Effects of Feeding Nutritional Formulas High in Carbohydrate or Monounsaturated Fat on Parameters of Carbohydrate and Lipid Metabolism of Patients with Non-Insulin Dependent Diabetes Mellitus

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ABSTRACT

Non-insulin dependent diabetes mellitus (NIDDM) is a common disorder of carbohydrate metabolism. The main characteristic of NIDDM is high blood glucose levels. People with NIDDM are at increased risk of microvascular (retinopathy, nephropathy and neuropathy) and macrovascular (coronary heart disease) complications. It has been found in insulin-dependent diabetic subjects that maintaining low blood glucose levels can reduce the risk of microvascular complications. Risk factors that have been identified in NIDDM subjects for heart disease have included high triglyceride levels, high levels of very-low density lipoprotein (VLDL) cholesterol and low levels of high-density lipoprotein (HDL) cholesterol. The current dietary recommendation for people with NIDDM is to consume a diet high in complex carbohydrate and fiber and low in fat. This recommendation has recently been challenged by some investigators who have suggested that high monounsaturated fat diets may be better.

Sixteen people with NIDDM who were in moderate metabolic control and had low to moderate blood lipid levels were randomized to receive either Ensure with Fiber® (high carbohydrate) or Glucerna® (high monounsaturated fat). The diets were followed for 28 days, with an average of about 90% of energy coming from the formula. Subjects performed finger prick blood glucose monitoring at home before and 2 hours after each meal for two days each week. Fasting blood samples were taken from the subjects on days 0, 7 and 28, and were used to measure selected indices of carbohydrate and lipid metabolism. It was found that the consumption of Glucerna® resulted in lower postprandial rises in blood glucose when compared to Ensure with Fiber® (p=0.000). Statistical analysis using
repeated measures ANOVA revealed significant time effects, in which a decrease in serum glucose (p=0.000), and plasma glucagon (p=0.002) were found. A different pattern of change between the groups was noted for plasma insulin levels (p=0.028) in which the Ensure with Fiber® group displayed a decrease in insulin over time. No significant effects were found for plasma triglycerides, total cholesterol or apo B cholesterol. There was a different pattern of change for HDL cholesterol between the two groups (p=0.004), explained by a significant decrease in the Glucerna® group. This result may be confounded by the significantly different baseline levels of HDL cholesterol between the two groups.

Overall, this study found that the only advantage of the high monounsaturated fat, Glucerna® diet over the high carbohydrate, Ensure with Fiber® diet, was the lower rise in postprandial blood glucose levels. This study found no benefit of Glucerna® on other indices of carbohydrate metabolism or on indices of lipid metabolism. More research is needed to understand the metabolic effects of high carbohydrate and high monounsaturated fat diets in NIDDM subjects.
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CHAPTER ONE
INTRODUCTION

RATIONALE

Non-insulin dependent diabetes mellitus (NIDDM) is a common disorder of carbohydrate metabolism. Most NIDDM patients have chronically high blood glucose levels and an altered lipid profile which includes elevated triglycerides and very-low density lipoprotein (VLDL) cholesterol and reduced high density lipoprotein (HDL) cholesterol (Reaven, 1987). As a result of these metabolic alterations, the diabetic population is at an increased risk for microvascular (nephropathy, retinopathy and neuropathy) and macrovascular (coronary heart disease) complications (Ross et al, 1992). Maintaining normal blood glucose levels may help to prevent the microvascular complications of diabetes (Diabetes Control and Complications Trial Research Group, 1993). Thus, control of blood sugars is a primary goal of the diabetic diet. As coronary heart disease is also a major concern in diabetes, the optimal diabetic diet should also aid in minimizing risk factors for this condition. In NIDDM patients, these risk factors include high triglycerides and VLDL cholesterol, and low HDL cholesterol (Laakso et al, 1993).

The current dietary recommendation for people with NIDDM is a diet high in complex carbohydrate and low in fat, especially saturated fat (Canadian Diabetes Association, 1989). Recent interest in diets high in monounsaturated fat has arisen from the results of studies which found high monounsaturated fat diets resulted in lower acute blood glucose levels and lower fasting triglycerides and VLDL cholesterol levels when compared
to diets high in carbohydrate. Higher HDL cholesterol levels have also been reported after consumption of high monounsaturated fat diets.

Nutritional formulas are designed as liquid diets providing complete nutrition. They can be used for enteral feeding or as oral supplements to the diet. Ensure with Fiber® (Abbott Laboratories, Columbus, Ohio) is a nutritional formula designed for the general population. It has a composition similar to that currently recommended for NIDDM patients; it provides 55% of calories from carbohydrate and 30.5% from fat. The carbohydrate is composed of hydrolyzed corn starch and sucrose. Soy polysaccharide is the source of dietary fiber. Fiber is included in the name of the product because Ensure® is also available without fiber. The fat source is high in polyunsaturated fatty acids. Linoleic acid (18:2n-6) represents 56.6% of the total fatty acids in Ensure with Fiber®.

Glucerna® (Abbott Laboratories, Columbus, Ohio) is a formula specially designed for the nutritional support of patients with abnormal glucose tolerance. It provides 33% of calories from carbohydrate and 50% from fat. The carbohydrate is composed of glucose polymers and fructose. Fructose has been shown to produce lower postprandial glucose and insulin responses than sucrose. Although it is not mentioned in the name of the product, Glucerna® also contains soy polysaccharide as a source of fiber. The amount of fiber in one can (235 ml) of Glucerna® is the same as in one can (235 ml) of Ensure with Fiber®. The fat source is high in monounsaturated fatty acids. Oleic acid (18:1n-9) represents 64.7% of the total fatty acids in Glucerna®.
PURPOSE AND OBJECTIVES
The purpose of this study was to assess the short term (7 days) and long
term (28 days) effects of feeding Ensure with Fiber® and Glucerna® at a
minimum intake of 80% of total energy requirements on indices of
carbohydrate and lipid metabolism in outpatients with NIDDM.

Specific objectives included:
1. To determine the effects of Ensure with Fiber® and Glucerna® on
   serum glucose and fructosamine, and plasma insulin and glucagon
   levels.

2. To determine the effects of Ensure with Fiber® and Glucerna® on
   plasma triglyceride and cholesterol levels (total cholesterol, HDL
   cholesterol and apoB cholesterol), the levels of oleic (18:1n-9) and
   linoleic (18:2n-6) fatty acids in plasma triglycerides, and the
   correlation of change in these fatty acids in plasma triglycerides to
   the change in cholesterol levels.
### NULL HYPOTHESES

Dietary intakes of 80% or more of total energy from Ensure with Fiber® or Glucerna® for 28 days by outpatients with NIDDM will not result in differences between groups in:

i) **indices of carbohydrate metabolism:**
   (levels of postprandial blood glucose, fasting serum glucose and fructosamine, and fasting plasma insulin and glucagon), or

ii) **indices of lipid metabolism:**
   (levels of fasting plasma triglycerides, total cholesterol, HDL cholesterol, apo B cholesterol, and levels of oleic acid (18:1n-9) and linoleic acid (18:2n-6) in plasma triglycerides)

Also, there will be no correlation between the levels of oleic acid (18:1n-9) and linoleic acid (18:2n-6) incorporated into plasma triglycerides, and the change in plasma cholesterol levels.
CHAPTER TWO
LITERATURE REVIEW

NORMAL CARBOHYDRATE AND LIPID METABOLISM

Carbohydrate Metabolism

Carbohydrate metabolism is regulated to provide glucose to tissues and to keep blood glucose at a desired level. Both low (hypoglycemia) and high (hyperglycemia) blood glucose levels are undesirable. The normal range for fasting blood glucose is 3.9 to 6.7 mmol/L (Malarkey and McMorrow, 1996) and blood glucose levels increase after a meal. Regulation of glucose metabolism is primarily mediated by hormones. Carbohydrate metabolism has been described by Berne and Levy (1993) and is summarized below. The major anabolic hormone is insulin, while the major catabolic hormone is glucagon. Other hormones such as cortisol and epinephrine provide minor catabolic effects. Both insulin and glucagon are produced in the pancreatic Islets of Langerhans, insulin in the β-cells and glucagon in the α-cells. Ingested carbohydrate is broken down to simple carbohydrate (primarily glucose), and absorbed into the blood stream. During fasting, glucose is provided by the breakdown of glycogen, stored in the muscle and liver, and by production of glucose in the liver.

The uptake of glucose into cells has been described by Bell et al, (1990). Except for the intestine and kidney, glucose is taken up into cells by facilitative glucose transport. Five different facilitative glucose transporters have been identified and are referred to as Glut 1-5. Glut 4 is the only one of these transporters that is insulin dependent, and is found primarily in skeletal muscle and adipose tissue.
During the fed state nutrients are absorbed from the gut into the bloodstream, and in response, insulin is released from the pancreas. A combination of neurohumoral agonists act to stimulate insulin secretion. Cholecystokinin, acetylcholine, and gastric inhibitory polypeptide act to enhance insulin secretion when plasma glucose is >6 mmol/L, but not when it is <4 mmol/L (Rasmussen et al, 1990). An increase in plasma glucose to 8-10 mmol/L causes enhanced insulin secretion in the absence of any of these neurohumoral agonists. Insulin circulates in the bloodstream and binds to receptors located on cell membranes. The insulin receptor is made up of two identical α and β subunits (Maron et al, 1984). The extracellular α subunit binds to the insulin molecule, which then sends a signal to the transmembrane and cytoplasmic β subunit. This β subunit contains intrinsic tyrosine kinase activity and undergoes autophosphorylation (Kahn et al, 1988). As a result, a cascade of phosphorylation events occurs that drives the actions of insulin. One of the major actions of insulin is to facilitate glucose transport into cells, the most predominant ones being skeletal muscle and adipose cells. The Glut 4 transport protein is contained in intracellular vesicles and upon stimulation of the cell by insulin, these transport proteins are brought to the cell membrane where they allow uptake of glucose (Birnbaum, 1992). The exact cellular mechanisms by which this occurs have yet to be determined. One of the major ways in which insulin exerts its other effects is by activation or deactivation of certain enzymes, as a result of the cascade of phosphorylation. Among these are the enzymes that regulate glycogen synthesis for the purpose of glucose storage. Insulin causes activation of glycogen synthetase and inactivation of glycogen phosphorylase, resulting in synthesis and storage of glycogen (Dent et al, 1990). Other effects of insulin include inhibition of adipose tissue lipolysis, muscle
proteolysis, and hepatic glycogenolysis and gluconeogenesis (Berne and Levy, 1993). All of these effects reflect the anabolic nature of the hormone insulin.

In the fasted state, when glucose is not readily available from the intestine, metabolism shifts towards catabolism. Glucagon is the major catabolic hormone and its actions are contrary to those of insulin. Glucagon is released from the α cells of the pancreatic islets in response to low blood glucose levels (Gerich, 1976). Glucagon promotes mobilization of glucose and increases blood glucose concentration. It acts primarily in the liver to stimulate glycogenolysis (Jaspan et al, 1977), gluconeogenesis (Cahill, 1970), and fatty acid oxidation (McGarry et al, 1976).

In summary carbohydrate metabolism is well regulated by hormones, the major ones being insulin and glucagon, to provide glucose to tissues and to keep blood glucose levels within normal limits.

Lipid Metabolism

Lipid metabolism involves a regulation of exogenous and endogenous lipids, including triglycerides, free fatty acids, free cholesterol, cholesterol esters and phospholipids. These substances are delivered to the tissues for energy, storage and structural and metabolic use. Lipid transport is regulated by lipoproteins, lipoprotein receptors, lipolytic enzymes and transfer proteins, which act together to maintain the balance of cholesterol and triglyceride in tissues and plasma.

The major storage site of triglyceride within the body is adipose tissue. The regulation of fatty acids in and out of adipose tissue has recently been described by Frayn et al, (1995) and is summarized below. Triglycerides of both exogenous and endogenous origin are delivered to adipose tissue by chylomicrons and very low density lipoproteins. Lipoprotein lipase (LPL) is
synthesized within the adipocyte and then exported to the luminal surface of the capillary endothelium. LPL interacts with the triglycerides and catalyzes the removal of the fatty acids from the glycerol backbone, with the subsequent transfer into adipose tissue or plasma. The fatty acids transferred into the adipocyte are then esterified and stored as triglyceride. Insulin may play a role in the activation of LPL and enhancement of esterification of the subsequently released fatty acids, allowing them to be stored within the adipocyte. Hormone sensitive lipase (HSL) is present within the adipocyte and acts to release fatty acids and glycerol from the adipose cell into the circulation. Insulin may inhibit HSL. In the fed state there is a net flux of fatty acids into the adipose cell, while in the fasted state there is a net flux of fatty acids out of the adipose cell. The free fatty acids released into the plasma travel bound to albumin. They can be taken up by other tissues, such as skeletal muscle, for energy, or they may be delivered to the liver where they may be esterified with glycerol to produce triglycerides, which then may be assembled into VLDL particles. In this way, triglycerides can be stored during times of excess and released in times of need for energy purposes.

Fat, including triglycerides, phospholipids and cholesterol, is insoluble in an aqueous environment. Thus, it must be transported in plasma particles called lipoproteins. Lipoproteins contain triglyceride and esterified cholesterol in the core, with protein and free cholesterol imbedded into the phospholipid outer layer. The presence of the polar head groups of the phospholipids give lipoproteins solubility in the aqueous environment of plasma and allows triglyceride and cholesterol to be transported in the blood. Lipoproteins are often classified by density or electrophoretic mobility. The names and density classifications of lipoproteins most commonly used are:
chylomicrons with a density <0.094 g/ml, very low density lipoproteins (VLDL) with a density of 0.94-1.006 g/ml, low density lipoproteins (LDL) with a density of 1.006-1.063 g/ml, and high density lipoproteins (HDL) with a density of 1.063-1.21 g/ml (Redgrave, 1975).

Chylomicrons are synthesized in the intestinal cells to transport dietary fat in the blood. The core of the chylomicron contains predominately triglyceride, with some cholesteryl ester as well. The relative composition can vary and will depend on the fat composition of the diet. In their review of chylomicron assembly and secretion, Hussain et al, (1996) noted that apo B 48 is the major apolipoprotein in chylomicrons and is required for chylomicron assembly. Nascent chylomicrons also contain apo C-II, apo C-III, apo A-I, and apo A-II (Wu et al, 1978). As the chylomicrons travel in the plasma, they interact with lipoprotein lipase, located on the capillary endothelial cell membranes. Apo C-II is a cofactor for lipoprotein lipase, and thus necessary for lipolysis of the chylomicron (Hussain et al, 1996). LPL cleaves fatty acids from the triglycerides and transfers them into the cells. As the chylomicron particle is progressively depleted of its triglyceride core, apo A-I and apo A-II are lost from the chylomicron, and possibly transferred to HDL. The chylomicron remnants, depleted of nearly all their triglyceride, acquire apolipoprotein E which aids in its catabolism (Mahley, 1988). At the liver the LDL receptor related protein (LPR) binds specifically with apo E and causes internalization of the remnant particle (Beisegel et al, 1989). The LDL receptor is also involved in the hepatic uptake of chylomicron remnants (Choi et al, 1991). In his review of lipoprotein metabolism, Illingworth (1993) concluded that chylomicrons are rapidly metabolized with a plasma half life of 5-15 minutes. The result of chylomicron metabolism is to deliver dietary
triglycerides to the tissues for energy or storage, and to deliver dietary cholesterol to the liver.

