatments for AUC values using the unlogged scale previously noted resulted in a difference in the PM AUC values between Breakfasts A and B versus Breakfast C for glucose, insulin and free fatty acids ranged from 6-7 %, 17-23 % and 6-8 %, respectively.

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DISCUSSION

A number of factors determine the glycemic response to foods: the quantity and quality of carbohydrates, food form, digestibility, sugars, fats, presence of anti-nutrients, and the second meal effect. The first objective of this study was to compare breakfast meal glycemic responses, influenced by glycemic loads of the meal as well as the presence of psyllium fiber. The second objective was to determine if second meal (lunch) glycemic responses were affected by the glycemic responses (glycemic load of the meal) or the amount of psyllium soluble fiber ingested at the first meal (breakfast) in subjects with Type 2 DM.

Previously published clinical studies have compared low and high glycemic index (GI) diets in individuals with Type 2 DM, with the number of subjects varying from 6-34 (Wolever et al, 1991; Wolever and Bolgonesi, 1996; Pastors et al, 1991; Liljeberg et al, 1999; Sierra et al, 2001; 2002; Wolever and Mehling, 2003). This trial, which included 45 subjects, is believed to be the largest clinical study thus far assessing the acute effects of glycemic load with and without fiber at breakfast on mid-day glycemic responses using subjects with Type 2 DM.

In this study, Breakfast A was designed to be a high glycemic load using farina cereal (food with a high GI). Breakfast B was similarly designed with farina cereal and to determine if the psyllium fiber (beverage exhibiting a low GI value) would influence glycemic responses in subjects with Type 2 DM. Breakfast C, in contrast, was designed to measure the impact of a breakfast with a lower percentage glycemic load. The low glycemic load of Breakfast C was achieved by a combined effect of low levels of

available carbohydrates/calorie intakes as well as the incorporation of psyllium fiber. Thus, Breakfast C was the treatment with the lowest glycemic load and also the lowest GI of the two breakfast foods in this study.

Jenkins et al, (1982) showed that consumption of foods with a low GI resulted in relatively small, slower increases in serum glucose levels, and insulin responses generally paralleled glycemic responses. Conversely, foods with a high GI caused large, rapid increases in blood glucose levels. For years, it has been reported that foods high in complex carbohydrates cause smaller increases in blood glucose than simple sugars, and similarly, that dietary fiber resulted in smaller increases in blood glucose than starchy foods. Hence, it would appear that the consumption of dietary fiber would benefit individuals with diabetes (Jenkins et al, 1976). Although the mechanism is unknown, it was hypothesized that foods high in dietary fiber improved glucose homeostasis by delaying gastric emptying, slowing/lowering the rate of intestinal absorption of glucose and/or altering hormone secretion and/or sensitivity to a carbohydrate load (Jenkins et al 1976, Sierra et al, 2001).

In individuals with diabetes, chronic elevation in blood glucose levels causes defects in insulin secretion and may exacerbate insulin resistance. Factors that increase blood glucose and insulin demand may aggravate the effects of insulin resistance, which increases the risk of diabetes. Conversely, factors that lower glycemia and insulin demand may protect against diabetes. Foods with a low GI, high fiber foods, and low carbohydrate loads reduce glycemia and insulin demand and may help protect against diabetes (Salmeron et al, 1997a,b; Jenkins, 1982; Pastors et al, 1991).

In general, foods high in fiber have low GI values. It is purported that the GI, by comparing the glucose-raising potential of equal amounts of carbohydrates, measured the quality of carbohydrates only and cannot capture the entire glucose raising potential of dietary carbohydrates. The concept of glycemic load, as an indicator of glucose response or insulin demand induced by total carbohydrate intake, was subsequently introduced in 1997 to simultaneously evaluate the quantity and quality of carbohydrates consumed (Liu et al, 1998). (The dietary glycemic load was calculated by multiplying the carbohydrate content of each food by its glycemic index; this value was then multiplied by the frequency of consumption and then the values were summed from all foods). Thus, the dietary glycemic load represents both the quality and quantity of carbohydrates and their interaction. Each unit of dietary glycemic load is the equivalent of 1 g carbohydrate from white bread or glucose (depending on whichever reference being used). A 10-year follow-up Nurses' Health prospective study showed that the relative risk (incidence-rate ratios) of Type 2 DM among subjects in the highest quintile of glycemic load compared with the lowest quintile (206 vs.117) was 1.98 (Liu et al, 2000).

In the past decade, an intense effort has been made to better understand the influence of source and amount of carbohydrate foods in the dietary management of Type 2 DM. It has been shown that reducing postprandial glucose and insulin concentrations are important in helping to achieve favorable outcomes in risk reduction and treatment (Jeppesen et al, 1997; Reaven, 1997). However, there continues to be a lack of consensus on the use of the quality of carbohydrate foods (glycemic load versus total fiber intake) in predicting glucose intolerance and the risk of Type 2 DM.

The glycemic load versus the GI concept was examined clinically in a chronic study among subjects with impaired glucose tolerance by Wolever and Mehring (2003). They reported that reducing the GI of the diet for four months reduced postprandial plasma glucose by the same amount as did reducing the glycemic load, or amount of carbohydrate intake. These two dietary maneuvers however, resulted in different effects on plasma insulin, triacylglcerols and free fatty acids levels where modest increases were associated with the low-GI diet. The insulin effect was not consistent with short-term studies showing that foods that elicit high glycemic responses also elicit high insulin responses regardless of whether the portions fed contained equal amounts of carbohydrates (Bjork et al, 2000) or equal amounts of energy (Holt et al, 1996). The authors hypothesized that lower postprandial insulin may have lessened the suppression of FFA mobilization from adipose tissue, and subsequently caused a rise in triacylglycerols. Additional research is needed to better delineate the utility of the glycemic load concept (amount and type of carbohydrate) in Type 2 DM.

This study further explored the utility of glycemic load concepts in influencing postprandial serum glucose, insulin and free fatty acid concentrations in subjects with Type 2 DM. An independent evaluation on the effect of a low GI food and psyllium fiber on glycemic responses however, was not conducted. Also, the three groups compared did not employ uniform food administration methods such as food form and ingestion time, and thus this may have contributed additional confounding factors. Also due to the number of treatment arms and subjects required for this study, another limitation was that a control loop cereal (without psyllium) was not included in this trial. Future studies should be designed to more specifically evaluate these two independent factors, e.g. low

GI and psyllium fiber, using similar food forms. The approach employed in this study used an orthogonal contrast design, and was aimed at securing knowledge of these variables both collectively and independently. The practical reality is that the execution of this design was less than ideal given that the primary driver of the total carbohydrate content of the breakfast treatments was caloric needs and not available carbohydrate consumed. While the glycemic responses are very consistent with previously reported glycemic responses irrespective of the approach employed, in retrospect, the use of smaller experiments investigating these variables independently would have been an ideal starting point for this larger study.

Breakfast Responses

It was expected that Breakfast B which contains psyllium fiber would induce lower glycemic responses than Breakfast A. Ten previous studies have been carried out with psyllium fiber in subjects with Type 1 (Florholmen et al, 1982), Type 2 (Sartor et al; 1981; Jarjis et al, 1984; Anderson et al, 1999; Pastors et al, 1991; Sierra et al, 2002) and healthy, normal subjects (Jarjis et al, 1984; Wolever et al, 1991, Frape and Jones, 1995; Sierra et al, 2001; Frost et al, 2003). In general, subjects administered with psyllium showed significant improvements in glycemic responses compared to placebo groups. Postprandial glycemia was significantly reduced after standardized breakfast with 3.6 g psyllium fiber (Florholmen et al, 1982), 6.6 g (Sartor et al, 1981; Anderson et al, 1999) or 5.1 g (Pastors et al, 1991) of fiber in patients with Type 2 DM, respectively.

In contrast, no significant differences were found after a 50 g glucose load administered with 3.5 g and 7 g psyllium fiber in both healthy and subjects with Type 2 DM (Jarjis et al, 1984). Consumption of low dose breakfast meals enriched with 1.7 g

(Frost et al, 2003) or 2.2 g psyllium (Frape and Jones, 1995) did not evoke measurable effects on glucose metabolism, measured by glycemic responses. However, Sierra et al (2001) found that a higher dose of psyllium, 10.5 g, administered with 50 g glucose load, showed significant improvements in glycemic responses in healthy subjects. When 3.5 g of psyllium was administered 4 times a day (before breakfast, lunch, afternoon snack and dinner) in a drink form, totaling 14 g/day, subjects with Type 2 DM had significantly lower glucose absorption (12.2 %) which was not associated with an important change in insulin levels (Sierra et al, 2002).

The findings of this research indicated that the morning (AM) serum postprandial glucose concentrations in subjects consuming Breakfast A (farina with no psyllium) were virtually similar to Breakfast B (farina with the psyllium drink post-meal). However, both Breakfasts A and B resulted in peak glucose concentrations of 14-15 % higher (on an unlogged scale) than for Breakfast C (loop ready-to eat cereal with psyllium soluble fiber), the breakfast with a lower glycemic load, at 60-90 minutes. Similarly, the morning area under the curve (AUC) values for serum postprandial glucose for Breakfast A was not significantly differently from Breakfast B. No measurable differences between the farina control and farina administered with psyllium were noted in my study. This could be partly attributed to several factors:

1) psyllium drink (6.6 g of psyllium in 240 cc of water) was ingested 20 minutes after consumption of farina and this 20 minutes lag may not have been long enough to allow proper mixing in stomach, thus psyllium fiber could not properly exert its effect. Wolever et al (1991) found that taking 20 g of psyllium in a beverage 20 minutes before

cereal consumption did not improve glycemic responses. Previous research using guar gum demonstrated that inadequate mixing of guar with food resulted in the lack of a therapeutic effect in the longer-term (Jenkins et al, 1978; 1986). The rationale for intimate mixing appears to be that viscous fibers act by delaying the rate of digestion and absorption due to the formation of an intraluminal gel within the small intestine (Holman et al, 1987);

2) the dosage of fiber used may not have been sufficiently high enough to demonstrate effect. Although previous work using ~ 6 g of psyllium was beneficial in reducing postprandial glucose and insulinemic effect (Florholmen et al, 1982; Sartor et al, 1981), a dose-response experiment conducted among these subjects under the conditions of this study would have been useful.

3) Soluble fiber may need to be incorporated with starches and sugars within a food to exert proper effect. It has been recognized that physiochemical properties of foods are also important in regulating glycemic responses (Wolever et al, 1991, Simpson et al, 1985). Research conducted to examine postprandial responses to various grain products, Juntunen et al (2002) found that lower insulinemic responses to rye breads and pasta than to wheat bread is not explained by the fiber content, type of cereal, or the rate of gastric emptying, but by the structural properties of the food. Thus, it is possible that both the food properties and the method of administration may have influenced the

lack of measurable difference between control and the farina breakfast with the psyllium drink;

4) Psyllium diluted in water may have offered less viscosity in the gastrointestinal tract than a cereal containing psyllium in dry form. Thus, the efficacy of psyllium in diluted hydrated form may have been reduced as compared to the dry form of psyllium. It has been hypothesized that viscous fibers such as psyllium fibers inhibit glucose absorption in the gastrointestinal tract to cause smaller increases in blood glucose. More highly viscous fibers were more effective in slowing the rate of glucose absorption and lowering blood glucose concentration (Jenkins and Jenkins, 1995). This suggests that viscosity plays an important role in glucose absorption in the gastrointestinal tract (Jenkins et al, 1978, 2000, Sierra et al, 2001).

In the future, it may be worthwhile to compare control loop cereal ± psyllium and a drink ± psyllium in a 2 x 2 factorial design to evaluate effects of the method of administration of psyllium on glycemic responses.

The AUC glucose values for both Breakfasts A and B both were 8-9 % higher (on an unlogged scale) ($p < 0.01$) than for Breakfast C, which could be biologically significant. Even a small physiological change can have enormous impact particularly since cardiovascular disease risk increases continuously as blood glucose increases within the normal range (Coutinho et al, 1999; Liu et al, 2000). Additionally, animal studies have shown that increases in mean blood glucose < 18 mg/100 ml can markedly affect β cell function via glucose toxicity (Leahy et al, 1987; 1988).

In this study, morning AM insulin responses were similar to the glycemic responses. Postprandial serum insulin concentrations after consuming Breakfasts A and B were 40-46 % higher than for the loop psyllium-containing cereal breakfast (Breakfast C) at the peak response time period of 60-90 minutes. The morning AUC values for insulin were not significantly different for Breakfast A versus B; however AUC values for insulin for after consuming Breakfast C was 27-32 % lower (on an unlogged scale) ($p < 0.05$) than after consuming Breakfasts A and B.

This elevated insulin secretion, associated with Breakfasts A and B, is consistent with other acute and chronic studies showing similar results with high carbohydrate load meals (Garg et al, 1994; Holt et al, 1996; Bjork et al, 2000). Additionally, as previously noted, prospective research shows that the consumption of high glycemic load meals is associated with the increased risk of developing Type 2 DM (Salmeron et al, 1997a,b). Thus, this study provides additional support that reducing the diet glycemic load may improve acute insulin responses postprandially. This is consistent with other short-term studies that suggest an improvement in insulin action and pancreatic function associated with low carbohydrate load meals (Bjork et al, 2000) and low-GI meals (Liljeberg et al, 1999).

It is important to note that dietary approaches using glycemic index, and glycemic load can have differing influence on postprandial plasma free fatty acid (FFA) responses (Wolever and Mehling, 2003). For example, in subjects with impaired glucose tolerance, reducing the GI of the diet for four months reduced postprandial plasma glucose by the same amount as by reducing carbohydrate intake (Wolever and Mehling,

2003). On the other hand, long-term replacements of dietary carbohydrate with MUFA (10% of energy intake) have been shown to raise postprandial FFA concentrations by > 30 % in subjects with Type 2 DM (Tsihlias et al, 2000). In my study, under fasting conditions, morning FFA concentrations were elevated in subjects with Type 2 DM, a confirmation of their fasting state at the beginning of the testing period. Elevated FFA concentrations are also associated with relative insulin resistance and fat mobilization occurring in the diabetic state (Sniderman et al, 1998; Gavin 1999).

FFA concentrations dropped at the beginning of the AM period and then increased sharply before declining below baseline levels. The morning AUC values for FFA were not significantly different between Breakfasts A and B, farina with and without psyllium fiber. However, AUC values for FFA for Breakfasts A and B were 15-16 % lower ($p< 0.05$) than after consuming Breakfast C containing the loop psyllium cereal. This may reflect the lowered insulin response to Breakfast C in comparison with Breakfasts A and B, where insulin concentrations were higher at the end of the AM period (Tsihlias et al, 2000).

Although the mechanism is not fully understood, research has shown that slowing carbohydrate absorption appears to influence the insulin sensitivity of adipose tissue *in vitro* and reduce FFA output in healthy individuals (Frost et al, 1996; 1998). Thus, while postprandial glucose and insulin concentrations were lower after consuming Breakfast C, this phenomenon of slowed rate of absorption did not manifest itself in a similar influence on FFA concentrations in subjects with Type 2 DM. This phenomenon would require further research to determine the level of reduction required and if replacing the

dietary carbohydrate with another macronutrient, e.g. fat, or monounsaturated fat would yield similar results.

We hypothesized that farina would induce higher glycemic responses than cereal loop with psyllium. Since Breakfasts B and C provided the same amount of psyllium fiber, we also hypothesized that the difference between Breakfasts B and C would suggest the effect of glycemic load on glycemic responses and that the differences between Breakfasts A and B could indicate psyllium fiber effects. A higher glycemic load and a higher fiber content may have independent mechanisms of actions, and should be further explored.

Thus, the overall conclusion from the AM morning results suggests that the lower glycemic load meal (versus fiber) had a more favorable influence on acute glycemic and insulinemic responses. The elevated FFA concentrations and AUC values were consistent with chronic observations among individuals with Type 2 DM where the reduction in carbohydrate load raised postprandial FFA concentrations (Tsihlias et al, 2000).

Lunch Responses

Previous research has shown that 5.1 – 12 g of viscous, soluble dietary fiber such as guar, psyllium, and pectin consumed in the first meal exhibited postprandial effects not only immediately following the first meal, but also resulted in residual effects that blunted postprandial glucose rise after meals eaten several hours after the fiber ingestion (Jenkins et al, 1980; 1982; Pastors et al, 1991; Anderson et al, 1999; Sierra et al, 2001). In an effort to determine whether or not there were any "carryover" or second meal effects of the glycemic load or psyllium fiber into the afternoon subsequent to the

consumption of a standard low-fat luncheon meal, the same biomarkers were examined in the afternoon as during the morning period.

The afternoon (PM) postprandial serum glucose concentrations were slightly lower (6-7 % on an unlogged scale) when subjects consumed Breakfasts A and B than when Breakfast C was consumed. Consistent with this, consumption of Breakfasts A and B containing farina cereal with and without psyllium soluble fiber resulted in higher post lunch insulin concentrations than did consumption of Breakfast C. Conversely, the post-lunch AUC values for insulin were also significantly different for Breakfast A versus Breakfast C, but not significantly different than after consuming Breakfast B. Thus, the PM glycemic and insulinemic responses did not parallel the morning responses given that the glucose response was similar across all breakfast types. The postlunch glycemic response was highest for Breakfast C, although not statistically different from Breakfasts A and B. Similarly, serum insulin was statistically higher (17-23 % on an unlogged scale) for Breakfast C as compared with Breakfasts A and B.

It has been well established that high-carbohydrate, low fat diets raise postprandial glucose and insulin concentrations (Jeppesen et al, 1997; Balkau et al, 1998), and they raise plasma insulin to the greatest extent in persons with insulin resistance (Wolever and Mehling, 2003). Thus, it is likely that the effects of the high carbohydrate or glycemic load of the farina breakfast elicited higher postprandial insulin responses in the AM morning meal and this phenomenon extended to the afternoon period. As a result, the insulin levels remained elevated postlunch, and potentially suppressed glucose concentrations to such an extent that they were lower than AM morning fasting concentrations.

The lunch response of higher glucose concentrations after the consumption of the low glycemic load breakfast meal fed in the morning led to a significantly higher postlunch insulin demand. These results suggest that the beneficial effects in reduced glucose and insulin concentrations associated with the low glycemic load treatment (Breakfast C) in the morning did not "carry over" or result in a second meal effect postlunch.

Similarly, there was no second meal effect after the standardized lunch for FFA concentrations and AUC values in response to the AM morning breakfast meals. At the end of the PM afternoon period, FFA concentrations were markedly lower than the morning fasting baseline levels. It has been reported that altering the amount and type of carbohydrate may influence FFA rebound in healthy subjects (Wolever and Miller, 1995). Thus, FFA may have inhibited insulin secretion and activity after the standardized lunch. It would be insightful to determine if triacylglycerol and lipid levels were affected in a similar manner as FFA concentrations by these different breakfast treatments, given that chronic ingestion of low-glycemic index meals is associated with a rise in triacylglcerols Wolever and Mehling, 2003). It cannot be concluded from the current body of literature that a similar phenomenon occurs with low glycemic load meals. Thus, any conclusion in this regard for low glycemic load meals is beyond the scope of this study, and should be explored in future work involving carbohydrate load versus dietary fiber intake.

Thus, it is concluded that the benefits of the low glycemic load containing psyllium soluble fiber observed in the morning breakfast meal did not carry over or influence the glucose, insulin and FFA responses after the standardized lunch meal was

consumed. While the expectation was that the presence of psyllium fiber in the gut from the morning meal would attenuate postprandial glucose response and absorption in the PM afternoon period, it is possible that the two forms of psyllium (post meal psyllium drink and psyllium incorporated in cereal loop) used in this study differentially affected intestinal viscosity, and thereby did not result in a second meal effect on postprandial glucose, insulin and FFA responses among subjects with Type 2 DM.

In this study, psyllium fiber consumption alone was not effective in influencing these postprandial results during both the morning (AM) and afternoon (PM) periods. This may be due to several reasons:

(1) the amount of fiber administered, e.g. 6.6 g of psyllium soluble fiber, in my study may have been insufficient to demonstrate an effect. This level of fiber was selected since previous research showed that a psyllium supplemental drink, in general, administered in the dosage of ~ 6 g of psyllium, was beneficial in reducing postprandial blood glucose and insulin responses in individuals with Type 2 DM (Florholmen et al, 1982; Sartor et al, 1981). However, the use of lower levels of dietary fiber, ~2-3 g, was not efficacious in a study conducted among healthy subjects (Frape and Jones, 1995). Other research examining the effects of guar gum demonstrated that levels of 12 g and higher, mixed in a 50 g glucose solution, were required to form a very viscous gel when added to water, and also to impair glucose absorption (Jenkins et al, 1976; 1977; 1978). The more viscous the gel formed, the greater the effect on glucose rise when fiber was mixed in a glucose solution.

(2) The difference in food form, e.g. a beverage versus incorporation into a cereal, also may have played a role in influencing the therapeutic effect. It is possible that the physiochemical properties of the psyllium in the two forms differentially affected intestinal viscosity. O'Connor et al (1981) has described the importance of viscosity in the efficacy of fiber formulations, and food form may be an important factor influencing results (Juntenun et al, 2002). While these data have not yet been reproducible, Wolever et al (1991) showed that psyllium only reduced the glycemic response when the fiber was incorporated into or sprinkled onto a flaked bran cereal. Further study is required in order to confirm this finding, as my study design does not allow for this analogy to be drawn. There was no effect when the psyllium was administered as a drink twenty minutes after the meal; and

(3) the average daily fiber intake reported by my subjects (i.e., 23 g of dietary fiber) may have been a confounding factor. According to the three-day dietary recall, subjects reported an average daily consumption substantially higher than the average fiber intake of 15 g reported in the Continuing Survey of Food Intake of Individuals (1994-1996). Consuming an additional 6.6 g of psyllium fiber on top of 23 g fiber intake/day may have less effect than the same 6.6 g as an addition to 15 g fiber intake/day. While the possibility exists that an adaptive high fiber intake may have masked the physiological effects on glucose absorption, it is possible that the reported amount of fiber consumed by these subjects is overstated. Thus, longer-term studies would be advisable to determine to

understand the role of psyllium soluble fiber and the amount required for effectual results in individuals with Type 2 DM.

(4) The variations in CHO load may not have been high enough to demonstrate a second meal effect. Second meal effects have been observed when the amount of CHO or fat consumed has been at least double the amount provided in a low CHO or low-fat test meal.

The present study was not specifically designed to evaluate the safety of psyllium husk fiber used adjunctively, as it had been previously used in subjects with Type 2 DM (Anderson et al, 1999; Sierra et al, 2002). It is worth noting however, that the breakfasts containing psyllium were well-tolerated with no side effects.

It should be noted that there were fairly large individual variations observed in postprandial FFA responses. This finding is consistent with previous research in healthy subjects where postprandial glycemic and insulinemic responses were widely varied when psyllium was administered (Sierra et al, 2001). This suggests that any dietary treatment using psyllium soluble fiber should be individualized in order to be most effective. Longer-term studies would be advisable to determine to better understand this phenomenon.

In conclusion, dietary management that can lead to risk reduction and reduced complications of associated chronic disease can greatly minimize disability and death among individuals with Type 2 DM. Results of this study indicate that the morning glycemic and insulinemic responses were affected by the glycemic load of the breakfast meal, and not by the psyllium soluble fiber source. This finding supports the American Diabetes Association position that "With regard to the glycemic effect of carbohydrates,

the total amount of carbohydrate.....is more important than the source or type" (Franz et al, 2002).

Further, the acute ingestion of psyllium soluble fiber at the morning meal did not result in second meal glucose tolerance after consuming a standardized lunch. These data would similarly suggest that the morning carbohydrate load may not exert metabolic influence on second meal tolerance, independent of its fiber content. Future research is needed to determine if chronic ingestion of low-glycemic index and glycemic load meals can be used to elicit different glycemic responses. Importantly, these results were achieved by using meals that patients with Type 2 DM might reasonably consume free-living, outside of a clinical setting.

Medical Nutrition Therapy in Type 2 DM must be individualized to reflect personal lifestyle and dietary goals. This approach of taking into consideration the metabolic effects of the glycemic response (glycemic load and the glycemic index) offers an additional dietary tool that may help improve the management and treatment of Type 2 DM.

Implications for Future Research Studies

In light of this current work on Type 2 DM, the following topics are suggested for further exploration in using psyllium:

1. Expand the current experiment by including control loop cereal, loop cereal with psyllium, control loop cereal with psyllium drink, and control loop cereal with placebo drink to more systemically investigate psyllium fiber effect and the method of administration on glycemic responses. This study design, which involved two different forms of psyllium and two different cereal types, proved to

be quite complex. Future work should employ a series of smaller experiments that are progressive in nature, and examine fiber and the method of administration. Separately, another experiment would then examine the glycemic load by changing the quantity and quality of carbohydrates. Under such conditions, the fat, protein and energy intake should be more tightly controlled.

2. Conduct a dose-response experiment by using one food form to examine baseline dietary fiber intake ranging from low (10-15 g) versus high (25-30 g). This would be useful in assessing both the acute and chronic benefits of varying levels of fiber. Additionally, it would also provide insights on whether or not this level of fiber supplementation is both achievable and tolerated over a specified time period among in individuals with Type 2 DM.
3. Examine the effect of high fiber, low GI diet treatments under different physiological conditions, e.g. healthy subjects versus subjects with Type 2 DM; obese versus non-obese subjects. It would also be interesting to examine these diet effects on subjects with pre-diabetic conditions (insulin resistance) where symptoms of overweight, high blood pressure, hyperlipidemia etc. are evident. The possibility exists that individuals with pre-diabetic conditions are more sensitive to the treatments employed, especially in the absence of oral glucose agents.
4. Examine effects of chronic consumption of psyllium on glycemic, insulinemic and lipid parameters in healthy subjects and subjects with Type 2 DM. It would be advantageous to see if any short-term changes observed would be extended longer-term. This could be achieved by administering 10-15 g psyllium per day

for four-six weeks. A random cross-over design could be employed where half of the subjects start on the acute phase and half on the chronic phase. This type of experiment would also allow for a more in-depth assessment of lipid profiles.

5. Employ a series of breakfast meal types to evaluate glycemic responses. Then select two diets to further develop studies on lunch responses. Consideration should be given to changing the fat and carbohydrate levels of the lunch meal versus holding lunch constant to determine if this influences the second meal effect.
6. Conduct an experiment to determine the influence of liquid versus the dry form of psyllium on glycemia and insulin response. Breakfast foods could be formulated and administered as nutrition bars with no liquid to minimize variances due to food form. Intestinal viscosity could also be measured in a parallel animal study or in patients with an ileostomy to determine if experimental conditions (various ways of delivering psyllium) influenced absorption rates.

APPENDIX A

UCRIHS and Sparrow Approval Forms

MICHIGAN STATE
UNIVERSITY

October 3, 2000

TO: Norman HORD
Food Science
2100 South Anthony Hall

RE: **IRB # 99-525** CATEGORY: FULL REVIEW
RENEWAL APPROVAL DATE: October 2, 2000

TITLE: BREAKFAST MEAL SOLUBLE FIBER CONTENT: IMPLICATIONS FOR METABOLIC CONTROL

The University Committee on Research Involving Human Subjects' (UCRIHS) review of this project is complete and I am pleased to advise that the rights and welfare of the human subjects appear to be adequately protected and methods to obtain informed consent are appropriate. Therefore, the **UCRIHS APPROVED THIS PROJECT'S RENEWAL.**

This letter also approves the increase in participant meal consumption

RENEWALS: UCRIHS approval is valid for one calendar year, beginning with the approval date shown above. Projects continuing beyond one year must be renewed with the green renewal form. A maximum of four such expedited renewal are possible. Investigators wishing to continue a project beyond that time need to submit it again for complete review.
REVISIONS: UCRIHS must review any changes in procedures involving human subjects, prior to initiation of the change. If this is done at the time of renewal, please use the green renewal form. To revise an approved protocol at any other time during the year, send your written request to the UCRIHS Chair, requesting revised approval and referencing the project's IRB# and title. Include in your request a description of the change and any revised instruments, consent forms or advertisements that are applicable.
PROBLEMS/CHANGES: Should either of the following arise during the course of the work, notify UCRIHS promptly: 1) problems (unexpected side effects, complaints, etc.) involving human subjects or 2) changes in the research environment or new information indicating greater risk to the human subjects than existed when the protocol was previously reviewed and approved.

If we can be of further assistance, please contact us at 517 355-2180 or via email: UCRIHS@pilot.msu.edu.

OFFICE OF RESEARCH AND GRADUATE STUDIES

Sincerely,

Ashir Kumar, M.D.
Interim Chair, UCRIHS

AK: bd

cc: Douglas Henry
111 Giltner Hall

MICHIGAN STATE
UNIVERSITY

June 30, 2000

Dr. David Wright
Office of Research and Graduate Studies
University Committee on Research Involving Human Subjects (UCRIHS)
Michigan State University
246 Administration Bldg.
East Lansing, MI 48824-1046

Dear Dr. Wright

This letter is to inform UCRIHS of a change in protocol for the project titled "*Breakfast Meal Soluble Fiber Content: Implications for Metabolic Control*" (**IRB# 99-525**). This change involves utilizing a different experimental design but utilizing the same food constituents as described. The study has not started and no subjects have signed "Consent Forms".

Subjects will be asked to consume three, rather than the original two, breakfast-lunch combinations. This will necessitate their participation for three days total. Each day of their participation will be at least 6 days apart. In addition, after each breakfast meal, subjects will be asked to consume an orange-flavored beverage. On one day of the study, this beverage will contain psyllium. As such, this beverage will be similar to Metamucil®, a commercially available product used to promote laxation. The other two days of the study, subjects will receive a placebo (non-psyllium-containing) orange-flavored beverage.

Please contact me if you have any further questions or concerns about this change in protocol. I appreciate your assistance in support of this project.

Best regards,

Norman Hord, PhD, MPH, RD
Assistant Professor

DEPARTMENT OF
FOOD SCIENCE AND HUMAN NUTRITION
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Consent Form
For Participants in the
Soluble Fiber Study in Individuals with Type 2 Diabetes

Investigators: Norman Hord, PhD, MPH, RD
(517) 353-9775

Douglas Henry, MD
(517) 355-6475

Lorraine Weatherspoon, PhD, RD
(517) 432-0813

PROJECT DESCRIPTION:

We plan to study the effects of the contents of a breakfast meal on the short-term control of important aspects of diabetes control such as blood sugar, blood fats and insulin production in adults who have Type 2 diabetes.

WHAT YOU WILL BE ASKED TO DO IF YOU PARTICIPATE:

Your voluntary participation in this study will entail eating a breakfast and lunch meal in the Department of Food Science and Human Nutrition at Michigan State University on three different days over a three week period. On the day before the actual study day (before each day of the study), you will receive a dinner meal from the study investigators. After this meal, you will be asked to fast until the breakfast meal is served at the Department of Food Science and Human Nutrition at Michigan State University. These meals are designed to test the ability of a breakfast ready-to-eat cereal which contains a special fiber ingredient called psyllium to improve blood sugar control in persons with Type 2 diabetes. Blood samples will be obtained after each meal from an indwelling catheter (a small plastic tube placed in your arm vein). This will mean that we not have to reinsert a needle every time we need a blood sample since it will be similar to having a small IV. These blood samples (5 milliliters, about a teaspoon each) will be drawn prior to and at thirty minute intervals after breakfast and lunch. A total of 14 blood draws will be made on each day of the study .

YOUR PARTICIPATION IN THIS PROJECT IS VOLUNTARY:

You may refuse to continue participation in this study at any time. There is no penalty for withdrawing from this study.

YOUR PARTICIPATION IN THE PROJECT WILL BE KEPT *CONFIDENTIAL (PRIVATE):

During and after this research, your privacy will be protected to the maximum extent of the law. Each individual will not be identified by name in any of the data analysis. Information with your name will be kept is a locked up filing cabinet which only the study investigators will have access to for the purposes of matching data. Every participant will be assigned an anonymous study number. Results of this study will not identify any individual.

Potential Benefits and Hazards:

RISK AND BENEFITS:
No personal or immediate benefit to you is expected by participation in this study. However, the results of the study may be beneficial for the dietary management of Type 2 diabetes in general. If desired, you may learn of the study results by asking the one of the investigators for the results to be made available to you when they are completed. Your participation in this study may therefore add further insight on the use of soluble fiber in the metabolic control of diabetes. Each participant will receive monetary compensation ($50.00 each day; $150.00 if the experiment is completed) for their participation in this study.

The consumption of a psyllium-based food may cause allergic reactions, intestinal blockage resulting from inadequate fluid intake, and abdominal cramping and/or diarrhea may be experienced. Anyone with psyllium allergies will be excluded from the study. Psyllium-containing products such as Metamucil® and specific ready-to-eat cereals from Kellogg Company are commonly consumed. This ingredient adds bulk to the stool to promote laxation (normal bowel movement function).

Pain, bruising, and discomfort can result from blood draws. These are similar to when the doctor orders routine blood tests during your medical annual examination. In order to minimize these risks, a small catheter (tube like used for the administration of I.V. fluids) will be inserted into a vein in your arm and blood samples will be drawn from this catheter.

RISK OF PHYSICAL INJURY:

If you are injured as a result of participating in this research project, Michigan State University will provide emergency medical care if necessary. You will not be held responsible for any medical expenses incurred as a result of this injury. All such medical expenses will be paid by Michigan State University or your insurer.

YOUR RIGHTS AS HUMAN RESEARCH PARTICIPANTS:

Participants in this study are welcome to call any of the study investigators with questions. Phone numbers for Drs. Hord, Weatherspoon, and Henry are listed at the top of the Consent Form. You are encouraged to contact Dr. David E. Wright, chair of the University Committee on Research Involving Human Subjects, at (517) 355-2180 with any questions regarding concerns raised by participation in this study. You are also encouraged to ask any one of the investigators about any specific questions regarding the research project.

STATEMENT OF AGREEMENT:

Please indicate your agreement to participate in the study and with your understanding of (with) the contents of this consent form by signing and dating below.

Signature_____
Date_____

Witness_____
Date_____

Dietary Study for Persons with Type 2 Diabetes

Individuals with Type 2 diabetes are needed to participate in a study to learn about the dietary management of Type 2 diabetes. This study will require you to be present for three complete days over a three week period. The study will require your participation on one day per week for each of the three weeks of the study. You will receive breakfast and lunch meals on these days as a part of the study.

Participants will be reimbursed $50.00 per day for each of three days of the study.

If interested, call Dr. Norm Hord at Michigan State University, (517) 353-9775.