VLDL particles are the primary carriers of endogenous triglyceride from the liver to the tissues, and also carry cholesterol esters in their core. Apolipoprotein B100 is necessary for the formation of VLDL. Each particle contains one apo B100 protein (Elovson et al, 1988). The levels of apo B mRNA do not reflect the changes in apo B secretion, thus it would appear the apo B production is not mediated at a transcriptional level (Pullinger et al, 1989). It is likely that the degradation of apo B is the major factor controlling how much of the protein is secreted (Frukawa et al, 1992). The apo B protein associates with lipids to form VLDL particles. In its core it contains triglycerides and cholesterol esters, of which triglycerides are the major component. The cholesterol can be synthesized in the liver or obtained by the liver by the uptake of remnant lipoproteins. Similarly, fatty acids may be synthesized or delivered to the liver. These fatty acids provide the substrate for hepatic triglyceride synthesis. The synthesis of triglyceride in the liver depends, to a large extent, on the metabolic hormonal status. Glucagon shifts metabolism of fatty acids away from esterification inducing their mitochondrial uptake and oxidation (Tiengo et al, 1977). In the postprandial state, insulin stimulates triglyceride synthesis. This was concluded by Sparks et al (1994) in their review of how insulin affects triglyceride rich lipoproteins. Although insulin stimulates lipogenesis, the acute presence of insulin acts to decrease VLDL secretion from the liver by enhancing intracellular degradation of apo B (Sparks et al, 1990). During transit in plasma, VLDL may transfer some of its triglyceride to HDL in exchange for cholesteryl esters, causing the VLDL particle to become cholesterol enriched (Tall, 1993). This process is mediated by the enzyme
cholesterol ester transfer protein (CETP). As VLDL particles are secreted into the plasma, they acquire apo C-II. Apo C-II is required for association with lipoprotein lipase. As the triglycerides are cleared from the core of the VLDL particle, apo C-II is then lost. After much of the triglyceride has been cleared from its core, the particle may follow one of two pathways. First, the VLDL remnant can be taken up by the liver by binding to the LDL receptor protein or the LRP. It has been suggested by Sehayek et al (1991) that the process of lipolysis may expose endogenous apo E associated with VLDL, thus making it susceptible to catabolism by the liver. VLDL particles are normally cleared more slowly than chylomicrons with a half life of 6 to 12 hours (Illingworth, 1993). The second alternative for the particle is to form an intermediate density lipoprotein (IDL) which is enriched in cholesteryl ester, and is then further transformed to become an LDL particle.

The metabolism of LDL has been described in the review by Illingworth, (1993). Since LDL particles are formed from VLDL after removal of triglyceride, the major apolipoprotein associated with them is also apo B-100. LDL particles are the major cholesterol carrying particles in the plasma. They deliver cholesterol from the liver to the peripheral tissues, where it is needed as a structural component of cell membranes. The catabolism of LDL can occur in the peripheral or hepatic tissues. The uptake of LDL occurs through the LDL receptor and results in the suppression of endogenous cholesterol synthesis, increased cholesteryl esterification and a reduced number of LDL receptors on the cell surface. Thus, this allows the cells to obtain the cholesterol they need in the proper amounts. The result of apo B-100 containing lipoproteins is that VLDL delivers triglyceride to the tissues, after which some of the VLDL remnants are converted to LDL which
LITERATURE REVIEW

is responsible for the forward cholesterol transport from the liver to the extrahepatic tissues.

Reverse cholesterol transport from the peripheral tissues to the liver is performed by HDL particles. The metabolism of HDL particles is very complex. HDL metabolism has been reviewed recently by Barrans et al, (1996) and is described below. Apo A-I is the major protein associated with HDL particles. The assembly of nascent HDL particles is not fully understood. It is possible that free apo A-I, secreted from the intestine and the liver may acquire lipids once they are in circulation. Pre-β HDL consist primarily of apo A-I and phospholipids, and are considered to be the first acceptors of cellular cholesterol (Castro et al, 1988). HDL particles obtain free cholesterol from the peripheral tissues through a process mediated by the enzyme lecithin:cholesterol acyl transferase (LCAT). LCAT catalyses the transfer of a fatty acid from lecithin to the hydroxyl residue on cholesterol, forming lysolecithin and cholesterol ester. In this manner, cholesterol can be picked up from cell membranes and deposited within the core of the HDL particle. As the HDL particle is filled with cholesterol ester, it gets bigger and more buoyant. Cholesterol esters can be transferred out of HDL by the enzyme cholesterol ester transferase protein (CETP) which allows exchange of core lipids between lipoproteins (Tall, 1993). It transfers cholesteryl esters from HDL to triglyceride rich particles such as VLDL, and in exchange, triglycerides may be transferred to HDL. In plasma, most of the CETP is found associated with HDL (Pattnaik et al, 1979). Hypertriglyceridemia can result in triglyceride enrichment of HDL due to an increased transfer of cholesterol ester and triglyceride between HDL and other lipoproteins (Tall, 1993). The composition of HDL can vary as it acquires cholesterol from tissues, delivers cholesterol to other lipoproteins and acquires triglycerides
from other lipoproteins. Triglycerides can be cleared from HDL by hepatic lipase. It is not clear how HDL is catabolised, but its cholesterol esters can be delivered to the liver.

Studies have shown that variations in lipid metabolism and plasma lipoproteins can affect the risk of atherosclerosis, which develops from cholesterol deposited in the arterial wall causing narrowing of the vessels. LDL particles play a major role in this process. The LDL particles associate with the arterial wall, where they are oxidized, making them susceptible to uptake by macrophages, and the formation of foam cells (Grundy, 1995). Accumulation of this LDL cholesterol within the arterial wall causes a fatty streak, which can later become a fibrous plaque. This proposed mechanism of pathology may explain why high levels of LDL cholesterol are independently and positively associated with increased coronary artery disease rates (Castelli, 1984). In contrast, HDL cholesterol is negatively associated with coronary artery disease (Castelli, 1984). This protective effect of HDL cholesterol may be partly due to its function in reverse cholesterol transport. This is not its only effect, however, as HDL has also been shown to protect LDL from oxidation (Pathasarathy et al, 1990), and it may have other functions that have yet to be discovered. Triglyceride levels have been suggested as a risk factor for atherosclerosis. However, the fact that it is cholesterol that accumulates in the atherosclerotic plaque, and not triglyceride, has led some investigators to question if triglycerides have a direct role in atherosclerosis. Speculation has been made, however, as to the possible contribution of triglyceride levels. One possible role it may have is that high triglyceride levels may promote the exchange of triglyceride for cholesterol ester from HDL, thus reducing the HDL cholesterol which has been shown to be protective against atherosclerosis (Tall, 1993). Another
possible action that high triglyceride levels may have in enhancing risk for coronary heart disease is through involvement in LDL metabolism. Shepherd, et al (1996) have proposed that when hepatic triglyceride levels are high, large triglyceride rich particles are formed which are delipidated into smaller VLDL remnants and smaller LDL particles. It has been demonstrated in vitro that smaller LDL particles have a distorted conformation of apo B that is less able to bind to receptors (Kleinman et al, 1987) but is more readily oxidized than larger particles (De Graaf et al, 1991), thus promoting the development of atherosclerosis. Therefore, it appears that high concentrations of LDL cholesterol, low concentrations of HDL cholesterol and possibly elevated triglyceride levels and small LDL particles may contribute to the risk of developing coronary artery disease.

DESCRIPTION OF DIABETES MELLITUS

Diabetes Mellitus is a disorder of carbohydrate metabolism. Insulin is either not available or not effective, resulting in elevated blood glucose levels. A diagnosis of diabetes mellitus can be made when one of the following is present (American Diabetes Association, 1993):

1) Unequivocal elevation of plasma glucose $\geq 200 \text{ mg/dl (11.0 mmol/L)}$ and classic symptoms of diabetes, including polydipsia, polyuria, polyphagia, and weight loss.

2) Fasting plasma glucose $\geq 140 \text{ mg/dl (7.7 mmol/L)}$ on two occasions.

3) Fasting plasma glucose $<140 \text{ mg/dl (7.7 mmol/L)}$ and two 75g oral glucose tolerance tests with the 2-hour plasma glucose $\geq 200 \text{ mg/dl (11.0 mmol/L)}$ and one intervening value $\geq 200 \text{ mg/dl (11.0 mmol/L)}$. 
The two major classifications of diabetes mellitus are insulin dependent diabetes mellitus (IDDM) and non-insulin dependent diabetes mellitus (NIDDM) (American Diabetes Association, 1993). Insulin dependent diabetes mellitus, also called Type-I or juvenile onset diabetes, is characterized by a complete lack of insulin production. The β-cells of the pancreas no longer produce insulin and exogenous insulin must be administered for survival. The onset of insulin-dependent diabetes mellitus is usually, but not exclusively, in childhood. Non-insulin dependent diabetes mellitus, also called Type-II or adult onset diabetes, is characterized by reduced insulin sensitivity and/or reduced secretion of insulin. The pancreas continues to produce some insulin, thus exogenous insulin is generally not required for survival. Insulin may, however, be administered to some patients to aid in blood glucose control. Non-insulin dependent diabetes mellitus usually has its onset in mid to late life. Impaired glucose tolerance is not a type of diabetes, yet it deserves mention at this point. With this condition blood glucose levels are above normal but not elevated to the level required for a diagnosis of diabetes. Impaired glucose tolerance of an individual may improve, stay the same, or worsen to develop into clinical diabetes.

The Canadian Diabetes Association has estimated that one in 20 Canadians has diabetes and that over 60,000 new cases are diagnosed each year in Canada (Canadian Diabetes Association, 1990). This same publication also indicated that the annual cost of diabetes to the Canadian Economy is more than $2.5 billion. Further information was published in 1994 by the Canadian Diabetes Association, which estimated that there were 1.4 million people with diabetes in Canada, which was equal to about 5% of the population, and that over 80% of these have NIDDM. It was also stated
that complications from diabetes make it the third major cause of death by disease in Canada, after heart disease and cancer. In addition, in 1994 it was estimated that over 100 million people have diabetes worldwide (Weiland 1994). Thus, it is evident that diabetes affects a large number of people.

**CARBOHYDRATE AND LIPID METABOLISM IN NIDDM**

**Carbohydrate Metabolism**

As previously stated, high blood glucose, or hyperglycemia, is the primary characteristic of NIDDM. Both fasting and postprandial glucose levels are above normal in most NIDDM patients (Ross et al, 1992). Measures of long term glucose concentrations are also usually elevated. Examples of these include hemoglobin A1C (HgbA1C) and fructosamine. Both of these are measures of the glycosylation of proteins. The hemoglobin protein remains in the blood stream for about 4 months, thus the glycosylation of it is indicative of the average blood glucose level for that period (Goldstein et al, 1995). Fructosamine is a collective term which refers to all the glycated serum proteins, the major one being albumin. These serum proteins remain in the circulation for one to three weeks, thus fructosamine is representative of the average blood glucose level for that period (Desjarlais et al, 1989).

The hyperglycemia found in NIDDM arises from several contributing factors. First, an increase in hepatic glucose production has been found in NIDDM patients with fasting hyperglycemia (Kolterman et al, 1981). Secondly, there is decreased insulin mediated glucose uptake in target tissues, the primary one being skeletal muscle (Kolterman et al, 1981). Finally, in many NIDDM subjects insulin secretion following a meal is
impaired (Reaven et al, 1977), and in addition, glucagon secretion may be increased (Unger et al, 1970).

Increased hepatic glucose production in NIDDM patients has often been observed. This may be a result of decreased sensitivity to the suppression of glucose production by insulin, to high glucagon levels, or to high levels of gluconeogenic precursors entering the liver. It has been demonstrated in diabetic rabbits that immunoneutralization of glucagon led to a dramatic decrease in plasma glucose (Brand et al, 1996). This suggests that glucagon plays a major role in the maintenance of fasting hyperglycemia in diabetes. The release of lactate and alanine from the skeletal muscle and glycerol from the adipose tissue is increased in NIDDM patients and the fractional conversions of lactate and glycerol to glucose are also increased (Consoli et al, 1990; Nurjihan et al, 1992). These findings show that there are increased gluconeogenic substrates available to the liver in NIDDM patients and that the liver is converting these at higher rates than normal to glucose.

Decreased insulin-mediated uptake of glucose into tissues also contributes to the hyperglycemia of NIDDM. This is often referred to as insulin resistance. The mechanism for this defect has been partially explored, although there is likely still much more that remains to be discovered. Insulin receptors isolated from NIDDM patients have reduced autophosphorylation-kinase activity when isolated from adipose cells (Freidenberg et al, 1987) and skeletal muscle (Caro et al, 1987). This defect may possibly result in decreased translocation of the Glut 4 glucose transporter protein to the membrane, thus resulting in less uptake of glucose. It has also been shown that this defect in adipocyte cells can be reversed when there is improvement in glycemia due to weight loss.
(Freidenberg et al, 1988). Based on this it could be proposed that hyperglycemia itself can contribute to insulin resistance. In fact, there is some evidence from animal models that incubation of cells under hyperglycemic conditions produces a state of insulin resistance (Muller et al, 1991). Although resistance to the glucose uptake aspect of insulin's action has received most of the attention in diabetes research, it has also been proposed that other actions of insulin may be compromised in NIDDM patients. Ronald Kahn has suggested that the term insulin resistance should be considered in broad terms as “existing whenever normal concentrations of insulin produce a less than normal biologic response” (Kahn, 1978).

NIDDM patients may have either normal or elevated fasting insulin levels, but insulin secretion following a meal is usually impaired. As the condition worsens β-cell function can deteriorate and even fasting insulin levels may be low. This is usually seen only when fasting glucose levels exceed 13.9 mmol/L (Ross et al 1992). This decreased postprandial insulin secretion contributes to the hyperglycemia of many NIDDM patients. The concept of glucose toxicity has received considerable attention by diabetes researchers. It has been observed that the primary mediators (cholecystokinin, acetylcholine, gastric inhibitory peptide, hyperglycemia) that enhance insulin secretion over brief periods, suppress secretion if present for long periods. The β-cell becomes less sensitive to these signals (Rasmussen et al, 1990). This was demonstrated in isolated pancreatic tissue which was perfused continuously with a high glucose concentration, resulting in reduced insulin secretion (Grodsky, 1989). Thus, chronically high glucose levels can make the β-cells less sensitive to stimulation, resulting in reduced insulin secretion. It has been proposed that efficient insulin secretion
requires a balanced pattern of signals and that they must be periodic rather than continuous (Rasmussen et al, 1990).

The fasting insulin concentration in patients with diabetes is often variable, being either normal or high. In patients with impaired glucose tolerance, a condition which may deteriorate and lead to NIDDM, fasting plasma insulin is typically high. Laakso et al (1990) found that in a group of subjects, those with normal glucose tolerance had similar fasting plasma insulin levels to those with NIDDM. However, subjects with impaired glucose tolerance had significantly higher fasting plasma insulin levels. The high insulin concentrations found in patients with impaired glucose tolerance may be indicative of decreased insulin sensitivity being compensated for by increased insulin secretion. Because of this compensation, blood glucose levels were not high enough for diagnosis of diabetes. The group of NIDDM patients in Laakso's study may have had normal fasting insulin levels because their β-cells could no longer maintain this high output of insulin. Since insulin resistance still remained, blood glucose levels were high and allowed for diagnosis of clinical diabetes. It has been hypothesized (Garg et al, 1992) that persistent hyperglycemia may lead to a reduction in insulin secretion, enhanced glucagon secretion and perhaps insulin resistance, which in turn may worsen the hyperglycemia.