MICHIGAN STATE
UNIVERSITY

October 4, 1999

TO: Norman HORD
Food Science
2100 South Anthony Hall

RE: IRB#99-525 CATEGORY: FULL

APPROVAL DATE: October 4, 1999

TITLE: BREAKFAST MEAL SOLUBLE FIBER CONTENT: IMPLICATIONS FOR METABOLIC CONTROL

The University Committee on Research Involving Human Subjects' (UCRIHS) review of this project is complete and I am pleased to advise that the rights and welfare of the human subjects appear to be adequately protected and methods to obtain informed consent are appropriate. Therefore, the UCRIHS approved this project.

RENEWALS: UCRIHS approval is valid for one calendar year, beginning with the approval date shown above. Projects continuing beyond one year must be renewed with the green renewal form. A maximum of four such expedited renewals possible. Investigators wishing to continue a project beyond that time need to submit it again for a complete review.
REVISIONS: UCRIHS must review any changes in procedures involving human subjects, prior to initiation of the change. If this is done at the time of renewal, please use the green renewal form. To revise an approved protocol at any other time during the year, send your written request to the UCRIHS Chair, requesting revised approval and referencing the project's IRB# and title. Include in your request a description of the change and any revised instruments, consent forms or advertisements that are applicable.
PROBLEMS/CHANGES: Should either of the following arise during the course of the work, notify UCRIHS promptly: 1) problems (unexpected side effects, complaints, etc:) involving human subjects or 2) changes in the research environment or new information indicating greater risk to the human subjects than existed when the protocol was previously reviewed and approved.

OFFICE OF
RESEARCH
AND
GRADUATE
STUDIES

If we can be of further assistance, please contact us at 517 355-2180 or via email: UCRIHS@pilot.msu.edu. Please note that all UCRIHS forms are located on the web: http://www.msu.edu/unit/vprgs/UCRIHS/

Sincerely,

David E. Wright, Ph.D.
DEW: bd
cc: Douglas Henry
Celeste A. Clark

Consent Form

For Participants in the

Soluble Fiber Study in Individuals with Type 2 Diabetes

Investigators: Norman Hord, PhD, MPH, RD
(517) 353-9775

Douglas Henry, MD
(517) 355-6475

Lorraine Weatherspoon, PhD, RD
(517) 432-0813

PROJECT DESCRIPTION:

We hope to study the effects of breakfast meal composition on the short-term metabolic control of Type 2 diabetes.

WHAT YOU WILL BE ASKED TO DO IF YOU PARTICIPATE:

Your voluntary participation in this study will entail consuming a breakfast and lunch meal in the Department of Food Science and Human Nutrition at Michigan State University on two different days in one week. Before each day of the study, you will receive their dinner meal from the study investigators. After this meal, you will be asked to fast until the breakfast meal is served at the Department of Food Science and Human Nutrition at Michigan State University. These meals are designed to test the ability of a breakfast ready-to-eat cereal to improve metabolic control in persons with Type 2 diabetes. Blood samples will be obtained after each meal from an indwelling catheter (a small plastic tube placed in your arm vein). These blood samples (5 milliliters, about a teaspoon each) will be drawn prior to and at thirty minute intervals after breakfast and lunch. A total of 13 blood draws will be made on each day of the study .

YOUR PARTICIPATION IN THIS PROJECT IS VOLUNTARY:
You may refuse to continue participation in this study at any time. There is no penalty for withdrawing from this study.

YOUR PARTICIPATION IN THE PROJECT WILL BE KEPT PRIVATE:

During and after this research, your privacy will be protected to the maximum extent of the law. **Results of this study will not identify any individual.**

Potential Benefits and Hazards:

RISK AND BENEFITS:
No benefit to you is expected by participation in this study. If desired, you may learn of the study results by asking the one of the investigators for the results to available to you when they are completed. Your participation in this study may add further insight on the use of soluble fiber in the metabolic control of diabetes. Each participant will receive monetary compensation ($75.00 each day; $150.00 if both days of the experiment are completed) for their contribution in this study.

The consumption of a psyllium-based food may cause allergic reactions, intestinal blockage resulting from inadequate fluid intake, and abdominal cramping and/or diarrhea may be experienced. Anyone with psyllium allergies will be excluded from the study. Psyllium-containing products such as Metamucil and specific ready-to-eat cereals from Kellogg Company are commonly consumed. This ingredient adds bulk to the stool to promote laxation (normal bowel function).

Pain, bruising, and discomfort can result from blood draws. In order to minimize these risks, a small catheter (tube like used for the administration of I.V. fluids) will be inserted into a vein in your arm and blood samples will be drawn from this catheter.

RISK OF PHYSICAL INJURY:

If you are injured as a result of participating in this research project, Michigan State University will provide emergency medical care if necessary. You will not be held responsible for any medical expenses incurred as a result of this injury. All such medical expenses will be paid by Michigan State University or your insurer.

YOUR RIGHTS AS HUMAN RESEARCH PARTICIPANTS:

Participants in this study are welcome to call any of the study investigators with questions. Phone numbers for Drs. Hord, Weatherspoon, and Henry are listed at the top of the Consent Form. You are encouraged to contact Dr. David E. Wright, chair of the University Committee on Research Involving Human Subjects, at (517) 355-2180 with any questions regarding concerns

raised by participation in this study. You are encouraged to ask one of the investigators about any specific questions regarding the research project.

STATEMENT OF AGREEMENT:

Please indicate your agreement with the contents of this consent form by signing and dating below.

Signature_____ **Date**_____

Witness_____ Date_____

UCRIHS APPROVAL FOR
THIS project EXPIRES:

OCT 4 - 2000

SUBMIT RENEWAL APPLICATION
ONE MONTH PRIOR TO
ABOVE DATE TO CONTINUE

SPARROW
HEALTH SYSTEM

October 5, 1999

Norman Hord, PhD, MPH, RD
Department of Food Science and
Human Nutrition
2100 Anthony Hall
Michigan State University
East Lansing MI 48824

RE: **Breakfast Meal Soluble Fiber Content: Implications for Metabolic Control (#0335)**

Dear Dr. Hord:

On behalf of the Sparrow Health System Institutional Research Review Committee, we received your application for the above-mentioned proposed study. On September 13, 1999 the Committee reviewed the protocol in your absence. According to your application, this protocol would study the recent data indicating that breakfast meal composition may exert important effects on glycemic and lipemic biomarkers beyond the immediate postprandial period for patients with Type II Diabetes Mellitus.

The Committee noted several areas that will need to be addressed before final approval can be given. They are 1) no dollar amount was identified as compensation for the patients, 2) *Section #17 on the application form* - no signature(s) identifying approval from managers of Medical Records, Laboratories, and Nursing Department, and 3) *Section #12 on the application form* -clarification on the subject population to be involved in this study (the current proposal identifies only low income persons and minorities as being targeted as the subject population). The consent form was found to be appropriate.

Therefore, effective September 13, 1999 **approval is granted pending** the Committee's receipt and review of the appropriately revised application form for the above-mentioned proposal. If the Committee finds the revisions acceptable, you will then be notified in writing of their formal approval.

Sincerely,

George S. Abela MD/sl

George S. Abela, MD, Chairperson
Institutional Research Review Committee
Sparrow Health System

/sl

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APPLICATION FOR APPROVAL OF A PROJECT
INVOLVING HUMAN SUBJECTS
INITIAL REVIEW (and 5 yr. renewal)
UCRIHS

University Committee on Research Involving Human Subjects
David E. Wright, Ph.D., Chair
246 Administration Building, Michigan State University
East Lansing, MI 48824-1046
PHONE (517) 355-2180 FAX (517) 353-2976
E-Mail - UCRIHS@pilot.msu.edu
WEB SITE - http://www.msu.edu/unit/vprgs/ucrihs/

Office Hours: M-F (8:00 A.M.-Noon & 1:00-5:00 P.M.)

DIRECTIONS: Please complete the questions on this application using the instructions and definitions found on the attached sheets. (revised 4/99)

1. Responsible Project Investigator:
(Faculty or staff supervisor)
Name: Norman Hord, PhD, MPH, RD
Social Security Number: 375-80-6350

Department: Food Science and Human Nutrition
College: Human Ecology

I accept responsibility for conducting the proposed research in accordance with the protections of human subjects as specified by UCRIHS, including the supervision of faculty and student co-investigators.
Signature: _____

Additional Investigator(s):

Name: Celeste A. Clark
SS# or Student ID#: A-29-22-1136

Name: _____
SS# or Student ID#: _____

Name: _____
SS# or Student ID#: _____

Name: _____
SS# or Student ID#: _____

2. Address: If there are more than two investigators, please indicate who should receive correspondence, and provide further addresses on a separate page.

Responsible Project Investigator
Norman Hord, PhD, MPH, RD
2100 Anthony Hall
MSU, E. Lansing, MI 48824
Phone #: 517-353-9775
Fax #: 517-353-1678
Email: hord@pilot.msu.edu

Additional Investigator(s)
1) Douglas Henry, MD
111 Giltner Hall
MSU, E. Lansing, MI 48824
Phone #: 517-355-8475
Fax #: 517-355-5125
Email: henry@psl.msu.edu

3. Title of Project: Breakfast Meal Soluble Fiber Content: Implications for Metabolic Control

FOR OFFICE USE ONLY
Subcommittee _____ Agenda _____

Additional Investigator(s)
2) Lorraine J. Weatherspoon Ph.D.; R.D.
334 A G.M. Trout FSHN Bldg
MSU, E. Lansing, MI 48824
Phone # 517- 432-0813
Fax # 517-353-8963
Email: weathe43@pilot.msu.edu

4. Have you ever received Preliminary Approval for this project?
No[x] Yes [ ]
If yes, what IRB # was assigned to it? _____

5. Funding (if any) Kellogg Company, unrestricted gift
MSU Contracts and Grants app. #_____ if applicable

6. Has this protocol been submitted to the FDA or are there plans to submit it to the FDA? No [x] Yes [ ]
If yes, is there an IND #? No [ ] Yes [ ] IND # _____

7. Does this project involve the use of Materials of Human Origin (e.g., human blood or tissue)?
No [ ] Yes [x]

8. When would you prefer to begin data collection? Fall, 1999
Please remember you may not begin data collection without UCRIHS approval.

9. Category (Circle a,b, or c below and specify category for a and b. See instructions pp. 4-7)

   (a) This proposal is submitted as EXEMPT from full review.
   Specify category or categories: 1-G
   b. This proposal is submitted for EXPEDITED review.
   Specify category or categories: _____
   c. This proposal is submitted for FULL sub-committee review.

10. Is this a Public Health Service funded, full review, multi-site project?
No [X] Yes [ ]
If yes, do the other sites have a Multiple Project Assurance IRB that will also review this project?
[ ] No. Please contact the UCRIHS office for further information about meeting the PHS/NIH/OPRR regulations.
[ ] Yes. Please supply a copy of that approval letter when obtained.

11. Project Description (Abstract): **Please limit your response to 200 words.**

Type 2 diabetes mellitus is a growing public health problem in developed nations, comprising 90-95% of all cases of people who have diabetes in the U.S. Medical complications related to poor control of diabetes include coronary heart disease, nephropathy, neuropathy, and retinopathy. Dietary treatment is an integral component of the medical management of this condition. Dietary soluble fiber has been shown to be efficacious in ameliorating metabolic disorders related to diabetes, including hyperglycemia and dyslipidemias. Recent data indicates that breakfast meal composition may exert important effects on glycemic and lipemic biomarkers beyond the immediate postprandial period. Breakfast meals containing moderate to high levels of fat have been shown to have adverse effects on oral glucose tolerance as well as serum insulin and c-reactive protein levels. We hypothesize that breakfast foods containing either psyllium, a soluble fiber source, or proprietary starch types will have a positive impact on glycemic and lipemic indices beyond the immediate postprandial period compared to a low fiber breakfast meal.

12. Procedures: **Please describe all project activities to be used in collecting data from human subjects. This also includes procedures for collecting materials of human origin and analysis of existing data originally collected from human subjects.**

Thirty six individuals with Type 2 diabetes will undergo an 8-10 hour fast after an isoenergetic meal and spend approximately six hours the following day in designated laboratory space at Michigan State University, Department of Food Science & Human Nutrition, where control and test meals will be provided. Serial blood samples will be collected via an indwelling catheter at 0, 30, 60, 90, 120, 150, and 180 minutes post breakfast and lunch meals. Subject identifier codes will be marked on each sample to ensure anonymity in sample handling, storage, and data analyses/reporting.

13. Subject Population: **Describe your subject population. (e.g., high school athletes, women over 50 w/breast cancer, small business owners)**

Patients with Type 2 Diabetes

a. **The study population may include (check each category where subjects may be included by design or incidentally):**

Minors	[ ]
Pregnant Women	[ ]
Women of Childbearing Age	[ ]
Institutionalized Persons	[ ]
Students	[ ]
Low Income Persons	[X]
Minorities	[X]
Incompetent Persons (or those with diminished capacity)	[ ]

b. Number of subjects (including controls) 36

c. How will the subjects be recruited? (Attach appropriate number of copies of recruiting advertisement, if any. See p. 13 of UCRIHS instructions)

Attached Advertisement will be posted at Sparrow Hospital and the MSU Clinics.

d. If you are associated with the subjects (e.g., they are your students, employees, patients), please explain the nature of the association.

Some of the subjects may be patients of the MSU clinical center.

e. If someone will receive payment for recruiting the subjects please explain the amount of payment, who pays it and who receives it.

No payment planned for recruitment.

f. Will the research subjects be compensated? [ ] No [X] Yes.
If yes, details concerning payment, including the amount and schedule of payments, must be explained in the informed consent.

See informed consent

g. Will the subjects incur additional financial costs as a result of their participation in this study? [X] No [ ] Yes. If yes, please include an explanation in the informed consent.

h. Will this research be conducted with subjects who reside in another country or live in a cultural context different from mainstream US society? [X] No [ ] Yes.

(1) If yes, will there be any corresponding complications in your ability to minimize risks to subjects, maintain their confidentiality and/or assure their right to voluntary informed consent as individuals?
[ ] No [ ] Yes.

(2) If your answer to h-1 is yes, what are these complications and how will you resolve them?

14. How will the subjects' privacy be protected? (See Instructions p. 8.)

During and after this research project, subject identifier codes will be used to insure anonymity, and privacy will be protected to the maximum extent of the law.

15. Risks and Benefits for subjects: (See Instructions p. 9.)

No benefit is implied by participation in the study. If desired, the subjects may learn of the study results. Their participation in this study may add further insight on the use of soluble fiber in the metabolic control of diabetes. The consumption of a psyllium-based food may cause allergic reactions (difficulty in breathing and swallowing, skin rash, or itching) if exposed to industrial use/dispensation of bulk psyllium; intestinal blockage resulting from inadequate fluid intake; some abdominal cramping and/or diarrhea may be experienced. Pain, bruising, and discomfort are possible with phlebotomy. In order to minimize discomfort, a small (22 guage) indwelling catheter will be placed in a forearm vein for repeated blood draws.

16. Consent Procedures (See Instructions pp. 9-13.)

If a subject is injured as a result of participating in this research project, Michigan State University will provide emergency medical care if necessary. Subjects will not be held responsible for any medical expenses incurred as a result of this injury. All such medical expenses will be paid by Michigan State University or the subject(s) insurer. In the event of an emergency, subjects should contact:

Dr. Douglas Henry Day: (517) 355-6475 Night: (517) 432-1661

Consent to participate in the study will be obtained by the primary investigator prior to the initiation of the study.

CHECKLIST: Check off that you have included each of these items. If not applicable, state N/A:

[X] Completed application

[X] The correct number of copies of the application and instruments, according to the category of review (See instructions p. 14.)

[X] Consent form (or script for verbal consent), if applicable

[X] Advertisement, if applicable

[X] One complete copy of the methods chapter of the research proposal

Want to Help??

Individuals with Type 2 diabetes are needed to participate in a study to learn about the dietary management of diabetes.

Participants will be reimbursed for their time.

If interested, call today: (517) 353-9775.

Consent Form

For Participants in the

Soluble Fiber Study in Individuals with Type 2 Diabetes

Co. Principal Investigators:

Norman Hord, PhD, MPH, RD
(517) 353-9775

Douglas Henry, MD
(517) 355-6475

Lorraine Weatherspoon, PhD, RD
(517) 432-0813

Project Description:

This project addresses the effect of breakfast meal composition on the metabolic control of Type 2 diabetes. Your voluntary participation in this study will entail an overnight fast, followed by eating breakfast and lunch meals in the Department of Food Science & Human Nutrition at Michigan State University. Blood samples will be obtained at periodic intervals after each meal. You may refuse to continue participation in this study at any time. During and after this research, your privacy will be protected to the maximum extent of the law.

Potential Benefits and Hazards:

No benefit to you is expected by participation in this study. If desired, the subjects may learn of the study results. Your participation in this study may add further insight on the use of soluble fiber in the metabolic control of diabetes.

The consumption of a psyllium-based food may cause allergic reactions if exposed to industrial use and dispensing of bulk psyllium; intestinal blockage resulting from inadequate fluid intake; some abdominal cramping and/or diarrhea may be experienced.

Pain, bruising, and discomfort can result from blood draws. In order to minimize these risk, a small catheter (tube like used for the administration of I.V. fluids) will be inserted into a vein in your arm and blood samples will be drawn from this catheter.

Risk of Physical Injury:

If you are injured as a result of participating in this research project, Michigan State University will provide emergency medical care if necessary. You will not be held responsible for any medical expenses incurred as a result of this injury. All such medical expenses will be paid by Michigan State University or your insurer.

Participant's Rights as Human Subjects of Research:

All research subjects are encouraged to contact Dr. David E. Wright, chair of the University Committee on Research Involving Human Subjects, at (517) 355-2180 with any questions regarding concerns raised by participation in this study.

Statement of Agreement:

Please indicate your agreement with the contents of this consent form by signing and dating below.

_____ _____

Signature Date

AA Kellogg Contact: Celeste A. Clark

Form Approved Through 2/28/01
OMB No. 0925-0001

Department of Health and Human Services
Public Health Service

Grant Application

Follow instructions carefully.
Do not exceed character length restrictions indicated on sample.

LEAVE BLANK—FOR PHS USE ONLY.

Type	Activity	Number
Review Group		Formerly
Council/Board (Month, Year)		Date Received

1. TITLE OF PROJECT
Breakfast Meal Soluble Fiber Content: Implications for Metabolic Control

2. RESPONSE TO SPECIFIC REQUEST FOR APPLICATIONS OR PROGRAM ANNOUNCEMENT ☐ NO ☐ YES *(If "Yes," state number and title)*
Number: Title:

3. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR — New Investigator ☐ YES

3a. NAME *(Last, first, middle)*
Hord, Norman Gary

3b. DEGREE(S)
PhD, MPH, MS

3c. SOCIAL SECURITY NO. [redacted]
Provide on Form Page 10C

3d. POSITION TITLE
Assistant Professor

3e. MAILING ADDRESS *(Street, city, state, zip code)*
2100E S. Anthony Hall
Michigan State University
East Lansing, MI 48824-1225

3f. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT
Food Science & Human Nutrition

3g. MAJOR SUBDIVISION
College of Human Ecology

3h. TELEPHONE AND FAX *(Area code, number and extension)*
TEL: 517-353-9775
FAX: 517-353-1676

E-MAIL ADDRESS: hord@pilot.msu.edu

4. HUMAN SUBJECTS ☐ No ☒ Yes
4a. If "Yes," Exemption no. or IRB approval date ☐ Full IRB or ☐ Expedited Review
4b. Assurance of compliance no.

5. VERTEBRATE ANIMALS ☒ No ☐ Yes
5a. If "Yes," IACUC approval date
5b. Animal welfare assurance no.

6. DATES OF PROPOSED PERIOD OF SUPPORT *(month, day, year—MM/DD/YY)*		7. COSTS REQUESTED FOR INITIAL BUDGET PERIOD		8. COSTS REQUESTED FOR PROPOSED PERIOD OF SUPPORT	
From	Through	7a. Direct Costs ($)	7b. Total Costs ($)	8a. Direct Costs ($)	8b. Total Costs ($)
08/01/99	07/31/01		$106,321.00		$150,000.00

9. APPLICANT ORGANIZATION
Name: Michigan State University
Address: 204 G. Malcolm Trout FSHN Building
Food Science & Human Nutrition
East Lansing, MI 48824-1224

10. TYPE OF ORGANIZATION
Public: ☐ Federal ☒ State ☐ Local
Private: ☐ Private Nonprofit
Forprofit: ☐ General ☐ Small Business

11. ORGANIZATIONAL COMPONENT CODE

12. ENTITY IDENTIFICATION NUMBER
DUNS NO. *(If available)*

Congressional District

13. ADMINISTRATIVE OFFICIAL TO BE NOTIFIED IF AWARD IS MADE
Name: Dr. Mark Uebersax
Title: Department Chair
Address: 204 G. Malcolm Trout FSHN Building
Food Science & Human Nutrition
East Lansing, MI 48824-1224
Telephone: 517-355-8474
Fax: 517-353-8963
E-mail: uebersax@pilot.msu.edu

14. OFFICIAL SIGNING FOR APPLICANT ORGANIZATION
Name: Same as 13
Title:
Address:
Telephone:
Fax:
E-mail:

15. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR ASSURANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application.

SIGNATURE OF PI / PD NAMED IN 3a. *(In ink. "Per" signature not acceptable.)*

DATE

16. APPLICANT ORGANIZATION CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Service terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

SIGNATURE OF OFFICIAL NAMED IN 14. *(In ink. "Per" signature not acceptable.)*

DATE

DESCRIPTION: State the application's broad, long-term objectives and specific aims, making reference to the health relatedness of the project. Describe concisely the research design and methods for achieving these goals. Avoid summaries of past accomplishments and the use of the first person. This description is meant to serve as a succinct and accurate description of the proposed work when separated from the application. If the application is funded, this description, as is, will become public information. Therefore, do not include proprietary/confidential information. DO NOT EXCEED THE SPACE PROVIDED.

Type 2 diabetes mellitus is a growing public health problem in developed nations, comprising 90-95% of all cases of people who have diabetes in the U.S. Medical complications related to poor control of diabetes include coronary heart disease, nephropathy, neuropathy, and retinopathy. Dietary treatment is an integral component of the medical management this condition. Dietary soluble fiber has been shown to be efficacious in ameliorating metabolic disorders related to diabetes, including hyperglycemia and dyslipidemias. Recent data indicates that breakfast meal composition may exert important effects on glycemic and lipemic biomarkers beyond the immediate postprandial period. Breakfast meals containing moderate to high levels of fat have been shown to have adverse effects on oral glucose tolerance as well as serum insulin and c-reactive protein levels. We hypothesize that breakfast foods containing either psyllium, a soluble fiber source, or proprietary starch types will have a positive impact on glycemic and lipemic indices beyond the immediate postprandial period compared to a low fiber breakfast meal. Critical biomarkers to be measured will include serial glucose, insulin, c-reactive peptide, and serum lipids. Fifty individuals with Type 2 diabetes will be fed, in a crossover design, three different breakfast meals on three separate occasions. A lunch meal of similar composition will be consumed after each of the breakfast meals. In order to test the hypothesis that the critical biomarkers will be modulated by psyllium- or novel starch-containing breakfasts, these parameters, as well as area-under-the-curve (AUC) glucose responses, will be calculated in the breakfast and lunch postprandial periods. It is anticipated, in support of this hypothesis, that psyllium- or novel starch-containing breakfasts will have salutary effects on AUC glucose, insulin, c-reactive peptide, and lipemic biomarkers compared to the control isocaloric meals of similar total carbohydrate content. These data may provide support for more specific dietary recommendations for improved control of Type 2 diabetes mellitus.

PERFORMANCE SITE(S) (organization, city, state)

Michigan State University, College of Human Ecology, Department of Food Science and Human Nutrition, East Lansing, MI 48824

KEY PERSONNEL. See instructions on Page 11. Use continuation pages as needed to provide the required information in the format shown below.

Name	Organization	Role on Project
Norman Hord,PhD, MPH, RD	MSU, FSHN	Co-Principal Investigator
Lorraine Weatherspoon,PhD, MPH, RD	MSU, FSHN	Co-Principal Investigator
Douglas Henry, MD	MSU, Physiology.	Co-Principal Investigator

DD Principal Investigator/Program Director (Last, first, middle): Hord, Norman, Gary

DETAILED BUDGET FOR INITIAL BUDGET PERIOD
DIRECT COSTS ONLY

FROM: 08/01/99 THROUGH: 07/31/01

PERSONNEL (Applicant organization only) NAME	ROLE ON PROJECT	TYPE APPT. (months)	% EFFORT ON PROJ.	INST. BASE SALARY	DOLLAR AMOUNT REQUESTED (omit cents) SALARY REQUESTED	FRINGE BENEFITS	TOTALS
Norman Hord	Principal Investigator	12	5	51,500.00	2575	806	$3381.
Lorraine Weatherspoon	Co-PI	12	5	56,000.00	2800	876	$3676
Douglas Henry	Co-PI	12	5	76,328.00	3816	1198	$5014
SUBTOTALS →					$9191.00	$2880.00	$12071

CONSULTANT COSTS	Certified Diabetes Educator $2500.00 Graduate Assistantship .(½ time) $18,000.00	20,500.00
EQUIPMENT (Itemize)	HPLC Columns	1500.00
SUPPLIES (Itemize by category)	Laboratory Analysis (Dr. Henry & Clinical Laboratory)	50,000.00
TRAVEL		3250.00
PATIENT CARE COSTS	INPATIENT OUTPATIENT Reimbursement	15,000.00
ALTERATIONS AND RENOVATIONS (Itemize by category)		
OTHER EXPENSES (Itemize by category)	Food 2000.00 Office Supplies 2000.00	4000.00
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		$ 106321.00
CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS FACILITIES AND ADMINISTRATION COSTS	
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD (Item 7a, Face Page) →		$ 106321.00

PHS 398 (Rev. 4/98) (Form Page 4) Page 3 DD
Number pages consecutively at the bottom throughout the application. Do not use suffixes such as 3a, 3b..

EE Principal Investigator/Program Director (Last, first, middle): Hord, Norman Gary

BUDGET FOR ENTIRE PROPOSED PERIOD OF SUPPORT
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS	INITIAL BUDGET PERIOD (from Form Page 4)	ADDITIONAL YEARS OF SUPPORT REQUESTED			
		2nd	3rd	4th	5th
PERSONNEL. *Salary and fringe benefits Applicant organization only*	$12,071.00	12,433.00			
CONSULTANT COSTS	20,500.00	18,000.00			
EQUIPMENT HPLC	1500.00				
SUPPLIES Laboratory Analysis	50,000.00	10,000.00			
TRAVEL/Publication	3250.00	3250.00			
PATIENT CARE COSTS — INPATIENT Reimbursment (Incentive)	15,000.00				
PATIENT CARE COSTS — OUTPATIENT					
ALTERATIONS AND RENOVATIONS					
OTHER EXPENSES Food Office Supplies	2000.00 2000.00				
SUBTOTAL DIRECT COSTS	106321.00	43683.00			
CONSORTIUM/ CONTRACTUAL COSTS — DIRECT					
CONSORTIUM/ CONTRACTUAL COSTS — F&A					
TOTAL DIRECT COSTS	106321.00	43683.00			

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PERIOD OF SUPPORT (from 6a, Face Page) → $ 150,004.00

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

RESOURCES

FACILITIES: Specify the facilities to be used for the conduct of the proposed research. Indicate the performance sites and describe capacities, pertinent capabilities, relative proximity, and extent of availability to the project. Under "Other," identify support services such as machine shop, electronics shop, and specify the extent to which they will be available to the project. Use continuation pages if necessary.

Laboratory: **Not applicable**

Clinical: The Endocrinology services at Michigan State University are comprised of the Pediatric and Adult Endocrine services. Patients with diabetes mellitus comprise the majority of visits. Approximately 250 pediatric patients (age 0 yr to 21 yr) and approximately 400 adult patients are enrolled at the combined clinics. These clinics are housed at the MSU clinical center and are supported by the clinical pathology laboratory (phlebotomy services are included), nursing, and secretarial support. The pediatric endocrinology clinic has a BAYER DCA2000 PLUS blood hemoglobin analizer.

Animal: **Not applicable**

Computer:

Office: **Not applicable**

Other:

MAJOR EQUIPMENT: List the most important equipment items already available for this project, noting the location and pertinent capabilities of each.
Not applicable

APPENDIX B

Advertisements

Do You Have Type 2 Diabetes?

Individuals with Type 2 diabetes are needed to participate in a study and gain a better understanding of how foods we eat help in managing diabetes. This study will require you to be present for three complete days in a three-week period. You will receive breakfast and lunch meals on these days as a part of the study.

Participants will be reimbursed $50.00 per day for each day of the study.

If interested please call Mrs. Sylvia Hindi at Michigan State University (517) 355-8468

Lansing State Journal, February 2001

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MICHIGAN STATE
UNIVERSITY

The State News, February 2001

APPENDIX C

Power Analysis (Modifications to Crossover and Statistical Considerations)

Modifications to the 2-period 2-treatment cross-over study

1. Changing the basic 2-treatment 2-period cross-over design to a 3-treatment 3-peiod design, involves several complications in the conduct of the study, let alone added costs of blood draws and analyses.

2. Analytical and design considerations in a cross-over study as based on the different sequences of treatments and order assignments to patients. For example, the original design has sequences AB and BA (Low-fiber to High fiber; High fiber to Low fiber). The new full design would have SIX sequences--ABC, CAB, BCA, BAC, CBA, ACB.

3. I surmised that not all comparisons would be needed and therefore some simplifications could be feasible. Suppose we are interested in only the comparisons of diets A with B; and A with C, with the B with C comparison of lesser interest.

We could use the sequences ABC, CAB, BAC, CBA for the first; and CAB, BCA, BAC, ACB for the second. This uses CAB and BAC twice. I think this is legitimate and would reduce our patient requirements.

4. For sample size one could use the argument I presented before. Conservatively, we would need about 13 subjects for EACH SEQUENCE, totaling 78.

5. Note the sample size calculation given earlier was based on conservative estimates. We had the AUC mean and SD from the Heilbron paper, and chose a 10% reduction to power the tests. Another important quantity in the calculation is the proportion of variation of within-subject to total variance--called the intracluster correlation (IC). This is anything between 0 and 1. I chose 0.5, but this could be quite conservative.

Would you, or anyone you know at Kellogg have some idea of what this could be? If this is quite large our sample size needs will come down quite a lot.

6. I looked at some data on a bioequivalence study (AUC measurements for formulations of verapamil) which was designed as a 4 period 2-treatment study. The IC was about 0.76.

If we use 0.75 instead of 0.5 that I previously used, it cuts our sample size by half. Therefore, for the new study we would need 42 patients.

STATISTICAL CONSIDERATIONS

The investigation is designed as a two period crossover study with two intervention diets. In the first period patients will be randomized to one of two diets (Low fiber (L) or High fiber (H)) and then crossed over to the other diet in the second period. There is a one week interval between treatments, which is adequate to ignore a carry over effect. Therefore two groups of patients are formed: Group I: Low fiber diet followed by the high fiber diet (LH) and Group II: High fiber diet followed by the low fiber diet (HL). Blood samples will be obtained from participants at various protocol times during the study. The precise conditions and procedures that will be adhered to in drawing blood specimens have been described elsewhere. In order to facilitate the discussion of the statistical analyses envisaged in this study we focus on a single measure that will be assessed in each group at each period. For example a primary outcome measure, the total area under the glucose curve (AUC) will be calculated from a series of readings from blood samples obtained at 30 minute intervals following the breakfast and lunch diets. For each period of the crossover study AUCs are computed for the post breakfast and post lunch periods. The primary objective is to compare the effect of diet (L and H) on AUC

Despite the random assignment of subjects to treatment groups (I or II) differences between them will be assessed on characteristics that are known or suspected of influencing the primary outcome measures. Where significant differences are found they will be controlled for in the subsequent analyses of primary outcomes. We will generally use *t*-tests for continuous variables and chi-square tests for categorical variables. For example, we will compare the groups at baseline on glucose, total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides. All tests will be two-sided at a 5% level of significance. Where simultaneous testing might appreciably inflate the type I error, appropriate control will be made using standard statistical techniques.

Analysis of outcomes

Let Y_{ijk} denote a response measure (eg., AUC) in the k-th subject receiving diet i (i=1 for low fiber, i=2 for high fiber) at period j (j=1, 2). A simple model incorporating diet, period and subject effects is $Y_{ijk} = \mu + \tau_i + \pi_j + c_k + \varepsilon_{ijk}$ where μ is the overall mean response, τ_i the additive effect of diet i, π_j the effect of period j, c_k the effect of subject k, and ε_{ijk} a pure measurement error. We take the diet and period effects, as well as the overall mean to be fixed effects, but regard c_k as a random effect (mean zero, variance σ_c^2) independent of the ε_{ijk} (with mean zero and variance σ_e^2). This is the standard model for the two period crossover design ignoring carryover effects. Note that we have focussed attention of a single response in each period. For the AUC two such measurements are available in each period. This, and covariate effects can be incorporated into this model with additional subscripts or right-hand side terms.

Assessing treatment effect

In the above model a test of the hypothesis $H_0: \tau_1 = \tau_2$ of no effect of diet on the response measure can be derived on the assumption of a normally distributed response. If skewness is present its effect might be mitigated by applying a suitable transformation such logarithmic the square-root to the original measure, and the analyses performed on the transformed variable. However, to keep this discussion straightforward we refer throughout to the original variable.

The relevant responses for the k-subject in group I are Y_{11k} and Y_{22k}; for the k-th subject in group II they are Y_{21k} and Y_{12k}. These are the response variables for periods 1 and 2 respectively. If $D_{1k} = Y_{11k} - Y_{22k}$ and

$D_{2k} = Y_{21k} - Y_{12k}$ then the independent samples $\{D_{1k}: 1 \le k \le n\}$ and $\{D_{2k}: 1 \le k \le n\}$ of n subjects in each of groups I and II lead to an estimate of the treatment effect $\tau_1 - \tau_2$, namely ½ $(\bar{D}_1 - \bar{D}_2)$, and a test of H_0 is based on the t-statistic $(\bar{D}_1 - \bar{D}_2)/(s_d(2/n)^{1/2})$ where s_d is the pooled estimate of the standard deviation. Under H_0 this statistic has a t distribution with $2(n-1)$ degrees of freedom.

Power and sample size

The total number of subjects $2n$ we will need to recruit for this crossover study will be determined by considerations of adequate statistical power to detect a clinically meaningful difference $\tau_1 - \tau_2$ in the test of the hypothesis H_0. Suppose our test at level α is designed to yield power $1-\beta$ to detect a specified $\tau_1 - \tau_2$. Then n is given approximately by $n = (z_{1-\alpha/2} + z_{1-\beta})^2 \sigma_e^2 / (\tau_1 - \tau_2)^2$ where $z_{1-\gamma}$ denotes the $100(1-\gamma)$ percentile of the standard normal distribution.