In summary, the disordered carbohydrate metabolism in NIDDM is a complex issue, involving several contributing factors including increased hepatic gluconeogenesis, decreased glucose uptake into tissues and impaired insulin secretion, particularly following a meal. As a result of this disordered metabolism, hyperglycemia often develops, which itself may perpetuate the disorder by decreasing β-cell responsiveness and possibly insulin sensitivity.
Lipid Metabolism

The plasma lipid profile is typically altered in NIDDM patients. These abnormalities have been reviewed by several authors, including Reaven (1987), Garg (1990), Dunn (1992) and Howard (1993). High concentrations of plasma triglycerides, and low concentrations of HDL cholesterol are often present. The levels of total cholesterol and LDL cholesterol of diabetic patients are usually similar to the general population.

Hypertriglyceridemia is very common in NIDDM with a reported 1.5 - 2 fold increase in triglycerides in people with diabetes compared to non-diabetic controls matched for age, sex and weight (Barrett-Connor et al, 1982; Brionnes et al, 1984). In fact, it has been observed that plasma triglyceride levels tend to increase with early changes in glucose metabolism, even before the patient has diabetes symptoms (Laakso et al, 1989). The hypertriglyceridemia is mainly due to increased VLDL-TG as a result of overproduction of triglyceride (Abrams et al, 1982), possibly due to increased uptake of glucose and free fatty acids in the liver. There is some evidence that the usual suppressive effect of insulin on the release of free fatty acids from adipose tissue is not optimally effective in NIDDM, (reviewed by Krentz and Nattrass, 1995), allowing increased flow of fatty acids to the liver. Although acute insulin release has an inhibitory effect on VLDL production due to increased degradation of apo B, chronic hyperinsulinemia, as seen in insulin resistant NIDDM patients, might override this effect (Lewis et al, 1993) such that apo B production is not inhibited. Thus, the increased apo B production combined with the lipogenic action of insulin and the increased availability of lipogenic substrates, may allow increased synthesis and secretion of VLDL and hence increased plasma triglyceride levels in individuals with NIDDM.
The HDL cholesterol levels of NIDDM patients are usually 25-30% lower than the levels of non-diabetic age and sex matched controls (Barrett-Connor et al, 1983). It has been observed through measurements of apo A-I kinetics that there is increased HDL clearance in NIDDM patients (Golay et al, 1987). It has also been suggested that non-enzymatic glycosylation of HDL apo A-I can accelerate its catabolism (Witzum et al, 1982). The HDL particles of people with NIDDM tend to be higher in triglyceride and lower in cholesterol compared to HDL particles of the general population (Syvanne et al, 1995). This is very likely a result of the hypertriglyceridemia found in NIDDM. In hypertriglyceridemia, the CETP mediated exchange of cholesterol and triglyceride between HDL and VLDL results in increased triglyceride concentration in the HDL particles and decreased HDL cholesterol concentration (Tall, 1993). Thus, hypertriglyceridemia is often associated with increased VLDL cholesterol and decreased HDL cholesterol.

Even though the plasma levels of LDL cholesterol are often normal in individuals with NIDDM (Barrett-Connor et al, 1983), the characteristics and metabolism of this particle may be altered. The LDL particles of people with NIDDM are smaller and denser than those found in the general population (Barakat et al, 1990). These abnormal LDL particles may contribute to the high risk of coronary heart disease in the diabetic population because they are less able to bind to receptors (Kleinman et al, 1987) and are more readily oxidized than larger particles (De Graaf et al, 1991). As reviewed by Dunn (1992) there appears to be decreased conversion of VLDL to LDL in NIDDM patients, and a decreased clearance of LDL by hepatic receptors, with the net result being a normal plasma level of LDL. From this, it appears that these particles remain in the plasma longer than normal and may have greater
opportunity for oxidation and possible development of coronary artery disease.

Some investigators have shown these lipoprotein abnormalities to be associated with hyperinsulinemia (Burchfield et al, 1990; Modan et al, 1988). Laakso et al (1990) suggested that the association of hyperinsulinemia with lipid abnormalities may be due to insulin resistance rather than hyperinsulinemia itself. Using a euglycemic clamp these investigators demonstrated that the decreased HDL cholesterol, and the increased VLDL triglyceride were associated with impaired insulin mediated glucose uptake (insulin resistance) regardless of the glucose tolerance status and independent of the fasting insulin level. There are three possible explanations for this association of insulin resistance and lipid levels (Reaven et al 1987). First, insulin resistance may cause changes in the lipid levels. Second, abnormal lipid levels could induce an impairment in insulin action. Third, the association of insulin resistance and abnormal lipid levels may be caused by other factors that influence both. There is some evidence to suggest that insulin resistance contributes to these lipid abnormalities, by increasing plasma triglyceride levels, which in turn may contribute to lower HDL cholesterol and perhaps the formation of small dense LDL particles. However, there is still much that needs to be understood about the mechanisms leading to these lipid abnormalities in NIDDM.

HEALTH RISKS OF NIDDM

The health risks of NIDDM are generally divided into two categories: microvascular and macrovascular (Ross et al, 1992). The former category includes diabetic complications such as nephropathy, neuropathy and retinopathy. The latter category describes cardiovascular disease and is the
main cause of death in this population. Statistical information regarding diabetes around the world (Weiland, 1994) states that half or more of all leg amputations are due to diabetes, that diabetes is the leading cause of kidney disease in Europe and the USA, the leading cause of blindness in the middle aged population in Europe, that diabetes more than doubles the risk of heart attack or stroke and is a major cause of impotence in men of all ages.

The Diabetes Control and Complication Trial (DCCT) Research Group (1993) demonstrated that by keeping blood sugars low, the development and progression of retinopathy, nephropathy and neuropathy was reduced in IDDM patients. Over the seven year period there was a 60% reduction in risk for these complications in the intensively treated group. No trial of this kind has been done in NIDDM, but the results might be applicable to this group since the complications observed are the same in both IDDM and NIDDM. In fact, in the Canadian Diabetes Association position statement based on the DCCT (1993), NIDDM patients are also recommended to strive for blood glucose control as close as possible to normal.

People with diabetes have about 2 - 3 times greater risk of cardiovascular disease than the general population, and this cannot be fully accounted for by the presence of other known risk factors such as obesity or age (Assman et al, 1988; Garcia et al, 1974). In addition, diabetes removes the usual protection that premenopausal women have against heart disease (Abbott et al, 1988). Thus, it is clear that reducing the risk of cardiovascular disease in NIDDM is an important health issue.

Some researchers believe that hyperinsulinemia is atherogenic. In healthy, non-diabetic subjects, hyperinsulinemia has been associated with heart disease (Ducimetiere et al, 1980). In addition, hyperinsulinemia is associated with increases in plasma triglycerides and decreases in HDL
cholesterol, which are themselves risk factors for heart disease (Laakso et al., 1993). It is debatable whether it is the high insulin level specifically that is atherogenic or the decreased insulin sensitivity that accompanies it. In diabetic patients, insulin levels are typically high until the pancreas can no longer sustain the output. At that point insulin levels decrease, but the insulin resistance is still present. Insulin may have several biological actions which could contribute to heart disease. As described by Stout (1990), these include that long term treatment with insulin can lead to lipid containing lesions and arterial wall thickening. Insulin stimulates lipid synthesis in arterial tissue. Insulin stimulates proliferation and migration of arterial smooth muscle cells and monocyte-macrophages. Lastly, insulin stimulates cholesterol synthesis and LDL binding in arterial smooth muscle cells and monocyte-macrophages. Despite these effects, studies have been inconsistent in finding an association between insulin and heart disease in NIDDM patients.

The characteristic plasma lipid profile of NIDDM, namely the hypertriglyceridemia, the HDL particles which are high in triglycerides and low in cholesterol and small dense LDL particles may contribute to the high risk of cardiovascular disease seen in this population. In a seven year prospective study of NIDDM patients it was found that high total and VLDL triglyceride, high VLDL cholesterol and low HDL cholesterol were powerful predictors of both fatal and nonfatal coronary heart disease events (Laakso et al, 1993). Another investigation, the Paris Prospective Study, demonstrated that hypertriglyceridemia was an independent risk factor for coronary heart disease in people with impaired glucose tolerance or diabetes (Fontbonne et al, 1989).
In summary, NIDDM patients are at risk of developing several complications both microvascular and macrovascular. The risk of developing these may be reduced by keeping blood glucose levels close to normal, improving insulin sensitivity, lowering plasma triglycerides and VLDL cholesterol and increasing HDL cholesterol.

**Diet in NIDDM Patients**

Issues related to the diet of NIDDM patients will be reviewed in the following section. These include the effect of weight loss, metabolic effects of dietary fructose, specific effects of dietary fat composition on lipid parameters, current dietary recommendations, and the effects of high carbohydrate diets, high monounsaturated fat diets, and formula feeding on parameters of glucose and lipid metabolism.

**Effect of Weight Loss on Metabolic Parameters in Patients with NIDDM**

Most NIDDM patients are overweight and insulin resistant (Tremblay, 1995). Insulin resistance may be associated with obesity, and in some cases may improve somewhat following weight loss (Friedenberg et al, 1988). Kelley et al (1993) reported a study in which a group of lean men with NIDDM had normal insulin sensitivity, but insulin secretion following a glucose load was impaired. Thus, these lean NIDDM patients had high glucose levels primarily due to insufficient insulin secretion following a meal, rather than insulin resistance. It may be debated as to whether normal insulin sensitivity allowed these patients to maintain a normal weight or whether the normal weight resulted in normal insulin sensitivity.

Many studies have been done to investigate the effect of weight loss on plasma lipids in the general population. A meta-analysis found that for
every kilogram of weight loss there is a 0.05 mmol/L decrease in total cholesterol, a 0.02 mmol/L decrease in LDL cholesterol, and a 0.015 mmol/L decrease in plasma triglycerides. There was a 0.007 mmol/L decrease in HDL during active weight loss and a 0.009 mmol/L increase after weight stabilization (Dattilo and Kris-Etherton 1992).

Moderate weight loss in NIDDM patients seems to have some effect on glucose and lipid parameters. Rabkin et al (1983) assessed the effect of such a weight loss in people with NIDDM. After six weeks subjects lost an average of 1.8 kg. Fasting serum glucose decreased significantly from 12.2 to 11.1 mmol/L and fasting serum triglycerides decreased significantly from 2.25 to 1.92 mmol/L. There was no change in LDL or HDL cholesterol levels. By twelve weeks, subjects had lost an average of 3.6 kg. Fasting serum glucose had decreased to 10.9 mmol/L and fasting serum triglycerides had decreased to 1.90 mmol/L. There was still no significant effect on cholesterol levels. Laitinen et al (1993) did a similar study with NIDDM patients. Over a three month period the subjects lost 3.3 kilograms and had a significant decrease in serum triglycerides and HgbA1C. There was no effect on total cholesterol or HDL cholesterol levels. Liu et al (1985) reported results of a study in which subjects consumed a hypocaloric liquid formula diet consisting of 40% fat, 40% carbohydrate and 20% protein for four weeks. There was a mean weight loss of 6.5 kg and a significant decrease in fasting glucose, triglycerides and total cholesterol. There was no change in HDL cholesterol or insulin levels. This study also showed that weight loss increased the glucose metabolic clearance rate in NIDDM patients. The improved glucose status may have been related to the fact that exercise and weight loss are associated with increased insulin binding (Neufeld et al, 1986). In summary, moderate weight loss in NIDDM patients tends to
decrease plasma glucose and triglyceride levels. The effects of small weight losses in NIDDM patients on cholesterol levels are less apparent.

**Metabolic Effects of Dietary Fructose**

The suitability of fructose in the diabetic diet has been debated. Due to its unique metabolism it may produce different metabolic effects than glucose. Fructose is a monosaccharide contained in several natural dietary sources such as fruits and honey. With modern technology, fructose can also be extracted from corn and used as a high fructose syrup to sweeten processed foods like soft drinks (Hanover and White, 1993). The major advantage of fructose is that it is sweeter than sucrose. The use of fructose may thus be advantageous for diabetic individuals because less of the carbohydrate could be consumed to receive the same sweetness as sucrose. As well, in NIDDM subjects fructose produces a much lower acute postprandial glycemic and insulin response than sucrose (Crapo et al, 1980). Additional studies have investigated the longer term effects of fructose consumption. Fructose supplementation for one week as 21% of energy intake (Bantle et al, 1986) and for two weeks as 24% of energy intake (Crapo et al, 1986) resulted in decreased postprandial blood glucose responses. There was no difference observed in the fasting serum glucose in either of these studies. McAteer et al (1987) studied the effects of fructose as 25% of the usual carbohydrate intake in NIDDM subjects for one month. Thus, if a subject consumed 55% of calories from carbohydrate, 13.8% of the total calories would come from fructose. Similar to the previous studies no change was observed in fasting plasma glucose, but postprandial glucose was lower at 30, 60 and 120 minutes. In a two month study where fructose was provided as 7.5-8.5% of energy intake in NIDDM subjects, Grigoresco et al
(1988) found no effect of fructose on fasting glucose or insulin levels. Postprandial glucose was not measured in this study. Osei et al. (1987) conducted a study in which NIDDM subjects consumed 60g of fructose per day for 3 months. Thus, for a 2400 kcal diet, 10% of the energy would come from fructose. These investigators did find a significant decrease in fasting plasma glucose and HgbA1c at the end of the three month period. In summary, the data available show that fructose may be beneficial in the short term in reducing postprandial glycemia. Fasting glucose levels may be lower after three months of consuming fructose, but do not seem to be affected in shorter periods of fructose feeding.

Despite the possible beneficial effects of fructose in diabetic diets, fructose consumption is not without its possible adverse effects. The most common negative consequences of fructose feeding in NIDDM are increased blood triglycerides and VLDL cholesterol levels (Hollenbeck, 1993). As previously discussed, these parameters are associated with increased risk for heart disease in NIDDM subjects and are thus undesirable (Laakso et al., 1993). The unique metabolism of fructose may lead to increased triglyceride production. After fructose is absorbed it is rapidly taken up by the liver, its primary site of metabolism (Mayes, 1993). The metabolism of fructose differs from that of glucose in the initial stages. Glucose is metabolized to fructose 6-P, which is then converted to fructose 1,6 bis-P by the enzyme phosphofructokinase. This is the rate limiting step in glucose metabolism. Fructose on the other hand, is converted directly to fructose 1-P by the enzyme fructokinase, and thus bypasses the rate limiting step in glucose metabolism. As a result of this difference, glucose metabolism is tightly regulated, but fructose is rapidly broken down in the liver, resulting in accumulation of large amounts of its metabolites. The byproducts of fructose
1-P are dihydroxyacetone-P and glyceraldehyde. These can either be converted to glucose or enter other metabolic pathways. The former is converted to glycerol-3P, which is available to be esterified with free fatty acids, forming triglycerides. Glyceraldehyde is able to enter the citric acid cycle and produce energy, lactate or acetyl-CoA which is a substrate for fatty acid synthesis. The increased amount of glycerol-3P and acetyl-CoA resulting from the rapid metabolism of fructose may lead to increased fatty acid synthesis. In fact it has been found that fructose feeding overrides the normal inhibitory effect of increased free fatty acids on fatty acid synthesis and it has been shown that fructose is more rapidly converted to lipid than is glucose (Zakim et al, 1969). Fructose causes increased esterification rather than oxidation of free fatty acids to triglycerides and consequently there is increased secretion of VLDL particles (Topping et al, 1976). It has been suggested by Mayes (1993) that this may lead to increased plasma cholesterol levels as well, since VLDL normally contains about 20% cholesterol.