For numerical evaluation of n we express the error variance σ_e^2 in terms of the variance of the response Y_{ijk}, $\sigma^2 = Var(Y_{ijk}) = \sigma_e^2 + \sigma_s^2$, and the intraclass correlation $r = \sigma_s^2/(\sigma_e^2 + \sigma_s^2)$ between the two responses in each subject, that is, between Y_{11k} and Y_{22k}, or Y_{21k} and Y_{12k}. The σ_e^2 in the above formula can be replaced by $\sigma^2(1-r)$. The presence of r therefore reduces the required sample size.

If we consider AUC as our response we can estimate its standard deviation σ from data reported by Heilbronn *et al.* (1999) in a study of three diet compositions on plasma lipids and glucose in type 2 diabetic patients. This study randomized patients to one of three energy-restricted diets and used a repeated measures parallel groups design. Assessments were made at week 0, 1 and 12. We will use the data for the high mono-unsaturated fat (MUFA) diet and high carbohydrate diet (HCARD) for weeks 0 and 1. The estimate of σ from these data is 11.3 for MUFA and 13.2 for HCARB. Conservatively we elect to use σ=12.0 in our calculations. The difference in AUC at week 1 between HCARB and MUFA is 6.4 (= 47.1–40.7). This is a 13.6% difference. We will assume our high fiber diet would yield a reduction of about 12% in AUC compared to the low fiber diet.

Calculating n

Suppose α=.05, β=.20 (that is, power=80%). The intracluster correlation r is assumed conservatively at 0.5. Therefore to detect a reduction of 12% from a value of 47 in AUC we will get n=18. This means 36 subjects must be recruited to this study. For a 10% reduction the total number of subjects increases to 52, but drops to 24 for a 15% reduction.

APPENDIX D

Communication from T. M. Wolever, Glycemic Testing, Ontario, Canada

Glycaemic Index Testing Inc.
135 Mavety St., Toronto,
Ontario, Canada M6P 2L8
Tel: (416) 978-5556
FAX: (416) 769-7210

KELLOGG COMPANY
FINAL REPORT

Psyllium Loops
vs
Malt-O-Meal

15 March, 2002

DISCLAIMER

GI Testing has taken due care to ensure the accuracy of the results provided in this report. However, the results of glycemic response tests in human subjects are subject to biological variability and may vary depending on the methods used. Thus, these results may not be able to be reproduced exactly either by GI Testing or by others.

SUMMARY

The glycemic index (GI) values of multi-grain psyllium cereal and malt-o-meal cereal were determined in 10 normal subjects (6 female, 4 male), aged 40±5 years and body mass index 23.2±0.9kg/m². Portions of breakfast cereal containing 50g available carbohydrate were fed to subjects on separate occasions in randomized order. Each subject also repeated the reference food of white bread 3 times. Breakfast cereals were fed with 250ml 2% butterfat milk, and, as a control, 250ml 2% milk was also fed with an additional 50g carbohydrate portion of white bread. The addition on milk to bread significantly reduced the incremental area under the glycemic response curve, although the reduction on a percentage basis, 18±12%, was not statistically significant. The GI values of psyllium cereal, 56±6, and malt-o-meal, 64±7, did not differ significantly from each other. For international standardization, it is recommended that GI values be expressed on the glucose standard, ie. the GI of glucose = 100. Based on this, the GI values of psyllium and malt-o-meal were 40±5 and 45±5, respectively.

METHODS

Subjects

Ten (10) healthy subjects (4 male and 6 female) aged 39.8±4.9 years with a body mass index of 23.2±0.9 were studied. The individual details are shown on the data sheet under "Subject Details". The ethnicity of the subjects was: 7 Caucasian, 1 African-American (ID #1), 1 Asian (ID #27) and 1 South Asian (ID #75).

Protocol

Subjects each underwent 6 treatments on separate days, with each subject performing a maximum of 2 tests per week. On each test day, subjects came to Glycaemic Index Testing Laboratory (55 Queen St. East, Suite 207) in the morning after a 10-14h overnight fast. After being weighed and having a fasting blood sample obtained by finger-prick, the subject then consumed a test meal within 10 minutes, and further blood samples were obtained at 15, 30, 45, 60, 90 and 120 minutes after the start of the test meal. Subjects were also given a drink of their choice of 1 or 2 cups of either water, coffee or tea, with or without 60ml of 2% milk. The drink chosen by each subject remained the same on each test day.

The tests meals consisted of portions of psyllium loop cereal and malt-o-meal containing 50g available carbohydrate served with 250ml 2% butterfat milk. Each subject repeated reference white bread containing 50g available carbohydrate on 3 occasions and also a test of bread plus 250ml 2% milk. Bread was baked in a bread maker in loaves containing 50g available carbohydrate. The ingredients for each loaf (250ml warm water, 334g all purpose flour, 7g sugar, 4g salt and 6.5g yeast) were placed into the bread maker according to instructions, and the machine turned on. After the loaf had been made, it was allowed to cool for an hour, and then weighed and after discarding the crust ends, the remainder was divided into portion sizes containing 50g available carbohydrate. These portions were frozen prior to use, and reheated in the microwave prior to consumption. Psyllium loop cereal was provided as a ready-to-eat cereal. Malt-o-Meal (70g) was cooked with 250ml milk as follows: 150ml 2%milk was added to the cereal and cooked for 1 minute in a microwave on high. The bowl was

removed and the cereal stirred and the remaining 100ml of 2% milk added and the cereal cooked for a further 1 minute in the microwave oven.

Composition of test cereals.

	Weight (g)	Fat (g)	Protein (g)	Total Carb (g)	Fiber (g)	Av Carb (g)
Psyllium cereal	91.7g	4.2	8.4	70.8	20.8	50.0
Malt-O-Meal (based on label)	70.0	1	5	52	2	50
Malt-O-Meal (based on Kellogg analysis)	70.0	1.1	8.5	53.0	2.1	50.9

Malt-O-Meal was fed based on label information (Kellogg analysis was not on file).

Blood samples (2-3 drops each) were collected into 5ml tubes containing a small amount of sodium fluoride/potassium oxalate, mixed by rotating the tube vigorously, and placed into a refrigerator. After the last blood sample was obtained subjects were offered a snack and then allowed to leave. Blood samples were then stored at -20°C prior to analysis of glucose using a YSI analyzer.

Data Analysis

Incremental area under the plasma glucose curves (IAUC) were calculated using the trapezoid rule and ignoring area beneath the baseline. The GI was calculated by expressing each subject's response IAUC for the test food as a percentage of the same subject's average response after reference white bread. The blood glucose concentrations and increments at each time and the IAUC values were subjected to repeated-measures analysis of variance (ANOVA) examining for the effect of test meal. After demonstration of significant heterogeneity, the significance of the differences between individual means was assessed using Tukey's test to adjust for multiple comparisons.

RESULTS

Blood Glucose Responses

On the same data page as "SUBJECT DETAILS" is shown the within-subject variation of IAUC and palatability for the 3 repeated tests of white bread. Order does not normally influence significantly the glycemic responses of repeated bread tests; the same was true here, although the p-value, 0.056 was nearly significant. The subject's mean IAUC values differed from each other as expected. The mean CV (coefficient of variation=100×SD/mean) for the 10 subject was 24%, which is typical for normal subjects. Palatability scores were not affected significantly by order, but the different subjects had highly significantly different perceptions about the palatability of the reference bread. The within-subject reproducibility of the palatability scores was similar to that for the glycemic responses, with a mean CV of 20%.

The 3 sheets headed "Blood Glucose Results" show the glycemic responses for each test meal plotted against the response to the mean reference bread result. The adding milk to bread resulted in a significantly lower blood glucose concentration at 60min and a significantly lower

IAUC than white bread alone. However the individual GI values were quite variable, ranging from 37 to 179, so that the mean GI value, 82, did not differ significantly from that for bread, 100.

Psyllium cereal elicited lower blood glucose concentrations than bread at 15 through 90 minutes, and significantly lower IAUC and GI values than bread alone. Malt-O-Meal elicited lower blood glucose concentrations than bread at 45 through 90 minutes, with significantly lower IAUC and GI values than bread.

Analysis of Variance

The sheets headed ANOVA show the results of the analysis of variance for palatability, each time point and the areas under the curve for the different treatments. Tukey's LSD is the least significant difference based on Tukey's test. Means which differ by more than this amount are statisticially significantly different.

Palatability: both cereals were rated as significantly less palatable than bread, but adding milk to bread did not differ significantly from bread alone.

Blood Glucose: the results are summarized on the sheet headed ANOVA-summary. Fasting glucose did not differ significantly between treatments. Blood glucose after psyllium cereal was significantly higher than malt-o-meal at 15 and 30min, but the mean IAUC and GI values did not differ between the 2 cereals. Blood glucose after psyllium was significantly lower than after bread plus milk at 15, 30, and 60 minutes, whereas blood glucose after malt-o-meal was significantly lower than that after bread and milk at only 45 and 90min. Both psyllium and malt-o-meal had a significantly lower IAUC than bread and milk, but only psyllium had a significantly lower GI value than bread and milk.

Discussion

The results were generally satisfactory. Adding milk to bread usually does not reduce the glycemic response compared to bread alone. Bread and milk has been tested 3 previous times by GI Testing in the past 4 years, and the mean GI values (n=10 subjects) were 81, 98 and 103. Thus, the present results are consistent within the previous range. The lower than expected glycemic response of bread and milk may be partly explained by the facts that it was always taken after the first white bread test, and often after the second, and there was a strong trend for reduced glycemic responses with time for the reference breads.

The GI of farina was lower than expected. We tested Nabisco Cream of Wheat previously and found a GI similar to that of bread. The lower response of malt-o-meal here could be a real difference, could be due to the fact that it was cooked with milk, or could be due to chance. The GI of the psyllium cereal was approximately what was expected based on previous studies with psyllium cereals. The GI of malt-o-meal tended to be lower than psyllium, but not significantly. However, the blood glucose after malt-o-meal increased more rapidly than after psyllium cereal, and only psyllium had a significantly lower GI than white bread plus milk.

To: Kellogg Company 15/03/02

SUBJECT DETAILS

ID	Sex	Age (y)	Height (cm)	Height (in)	Weight (kg)	Weight (lb)	BMI
1	M	35	173	68.1	85.9	189	28.70
27	F	24	170.2	67.0	58.6	129	20.23
31	F	53	162	63.8	58.6	129	22.33
33	F	37	165	65.0	60	132	22.04
38	M	20	187.5	73.8	90.6	199	25.77
39	F	70	161.3	63.5	55.5	122	21.33
43	F	53	167.6	66.0	63.9	141	22.75
44	M	45	182.9	72.0	83.9	185	25.08
74	F	30	162	63.8	51.5	113	19.62
75	M	31	177.8	70.0	75.5	166	23.88
Mean		39.8	170.9	67.3	68.4	150.5	23.17
SEM		4.9	2.9	1.2	4.5	9.9	0.87

Within-subject variation of standard tests

INCREMENTAL AREA UNDER THE CURVE

ID	WB#1	WB#2	WB#3	Mean	SD	CV
1	105.2	158.0	128.0	130.4	26.5	20.3
27	278.9	210.5	95.8	195.1	92.5	47.4
31	366.5	413.2	344.0	374.6	35.3	9.4
33	221.8	181.6	160.9	188.1	31.0	16.5
38	147.9	236.5	118.1	167.5	61.6	36.8
39	310.4	288.9	308.8	302.7	12.0	4.0
43	374.0	272.1	351.4	332.5	53.5	16.1
44	131.4	135.1	148.5	138.3	9.0	6.5
74	267.8	131.6	101.3	166.9	88.7	53.1
75	179.0	105.5	104.0	129.5	42.9	33.1
Mean	238.3	213.3	186.1	212.6	45.3	24.3
SEM	30.5	29.5	33.2	28.5	9.2	5.5

ANOVA	Source	SS	df	MS	F	p
	Order	15192.52	2	7596.26	3.38676	0.05644
	Subject	212246.4	9	23582.9	10.5143	0.00002
	Error	40372.75	18	2242.93		
	Total	267811.7				

PALABILITY

ID	WB#1	WB#2	WB#3	Mean	SD	CV
1	87.0	82.5	96.0	89.2	8.0	8.9
27	70.0	63.0	70.0	67.7	4.0	6.0
31	89.5	80.0	80.5	83.3	5.3	6.4
33	89.5	94.5	92.0	92.0	2.5	2.7
38	83.0	79.0	78.0	80.0	2.6	3.3
39	26.5	18.5	39.5	28.2	10.6	37.6
43	39.5	36.5	22.5	32.8	9.1	27.6
44	5.0	6.5	24.5	12.0	10.9	90.4
74	43.0	41.0	52.5	45.5	6.1	13.5
75	80.0	71.0	80.0	77.0	5.2	6.7
Mean	61.3	57.3	63.8	60.8	6.4	20.3
SEM	9.6	9.4	8.6	9.1	1.0	8.6

ANOVA	Source	SS	df	MS	F	p
	Order	215.5167	2	107.758	2.47937	0.11192
	Subject	22280.03	9	2475.56	56.9591	2E-011
	Error	782.3167	18	43.462		
	Total	23277.87				

Dates (dd/mm/yy) of White Bread tests

ID	WB#1	WB#2	WB#3
1	30/01/02	14/02/02	27/02/02
27	29/01/02	07/02/02	19/02/02
31	29/01/02	13/02/02	19/02/02
33	23/01/02	05/02/02	14/02/02
38	05/02/02	13/02/02	19/02/02
39	17/01/02	23/01/02	05/02/02
43	17/01/02	24/01/02	05/02/02
44	17/01/02	30/01/02	13/02/02
74	23/01/02	07/02/02	27/02/02
75	19/02/02	26/02/02	07/05/02

Glycaemic Index Testing Inc. 135 Mavety St, Toronto, Ont, Canada M6P 2L8 Tel: (416) 978-5556 FAX (416) 769-7210

To: Kellogg Company

Blood Glucose Results

15/03/02

White Bread + Milk

ID	Pal	0min	15min	30min	45min	60min	90min	120min	AUC	GI
1	41.0	5.08	5.52	8.35	8.92	7.62	6.48	6.48	233.4	179.0
27	62.5	4.27	5.07	6.54	5.46	4.53	4.48	5.32	91.8	47.1
31	88.0	4.72	5.06	7.15	7.92	6.03	7.31	6.92	274.7	73.3
33	93.5	4.19	5.86	6.65	6.24	5.55	5.44	4.91	171.6	91.2
38	75.0	4.16	4.84	6.79	6.50	4.96	4.27	4.74	114.8	68.5
39	41.0	5.08	5.52	8.35	8.92	7.62	6.48	6.48	233.4	77.1
43	34.5	4.48	5.39	7.46	6.58	7.14	7.26	6.42	262.2	78.9
44	21.5	4.41	4.45	6.33	7.39	6.99	4.63	4.14	136.9	99.0
74	54.0	4.26	4.36	5.77	4.40	3.92	5.52	4.43	61.8	37.0
75	72.5	4.40	4.57	6.03	5.39	4.60	5.19	5.66	89.0	66.7
Mean	58.4	4.51	5.06	6.94	6.77	6.10	5.71	5.55	167.0	82.0
SEM	7.6	0.11	0.16	0.28	0.48	0.49	0.35	0.31	24.9	12.2
p	0.669	0.339	0.529	0.346	0.194	0.012	0.425	0.272	0.048	0.175

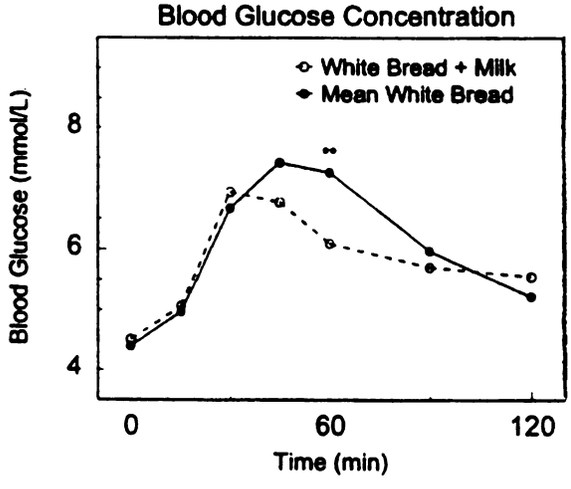
INCREMENTS

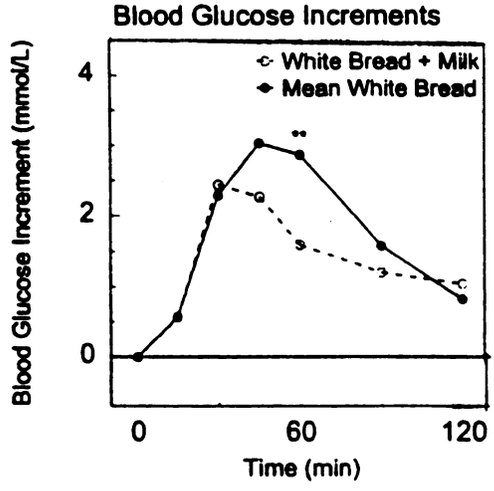
0min	15min	30min	45min	60min	90min	120min
0	0.44	3.27	3.84	2.54	1.40	1.40
0	0.80	2.27	1.19	0.26	0.21	1.05
0	0.34	2.43	3.20	3.31	2.59	2.20
0	1.67	2.46	2.05	1.36	1.25	0.72
0	0.68	2.63	2.34	0.80	0.11	0.58
0	0.44	3.27	3.84	2.54	1.40	1.40
0	0.91	2.96	2.10	2.66	2.78	1.94
0	0.04	1.92	2.98	2.58	0.22	-0.27
0	0.10	1.51	0.14	-0.34	1.26	0.17
0	0.17	1.63	0.99	0.20	0.79	1.26
0	0.56	2.44	2.27	1.59	1.20	1.05
	0.15	0.20	0.39	0.41	0.29	0.24
	0.898	0.454	0.081	0.002	0.148	0.351

Mean White Bread

ID	Pal	0min	15min	30min	45min	60min	90min	120min	AUC
1	89.2	4.11	4.33	6.32	6.47	6.12	4.42	4.26	130.4
27	67.7	4.38	4.99	6.83	7.36	7.20	5.41	5.01	195.1
31	83.3	4.90	5.06	7.25	9.42	9.90	8.97	7.17	374.6
33	92.0	4.01	5.29	7.10	6.76	5.97	5.13	4.19	188.1
38	80.0	3.93	5.09	6.12	6.71	6.11	4.70	4.08	167.5
39	28.2	4.97	5.40	7.97	8.91	8.69	7.52	7.12	302.7
43	32.8	4.61	5.63	8.28	8.89	8.71	7.45	6.00	332.5
44	12.0	4.48	4.48	5.13	6.65	7.43	5.45	4.48	138.3
74	45.5	4.09	4.66	5.67	6.32	5.92	5.56	5.16	166.9
75	77.0	4.49	4.73	6.08	6.73	6.51	5.13	4.75	129.5
Mean	60.8	4.40	4.97	6.68	7.42	7.26	5.97	5.22	212.6
SEM	9.1	0.11	0.13	0.32	0.37	0.44	0.47	0.37	28.5

0min	15min	30min	45min	60min	90min	120min
0	0.22	2.21	2.36	2.01	0.31	0.15
0	0.61	2.46	2.99	2.63	1.03	0.63
0	0.16	2.35	4.53	5.00	4.07	2.28
0	1.29	3.09	2.75	1.96	1.12	0.19
0	1.16	2.18	2.78	2.18	0.77	0.15
0	0.42	3.00	3.94	3.72	2.55	2.15
0	1.02	3.67	4.28	4.09	2.84	1.39
0	0.00	0.65	2.17	2.95	0.97	0.00
0	0.58	1.58	2.23	1.83	1.46	1.07
0	0.24	1.59	2.24	2.02	0.64	0.26
0	0.57	2.28	3.03	2.86	1.58	0.83
	0.14	0.27	0.28	0.34	0.38	0.27





Glycaemic Index Testing Inc. 135 Mavety St, Toronto, Ont, Canada M6P 2L8 Tel: (416) 978-5556 FAX: (416) 769-7210

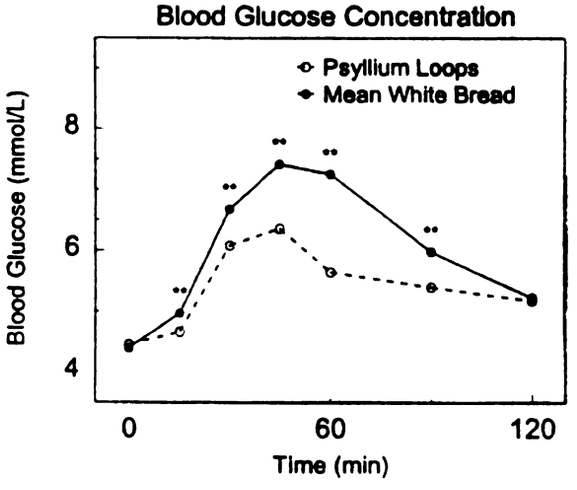
To: Kellogg Company

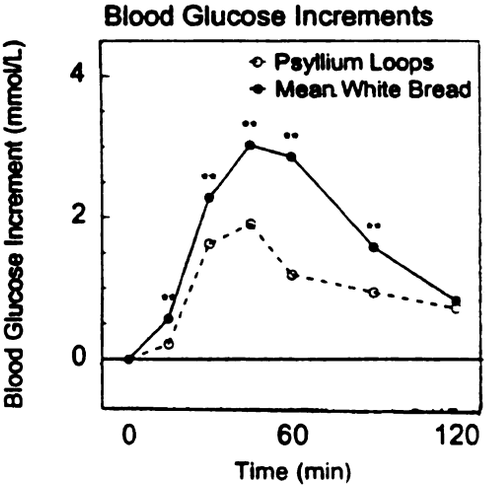
Blood Glucose Results

15/03/02

Psyllium Loops											INCREMENTS						
ID	Pal	0min	15min	30min	45min	60min	90min	120min	AUC	GI	0min	15min	30min	45min	60min	90min	120min
1	61.0	4.13	4.34	5.48	5.46	4.80	5.17	4.36	93.1	71.4	0	0.21	1.35	1.33	0.67	1.04	0.23
27	16.0	4.24	4.34	6.48	6.65	5.54	4.70	4.93	124.7	63.9	0	0.10	2.24	2.41	1.30	0.46	0.69
31	33.0	4.93	4.98	5.86	7.64	8.19	8.27	6.63	254.4	67.9	0	0.05	0.93	2.71	3.26	3.34	1.70
33	44.0	4.19	5.27	6.96	6.70	5.55	5.37	5.32	178.4	94.8	0	1.08	2.77	2.51	1.36	1.18	1.13
38	51.5	4.28	4.73	6.03	5.46	4.54	4.36	4.96	89.5	41.5	0	0.45	1.75	1.18	0.26	0.08	0.70
39	23.0	4.92	5.44	7.72	8.29	7.36	6.24	6.01	211.2	69.8	0	0.52	2.80	3.37	2.44	1.32	1.09
43	69.5	4.73	5.07	6.25	6.69	6.12	5.88	5.28	131.3	39.5	0	0.34	1.52	1.96	1.39	1.15	0.55
44	6.0	4.35	4.14	5.32	5.55	5.10	4.36	4.59	52.0	37.6	0	-0.21	0.97	1.20	0.75	0.01	0.24
74	11.0	4.48	4.06	5.29	5.88	4.75	4.84	5.26	59.7	35.8	0	-0.42	0.81	1.40	0.27	0.36	0.78
75	42.0	4.28	4.28	5.39	5.26	4.47	4.75	4.37	51.1	39.5	0	0.00	1.11	0.98	0.19	0.47	0.09
Mean	35.7	4.45	4.67	6.08	6.36	5.64	5.39	5.17	122.5	56.2	0	0.21	1.63	1.91	1.19	0.94	0.72
SEM	6.8	0.10	0.16	0.25	0.32	0.40	0.37	0.23	22.6	6.4		0.13	0.24	0.26	0.32	0.31	0.16
p	0.017	0.408	0.006	0.020	0.001	0.000	0.027	0.824	0.000	0.000		0.015	0.016	0.000	0.000	0.018	0.601

Mean White Bread																
ID	Pal	0min	15min	30min	45min	60min	90min	120min	AUC	0min	15min	30min	45min	60min	90min	120min
1	89.2	4.11	4.33	6.32	6.47	6.12	4.42	4.26	130.4	0	0.22	2.21	2.36	2.01	0.31	0.15
27	67.7	4.38	4.99	6.83	7.36	7.20	5.41	5.01	195.1	0	0.61	2.46	2.99	2.83	1.03	0.63
31	83.3	4.90	5.06	7.25	9.42	9.90	8.97	7.17	374.6	0	0.16	2.35	4.53	5.00	4.07	2.28
33	92.0	4.01	5.29	7.10	6.76	5.97	5.13	4.19	188.1	0	1.29	3.09	2.75	1.96	1.12	0.19
38	80.0	3.93	5.09	6.12	6.71	6.11	4.70	4.08	167.5	0	1.16	2.18	2.78	2.18	0.77	0.15
39	28.2	4.97	5.40	7.97	8.91	8.69	7.52	7.12	302.7	0	0.42	3.00	3.94	3.72	2.55	2.15
43	32.8	4.61	5.63	8.28	8.89	8.71	7.45	6.00	332.5	0	1.02	3.67	4.28	4.09	2.84	1.39
44	12.0	4.48	4.48	5.13	6.65	7.43	5.45	4.48	138.3	0	0.00	0.65	2.17	2.95	0.97	0.00
74	45.5	4.09	4.68	5.67	6.32	5.92	5.56	5.16	166.9	0	0.58	1.58	2.23	1.83	1.46	1.07
75	77.0	4.49	4.73	6.08	6.73	6.51	5.13	4.75	129.5	0	0.24	1.59	2.24	2.02	0.64	0.26
Mean	60.8	4.40	4.97	6.68	7.42	7.26	5.97	5.22	212.6	0	0.57	2.28	3.03	2.66	1.58	0.83
SEM	9.1	0.11	0.13	0.32	0.37	0.44	0.47	0.37	28.5		0.14	0.27	0.28	0.34	0.38	0.27





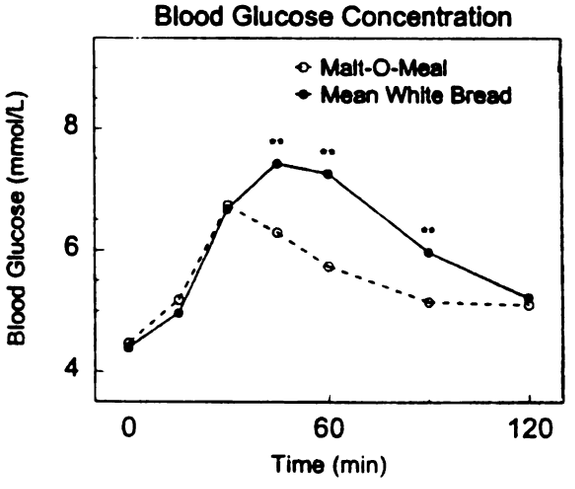
To: Kellogg Company

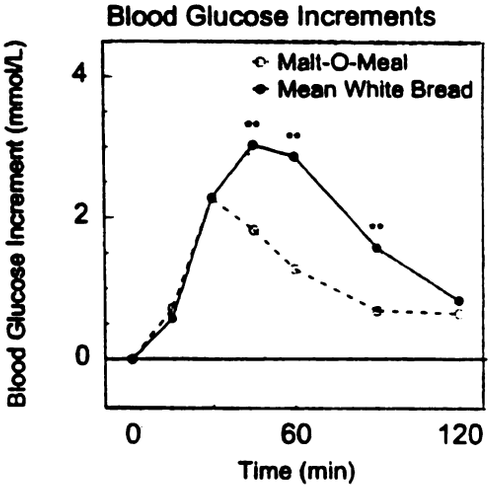
Blood Glucose Results

15/03/02

Malt-O-Meal											INCREMENTS						
ID	Pal	0min	15min	30min	45min	60min	90min	120min	AUC	GI	0min	15min	30min	45min	60min	90min	120min
1	6.5	4.00	4.61	5.89	5.13	4.89	3.81	4.28	74.6	57.2	0	0.61	1.89	1.13	0.89	-0.19	0.28
27	35.0	4.51	5.27	7.39	6.68	5.30	4.72	5.08	119.8	61.4	0	0.76	2.88	2.17	0.79	0.21	0.57
31	47.5	5.05	5.25	6.74	7.46	7.02	6.52	6.32	172.0	45.9	0	0.20	1.69	2.41	1.97	1.47	1.27
33	14.0	4.15	5.39	6.75	5.22	4.97	5.02	5.22	134.2	71.3	0	1.24	2.60	1.07	0.82	0.87	1.07
38	14.0	4.25	4.80	5.19	4.45	4.09	4.58	4.89	42.6	25.4	0	0.55	0.94	0.20	-0.16	0.33	0.64
39	18.0	5.68	6.55	8.27	8.91	8.49	7.17	6.66	221.5	73.2	0	0.87	2.59	3.23	2.81	1.49	0.88
43	73.5	4.64	5.73	7.52	7.52	7.20	6.11	5.51	217.5	65.4	0	1.09	2.88	2.88	2.56	1.47	0.87
44	60.5	4.17	4.45	6.47	7.11	5.92	4.72	4.44	142.7	103.2	0	0.28	2.30	2.94	1.75	0.55	0.27
74	15.0	3.89	4.31	5.74	4.42	4.48	4.29	4.05	69.7	41.8	0	0.42	1.85	0.53	0.59	0.40	0.16
75	3.0	4.33	5.47	7.42	6.04	5.03	4.66	4.70	117.3	90.6	0	1.14	3.09	1.71	0.70	0.23	0.37
Mean	28.7	4.47	5.18	6.74	6.29	5.74	5.15	5.11	131.2	63.5	0	0.72	2.27	1.83	1.27	0.68	0.64
SEM	7.7	0.17	0.21	0.30	0.47	0.44	0.34	0.26	19.0	7.3		0.12	0.21	0.34	0.30	0.19	0.12
p	0.059	0.476	0.169	0.816	0.003	0.000	0.005	0.594	0.002	0.001		0.285	0.980	0.003	0.000	0.003	0.423

Mean White Bread																	
ID	Pal	0min	15min	30min	45min	60min	90min	120min	AUC		0min	15min	30min	45min	60min	90min	120min
1	89.2	4.11	4.33	6.32	6.47	6.12	4.42	4.26	130.4		0	0.22	2.21	2.36	2.01	0.31	0.15
27	67.7	4.38	4.99	6.83	7.36	7.20	5.41	5.01	195.1		0	0.61	2.46	2.99	2.83	1.03	0.63
31	83.3	4.90	5.06	7.25	9.42	9.90	8.97	7.17	374.6		0	0.16	2.35	4.53	5.00	4.07	2.28
33	92.0	4.01	5.29	7.10	6.76	5.97	5.13	4.19	188.1		0	1.29	3.09	2.75	1.96	1.12	0.19
38	80.0	3.93	5.09	6.12	6.71	6.11	4.70	4.08	167.5		0	1.16	2.18	2.78	2.18	0.77	0.15
39	28.2	4.97	5.40	7.97	8.91	8.69	7.52	7.12	302.7		0	0.42	3.00	3.94	3.72	2.55	2.15
43	32.8	4.61	5.63	8.28	8.89	8.71	7.45	6.00	332.5		0	1.02	3.67	4.28	4.09	2.84	1.39
44	12.0	4.48	4.48	5.13	6.65	7.43	5.45	4.48	138.3		0	0.00	0.65	2.17	2.95	0.97	0.00
74	45.5	4.09	4.68	5.67	6.32	5.92	5.56	5.16	166.9		0	0.58	1.58	2.23	1.83	1.46	1.07
75	77.0	4.49	4.73	6.08	6.73	6.51	5.13	4.75	129.5		0	0.24	1.59	2.24	2.02	0.64	0.26
Mean	60.8	4.40	4.97	6.68	7.42	7.26	5.97	5.22	212.6		0	0.57	2.28	3.03	2.86	1.58	0.83
SEM	9.1	0.11	0.13	0.32	0.37	0.44	0.47	0.37	28.5			0.14	0.27	0.28	0.34	0.38	0.27





Glycaemic Index Testing Inc. 135 Mavety St, Toronto, Ont, Canada M6P 2L8 Tel: (416) 978-5556 FAX (416) 769-7210

ANOVA - Palatability

Palatability

ID	MOM	Psyl	WB+M	RefWB
1	6.5	61.0	41.0	89.2
27	35.0	16.0	62.5	67.7
31	47.5	33.0	88.0	83.3
33	14.0	44.0	93.5	92.0
38	14.0	51.5	75.0	80.0
39	18.0	23.0	41.0	28.2
43	73.5	69.5	34.5	32.8
44	60.5	6.0	21.5	12.0
74	15.0	11.0	54.0	45.5
75	3.0	42.0	72.5	77.0
Mean	28.7	35.7	58.4	60.8
SEM	7.7	6.8	7.6	9.1

ANOVA

Source	SS	df	MS	F	p
Foods	7758.9854	3	2586.3285	4.51	0.0108549
Subj	6599.909	9	733.32323	1.28	0.2924266
Error	15472.494	27	573.05532		
Total	29831.388			Tukey's LSD	
				14.38	

Expressed as % of Mean WB Value

ID	MOM	Psyl	WB+M	RefWB
1	7.3	68.4	46.0	100.0
27	51.7	23.6	92.4	100.0
31	57.0	39.6	105.6	100.0
33	15.2	47.8	101.6	100.0
38	17.5	64.4	93.8	100.0
39	63.9	81.7	145.6	100.0
43	223.9	211.7	105.1	100.0
44	504.2	50.0	179.2	100.0
74	33.0	24.2	118.7	100.0
75	3.9	54.5	94.2	100.0
Mean	97.8	66.6	108.2	100
SEM	49.5	17.1	11.1	0

ANOVA

Source	SS	df	MS	F	p
Foods	9998.8731	3	3332.9577	0.54	0.6576082
Subj	91998.7	9	10222.078	1.66	0.1475561
Error	166002.23	27	6148.2306		
Total	267999.8			Tukey's LSD	
				47.11	

To: Kellogg Company

ANOVA - Summary

15/03/02

FASTING Glucose		LSD ns
MOM	4.47	
Psyl	4.45	
WB+M	4.51	
RefWB	4.40	
0		
0		
0		
0		
0		
0		
0		
0		
0		
0		
0		
0		
0		
0		
0		

15min Glucose		LSD 0.18
MOM	5.18	
Psyl	4.67	
WB+M	5.06	
RefWB	4.97	
0	ERR	
0		
0		
0		
0		
0		
0		
0		
0		
0		
0		
0		
0		
0		
0		