Although the metabolism of fructose suggests the possibility of increased triglyceride and cholesterol levels, several studies have investigated the effect of dietary fructose on blood lipids and the results have been quite variable. In subjects with carbohydrate induced hypertriglyceridemia, consumption of 54% of energy as fructose for two to five weeks, resulted in a significant increase in plasma triglycerides and cholesterol (Kaufmann et al, 1966). The amount of fructose used in this study (54% of energy), however, is much higher than most people would consume. In another study of people with high plasma triglycerides, consumption of 9% or 17% of energy as fructose for two weeks resulted in no difference in plasma triglycerides or cholesterol levels from baseline (Turner et al, 1979). Halfrish et al (1983), observed that hyperinsulinemic men
consuming 7.5% of energy as fructose for five weeks had an increase in fasting plasma triglycerides and cholesterol from baseline. The increase in cholesterol but not triglycerides was seen in non-hyperinsulinemic men as well. Reiser et al (1989) found that even non-hyperinsulinemic men had an increase in plasma triglycerides after consuming fructose as 20% of energy for five weeks. Thus it seems that there is a dose response to the adverse effects of dietary fructose.

A few studies have investigated the effect of dietary fructose on blood lipids specifically in the diabetic population. In 1986, Bantle et al investigated subjects with NIDDM who consumed 21% of energy as fructose for eight days. No change was seen in plasma lipid parameters at the end of the study. The short study duration may not have allowed enough time for changes to occur. In 1992, Bantle et al did a similar study in which NIDDM subjects consumed 20% of energy as fructose for four weeks. This time it was found that there was an increase in plasma cholesterol but there was still no significant change in triglycerides. Grigoresco et al (1988) performed a two month crossover study in which NIDDM subjects consumed 30 grams of fructose in exchange for an isocaloric amount of starch. This resulted in 7.5-8.6% of energy coming from fructose. No significant change was found in cholesterol, however, there was a significant increase in plasma triglycerides in subjects on the fructose diet. The authors concluded that this was not a deleterious effect, however, because the triglycerides were still within the normal range. In a study by Crapo et al (1986) in which NIDDM subjects consumed 24% of energy as fructose for two weeks, there was no overall significant change in plasma cholesterol or triglycerides. However, five of the seven subjects who had high baseline triglyceride levels (>150 mg/dl or 1.7 mmol/L) had a 13% increase in triglycerides. This may suggest that some
individuals (especially those with higher triglyceride levels) may be more susceptible to the adverse effects of fructose. Osei et al (1987), and McAteer et al (1987), whose studies have been previously discussed, found no significant effect of fructose on blood lipids in NIDDM patients.

There may be several reasons for these conflicting results. The studies have used different diets. The percent of energy derived from fructose differed, as did the ratio of carbohydrate, fat and protein, the ratio of saturated, polyunsaturated, and monounsaturated fat and the cholesterol content. Many of the studies used small sample sizes, and the subject characteristics have been different. It appears that this is not a simple issue and there is still more that needs to be understood about the metabolic effects of dietary fructose in NIDDM patients. Many studies have found lower postprandial blood glucose in response to dietary fructose. Several studies have found no effect of fructose on lipid parameters. However, a few investigators have reported increased triglycerides and cholesterol from dietary fructose.

Specific Effects of Fat Composition on Lipid Parameters

It has been recognized since the 1950's that different types of fat have differing effects on plasma lipid levels. This topic has been reviewed by Zollner and Tato (1992). Specifically, early studies found that saturated fatty acids increased serum cholesterol, and polyunsaturated fatty acids decreased serum cholesterol. Some studies found a drop in cholesterol with monounsaturated fat, however, this was not as great a decrease as was seen with the polyunsaturated fat. Further investigation has found that diets high in linoleic acid (polyunsaturated fat) decrease both LDL and HDL cholesterol. The decrease in cholesterol from oleic acid (monounsaturated
fat) has been found to be due to LDL cholesterol, with no change in HDL cholesterol.

Several mechanisms may be responsible for these metabolic responses. Substitution of monounsaturated fat (Daumerie et al, 1992) or polyunsaturated fat (Woolett et al, 1992) in place of saturated fat has been shown to result in increased LDL receptor activity. This increased uptake of LDL may cause a reduction in the plasma LDL cholesterol. Similarly, Kurishima et al (1995) found that both linoleic and oleic acid prevented the hepatic LDL receptor suppression that was observed with a cholesterol supplemented diet alone. With regard to HDL cholesterol, Shepherd et al (1978) found a decrease in the synthesis rate of apo A-1 in subjects consuming diets high in polyunsaturated fat compared to diets high in saturated fat. This is reflective of the decrease in HDL cholesterol often found when saturated fat is replaced by polyunsaturated fat. Osada et al (1991) found that rats consuming a diet high in monounsaturated fat expressed significantly more hepatic apo A-1 mRNA. Since this protein is incorporated into HDL particles, the increased production of it may lead to greater HDL particles in the circulation, and thus greater concentrations of HDL cholesterol in response to monounsaturated fat. These are some of the ways in which dietary fatty acids affect plasma lipids. Other mechanisms may also contribute.
Dietary Recommendations for NIDDM

The optimal diet for diabetes mellitus has been a topic of controversy over the years. From 1940 to 1970 the recommendation was a high fat diet, limiting the amount of carbohydrate consumed (Vinik and Wing, 1992). Since 1971 the high fat diet has been abandoned and replaced with a diet high in complex carbohydrates and fiber, and low in total fat, saturated fat and cholesterol. The current recommendations, published in 1989 are described as follows (Canadian Diabetes Association, 1989): energy should be consumed in amounts suitable to achieve a healthy body weight; protein consumption should be approximately 0.8/kg of ideal body weight; up to 30% of calories may come from fat, of which less than 10% should be saturated fat, up to 10% polyunsaturated fat and the remainder monounsaturated fat; carbohydrate should supply the remaining calories, (45-55% of energy) and approximately 40g of fiber per day is recommended.

Although high carbohydrate diets are currently recommended to many NIDDM patients, the wisdom of this has been questioned by some investigators. Most diabetes researchers agree that it is logical to recommend a low intake of saturated fat as this population is at a high risk for cardiovascular disease. A controversy however arises as to which macronutrient should replace those calories. Most researchers agree that it is not desirable to increase the protein content of the diet, as this would place an extra burden on the kidney in a population already at high risk for nephropathy. Higher fat diets enriched with monounsaturated fatty acids have been suggested by several research groups as an appropriate substitute for some of the saturated fat and carbohydrate in the diet of NIDDM patients.
Comparison of Effects of High Carbohydrate and High Monounsaturated Fat Diets on Glucose and Lipid Levels

Several studies in the last few years have investigated the effects of diets varying in carbohydrate and fat content in NIDDM patients on parameters of carbohydrate and lipid metabolism. Many of the studies have been done by Garg and his group in Dallas, Texas, and have compared high carbohydrate diets to high monounsaturated fat diets (Garg et al, 1988; Garg et al 1992a; Garg et al 1992b; Garg et al 1994). Although this group has done much of this work, other groups have contributed to the area as well, (Rasmussen et al, 1993; Campbell et al, 1994; Lerman-Garber et al, 1994; Lerman-Garber et al, 1995). The diets in these studies have varied from 20-32% of calories from fat in the high carbohydrate diets, and from 37-50% of calories as fat in the high monounsaturated fat diets. The duration of the studies have ranged from 2 to 14 weeks and have all been crossover designs. The high monounsaturated fat diets have generally resulted in lower day-long blood glucose and insulin levels and lower fasting plasma triglyceride and VLDL cholesterol levels. No difference has been found in total cholesterol or LDL cholesterol, however, some studies have demonstrated higher HDL cholesterol levels after the high monounsaturated fat diet. Thus, the high carbohydrate dietary regimen, while intended to reduce the risk of cardiovascular disease may actually increase that risk, in comparison to a high monounsaturated fat diet. Tables 1 and 2 briefly describe the previous study diets and the findings.
**TABLE 1:**
Duration and Diet Composition of Studies Comparing High Carbohydrate Diets to High Monounsaturated Fat Diets

<table>
<thead>
<tr>
<th>Study</th>
<th>n</th>
<th>Duration of Diet</th>
<th>High Carbohydrate Diet</th>
<th>High Monounsaturated Fat Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(days)</td>
<td>CHO (%)</td>
<td>Fat (%)</td>
</tr>
<tr>
<td>Garg et al, 1988</td>
<td>10</td>
<td>28</td>
<td>60</td>
<td>25</td>
</tr>
<tr>
<td>Garg et al, 1992 (a)</td>
<td>10</td>
<td>28</td>
<td>65</td>
<td>20</td>
</tr>
<tr>
<td>Garg et al, 1992 (b)</td>
<td>8</td>
<td>21</td>
<td>60</td>
<td>25</td>
</tr>
<tr>
<td>Garg et al, 1994</td>
<td>42</td>
<td>42/98</td>
<td>55</td>
<td>30</td>
</tr>
<tr>
<td>Rasmussen et al, 1993</td>
<td>15</td>
<td>21</td>
<td>49</td>
<td>32</td>
</tr>
<tr>
<td>Campbell et al, 1994</td>
<td>10</td>
<td>14</td>
<td>55</td>
<td>22</td>
</tr>
<tr>
<td>Lerman-Garber, 1994</td>
<td>12</td>
<td>28</td>
<td>60</td>
<td>20</td>
</tr>
<tr>
<td>Lerman-Garber, 1995</td>
<td>13</td>
<td>42</td>
<td>60</td>
<td>20</td>
</tr>
</tbody>
</table>

CHO = carbohydrate, S = saturated, M = monounsaturated, P = polyunsaturated, % = percent of total energy
a = all subjects followed the diets for 42 days and half of the subjects continued their second dietary period for 98 days
b = range of washout period
c = median
* = not presented
TABLE 2:
Comparison of the Effects of a High Monounsaturated Fat Diet and a High Carbohydrate Diet on Plasma Glucose and Lipid Levels

<table>
<thead>
<tr>
<th>Study</th>
<th>PP Glucose</th>
<th>Fasting Glucose</th>
<th>TG</th>
<th>T-C</th>
<th>HDL-C</th>
<th>VLDL-C</th>
<th>LDL-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Garg et al, 1988</td>
<td>↓</td>
<td>*</td>
<td>↓</td>
<td></td>
<td>↑</td>
<td>↓</td>
<td></td>
</tr>
<tr>
<td>Garg et al, 1992 (a)</td>
<td>*</td>
<td>-</td>
<td>↓</td>
<td>↓</td>
<td></td>
<td>↓</td>
<td></td>
</tr>
<tr>
<td>Garg et al, 1992 (b)</td>
<td>-</td>
<td>-</td>
<td>↓</td>
<td></td>
<td>↑</td>
<td>↓</td>
<td></td>
</tr>
<tr>
<td>Garg et al, 1994</td>
<td>↓</td>
<td>-</td>
<td>↓</td>
<td></td>
<td></td>
<td>↓</td>
<td></td>
</tr>
<tr>
<td>Rasmussen et al, 1993</td>
<td>↓</td>
<td>↓</td>
<td></td>
<td></td>
<td></td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Campbell et al, 1994</td>
<td>↓</td>
<td>-</td>
<td>↓</td>
<td></td>
<td></td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Lerman-Garber, 1994</td>
<td>-</td>
<td>-</td>
<td>↓</td>
<td></td>
<td></td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Lerman-Garber, 1995</td>
<td>*</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PP=postprandial, TG-triglyceride, T-C = total cholesterol, HDL-C = high density lipoprotein cholesterol, VLDL-C = very low density lipoprotein cholesterol, LDL-C = low density lipoprotein cholesterol
↓ = lower after the high monounsaturated fat diet
- = same after the high monounsaturated fat diet
↑ = higher after the high monounsaturated fat diet
* = not presented
The most consistent findings of these previous studies has been lower postprandial blood glucose, fasting triglyceride and VLDL cholesterol levels after a high monounsaturated fat diet compared to a high carbohydrate diet. The lower postprandial blood glucose is likely due to the fact that less carbohydrate is ingested in a meal high in monounsaturated fat. The mechanisms for lower triglyceride levels are not fully known, however, some aspects have been explored. Chen et al (1995) demonstrated that high carbohydrate diets result in greater postprandial accumulation of plasma triglycerides and that this was due to increased VLDL-TG production and reduced clearance of VLDL-TG. The study also found that there was an increase in postprandial lipoprotein lipase activity on the high carbohydrate diet, which may indicate that there was increased removal of triglycerides from the plasma into the cells, but that this was not enough to compensate for the increase in triglycerides of endogenous origin competing with chylomicron-TG for uptake by lipoprotein lipase. Blades and Garg (1995) conducted a similar study, in which they found no difference between a high carbohydrate or a high monounsaturated fat diet on the activities of lipoprotein lipase or hepatic lipase. From this they concluded that the increase in plasma triglyceride concentrations from a high carbohydrate diet was not a result of decreased uptake of fatty acids in the skeletal and adipose tissues, but must be due to increased hepatic secretion of VLDL triglycerides. The reason for this increase in VLDL triglycerides is not conclusively known, although some aspects have been explored. Several things may contribute to this effect. Perhaps high carbohydrate diets result in greater hyperglycemia, providing the liver with more substrate for fatty acid synthesis. High carbohydrate diets may result in higher insulin levels, especially in the postprandial state, which may affect triglyceride synthesis and secretion. As
already discussed, insulin increases triglyceride synthesis within the liver (Sparks et al, 1994). In chronic hyperinsulinemia, and therefore probable insulin resistance, the normal inhibitory effect of insulin on apo B secretion is not effective (Lewis et al, 1993). Thus, it is possible that high carbohydrate diets may result in greater postprandial levels of insulin, and as a result, greater synthesis of triglyceride and release of VLDL into the circulation. This may contribute to the higher levels of triglycerides found after high carbohydrate diets. More research is required to confirm these mechanisms and/or to investigate other possible mechanisms to explain the increased triglyceride levels after high carbohydrate intakes in NIDDM subjects. The lower VLDL cholesterol levels appear to be related to the decrease in fasting triglycerides. Two studies found a increase in HDL cholesterol after the high monounsaturated fat diet. This might be due to the effects of fatty acids on lipoproteins as described in the previous section. Another possible contributing factor could be that as triglycerides decrease, there is less action of CETP taking cholesterol out of the HDL particles. These are some of the possible factors contributing to the metabolic effects of high carbohydrate and high monounsaturated fat diets in NIDDM subjects.