30min Glucose		LSD 0.36
MOM	6.74	
Psyl	6.08	
WB+M	6.94	
RefWB	6.68	
0		
0		
0		
0		
0		
0		
0		
0		
0		
0		
0		
0		
0		
0		
0		

45min Glucose		LSD 0.51
MOM	6.29	
Psyl	6.36	
WB+M	6.77	
RefWB	7.42	
0		
0		
0		
0		
0		
0		
0		
0		
0		
0		
0		
0		
0		
0		
0		

60min Glucose		LSD 0.41
MOM	5.74	
Psyl	5.64	
WB+M	6.10	
RefWB	7.26	
0		
0		
0		
0		
0		
0		
0		
0		
0		
0		
0		
0		
0		
0		
0		

90min Glucose		LSD 0.35
MOM	5.15	
Psyl	5.39	
WB+M	5.71	
RefWB	5.97	
0		
0		
0		
0		
0		
0		
0		
0		
0		
0		
0		
0		
0		
0		
0		

120min Glucose		LSD ns
MOM	5.11	
Psyl	5.17	
WB+M	5.55	
RefWB	5.22	
0		

Incremental AUC		LSD 24.76
MOM	131.2	
Psyl	122.5	
WB+M	167.0	
RefWB	212.6	
0		

Glycaemic Index		LSD 24.69
MOM	63.5	
Psyl	56.2	
WB+M	82.0	
RefWB	100.0	
0		

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To: Kellogg Company

ANOVA - Fasting Glucose

16/03/02

Fasting Glucose

ID	MOM	Psyl	WB+M	RefWB
1	4.0	4.1	5.1	4.1
27	4.5	4.2	4.3	4.4
31	5.1	4.9	4.7	4.9
33	4.2	4.2	4.2	4.0
38	4.3	4.3	4.2	3.9
39	5.7	4.9	5.1	5.0
43	4.6	4.7	4.5	4.6
44	4.2	4.4	4.4	4.5
74	3.9	4.5	4.3	4.1
75	4.3	4.3	4.4	4.5
Mean	4.47	4.45	4.51	4.4
SEM	0.17	0.1	0.11	0.11

ANOVA

Source	SS	df	MS	F	p
Foods	0.0597208	3	0.0199069	0.34	0.7939964
Subj	4.1448725	9	0.4605414	7.95	0.0000122
Error	1.5643042	27	0.0579372		
Total	5.7688975			Tukey's LSD	
				0.14	

Expressed as % of Mean WB Value

ID	MOM	Psyl	WB+M	RefWB
1	97.3	100.5	123.6	100.0
27	103.0	96.9	97.6	100.0
31	103.1	100.7	96.4	100.0
33	103.6	104.6	104.6	100.0
38	108.1	108.8	105.8	100.0
39	114.2	98.9	102.1	100.0
43	100.6	102.5	97.1	100.0
44	93.1	97.1	98.4	100.0
74	95.0	109.4	104.1	100.0
75	96.4	95.3	98.0	100.0
Mean	101.45	101.48	102.77	100
SEM	2.03	1.54	2.56	0

ANOVA

Source	SS	df	MS	F	p
Foods	38.302094	3	12.767365	0.41	0.7452095
Subj	339.1333	9	37.681478	1.22	0.3249506
Error	835.27755	27	30.936205		
Total	1212.7129			Tukey's LSD	
				3.34	

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To: Kellogg Company

ANOVA - 15min Glucose

16/03/02

15min Glucose

ID	MOM	Psyl	WB+M	RefWB
1	4.6	4.3	5.5	4.3
27	5.3	4.3	5.1	5.0
31	5.3	5.0	5.1	5.1
33	5.4	5.3	5.9	5.3
38	4.8	4.7	4.8	5.1
39	6.6	5.4	5.5	5.4
43	5.7	5.1	5.4	5.6
44	4.5	4.1	4.5	4.5
74	4.3	4.1	4.4	4.7
75	5.5	4.3	4.6	4.7
Mean	5.18	4.67	5.06	4.97
SEM	0.21	0.16	0.16	0.13

ANOVA

Source	SS	df	MS	F	p
Foods	1.4723275	3	0.4907758	5.26	0.0054612
Subj	7.6614358	9	0.8512706	9.13	0.0000034
Error	2.5180475	27	0.093261		
Total	11.651811			Tukey's LSD	
				0.18	

Expressed as % of Mean WB Value

ID	MOM	Psyl	WB+M	RefWB
1	106.5	100.2	127.5	100.0
27	105.6	87.0	101.6	100.0
31	103.8	98.4	100.0	100.0
33	101.8	99.6	110.7	100.0
38	94.2	92.9	95.0	100.0
39	121.4	100.8	102.3	100.0
43	101.7	90.0	95.7	100.0
44	99.3	92.3	99.3	100.0
74	92.2	86.8	93.2	100.0
75	115.7	90.6	96.7	100.0
Mean	104.21	93.86	102.2	100
SEM	2.82	1.73	3.22	0

ANOVA

Source	SS	df	MS	F	p
Foods	603.01711	3	201.0057	4.98	0.0070718
Subj	823.42711	9	91.491901	2.27	0.0486389
Error	1090.5553	27	40.390935		
Total	2516.9995			Tukey's LSD	
				3.82	

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To: Kellogg Company

ANOVA - 30min Glucose

16/03/02

30min Glucose

ID	MOM	Psyl	WB+M	RefWB
1	5.9	5.5	8.4	6.3
27	7.4	6.5	6.5	6.8
31	6.7	5.9	7.2	7.3
33	6.8	7.0	6.7	7.1
38	5.2	6.0	6.8	6.1
39	8.3	7.7	8.4	8.0
43	7.5	6.3	7.5	8.3
44	6.5	5.3	6.3	5.1
74	5.7	5.3	5.8	5.7
75	7.4	5.4	6.0	6.1
Mean	6.74	6.08	6.94	6.68
SEM	0.3	0.25	0.28	0.32

ANOVA

Source	SS	df	MS	F	p
Foods	4.1402408	3	1.3800803	3.74	0.0227796
Subj	19.911211	9	2.2123568	6.00	0.0001316
Error	9.9591008	27	0.3688556		
Total	34.010553			Tukey's LSD	
				0.36	

Expressed as % of Mean WB Value

ID	MOM	Psyl	WB+M	RefWB
1	93.1	86.7	132.1	100.0
27	108.1	94.8	95.7	100.0
31	93.0	80.8	98.6	100.0
33	95.1	98.0	93.7	100.0
38	84.9	98.6	111.0	100.0
39	103.7	96.8	104.7	100.0
43	90.8	75.5	90.1	100.0
44	126.0	103.6	123.3	100.0
74	101.2	93.3	101.8	100.0
75	122.0	88.6	99.1	100.0
Mean	101.8	91.68	105.01	100
SEM	4.27	2.76	4.25	0

ANOVA

Source	SS	df	MS	F	p
Foods	969.84865	3	323.28288	3.53	0.0280846
Subj	1482.6368	9	164.73742	1.80	0.1149283
Error	2472.7397	27	91.582954		
Total	4925.2252			Tukey's LSD	
				5.75	

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To: Kellogg Company

ANOVA - 45min Glucose

16/03/02

45min Glucose

ID	MOM	Psyl	WB+M	RefWB
1	5.1	5.5	8.9	6.5
27	6.7	6.7	5.5	7.4
31	7.5	7.6	7.9	9.4
33	5.2	6.7	6.2	6.8
38	4.5	5.5	6.5	6.7
39	8.9	8.3	8.9	8.9
43	7.5	6.7	6.6	8.9
44	7.1	5.6	7.4	6.6
74	4.4	5.9	4.4	6.3
75	6.0	5.3	5.4	6.7
Mean	6.29	6.36	6.77	7.42
SEM	0.47	0.32	0.48	0.37

ANOVA

Source	SS	df	MS	F	p
Foods	8.0868667	3	2.6956222	3.68	0.0242366
Subj	42.733561	9	4.7481735	6.48	0.0000704
Error	19.785283	27	0.7327883		
Total	70.605711			Tukey's LSD	
				0.51	

Expressed as % of Mean WB Value

ID	MOM	Psyl	WB+M	RefWB
1	79.3	84.4	137.9	100.0
27	90.7	90.3	74.2	100.0
31	79.2	81.1	84.0	100.0
33	77.3	99.2	92.4	100.0
38	66.3	81.4	96.9	100.0
39	100.0	93.0	100.1	100.0
43	84.6	75.2	74.0	100.0
44	107.0	83.5	111.2	100.0
74	69.9	93.0	69.6	100.0
75	89.8	78.2	80.1	100.0
Mean	84.39	85.92	92.02	100
SEM	4.03	2.41	6.59	0

ANOVA

Source	SS	df	MS	F	p
Foods	1507.8945	3	502.6315	3.21	0.0387022
Subj	1667.6682	9	185.29647	1.18	0.344314
Error	4226.4815	27	156.53635		
Total	7402.0442			Tukey's LSD	
				7.52	

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To: Kellogg Company

ANOVA - 60min Glucose

16/03/02

60min Glucose

ID	MOM	Psyl	WB+M	RefWB
1	4.9	4.8	7.6	6.1
27	5.3	5.5	4.5	7.2
31	7.0	8.2	8.0	9.9
33	5.0	5.6	5.6	6.0
38	4.1	4.5	5.0	6.1
39	8.5	7.4	7.6	8.7
43	7.2	6.1	7.1	8.7
44	5.9	5.1	7.0	7.4
74	4.5	4.8	3.9	5.9
75	5.0	4.5	4.6	6.5
Mean	5.74	5.64	6.1	7.26
SEM	0.44	0.4	0.49	0.44

ANOVA

Source	SS	df	MS	F	p
Foods	16.488578	3	5.4961926	12.03	0.0000349
Subj	58.506035	9	6.5006705	14.23	4.04E-008
Error	12.337447	27	0.4569425		
Total	87.33206			Tukey's LSD	
				0.41	

Expressed as % of Mean WB Value

ID	MOM	Psyl	WB+M	RefWB
1	79.9	78.4	124.5	100.0
27	73.6	76.9	62.9	100.0
31	70.9	82.7	81.1	100.0
33	83.3	93.0	93.0	100.0
38	66.9	74.3	81.1	100.0
39	97.7	84.7	87.7	100.0
43	82.7	70.3	82.0	100.0
44	79.6	68.6	94.0	100.0
74	75.7	80.2	66.2	100.0
75	77.3	68.7	70.7	100.0
Mean	78.76	77.79	84.33	100
SEM	2.66	2.46	5.57	0

ANOVA

Source	SS	df	MS	F	p
Foods	3162.2984	3	1054.0995	10.97	0.000069
Subj	1383.1679	9	153.68532	1.60	0.1656866
Error	2594.9973	27	96.111012		
Total	7140.4636			Tukey's LSD	
				5.89	

ANOVA - 90min Glucose

90min Glucose

ID	MOM	Psyl	WB+M	RefWB
1	3.8	5.2	6.5	4.4
27	4.7	4.7	4.5	5.4
31	6.5	8.3	7.3	9.0
33	5.0	5.4	5.4	5.1
38	4.6	4.4	4.3	4.7
39	7.2	6.2	6.5	7.5
43	6.1	5.9	7.3	7.5
44	4.7	4.4	4.6	5.5
74	4.3	4.8	5.5	5.6
75	4.6	4.8	5.2	5.1
Mean	5.15	5.39	5.71	5.97
SEM	0.34	0.37	0.35	0.47

ANOVA

Source	SS	df	MS	F	p
Foods	3.8802542	3	1.2934181	3.77	0.0220673
Subj	44.887508	9	4.9875009	14.55	3.17E-008
Error	9.2537375	27	0.342731		
Total	58.0215			Tukey's LSD	
				0.35	

Expressed as % of Mean WB Value

ID	MOM	Psyl	WB+M	RefWB
1	86.2	117.0	146.6	100.0
27	87.2	86.9	82.8	100.0
31	72.7	92.2	81.5	100.0
33	97.9	104.7	106.0	100.0
38	97.4	92.7	90.8	100.0
39	95.3	83.0	86.2	100.0
43	82.0	78.9	97.4	100.0
44	86.6	80.0	85.0	100.0
74	77.2	87.1	99.3	100.0
75	88.9	92.7	101.2	100.0
Mean	87.15	91.51	97.69	100
SEM	2.64	3.7	6.06	0

ANOVA

Source	SS	df	MS	F	p
Foods	1027.3687	3	342.45622	3.18	0.0399156
Subj	2251.6466	9	250.18296	2.32	0.0436978
Error	2907.1358	27	107.6717		
Total	6186.1511			Tukey's LSD	
				6.23	

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To: Kellogg Company

ANOVA - 120min Glucose

16/03/02

120min Glucose

ID	MOM	Psyl	WB+M	RefWB
1	4.3	4.4	6.5	4.3
27	5.1	4.9	5.3	5.0
31	6.3	6.6	6.9	7.2
33	5.2	5.3	4.9	4.2
38	4.9	5.0	4.7	4.1
39	6.6	6.0	6.5	7.1
43	5.5	5.3	6.4	6.0
44	4.4	4.6	4.1	4.5
74	4.1	5.3	4.4	5.2
75	4.7	4.4	5.7	4.7
Mean	5.11	5.17	5.55	5.22
SEM	0.26	0.23	0.31	0.37

ANOVA

Source	SS	df	MS	F	p
Foods	1.1703275	3	0.3901092	1.38	0.2690793
Subj	24.068445	9	2.6742716	9.48	0.0000024
Error	7.6132475	27	0.2819721		
Total	32.85202			Tukey's LSD	
				0.32	

Expressed as % of Mean WB Value

ID	MOM	Psyl	WB+M	RefWB
1	100.4	102.3	152.0	100.0
27	101.4	98.4	106.2	100.0
31	88.1	92.4	96.5	100.0
33	124.5	126.9	117.1	100.0
38	119.9	122.1	116.2	100.0
39	92.1	84.4	91.0	100.0
43	91.8	88.0	107.0	100.0
44	99.1	102.5	92.4	100.0
74	78.4	101.9	85.8	100.0
75	99.0	92.1	119.2	100.0
Mean	99.48	101.08	108.34	100
SEM	4.38	4.37	6.12	0

ANOVA

Source	SS	df	MS	F	p
Foods	511.79686	3	170.59895	1.32	0.2882956
Subj	3334.1247	9	370.4583	2.87	0.0164011
Error	3488.9866	27	129.22173		
Total	7334.9082			Tukey's LSD	
				6.83	

To: Kellogg Company

ANOVA - Area Under the Curve

16/03/02

Area Under the Curve

ID	MOM	Psyl	WB+M	RefWB
1	74.6	93.1	233.4	130.4
27	119.8	124.7	91.8	195.1
31	172.0	254.4	274.7	374.6
33	134.2	178.4	171.6	188.1
38	42.6	69.5	114.8	167.5
39	221.5	211.2	233.4	302.7
43	217.5	131.3	262.2	332.5
44	142.7	52.0	136.9	138.3
74	69.7	59.7	61.8	166.9
75	117.3	51.1	89.0	129.5
Mean	131.2	122.5	167	212.6
SEM	19	22.6	24.9	28.5

ANOVA

Source	SS	df	MS	F	p
Foods	50325.106	3	16775.035	9.88	0.0001443
Subj	161739.26	9	17971.029	10.58	0.0000008
Error	45858.812	27	1698.4745		
Total	257923.18			Tukey's LSD	
				24.76	

Glycaemic Index

ID	MOM	Psyl	WB+M	RefWB
1	57.2	71.4	179.0	100.0
27	61.4	63.9	47.1	100.0
31	45.9	67.9	73.3	100.0
33	71.3	94.8	91.2	100.0
38	25.4	41.5	68.5	100.0
39	73.2	69.8	77.1	100.0
43	65.4	39.5	78.9	100.0
44	103.2	37.6	99.0	100.0
74	41.8	35.8	37.0	100.0
75	90.6	39.5	68.7	100.0
Mean	63.5	56.2	82	100
SEM	7.3	6.4	12.2	0

ANOVA

Source	SS	df	MS	F	p
Foods	11590.989	3	3863.6631	7.19	0.001065
Subj	7369.9151	9	818.87945	1.52	0.1896558
Error	14503.48	27	537.16592		
Total	33464.384			Tukey's LSD	
				13.93	

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APPENDIX E

Farina

(Proximate Analysis – Rounded for Carton Copy)

FARINA

Nutrition Facts	
Serving Size	***3 tbsp (33g) makes 1 cup***
Amount/Serving	
Calories	*120*
Fat Calories	5
	%DV*
Total Fat ***0.5 g***	***1%***
Saturated Fat 0 g	***0%***
Cholesterol ***0 mg***	***0%***
Sodium ***0 mg***	***0%***
Total Carbohydrate ***25 g***	***8%***
Fiber 1 g	***5%***
Soluble Fiber 0.5 g	
Insoluble Fiber 0.5 g	
Sugars 0 g	
Protein ***4 g***	

Vitamin A	***0%***	***Vitamin C***	***0%***
Calcium	***10%***	***Iron***	***50%***
Thiamin	***10%***	***Riboflavin***	***4%***
Niacin	***6%***	***Folic Acid***	***10%***

**** Percent Daily Values (DV) are based on a 2,000 calorie diet.***

Ingredients: ***Enriched farina (farina, iron, niacin, thiamin mononitrate, riboflavin, folic acid), calcium carbonate, ferric phosphate, niacinamide, thiamin mononitrate (vitamin B_1), riboflavin (vitamin B_2), folic acid.***

For individuals with food allergies, this product contains wheat ingredients.