It is widely believed that high carbohydrate diets increase peripheral insulin sensitivity, and thus are beneficial to NIDDM patients. The evidence for this has been criticized, however, because the diets used in the initial studies were very high in carbohydrate or fat and the methods of measuring insulin action were not very sensitive. Two recent studies have utilized a euglycemic hyperinsulinemic clamp and provided diets with realistic ratios of the percentages of energy coming from carbohydrate and fat. Garg et al (1992b) compared a diet with 35% carbohydrate and 50% fat to a diet with 60% carbohydrate and 25% fat. Although glucose disposal (uptake of glucose
into the tissues) was higher during the high monounsaturated fat diet, this difference was not significant. Parillo et al (1992) compared a diet with 40% carbohydrate and 40% fat to a diet with 60% carbohydrate and 20% fat. The high monounsaturated fat diet resulted in significantly higher glucose disposal. This finding suggests that high carbohydrate diets do not increase peripheral insulin sensitivity, and that diets high in monounsaturated fat may actually be better at improving insulin sensitivity.

The above findings suggest that a diet high in monounsaturated fat may be better than a diet high in carbohydrate for some patients with NIDDM. In March of 1994, Garg published a commentary in the journal "Diabetes Care" suggesting that the dietary recommendations for people with diabetes should be changed to increase the intake of monounsaturated fat (Garg, 1994). The evidence available suggests that these diets may be beneficial for NIDDM patients, both in optimizing glycemic response and also in relation to optimizing the lipoprotein profile. Thus, high monounsaturated fat diets might provide a good alternative to the widely recommended high carbohydrate diets for people with NIDDM. As many people with NIDDM would benefit from weight loss, it would be important not to increase the energy content of the diet if a patient were to follow this dietary advice.

Formula Feeding

Nutritional formulas are used to replace food completely or to supplement an oral diet. They are used commonly in hospitals as enteral feeding for patients who are unable to tolerate oral food. They are also commonly given to hospitalized patients as a drink to provide extra nutrition. Many of these nutritional formulas are also commercially available and can be consumed by people as an addition to their regular diet. This may be
helpful for people who are unable to consume an adequate diet on solid foods alone.

Studies have shown that formula feeding results in faster gastric emptying times and quicker nutrient absorption than regular solid food. (Stevens et al, 1979). In diabetic patients this may compromise blood glucose control, leading to postprandial hyperglycemia. Maintaining glucose control is important. In addition to increasing the risk of diabetic complications, uncontrolled hyperglycemia can result in diuresis and dehydration and may also interfere with wound healing and impair leukocyte function (Campbell et al, 1991). These are undesirable effects, especially for sick patients who are already vulnerable.

Glucerna® is an enteral feeding product designed to reduce hyperglycemia in diabetic or glucose intolerant patients. It has less carbohydrate and more fat (predominately monounsaturated) than the commonly used enteral formulas. The fiber source in Glucerna® is soy polysaccharide, which is 10% soluble and 90% insoluble. Soluble fibers may help decrease postprandial blood glucose, however large amounts of soluble fibers cannot be added to formulas because they are gel forming. Several short term studies have compared the glucose response from Glucerna® to other nutritional formulas. These studies have been done with IDDM patients, NIDDM patients and patients with stress induced hyperglycemia due to head injury. Most of these studies have compared Glucerna® to Ensure HN® (Abbott Laboratories, Columbus, Ohio), which is a high carbohydrate formula with no added fiber.

The first study, which was done with IDDM patients, demonstrated that Glucerna® resulted in a significantly lower acute serum glucose response than Ensure HN® (Davidson et al, 1989). Ten subjects were connected to a
biostator (an artificial endocrine pancreas) to maintain blood glucose levels at 150 mg/dL. Insulin was delivered at a rate of 0.1 milliunits/kg/minute. The subjects consumed the formula slowly for 4 hours to mimic continuous tube feeding, and blood glucose was measured every 30 minutes. It was found that Glucerna® resulted in a lower serum glucose response than Ensure HN®, as assessed by the area under the glucose curve.

A similar 4 hour study was done with 10 obese NIDDM patients (Harley et al, 1989). These subjects ingested 20 ml of Glucerna® or Ensure HN® every 15 minutes for 4 hours and serum glucose measurements were taken every 30 minutes. This study found that Glucerna® resulted in significantly lower peak blood glucose levels and total glucose excursions (as measured by the area under the glucose curve) than Ensure HN®.

Galkowski et al, (1989) conducted a study with elderly nursing home NIDDM patients. These patients are often given nutritional formulas as snacks. Five patients were assigned to receive in random order an isocaloric snack of solid food, Ensure HN® or Glucerna®. The postprandial glucose response was similar for the food snack and the Glucerna®, but the Ensure HN® resulted in significantly higher postprandial serum glucose responses. This suggests that when a liquid supplement is required for these patients, Glucerna® will produce less of a blood glucose rise than the higher carbohydrate formula Ensure HN®.

A study using Glucerna® was also done with patients prone to hyperglycemia due to acute head injury (Grahm et al, 1989). These patients were continuously tube fed into the small bowel for 7 days with either Glucerna® or Vital HN® (Abbott Laboratories, Columbus, Ohio). Vital HN® is a high nitrogen elemental formula, which contains 16.7 % of calories from protein, 73.9% from carbohydrate (no fiber) and only 9.4% from fat. The
Glucerna® feeding resulted in significantly lower day-long blood glucose levels than Vital HN®. In addition, all the patients receiving Vital HN® required daily insulin therapy, while none of the patients receiving Glucerna® required administration of insulin.

The above studies have compared Glucerna® to high carbohydrate formulas that do not contain fiber. Thus, a question that one may ask is whether the results would be similar if a high carbohydrate formula with fiber was used. Peters et al, (1992) compared Glucerna® to 4 other formulas: Ensure HN®, Enrich® (Abbott Laboratories, Columbus, Ohio), Compleat Modified® (Sandoz Nutrition Corporation, Minneapolis), and Pulmocare® (Abbott Laboratories, Columbus, Ohio). The first formula has been previously described as a high carbohydrate formula without fiber. Enrich® (now called Ensure with Fiber®) is a high carbohydrate formula with the same type and amount of fiber as Glucerna®. Compleat Modified® is a high carbohydrate formula with fiber. Pulmocare® is a formula designed for the special needs of patients with chronic obstructive pulmonary disease. Like Glucerna®, it is a high fat, low carbohydrate formula, however it does not contain fiber and is high in polyunsaturated fat rather than monounsaturated fat. Five IDDM subjects consumed each of these five products in random order. Subjects consumed 20 ml of the formula every 15 minutes for a total of 4 hours, and a measurement of blood glucose was taken every 30 minutes. The glucose response was lower for Glucerna® than for Ensure HN®, Enrich® and Compleat Modified®. There was no statistically significant difference in blood glucose levels between Pulmocare® and Glucerna®. These results indicate that the low carbohydrate, high fat content of the formulas has much more of an impact on acute blood glucose levels than the fiber in the formulas.
A final study done with Glucerna® measured both glucose and lipid levels (Abbruzzese et al, 1993). This study was done with elderly NIDDM patients receiving total enteral nutrition. Subjects were maintained on their usual high carbohydrate formula for 28 days, and then were switched to Glucerna® for 42 days. During the period of Glucerna® feeding these subjects had reduced circadian amplitudes in blood glucose. The abstract also stated that the Glucerna® feeding “reduced serum lipids and had no adverse effects on successful glycemic management of HgbA1C”. Results of statistical testing were not available, but the data indicated a decrease in fasting glucose, triglycerides, total, LDL and VLDL cholesterol and an increase in HDL cholesterol from Glucerna® (personal communication, Abbott Laboratories).

**SUMMARY**

This review of the literature suggests that diets high in monounsaturated fat may have more beneficial effects on plasma glucose and lipid levels in people with NIDDM, than high carbohydrate diets. The main reasons for this are the lower postprandial blood glucose levels, lower plasma triglycerides and VLDL cholesterol levels and in some cases, higher HDL cholesterol levels found after high monounsaturated fat diets. These findings are taken from a small number of studies and need to be confirmed in future investigations. Several studies have demonstrated that Glucerna® results in lower acute blood glucose response than higher carbohydrate formulas. Only one study has investigated the effects of Glucerna® on blood lipids, and this was done in elderly, chronically ill patients who required enteral feeding. The present study examined the effects of a high carbohydrate diet (Ensure with Fiber®) and a high monounsaturated fat diet (Glucerna®) on parameters
of carbohydrate and lipid metabolism in outpatients with NIDDM who were in good metabolic control and who had moderate lipid levels.
CHAPTER THREE

METHODS

EXPERIMENTAL DESIGN

This study utilized a randomized controlled design. Subjects with NIDDM were randomized to receive either Ensure with Fiber\textsuperscript{®} or Glucerna\textsuperscript{®} as their main source of energy for 28 days. Up to twenty percent of their daily energy intake could come from regular solid food, referred to in this report as mini-meals. These mini-meals were specifically designed to provide a similar macronutrient composition and a similar ratio of saturated, monounsaturated and polyunsaturated fatty acids as the respective formulas. The subjects recorded measurements of blood glucose using their own blood glucose monitoring equipment just before, and two hours after each meal for two days each week for the four weeks of the study. As well, fasting blood samples were taken on the mornings of day 0, 7 and 28, for analysis of parameters of carbohydrate and lipid metabolism.

SUBJECT RECRUITMENT

Recruitment posters were placed in seven diabetes clinics, four endocrinologists offices, thirty seven general practitioners offices and in the Diabetes Resource Center (Appendix A). One hundred and sixty one letters were sent to former patients of the Shaughnessy Hospital Diabetes Clinic inviting them to participate in the study. Information sheets about the study were given to eligible participants who attended the Vancouver Hospital Diabetes Clinic (Appendix B). Brief presentations about the study were given to patients at diabetes clinics at St. Paul’s Hospital and Richmond General Hospital. At St. Paul’s Hospital this was done about twice a week.
from January until April, and at Richmond General Hospital, twice a month from March to November. Newspaper advertisements were also used for recruitment purposes (Appendix C). Two advertisements appeared in the Vancouver Sun and the Province, one in the Vancouver Courier and one in the UBC Reports. One advertisement was placed in the Canadian Diabetes Association Greater Vancouver branch newsletter.

**ELIGIBILITY CRITERIA**

Individuals were eligible to participate in the study if they met the following inclusion criteria. Subjects had non-insulin dependent diabetes mellitus which had been diagnosed by a physician. Subjects were not taking insulin and were 18-70 years old. Subjects had a stable body weight defined as less than a 5% change in weight in the last three months. The stability of body weight was used because weight change can have an effect on glucose and lipid metabolism. Subjects had a BMI between 19 and 36. It is recognized that this is a wide range, however many people with non-insulin dependent diabetes are overweight. The upper limit was set to exclude people with morbid obesity. Subjects had no recent history of ketosis and no significant medical conditions other than diabetes which would impact on glucose or lipid metabolism. Subjects were required to monitor blood glucose at home, and have a support person who would provide encouragement throughout the study. Subjects were not taking steroids, β-blockers or any lipid lowering medications. As well, plasma triglycerides had to be less than or equal to 4.5 mmol/L, plasma cholesterol less than or equal to 6.5 mmol/L and HgbA1C less than or equal to 0.09%. The diabetic population typically has elevated plasma lipids and HgbA1C, thus normal values could not be used for inclusion criteria. These upper limits were set to exclude individuals with
very high values. All medical information was verified by the subject’s physician. Before the subjects entered the study they performed a taste test of both formulas, consuming four cans of each formula over a period of eight hours on two separate days. If they tolerated the formulas and were willing to be randomized to receive either formula, subjects were then enrolled into the study. A consent form was signed by each participant. (Appendix D).

**ASSESSMENT OF ENERGY REQUIREMENTS**

Before starting the formula diet, subjects were asked to keep a four day food record. Subjects were verbally instructed on how to do this. As well, they received written instructions and an example of a recorded meal was provided (Appendix E). Nutritional analysis of the food records was done using Food Processor II (ESHA Research, Salem Oregon) and the energy content was assumed to be their usual energy intake. As a second assessment of energy requirements, the Harris-Benedict equation was calculated (Harris and Benedict, 1919). This equation was developed to estimate energy needs and is shown below.

Males: 66 + (13.7 x Wt (kg)) + (5 x Ht (cm)) - (6.8 x age (years))

Females: 655 + (9.6 x Wt (kg)) + (1.8 x Ht (cm)) - (4.7 x age (years))

These equations provide an estimate of basal energy requirements. They were multiplied by an activity factor of 1.3 or 1.5 (based on the self reported activity levels of the subjects) to obtain an estimate of total energy requirements. The results of the four day food record and the Harris Benedict equation were averaged and the mean was used as the estimated energy requirement of the individual. Because weight change may have an effect on blood glucose and lipids, it was intended that subjects would
maintain their weight throughout the study. Subjects were weighed at each weekly visit and food intake was adjusted if there was weight gain or loss.

**STUDY DIETS**

Subjects were randomly assigned to receive one of the two formula diets, Ensure with Fiber® or Glucerna®. It was thought that a complete liquid diet would be very difficult to adhere to for four weeks, thus mini-meals were designed which would have a similar macronutrient composition and ratio of saturated, monounsaturated and polyunsaturated fatty acids as their respective formulas. Up to twenty percent of energy intake was permitted to come from these mini-meals. Each subject was provided with a meal plan outlining how many cans of formula and how many mini-meals he or she should consume each day.

Information about the nutritional composition of the diets is provided in Tables 3 to 8. The source of nutrients differs between the two formulas and is shown in Table 3. Ensure with Fiber® contains maltodextran and sucrose as its source of carbohydrate, while Glucerna® contains glucose polymers and fructose. Both formulas contain soy polysaccharide as a source of dietary fiber. Ensure with Fiber® contains corn oil as its source of fat, while Glucerna® contains high-oleic safflower oil and nonhydrogenated soybean oil. Ensure with Fiber® contains sodium and calcium caseinates and soy protein as a source of protein, while Glucerna® contains only sodium and calcium caseinates.
**TABLE 3:**  
*Nutrient Sources of Ensure with Fiber® and Glucerna®*

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Ensure with Fiber® (%)</th>
<th>Glucerna® (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>maltodextran</td>
<td>58.0</td>
<td>glucose polymers 53.3</td>
</tr>
<tr>
<td>sucrose</td>
<td>32.0</td>
<td>fructose 21.3</td>
</tr>
<tr>
<td>soy polysaccharide</td>
<td>10.0</td>
<td>soy polysaccharide 25.4</td>
</tr>
<tr>
<td>Fat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>corn oil</td>
<td>100.0</td>
<td>high oleic safflower oil 85.0</td>
</tr>
<tr>
<td>nonhydrogenated soy oil</td>
<td>15.0</td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na and Ca caseinates</td>
<td>84.0</td>
<td>Na and Ca caseinates 100.0</td>
</tr>
<tr>
<td>soy protein</td>
<td>16.0</td>
<td></td>
</tr>
</tbody>
</table>

The nutrient composition per can of formula is shown below in Table 4.

**TABLE 4:**  
*Nutrient Composition per Can of Formula (235 ml)*

<table>
<thead>
<tr>
<th></th>
<th>Ensure with Fiber®</th>
<th>Glucerna®</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories (kcal)</td>
<td>260</td>
<td>237</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>38.3</td>
<td>22.2</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>8.8</td>
<td>13.2</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>9.4</td>
<td>9.9</td>
</tr>
<tr>
<td>Fiber (g)</td>
<td>3.3</td>
<td>3.3</td>
</tr>
</tbody>
</table>
Table 5 shows the nutrient breakdown of the formulas as a percentage of total energy.

**TABLE 5:**
Nutrient Breakdown of the Formulas as a Percentage of Total Energy

<table>
<thead>
<tr>
<th></th>
<th>Ensure with Fiber®</th>
<th>Glucerna®</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate</td>
<td>55.0</td>
<td>33.3</td>
</tr>
<tr>
<td>Fat</td>
<td>30.5</td>
<td>50.0</td>
</tr>
<tr>
<td>Protein</td>
<td>14.5</td>
<td>16.7</td>
</tr>
</tbody>
</table>

The mean nutrient breakdown of the mini-meal recipes as a percentage of energy is shown below in Table 6. This is the average composition of all of the mini-meal recipes for each diet group. As can be seen, the composition is similar to that of the formulas.

**TABLE 6:**
Mean Nutrient Breakdown of Mini-Meal Recipes as a Percentage of Total Energy

<table>
<thead>
<tr>
<th></th>
<th>Ensure with Fiber® Group Meals</th>
<th>Glucerna® Group Meals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate</td>
<td>54.7</td>
<td>34.0</td>
</tr>
<tr>
<td>Fat</td>
<td>29.9</td>
<td>50.5</td>
</tr>
<tr>
<td>Protein</td>
<td>15.1</td>
<td>15.5</td>
</tr>
</tbody>
</table>
The fatty acid profile of the study formulas as a percentage of total fatty acids is shown below in Table 7

**TABLE 7:**
**Fatty Acid Profile of the Formulas as a Percentage of Total Fatty Acids**

<table>
<thead>
<tr>
<th>Fat Type</th>
<th>Ensure with Fiber®</th>
<th>Glucerna®</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated</td>
<td>16</td>
<td>10</td>
</tr>
<tr>
<td>Monounsaturated</td>
<td>26</td>
<td>64</td>
</tr>
<tr>
<td>Polyunsaturated</td>
<td>58</td>
<td>26</td>
</tr>
</tbody>
</table>

The mean fatty acid profile of the mini-meal recipes is shown below in Table 8. This is the average fatty acid profile of all of the mini-meal recipes for each diet group. As can be seen, the composition is similar to, although not exactly the same as that of the formulas.

**TABLE 8:**
**Mean Fatty Acid Profile of the Mini-Meal Recipes as a Percentage of Total Fatty Acids**

<table>
<thead>
<tr>
<th>Fat Type</th>
<th>Ensure with Fiber® Group Meals</th>
<th>Glucerna® Group Meals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated</td>
<td>20.2</td>
<td>16.3</td>
</tr>
<tr>
<td>Monounsaturated</td>
<td>33.7</td>
<td>52.3</td>
</tr>
<tr>
<td>Polyunsaturated</td>
<td>41.6</td>
<td>25.6</td>
</tr>
</tbody>
</table>
METHODS

Table 9 shows the fatty acid profile of the formulas as a percentage of total energy.

**TABLE 9:**
Fatty Acid Profiles of the Formulas as a Percentage of Total Energy

<table>
<thead>
<tr>
<th>Fat Type</th>
<th>Ensure with Fiber&lt;sup&gt;®&lt;/sup&gt;</th>
<th>Glucerna&lt;sup&gt;®&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Monounsaturated</td>
<td>8</td>
<td>32</td>
</tr>
<tr>
<td>Polyunsaturated</td>
<td>18</td>
<td>13</td>
</tr>
</tbody>
</table>

**SUBJECT APPOINTMENTS**

Subjects met with the investigators once a week during the study. This meeting occurred on Monday or Tuesday mornings. On the first visit the diet was carefully explained and each subject was given a meal plan outlining the number of cans of formula and the number of mini-meals to consume each day. It was emphasized that the mini-meals were designed specifically to be similar to the assigned formula and that the recipes must be followed exactly. If subjects chose to have mini-meals, the specific kind of margarine, oil, mayonnaise and meat required for their diet was provided for them. Subjects were provided with a food diary in which they were instructed to record all the formula and food that they consumed for the 28 day study period. On each subsequent visit this diary was reviewed with them. In this diary they were also instructed to record their home blood glucose monitoring results. On the first and last visit, skinfold and hip and
waist circumference measurements were recorded. On the first visit, height was recorded, and on every visit weight was recorded. If an individual's weight had changed, food intake was adjusted in an attempt to restore the weight to what it had been initially. On days 0, 7 and 28 a fasting blood sample was taken. Between visits subjects were telephoned to resolve any questions or concerns. The frequency of these phone calls varied among subjects, but for most of them it was twice a week.

ANTHROPOMETRIC MEASUREMENTS

Anthropometric measurements were taken primarily for descriptive purposes, but also to assess if changes in weight, body fat or body fat distribution occurred. Measurements were taken according to published techniques (Gibson, 1990). Body mass index was calculated from weight and height (kg/m²). Weight was recorded at each weekly visit. At the beginning and the end of the study, circumference and skinfold measurements were done. Two girth measurements were taken, waist circumference and hip circumference, from which the waist to hip ratio was calculated. Skinfold measurements were taken at four sites: triceps, biceps, subscapular and suprailiac. These provide a measure of subcutaneous fat. Measurements were taken in triplicate and the mean of the closest two values was used, as recommended by Gibson (1990). Percent body fat was estimated from the sum of the four skinfolds using published tables (Durnin and Womersley, 1974).
METHODS

POSTPRANDIAL BLOOD GLUCOSE MONITORING

One of the goals of this study was to assess the effects of Ensure with Fiber® and Glucerna® on postprandial blood glucose levels. To achieve this, subjects were asked to take a finger prick blood glucose measurement before and two hours after each meal for two days each week of the study. This occurred on Wednesdays and Saturdays. The subjects had their own glucose monitoring equipment and they were familiar with its use. There are several brands available, thus different kinds of glucose monitoring equipment were used by the subjects. It is recognized that this measure was not controlled. Subjects performed the test the way they usually do, and the results may be subject to user or equipment error. In spite of this, it was felt that this information was valuable and that this was the only practical way of obtaining it in an outpatient study.

COLLECTION OF A FASTING BLOOD SAMPLE

On day 0, 7 and 28 of the study, subjects had a fasting blood sample taken. Subjects were instructed not to eat for 12 hours prior to each blood draw. The subjects were taken to the outpatient medical laboratory at the Vancouver Hospital and Health Sciences Center, University Site, where blood was drawn by a trained phlebotomist by venipuncture. Five milliliters of blood was taken and placed in a serum separation tube for determination of glucose and fructosamine. Another 5 ml of blood was taken and placed in a tube prepared with ethylene diamine tetra acetic acid (EDTA) and trasylol. From this, plasma insulin and glucagon was measured. In addition, a 5 ml EDTA tube was filled for determination of plasma lipids. Blood was centrifuged immediately and the plasma and red blood cells were separated. Blood was kept on ice and held for pick up by the investigators. The samples
were then frozen at -70° C until subsequent analysis was performed, with the exception of serum glucose (see below).

**SERUM GLUCOSE DETERMINATION**

Serum glucose was determined on fresh serum samples. Analysis was done on the morning of the blood collection by an enzymatic method using a test kit from Kodak Ektachem (Rochester, New York). A 10 ul drop of the serum sample was placed on a slide where it spread evenly over the surface. This slide was placed on top of a layer of reagent solution containing glucose oxidase, peroxidase, 4-aminoantipyrine hydrochloride and 1,7-dihydroxynaphthalene. When the serum sample comes in contact with the reagent, glucose is oxidized to gluconic acid by glucose oxidase, producing hydrogen peroxide as a byproduct. In the presence of peroxidase, hydrogen peroxide reacts with 4-aminoantipyrine hydrochloride and 1,7-dihydroxynaphthalene to produce a red colored dye. The intensity of the dye is directly proportional to the concentration of glucose in the serum sample.

**SERUM FRUCTOSAMINE DETERMINATION**

Fructosamine was determined by a kinetic reduction test using a test kit from Boehringer Mannheim. A reagent, containing a buffer, nitroblue tetrazolium (NBT) and uricase, was added to the serum samples. In this alkaline medium (reagent at pH 10.3) fructosamine from the serum is present in the enol form which reduces NBT to formazan. The concentration of formazan in the samples is determined by measuring the absorbance in a spectrophotometer and comparing it to a standard curve obtained during the same assay.
PLASMA INSULIN AND GLUCAGON DETERMINATION

Blood for insulin and glucagon analyses was collected in tubes in which EDTA was added to the plasma as an anticoagulant and trasylol, a proteolytic enzyme inhibitor was added to prevent the breakdown of glucagon. Both insulin and glucagon were measured by radioimmunoassay (RIA). This technique measures the concentration of antigen molecules using a radioactive label that quantitates the amount of antigen by determination of the extent to which it combines with its antibody. In this case, the antigen is insulin or glucagon. Antibodies are made to the antigen, and radioactively labeled antigen is used in the procedure. A plasma sample containing the antigen is combined with the antibody and the radiolabelled antigen. The antigen from the plasma and the added radioactive antigen will compete for binding to the antibody, and will form an equilibrium. Separating the free from the bound antigen and calculating the percent radioactive antigen bound to the antibody, the concentration of the antigen in the test sample can be calculated by comparing it to a standard curve.

The insulin determination was done using a kit from Immunocorp Sciences Inc. (Montreal, Quebec), catalogue number KTSP-11001. Plasma samples and standards were added to separate tubes coated with insulin antibody. Radioactive insulin (\(^{125}\text{I}\)-insulin) was then added to all the tubes. After an 18 hour incubation period, the liquid was decanted from the tubes and the radioactivity was measured in a gamma counter. Results of the test samples were read off the standard curve. With any RIA assay it is important that the antibody used reacts only with the antigen of interest. The anti-insulin antibody used in this kit has insignificant cross-reactivity with human pro-insulin, C-peptide and glucagon.
METHODS

The glucagon determination was done using a kit from ICN Biomedicals Inc. (Carson, CA), catalogue number 07-152101. The anti-glucagon antibody and the radioactive glucagon (\(^{125}\)I-glucagon) were added to the standards and the plasma samples and incubated for 16 hours. After incubation, a second antibody was added which binds to the first antibody. The tubes were centrifuged and all of the antibody and bound antigen precipitated out of the solution. The supernatant was decanted and the precipitate measured in a gamma counter. The concentration of glucagon in the test samples was derived by comparing the absorbance results to the standard curve. The cross-reactivity of the glucagon antibody was checked with the following compounds: gut glucagon, porcine insulin, porcine gastrin and human ACTH and the cross reactivity found was 0.0013, 0.0005, 0.0005, and 0.0002 percent, respectively.

PLASMA TOTAL CHOLESTEROL DETERMINATION

Total cholesterol was determined by an enzymatic method using a kit from Diagnostic Chemicals Limited (Charlottetown, PEI), catalogue number 225-26. A reagent containing phosphate buffer, 4-aminoantipyrine, peroxidase, cholesterol esterase, cholesterol oxidase and phenol was added to the test samples and cholesterol standards. The cholesterol esters in the sample are hydrolyzed to free cholesterol by cholesterol esterase which is present in the reagent solution. The free cholesterol is then oxidized by cholesterol oxidase, also present in the reagent, to cholesten-3-one with the simultaneous production of hydrogen peroxide. The hydrogen peroxide produced, reacts with 4-aminoantipyrine and phenol in the presence of peroxidase, to yield a chromogen with maximum absorbance at 505 nm. The intensity of the color produced is directly proportional to the concentration of
total cholesterol in the sample. The absorbance results of the samples are compared to a standard curve run during the same assay.

**Plasma HDL Cholesterol Determination**

For determination of plasma HDL cholesterol, the apo B proteins (and the lipoproteins associated with them) were precipitated from the sample using the heparin manganese method (Warnick and Albers 1978). In the presence of heparin and manganese ions apo-B containing lipoproteins form a crosslinked matrix which precipitates from plasma. The cholesterol remaining in the sample is associated with the high density lipoprotein. The same kit for total cholesterol determination described above was used to measure the HDL cholesterol from this supernatant.

**Plasma Apo B Cholesterol Determination**

The plasma apo B cholesterol was not measured directly. It was estimated by subtracting the HDL cholesterol from the total cholesterol.

**Plasma Triglyceride Determination**

Triglyceride determination was done by an enzymatic method using a kit from Diagnostic Chemicals Limited (Charlottetown PEI), catalogue number 210-78. Samples were combined with a reagent containing ATP, 3,5-dichloro-2-hydroxy-benzenesulfonic acid (DHBS), 4-aminoantipyrine, peroxidase, glycerol phosphate oxidase, glycerol kinase and lipase. This was mixed and allowed to sit for thirty minutes. During this time the triglycerides present in the plasma sample were hydrolyzed to glycerol and free fatty acids by lipase. In the presence of ATP and glycerol kinase, the glycerol is converted to glycerol-1-phosphate. The glycerol-1-phosphate is
then oxidized by glycerol phosphate oxidase to yield hydrogen peroxide. The condensation of hydrogen peroxide with DHBS and 4-aminoantipyrine in the presence of peroxidase produces a red colored quinoneimeine dye which absorbs at 515 nm. The intensity of the color is directly proportional to the amount of triglyceride in the sample. The absorbance results of the samples were compared to those of a standard curve done at the same time.

DETERMINATION OF LEVELS OF LINOLEIC (18:2N-6) AND OLEIC (18:1N-9) FATTY ACIDS IN PLASMA TRIGLYCERIDES

The levels of linoleic and oleic fatty acids in plasma triglycerides, as a percentage of total fatty acids, were determined. All the lipids were first extracted from the plasma. This was done by mixing the plasma with a solution of chloroform, methanol and saline in a 6/3/2 ratio and centrifuging for 5 minutes. The lower organic layer, containing all of the lipid soluble components was then removed. This organic layer was then filtered through a paper rinsed with chloroform and then dried down under nitrogen. The lipid sample was spotted on a thin layer chromatography (TLC) plate (PK6F Silica Gel, 500 µm thick). These plates were then placed in a TLC tank with a solvent system of hexane, diethyl ether and glacial acetic acid in a ratio of 60/40/2. Due to differing polarities, the cholesterol esters, triglycerides, fatty acids, free cholesterol and phospholipid components migrate to different regions on the plate. These components were then scraped off the plates and set aside. To determine the proportions of different fatty acids in the triglyceride component, the fatty acids must first be methylated so that they can be detected in a gas chromatograph. This methylation was done according to a procedure described by Morrison and Smith (1964). The extracted lipids were mixed with a solution of 14% boron-tri-floride in
methanol, benzene, and methanol in a ratio of 35/20/55 and the reaction proceeded at 100°C for 30 minutes. After this, 3 ml of saline and 4 ml of pentane were added to the tubes, and then they were centrifuged. The methylated fatty acids, being more soluble in pentane than in the other components move to the pentane layer. The upper pentane layer was then removed and dried down under nitrogen, leaving behind the plasma triglycerides with the methylated fatty acids. These samples were then separated and quantitated using a gas chromatograph containing a capillary column. The fatty acid peaks were identified by comparing to known standards, and the levels of linoleic (18:2n-6) and oleic (18:1n-9) acids, as a percentage of total fatty acids, were then calculated.