APPENDIX F

Psyllium Loop Cereal

Proximate Analysis (Rounded for Carton Copy) May 2, 2001

MULTI-GRAIN PSYLLIUM CEREAL
(To deliver 3.3 g Psyllium Seed Husk Fiber)

Nutrition Facts

Serving Size	*2/3 cup (22g)*
Amount/Serving	
Calories	*80*
Fat Calories	10
	%DV*
Total Fat ***1 g***	*2%*
Saturated Fat 0 g	*0%*
Cholesterol ***0 mg***	*0%*
Sodium ***150 mg***	*6%*
Total Carbohydrate ***17 g***	*6%*
Fiber 5 g	*20%*
Soluble Fiber 3 g	
Insoluble Fiber 2 g	
Sugars 2 g	
Protein ***2 g***	

Vitamin A	***0%***	***Vitamin C***	***0%***
Calcium	***0%***	***Iron***	***2%***
Thiamin	***5%***	***Riboflavin***	***5%***
Niacin	***5%***	***Vitamin B_6***	***5%***
Folic Acid	***5%***		

**** Percent Daily Values (DV) are based on a 2,000 calorie diet.***

Ingredients: ***Whole oat flour, psyllium seed husk, yellow corn meal, modified corn flour, sugar, wheat bran, wheat germ flour, salt, rice flour, baking soda, turmeric for color, citric acid, soy lecithin, niacinamide, pyridoxine hydrochloride (vitamin B_6), riboflavin (vitamin B_2), thiamin hydrochloride (vitamin B_1), folic acid.***

For individuals with food allergies, this product contains psyllium and wheat ingredients.

APPENDIX G

Psyllium Drink

Proximate Analysis (Rounded For Carton Copy) May 2, 2001

SUGAR-FREE PSYLLIUM BEVERAGE
(7.4 g Dry Powder with 230 g Water)

(To deliver 3.3 g Psyllium Seed Husk Fiber)

Nutrition Facts

Serving Size	*1 cup (240 ml)*
Amount/Serving	
Calories	*15*
Fat Calories	0
	%DV*
Total Fat ***0 g***	***0%***
Saturated Fat 0 g	***0%***
Cholesterol ***0 mg***	***0%***
Sodium ***25 mg***	***1%***
Total Carbohydrate ***6 g***	***2%***
Fiber 5 g	***20%***
Soluble Fiber 3 g	
Insoluble Fiber 2 g	
Sugars 0 g	
Protein ***0 g***	

Vitamin A	***0%***	***Vitamin C***	***10%***
Calcium	***10%***	***Iron***	***0%***

**** Percent Daily Values (DV) are based on a 2,000 calorie diet.***

Ingredients: ***Water, psyllium seed husk, maltodextrin, citric acid, natural tangerine orange flavor with other natural flavors, tricalcium phosphate, salt, sucralose (artificial sweetener), ascorbic acid (vitamin C), yellow #6 lake.***

For individuals with food allergies, this product contains psyllium ingredients.

APPENDIX H

Placebo Beverage

Proximate Analysis (Rounded for Carton Copy) May 2, 2001

SUGAR-FREE PLACEBO BEVERAGE

(2.5 g Dry Powder and Sucralose with 240 g Water)

Nutrition Facts			
Serving Size			*1 cup (240 ml)*
Amount/Serving			
Calories			5
Fat Calories			0
			%DV*
Total Fat ***0 g***			***0%***
Saturated Fat 0 g			***0%***
Cholesterol ***0 mg***			***0%***
Sodium ***15 mg***			***1%***
Total Carbohydrate ***2 g***			***1%***
Fiber 0 g			***0%***
Sugars 0 g			
Protein ***0 g***			
Vitamin A	***0%***	***Vitamin C***	***0%***
Calcium	***0%***	***Iron***	***0%***

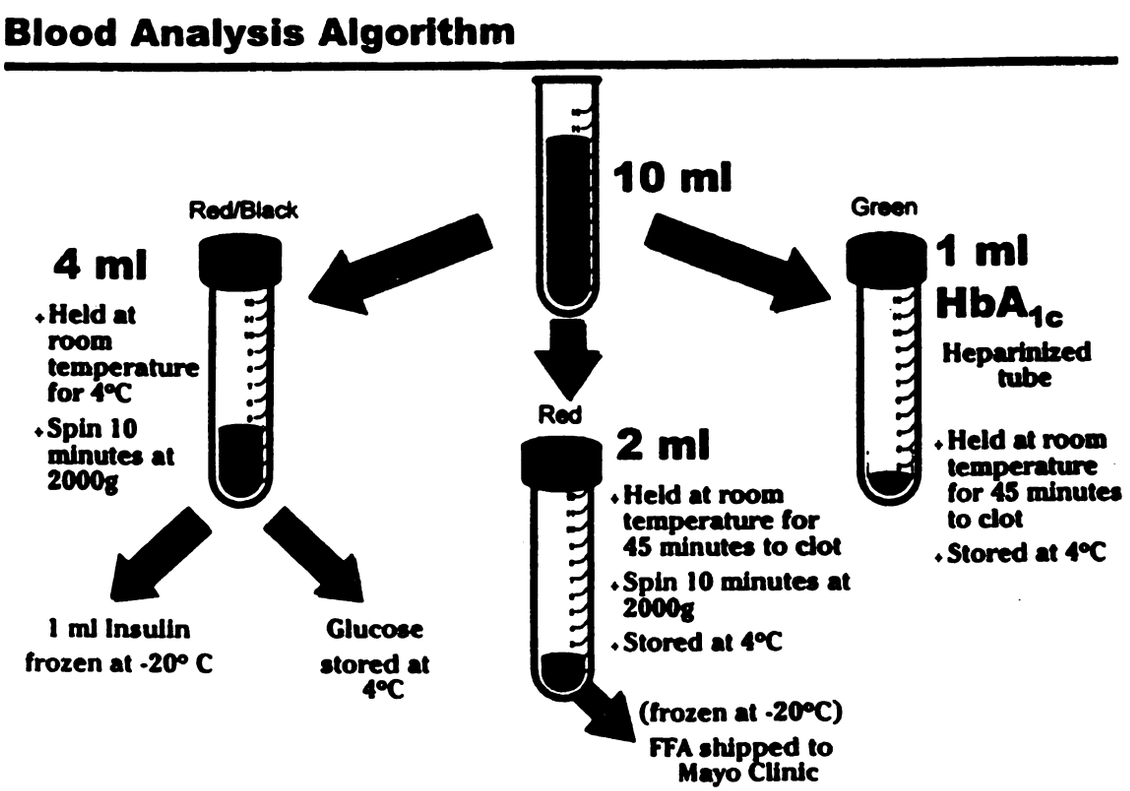
**** Percent Daily Values (DV) are based on a 2,000 calorie diet.***

Ingredients: ***Water, maltodextrin, citric acid, sucralose (artificial sweetener), sodium citrate, natural orange flavor, yellow #6 lake.***

APPENDIX I

Blood Algorithm

Figure 5.


Blood Analysis Algorithm
10 ml
Red/Black
4 ml
• Held at room temperature for 4°C
• Spin 10 minutes at 2000g
1 ml Insulin frozen at -20° C
Glucose stored at 4°C
Red
2 ml
• Held at room temperature for 45 minutes to clot
• Spin 10 minutes at 2000g
• Stored at 4°C
(frozen at -20°C)
FFA shipped to Mayo Clinic
Green
1 ml
HbA1c
Heparinized tube
• Held at room temperature for 45 minutes to clot
• Stored at 4°C

APPENDIX J

Q-Q Plot

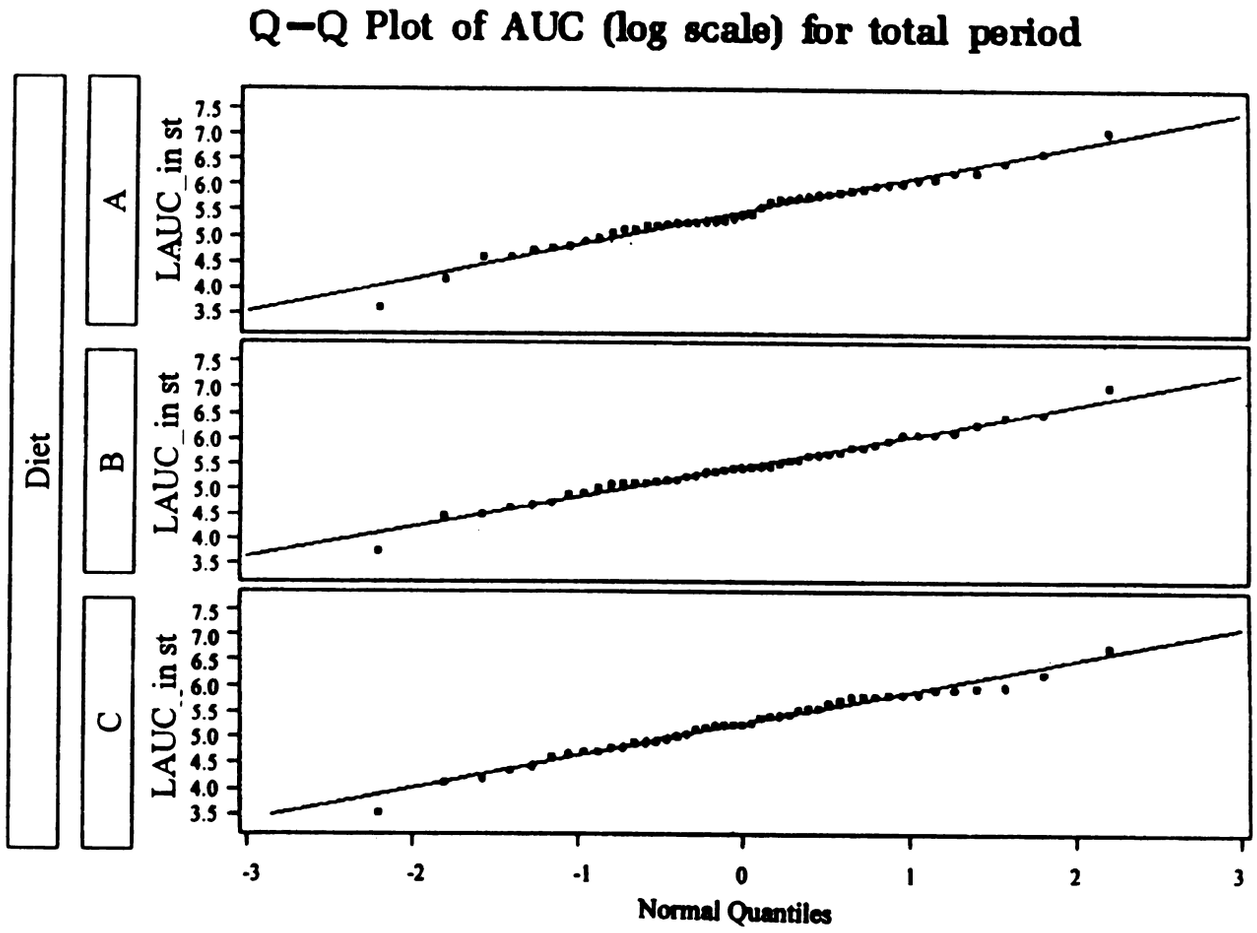


Figure 6. Example of a quantile-quantile (Q-Q) plot of log-transformed AUC for insulin for the whole period (LAUC_inst) against the quantiles of the normal distribution. A separate plot is shown for each diet. Data for glucose and free fatty acids for the whole period showed a similar profile, and thus provided assurances on normality.

APPENDIX K

Glucose, Insulin and Free Fatty Acid Concentrations

Table 13.

Mean tasting and postprandial serum glucose, insulin, and free fatty acid concentrations during the AM and PM periods in subjects with Type 2 Diabetes Mellitus (n=45) [1,2]

Time/SEM	Breakfast A			Breakfast B			Breakfast C		
Morning	Glucose (mg/dℓ)	Insulin (uU/dl)	FFA (mg/dℓ)	Glucose (mg/dℓ)	Insulin (uU/dl)	FFA (mg/dℓ)	Glucose (mg/dℓ)	Insulin (uU/dl)	FFA (mg/dℓ)
0	131	11	533	132	12	516	133	11	550
SEM	5	1	49	5	1	32	4	1	34
30	215	46	374	216	51	347	186	35	409
SEM	7	5	28	8	6	24	6	4	31
60	230	74	564	228	64	567	196	38	721
SEM	8	16	44	9	8	37	7	4	69
90	230	69	615	228	63	579	200	45	372
SEM	10	8	57	9	7	47	8	5	35
120	213	61	314	210	58	276	191	38	302
SEM	10	7	28	11	7	21	8	4	21
150	192	52	278	191	46	229	173	31	224
SEM	10	7	32	11	5	17	8	4	20
180	151	30	202	155	27	190	149	23	360
SEM	9	5	25	10	4	17	7	2	35
210	134	22	318	138	21	315	138	18	346
SEM	8	3	34	9	3	28	7	2	25
Afternoon									
285	121	23	334	121	34	324	124	23	346
SEM	6	2	24	7	8	28	6	4	25
300	137	52	677	138	40	578	143	34	727
SEM	6	14	53	7	5	46	6	4	51
330	147	44	708	146	43	669	151	40	733
SEM	6	5	52	6	4	54	6	5	60
360	149	43	742	147	45	705	150	41	762
SEM	7	4	60	6	4	58	6	5	64
390	141	45	404	140	42	348	147	47	428
SEM	6	8	30	6	5	30	7	9	40
420	131	37	403	127	41	365	133	33	391
SEM	7	4	44	6	7	30	6	3	28
450	114	24	299	112	26	272	113	31	293
SEM	6	3	21	6	3	20	6	7	23

[1] Mean values and standard errors of means at different intervals from time 0 to 450 minutes.
[2] Meal types consist of (1) Breakfast A including farina with no psyllium soluble fiber, (2) Breakfast B including farina plus a psyllium drink 20 minutes postmeal, and (3) Breakfast C including a ready-to-eat psyllium loop cereal.

APPENDIX L

Summary Comparisons for AUC Values

Table 14.

First meal response area under curve values for insulin, glucose, and free fatty acids by treatment and by week during the AM breakfast period (n=15) [1,2,3]

Variable	Week 1	Week 2	Week 3
Breakfast A			
Glucose (mg/ml)	6.53 ± 0.05	6.33 ± 0.08	6.48 ± 0.08
Insulin (mIU/ml)	4.86 ± 0.18	4.78 ± 0.20	4.93 ± 0.18
Free fatty acid (mg/dℓ)	7.00 ± 0.13	6.93 ± 0.12	7.08 ± 0.10
Breakfast B			
Glucose (mg/dℓ)	6.49 ± 0.09	6.45 ± 0.07	6.37 ± 0.08
Insulin (mIU/ml)	4.76 ± 0.1	4.95 ± 0.16	4.66 ± 0.19
Free fatty acid (mg/dℓ)	7.03 ± 0.09	7.06 ± 0.10	6.85 ± 0.09
Breakfast C			
Glucose (mg/dl)	6.36 ± 0.06	6.40 ± 0.08	6.32 ± 0.08
Insulin (mIU/ml)	4.50 ± 0.21	4.29 ± 0.15	4.63 ± 0.15
Free fatty acid (mg/dℓ)	7.22 ± 0.12	7.09 ± 0.09	7.17 ± 0.13

[1] Area under curve values above the fasting values for a 4-hour period after each meal obtained with the various treatments.
[2] No significant differences between weeks.
[3] Meal types consist of (1) Breakfast A including farina with no psyllium soluble fiber, (2) Breakfast B including farina plus a psyllium drink 20 minutes postmeal, and (3) Breakfast C including a ready-to-eat psyllium loop cereal.

Table 15.

Second-meal area under curve values for insulin, glucose, and free fatty acids by treatment and by week during the lunch period (n=15) [1,2,3]

Variable	Week 1	Week 2	Week 3
Breakfast A			
Glucose (mg/dl)	5.94 ± 0.05	5.92 ± 0.06	5.98 ± 0.09
Insulin (mIU/ml)	4.53 ± 0.16	4.42 ± 0.15	4.51 ± 0.20
Free fatty acid (mg/dℓ)	7.07 ± 0.12	7.19 ± 0.12	7.16 ± 0.10
Breakfast B			
Glucose (mg/dl)	5.91 ± 0.08	5.91 ± 0.08	5.99 ± 0.07
Insulin (mIU/ml)	4.44 ± 0.13	4.79 ± 0.17	4.33 ± 0.14
Free fatty acid (mg/dℓ)	7.10 ± 0.12	6.99 ± 0.15	7.08 ± 0.10
Breakfast C			
Glucose (mg/dl)	6.01 ± 0.07	6.00 ± 0.08	5.92 ± 0.05
Insulin (mIU/ml)	4.35 ± 0.18	4.42 ± 0.16	4.60 ± 0.17
Free fatty acid (mg/dℓ)	7.25 ± 0.09	7.08 ± 0.08	7.21 ± 0.14

[1] Area under curve values above fasting and after both breakfast and lunch meals obtained with the various treatments.
[2] No significant differences between weeks.
[3] Meal types consist of (1) Breakfast A including farina with no psyllium soluble fiber, (2) Breakfast B including farina plus a psyllium drink 20 minutes postmeal, and (3) Breakfast C including a ready-to-eat psyllium loop cereal.

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