**STATISTICAL ANALYSIS**

Differences in subject characteristics and postprandial blood glucose responses between the two groups were assessed by two tailed independent t-tests using Excel 5 for windows (Microsoft Inc., Indianapolis IN). The remaining analyses were done using the BMPD statistical software package. Body weight was assessed using a 2x5 RM ANOVA. A 2x2 RM ANOVA was used to assess serum fructosamine results and a 2x3 RM ANOVA for unbalanced models was used to analyze the remaining parameters of glucose and lipid metabolism (glucose, insulin, glucagon, triglycerides, total cholesterol, HDL cholesterol, apo B cholesterol and percentages of oleic (18:1n-9) and linoleic (18:2n-6) fatty acids in plasma triglycerides). When significant effects were found post hoc t-tests were done to assess where differences occurred. Lastly, correlation coefficients were obtained for the change in cholesterol levels and the change in the percentages of 18:1n-9 and 18:2n-6 in plasma triglycerides.
SCOPE OF THE STUDY

This thesis was part of a larger study which also investigated the fatty acid composition of the lipid fractions and lipid hydroperoxides. The estimated sample size required was 16-18 subjects per group. Because subject recruitment was difficult, data for the first 16 subjects are presented in this thesis. The larger study was completed at another site.

ETHICAL APPROVAL

This study received approval from the University of British Columbia Clinical Screening Committee for Research Involving Human Subjects (Appendix F).
CHAPTER FOUR

RESULTS

SUBJECT RECRUITMENT

A total of 113 people inquired about this study, 16 of whom actually participated. Table 10 shows the number of people who inquired about the study after learning about it from various sources. As can be seen from this table, the newspaper advertisements and recruitment posters were the most successful recruitment efforts.

TABLE 10:
Number of People Inquiring About the Study

<table>
<thead>
<tr>
<th>Source</th>
<th>Number of People</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newspaper advertisement</td>
<td>42</td>
</tr>
<tr>
<td>Poster at doctors offices</td>
<td>23</td>
</tr>
<tr>
<td>Poster at diabetes clinics</td>
<td>17</td>
</tr>
<tr>
<td>Source not recorded</td>
<td>14</td>
</tr>
<tr>
<td>Recruitment letter</td>
<td>7</td>
</tr>
<tr>
<td>Presentation at diabetes clinics</td>
<td>7</td>
</tr>
<tr>
<td>Heard from a friend</td>
<td>2</td>
</tr>
<tr>
<td>Poster at Canadian Diabetes Association</td>
<td>1</td>
</tr>
</tbody>
</table>
The reasons why people did not participate in the study are presented in Table 11. The most common reasons were that they were not diabetic, were on insulin treatment, the university was too far to travel to, or they were simply no longer interested once they heard what the study involved.

**TABLE 11:**
Reasons for not Entering the Study

<table>
<thead>
<tr>
<th>Reasons</th>
<th>Number of People</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not diabetic</td>
<td>16</td>
</tr>
<tr>
<td>No longer interested</td>
<td>14</td>
</tr>
<tr>
<td>Taking insulin</td>
<td>11</td>
</tr>
<tr>
<td>Too far to travel</td>
<td>11</td>
</tr>
<tr>
<td>Could not make time commitment</td>
<td>7</td>
</tr>
<tr>
<td>BMI not between 19 - 36 (kg/m²)</td>
<td>7</td>
</tr>
<tr>
<td>Other health concerns</td>
<td>6</td>
</tr>
<tr>
<td>Triglycerides &gt; 4.5 (mmol/L)</td>
<td>6</td>
</tr>
<tr>
<td>HgB&lt;sub&gt;A1C&lt;/sub&gt; &gt;0.09 (%)</td>
<td>5</td>
</tr>
<tr>
<td>Taking lipid lowering medication or β-blockers</td>
<td>5</td>
</tr>
<tr>
<td>Could not tolerate the formulas</td>
<td>3</td>
</tr>
<tr>
<td>Weight unstable</td>
<td>2</td>
</tr>
<tr>
<td>Did not return phone call</td>
<td>2</td>
</tr>
<tr>
<td>Cholesterol &gt; 6.5 (mmol/L)</td>
<td>1</td>
</tr>
</tbody>
</table>
RESULTS

How those subjects who did participate heard about the study is shown in Table 12. The posters in diabetes clinics attracted most of the subjects.

**TABLE 12:**
*How Subjects Heard About the Study*

<table>
<thead>
<tr>
<th>Source</th>
<th>Number of People</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poster in diabetes clinic</td>
<td>7</td>
</tr>
<tr>
<td>Newspaper advertisement</td>
<td>4</td>
</tr>
<tr>
<td>Poster in doctors office</td>
<td>3</td>
</tr>
<tr>
<td>Recruitment letter</td>
<td>2</td>
</tr>
</tbody>
</table>

**SUBJECT CHARACTERISTICS**

Sixteen people entered this study. Eight subjects followed the Ensure with Fiber® diet, and eight followed the Glucerna® diet. One subject in the Ensure with Fiber® diet group withdrew from the study on day 15 for personal reasons. Her blood tests from days 0 and 7 are included in the statistical analyses. All the subjects had non-insulin dependent diabetes mellitus which had been documented by a physician. Many of the subjects had other health problems and these are shown in Appendix G. In addition, many were also on medications. A list of medications that each subject took on a regular basis throughout the study is provided in Appendix H. Six subjects in each group took oral hypoglycemic medication to control blood glucose levels. Table 13 shows a list of selected subject characteristics. It can be seen from this table that the subjects were well matched in these
characteristics, and there were no significant differences between groups. Figure 1 shows the activity level of the subjects.

**TABLE 13:**
Subject Characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Ensure with Fiber*</th>
<th>Glucerna*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>57.9 ± 11.9 *</td>
<td>59.0 ± 4.2</td>
</tr>
<tr>
<td>Duration of NIDDM (yrs)</td>
<td>6.1 ± 3.2</td>
<td>6.1 ± 8.3</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>79.9 ± 13.0</td>
<td>79.9 ± 16.1</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>171.8 ± 5.9</td>
<td>172.5 ± 11.1</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>26.5 ± 2.9</td>
<td>26.5 ± 3.9</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>6/2</td>
<td>5/3</td>
</tr>
<tr>
<td>Smoking Status (Y/N)</td>
<td>1/7</td>
<td>1/7</td>
</tr>
</tbody>
</table>

*mean ± standard deviation

**FIGURE 1:**
Activity Level of Subjects*

*Activity was classified as at least 20 minutes per day. Intensity was not assessed.
**RESULTS**

**PRESTUDY FOOD RECORDS**

The results from the four day food records completed just prior to the study are shown in Table 14. Two subjects in the Ensure with Fiber\textsuperscript{®} group and two in the Glucerna\textsuperscript{®} group did not complete a food record. No significant differences were found between the two groups in energy intake or in the percentage of calories coming from carbohydrate, fat or protein from these baseline food records. The subjects in the Ensure with Fiber\textsuperscript{®} group reported consuming significantly more fiber than those in the Glucerna\textsuperscript{®} group.

**TABLE 14:**
*Baseline Dietary Intake*

<table>
<thead>
<tr>
<th></th>
<th>Ensure with Fiber\textsuperscript{®} (n=6)</th>
<th>Glucerna\textsuperscript{®} (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories (kcal)</td>
<td>1848 ± 696 *</td>
<td>1657 ± 216</td>
</tr>
<tr>
<td>Carbohydrate (%)</td>
<td>52 ± 9</td>
<td>50 ± 9</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>27 ± 11</td>
<td>30 ± 7</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>20 ± 4</td>
<td>20 ± 4</td>
</tr>
<tr>
<td>Fiber (g) (a)</td>
<td>23 ± 5</td>
<td>14 ± 6</td>
</tr>
</tbody>
</table>

\*mean ± standard deviation

\(a\) p<0.05

Table 15 shows the approximate composition of fat in the prestudy dietary intakes. The accuracy of these data may be questioned because some assumptions had to be made about the types of oil and margarines used by the subjects. Therefore, these data should be interpreted only as an estimate.
of fat intake and composition. There were no significant differences between groups in the percentage of saturated, monounsaturated or polyunsaturated fat or in cholesterol intake.

**TABLE 15:**
*Baseline Dietary Fat Intake*

<table>
<thead>
<tr>
<th></th>
<th>Ensure with Fiber® (n=6)</th>
<th>Glucerna® (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated (%)</td>
<td>35.5 ± 3.8 *</td>
<td>32.2 ± 5.0</td>
</tr>
<tr>
<td>Monounsaturated (%)</td>
<td>37.0 ± 3.8</td>
<td>35.7 ± 2.8</td>
</tr>
<tr>
<td>Polyunsaturated (%)</td>
<td>17.9 ± 2.0</td>
<td>22.6 ± 5.7</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
<td>250 ± 134</td>
<td>237 ± 167</td>
</tr>
</tbody>
</table>

*mean ± standard deviation

**DIETARY INTAKE THROUGHOUT THE STUDY**

The subjects were asked to consume at least 80% of daily calories from the assigned formula, with the remainder of calories coming from the appropriate mini-meals. Table 16 shows that on average each group consumed about 90% of calories from formula. This corresponded to an average daily intake of 7.3 cans of formula per day. There was little change in the percent of calories coming from formula during the study period, thus results are shown as an average over the four weeks of the study. There was no significant difference in the mean percent of calories consumed from formula between groups.
RESULTS

TABLE 16: Percent of Total Daily Calories from Formula

<table>
<thead>
<tr>
<th></th>
<th>Ensure with Fiber\textsuperscript{®} (n=8)</th>
<th>Glucerna\textsuperscript{®} (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± standard deviation</td>
<td>90.8 ± 8.9</td>
<td>90.6 ± 6.8</td>
</tr>
<tr>
<td>Range</td>
<td>76.5 to 100</td>
<td>80.3 to 100</td>
</tr>
</tbody>
</table>

COMPLIANCE WITH THE STUDY PROTOCOL

All the subjects kept a daily record which indicated the number of cans of formula and the amount and type of any food that was consumed. This record was reviewed with the subjects on a weekly basis. The records and reviews indicated that subjects adhered to the study protocol. Some minor deviations were noted and these are included in Appendix I. As a second check of dietary compliance the subjects were asked to bring back the labels from each can of formula that they had consumed. The number of cans reported and the number of labels returned are shown in Tables 17 and 18 for the Ensure with Fiber\textsuperscript{®} and Glucerna\textsuperscript{®} groups, respectively. Most of the subjects had very few missing labels. As noted in Table 18, one subject misunderstood the instructions and threw out her labels in the first week. As a result she had 28 missing labels for the study. A few other subjects were forgetful, reporting that they sometimes threw out labels by mistake.
### TABLE 17:
**Ensure with Fiber® Group - Return of Formula Labels**

<table>
<thead>
<tr>
<th>Cans Recorded</th>
<th>Labels Returned</th>
<th>Missing</th>
</tr>
</thead>
<tbody>
<tr>
<td>233</td>
<td>226</td>
<td>7</td>
</tr>
<tr>
<td>162</td>
<td>161</td>
<td>1</td>
</tr>
<tr>
<td>89</td>
<td>85</td>
<td>4</td>
</tr>
<tr>
<td>166</td>
<td>166</td>
<td>0</td>
</tr>
<tr>
<td>214</td>
<td>211</td>
<td>3</td>
</tr>
<tr>
<td>301</td>
<td>291</td>
<td>10</td>
</tr>
<tr>
<td>242</td>
<td>242</td>
<td>0</td>
</tr>
<tr>
<td>182</td>
<td>182</td>
<td>0</td>
</tr>
</tbody>
</table>

### TABLE 18:
**Glucerna® Group - Return of Formula Labels**

<table>
<thead>
<tr>
<th>Cans Recorded</th>
<th>Labels Returned</th>
<th>Missing</th>
</tr>
</thead>
<tbody>
<tr>
<td>176</td>
<td>175</td>
<td>1</td>
</tr>
<tr>
<td>299</td>
<td>299</td>
<td>0</td>
</tr>
<tr>
<td>177</td>
<td>177</td>
<td>0</td>
</tr>
<tr>
<td>199</td>
<td>198</td>
<td>1</td>
</tr>
<tr>
<td>212</td>
<td>212</td>
<td>0</td>
</tr>
<tr>
<td>144</td>
<td>116</td>
<td>28 *</td>
</tr>
<tr>
<td>223</td>
<td>212</td>
<td>11</td>
</tr>
<tr>
<td>216</td>
<td>216</td>
<td>0</td>
</tr>
</tbody>
</table>

* Subject reported that she threw out labels in the first week of the study
**RESULTS**

**Body Weight**

Subjects were weighed at each weekly visit and some small changes were observed, most often with subjects losing weight. Table 19 shows the mean body weights of the subjects for males and females in each group on each study visit. Body weight was analyzed with a repeated measures ANOVA. There was a significant time effect for body weight, with both diet groups losing an average of about 1 kg during the study.

**Table 19:**
Body Weight of Subjects over Study Period

<table>
<thead>
<tr>
<th>Day</th>
<th>Ensure with Fiber®</th>
<th>Glucerna®</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males (n=6)</td>
<td>Females (n=2,1)**</td>
</tr>
<tr>
<td>0*</td>
<td>81.7 ± 14.8 *</td>
<td>73.0 - 75.3***</td>
</tr>
<tr>
<td>7</td>
<td>81.0 ± 13.6</td>
<td>71.0 - 74.1</td>
</tr>
<tr>
<td>14</td>
<td>81.8 ± 14.0</td>
<td>73.8</td>
</tr>
<tr>
<td>21</td>
<td>81.5 ± 14.4</td>
<td>73.4</td>
</tr>
<tr>
<td>28</td>
<td>80.7 ± 14.1</td>
<td>72.6</td>
</tr>
</tbody>
</table>

*mean ± standard deviation in kg  
**n=2 for days 0 and 7; n=1 for days 14, 21 and 28  
***range for 2 subjects  
*a time effect, p<0.01

Because of the significant time effect found for body weight, the statistical significance of this weight change on the study parameters was investigated, and the possibility of using weight change as a covariate in the
statistical analyses was considered. None of the correlations between the amount of weight change and the change in each study parameter was found to be significant. For this reason, weight change was not used as a covariate in the statistical analysis of the blood work. However, it should be noted that weight change in some individuals may have influenced their own study parameters, without significantly affecting the group as a whole.

ANTHROPOMETRIC MEASUREMENTS

Anthropometric measurements were taken on the first and last day of the study. The results are presented in Tables 20 and 21 as waist to hip ratio, sum of skinfolds (biceps, triceps, subscapular and suprailiac) and percent body fat (calculated from the sum of skinfolds) for the male and female subjects, respectively. The results for one female subject from the Ensure with Fiber® group are excluded because she dropped out of the study prior to day 28.

MISSING DATA

There are some missing data in this study. As previously mentioned, one of the subjects in the Ensure with Fiber® group withdrew from the study, thus only the day 0 and day 7 results are available for her. Also in the Ensure with Fiber® group, blood samples were not drawn for two of the subjects on day 7 because of bruising from the first blood draw. For a fourth subject in the Ensure with Fiber® group, the day 7 blood sample aliquoted for the lipid analyses was lost. For one subject in the Glucerna® group the blood sample was too small for measurement of HDL cholesterol, and thus apo B cholesterol could not be determined either.
### TABLE 20:
**Anthropometric Measurements of Male Subjects**

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Day 0</th>
<th>Day 28</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waist to hip ratio (cm/cm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ensure with Fiber®</td>
<td>0.92 ±0.03 *</td>
<td>0.92 ±0.02</td>
<td>0.00 ±0.02</td>
</tr>
<tr>
<td>Glucerna®</td>
<td>0.95 ±0.03</td>
<td>0.95 ±0.04</td>
<td>0.00 ±0.03</td>
</tr>
<tr>
<td>Sum of skinfolds (mm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ensure with Fiber®</td>
<td>55.0 ±15.1</td>
<td>55.0 ±16.6</td>
<td>0.0 ±4.5</td>
</tr>
<tr>
<td>Glucerna®</td>
<td>63.8 ±11.4</td>
<td>63.2 ±7.6</td>
<td>0.5 ±5.3</td>
</tr>
<tr>
<td>Percent body fat (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ensure with Fiber®</td>
<td>26.3 ±5.1</td>
<td>26.3 ±5.6</td>
<td>0.0 ±1.1</td>
</tr>
<tr>
<td>Glucerna®</td>
<td>29.6 ±3.3</td>
<td>29.5 ±2.2</td>
<td>0.1 ±1.2</td>
</tr>
</tbody>
</table>

* mean ± standard deviation in the Ensure with Fiber® group (n=6) and in the Glucerna® group (n=5)

### TABLE 21:
**Anthropometric Measurements of Female Subjects**

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Day 0</th>
<th>Day 28</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waist to hip ratio (cm/cm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ensure with Fiber®</td>
<td>0.85 *</td>
<td>0.86</td>
<td>0.01</td>
</tr>
<tr>
<td>Glucerna®</td>
<td>0.82 ±0.07</td>
<td>0.83 ±0.03</td>
<td>0.01 ±0.04</td>
</tr>
<tr>
<td>Sum of skinfolds (mm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ensure with Fiber®</td>
<td>89.2</td>
<td>79.7</td>
<td>-9.5</td>
</tr>
<tr>
<td>Glucerna®</td>
<td>72.2 ±8.4</td>
<td>73.3 ±12.5</td>
<td>1.2 ±6.4</td>
</tr>
<tr>
<td>Percent body fat (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ensure with Fiber®</td>
<td>41.1</td>
<td>39.6</td>
<td>-1.5</td>
</tr>
<tr>
<td>Glucerna®</td>
<td>37.3 ±3.1</td>
<td>37.4 ±3.7</td>
<td>0.1 ±1.1</td>
</tr>
</tbody>
</table>

* mean ± standard deviation in the Ensure with Fiber® group (n=1) and in the Glucerna® group (n=3)
RESULTS

STATISTICAL ANALYSES

To analyze differences in postprandial blood glucose responses, the mean difference in blood glucose from before and after all meals was compared between the groups with an independent two tailed t-test. The remainder of the study results were analyzed by repeated measures ANOVA. Because of missing data, a choice had to be made about how to proceed with the statistical analyses. If subjects with missing data were excluded the sample size would have been reduced considerably. Since this was not desirable, a repeated measures ANOVA was done with an unbalanced model statistical package (BMPD Statistical Software, Program 5V - unbalanced repeated measures models). This method uses regression analysis, based on the data available, to predict what the missing values would be. When all the missing data points are assigned values, a repeated measures ANOVA is performed on the data. Results are presented in the following tables as means and standard deviations at each time point, with any statistically significant effects indicated. The means presented are based only on the data that were collected, and do not include assigned values predicted in the ANOVA. Thus, it should be noted that some of the means may be influenced by missing data. The largest influence would be on the Ensure with Fiber® group day 7 means, as this is where most of the missing data occur. To obtain a clearer picture of individual responses to the formulas, the reader is referred to the charts in Appendix J. Post hoc analysis was done when the repeated measures ANOVA indicated a significant effect. Differences between the groups (group effects) were assessed by independent t-tests, while differences within the groups were assessed by paired t-tests. These included the change over time when both groups were considered together.
RESULTS

(time effects) and differences in the pattern of change over time between the groups (group by time interactions).

The subject who dropped out of the study had much higher HDL cholesterol concentrations than those of the rest of the Ensure with Fiber\textsuperscript{®} group. Thus, since her value is missing for day 28, the means of the group data for day 0, 7 and 28 do not accurately reflect the group means for HDL cholesterol throughout the study. Thus, for clarity in the tables and figures, her data were not included in the results for HDL cholesterol. It should be noted however, that this does not affect the conclusions for this parameter, as a significant group by time interaction was observed whether or not her data were included in the analysis. The value for fructosamine for this subject was also excluded from the statistical analysis because results were available for only one of the two time points.
PARAMETERS OF CARBOHYDRATE METABOLISM

Postprandial Blood Glucose

The postprandial blood glucose results are displayed in Figures 2 to 4. Some subjects missed a few measurements, thus the number of entries are slightly different between the groups and at different time points. Taken as an average over the whole study, (considering all meals and all time points), the rise in blood glucose after a meal was significantly lower for the Glucerna® group than for the Ensure with Fiber® group, as assessed by a two tailed independent t-test (p<0.001). This difference in postprandial rises in blood glucose is illustrated below in Figure 2. Graphical information showing the blood glucose levels before and after each meal throughout the study is provided in Figures 3 and 4.

FIGURE 2:
Postprandial Rises in Blood Glucose Levels*

* Values represent means of all blood glucose measurements just before and two hours after each meal. These means are based on 174 entries for the Ensure with Fiber® group and 188 entries for the Glucerna® group.

**p<0.001
**FIGURE 3:** Postprandial Blood Glucose for the Ensure with Fiber® Group*

* all points represent means ± standard deviations based on 14 to 16 measurements

* = mean blood glucose just before the meal

■ = mean blood glucose 2 hours after the meal
FIGURE 4: 
Postprandial Blood Glucose for the Glucerna® Group*

**BREAKFAST**

**LUNCH**

**SUPPER**

* all points represent means ± standard deviations based on 13 to 16 measurements

● = mean blood glucose just before the meal

■ = mean blood glucose 2 hours after the meal
Fasting Parameters of Carbohydrate Metabolism

Table 22 shows the results of the fasting blood tests for parameters of carbohydrate metabolism.

**TABLE 22:**
Fasting Parameters of Carbohydrate Metabolism

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum glucose (mmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ensure with Fiber®</td>
<td>8.5 ±1.8 (8)*</td>
<td>7.9 ±1.9 (6)</td>
<td>7.7 ±1.8 (7)</td>
</tr>
<tr>
<td>Glucerna®</td>
<td>7.9 ±1.2 (8)</td>
<td>7.3 ±1.1 (8)</td>
<td>6.7 ±0.9 (8)</td>
</tr>
<tr>
<td>Serum fructosamine (μmol/L)</td>
<td></td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>Ensure with Fiber®</td>
<td>305 ±33 (7)</td>
<td>**</td>
<td>310 ±61 (7)</td>
</tr>
<tr>
<td>Glucerna®</td>
<td>280 ±26 (8)</td>
<td>**</td>
<td>267 ±35 (8)</td>
</tr>
<tr>
<td>Plasma insulin (pmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ensure with Fiber®</td>
<td>168 ±65 (8)</td>
<td>151 ±58 (6)</td>
<td>148 ±67 (7)</td>
</tr>
<tr>
<td>Glucerna®</td>
<td>129 ±24 (8)</td>
<td>132 ±26 (8)</td>
<td>126 ±34 (8)</td>
</tr>
<tr>
<td>Plasma glucagon (pmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ensure with Fiber®</td>
<td>298 ±249 (8)</td>
<td>210 ±227 (6)</td>
<td>193 ±168 (7)</td>
</tr>
<tr>
<td>Glucerna®</td>
<td>171 ±97 (8)</td>
<td>134 ±118 (8)</td>
<td>102 ±55 (8)</td>
</tr>
</tbody>
</table>

* mean ± standard deviation (n)
** not measured at this time point
a time effect, p<0.001
b time effect, p<0.05
c group by time interaction, p<0.05
d time effect, p<0.01
Table 23 shows the p values resulting from post hoc analysis of significant effects from the repeated measures ANOVA for fasting parameters of carbohydrate metabolism.

**TABLE 23:**
P values from Post Hoc Analysis of Parameters of Carbohydrate Metabolism*

<table>
<thead>
<tr>
<th>EFFECTS</th>
<th>TIME POINTS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DAY 0-7</td>
</tr>
<tr>
<td><strong>TIME EFFECTS</strong></td>
<td></td>
</tr>
<tr>
<td>Serum Glucose</td>
<td>0.052*</td>
</tr>
<tr>
<td>Plasma Insulin</td>
<td>0.263</td>
</tr>
<tr>
<td>Plasma Glucagon</td>
<td>0.011*</td>
</tr>
<tr>
<td><strong>GROUP BY TIME INTERACTIONS</strong></td>
<td></td>
</tr>
<tr>
<td>Plasma Insulin</td>
<td></td>
</tr>
<tr>
<td>Ensure with Fiber</td>
<td>0.117</td>
</tr>
<tr>
<td>Glucerna</td>
<td>0.566</td>
</tr>
</tbody>
</table>

Time effects and group by time interactions were assessed by paired t-tests
* significant at p<0.05

There was a significant time effect for fasting serum glucose, with a mean decrease over time (p<0.001). This decrease in serum glucose found when the results of the two groups were combined was significant at all time intervals. There was no significant group effect or group by time interaction for the fasting serum glucose results. There was no significant group effect, time effect or group by time interaction for fasting serum fructosamine. There was a significant time effect (p <0.05) and group by time interaction
RESULTS

(p<0.05) for fasting plasma insulin, caused by a decrease in the Ensure with Fiber® group, which was significant in the interval from day 0 to 28. There was no effect of the groups on plasma insulin independent of time. A significant time effect was found for fasting plasma glucagon (p< 0.01). When both diet groups were combined, there was a significant decrease in fasting plasma glucagon from day 0 to 7. The plasma glucagon remained significantly lower throughout the study, leading to a significant difference between the day 0 and day 28 values as well. There was no significant group effect or group by time interaction for fasting plasma glucagon.

PARAMETERS OF LIPID METABOLISM

Table 24 shows the results of the fasting blood tests for parameters of lipid metabolism. Table 25 shows the p values resulting from post hoc analysis of significant effects found from the repeated measures ANOVA for fasting parameters of lipid metabolism.
### TABLE 24:
*Parameters of Lipid Metabolism*

<table>
<thead>
<tr>
<th>Plasma measurements</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Triglycerides (mmol/L)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ensure with Fiber®</td>
<td>2.05 ± 1.28 (8)*</td>
<td>1.35 ± 0.77 (6)</td>
<td>2.17 ± 1.61 (7)</td>
</tr>
<tr>
<td>Glucerna®</td>
<td>1.13 ± 0.80 (8)</td>
<td>1.00 ± 0.63 (8)</td>
<td>1.22 ± 0.88 (8)</td>
</tr>
<tr>
<td><strong>Total cholesterol (mmol/L)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ensure with Fiber®</td>
<td>4.76 ± 0.88 (8)</td>
<td>4.29 ± 1.42 (6)</td>
<td>4.28 ± 1.22 (7)</td>
</tr>
<tr>
<td>Glucerna®</td>
<td>5.04 ± 0.90 (8)</td>
<td>4.92 ± 1.56 (8)</td>
<td>5.25 ± 1.62 (8)</td>
</tr>
<tr>
<td><strong>HDL cholesterol (mmol/L)</strong></td>
<td>a,b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ensure with Fiber®</td>
<td>0.98 ± 0.26 (7)</td>
<td>0.88 ± 0.23 (5)</td>
<td>1.14 ± 0.35 (7)</td>
</tr>
<tr>
<td>Glucerna®</td>
<td>1.45 ± 0.33 (8)</td>
<td>1.42 ± 0.28 (7)</td>
<td>1.31 ± 0.38 (8)</td>
</tr>
<tr>
<td><strong>Apo B cholesterol (mmol/L)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ensure with Fiber®</td>
<td>3.57 ± 0.96 (8)</td>
<td>3.01 ± 1.45 (6)</td>
<td>3.22 ± 1.69 (7)</td>
</tr>
<tr>
<td>Glucerna®</td>
<td>3.59 ± 0.68 (8)</td>
<td>3.41 ± 1.46 (7)</td>
<td>3.94 ± 1.37 (8)</td>
</tr>
<tr>
<td><strong>% 18:1n-9 in triglycerides</strong></td>
<td>c,d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ensure with Fiber®</td>
<td>43.8 ± 2.3 (8)</td>
<td>39.5 ± 1.7 (6)</td>
<td>36.4 ± 3.1 (7)</td>
</tr>
<tr>
<td>Glucerna®</td>
<td>42.6 ± 0.9 (8)</td>
<td>47.9 ± 1.3 (8)</td>
<td>47.8 ± 1.2 (8)</td>
</tr>
<tr>
<td><strong>% 18:2n-6 in triglycerides</strong></td>
<td>b,c,e</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ensure with Fiber®</td>
<td>17.1 ± 4.7 (8)</td>
<td>24.4 ± 2.5 (6)</td>
<td>26.3 ± 5.9 (7)</td>
</tr>
<tr>
<td>Glucerna®</td>
<td>15.9 ± 4.0 (8)</td>
<td>17.4 ± 0.7 (8)</td>
<td>17.0 ± 2.2 (8)</td>
</tr>
</tbody>
</table>

* mean ± standard deviation (n)

a group effect, p<0.01
b group by time interaction, p<0.01
c group effect, p<0.001
d group by time interaction, p<0.001
e time effect, p<0.001
### TABLE 25:
P values from Post Hoc Analysis of Parameters of Lipid Metabolism

<table>
<thead>
<tr>
<th>EFFECTS</th>
<th>TIME POINTS</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DAY 0</td>
<td>DAY 7</td>
<td>DAY 28</td>
<td></td>
</tr>
<tr>
<td><strong>GROUP EFFECTS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL Cholesterol</td>
<td>0.008*</td>
<td>0.011*</td>
<td>0.384</td>
<td></td>
</tr>
<tr>
<td>Oleic Acid</td>
<td>0.125</td>
<td>0.005*</td>
<td>0.000*</td>
<td></td>
</tr>
<tr>
<td>Linoleic Acid</td>
<td>0.589</td>
<td>0.043*</td>
<td>0.005*</td>
<td></td>
</tr>
<tr>
<td><strong>TIME EFFECTS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linoleic Acid</td>
<td>0.019*</td>
<td>0.863</td>
<td>0.018*</td>
<td></td>
</tr>
<tr>
<td><strong>GROUP BY TIME INTERACTIONS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL Cholesterol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ensure with Fiber®</td>
<td>0.949</td>
<td>0.345</td>
<td>0.226</td>
<td></td>
</tr>
<tr>
<td>Glucerna®</td>
<td>0.799</td>
<td>0.310</td>
<td>0.014*</td>
<td></td>
</tr>
<tr>
<td>Oleic Acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ensure with Fiber®</td>
<td>0.062</td>
<td>0.323</td>
<td>0.006*</td>
<td></td>
</tr>
<tr>
<td>Glucerna®</td>
<td>0.003*</td>
<td>0.723</td>
<td>0.003*</td>
<td></td>
</tr>
<tr>
<td>Linoleic Acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ensure with Fiber®</td>
<td>0.035*</td>
<td>0.693</td>
<td>0.018*</td>
<td></td>
</tr>
<tr>
<td>Glucerna®</td>
<td>0.116</td>
<td>0.213</td>
<td>0.300</td>
<td></td>
</tr>
</tbody>
</table>

Group effects were assessed by independent t-tests. Time effects and group by time interactions were assessed by paired t-tests.
*significant at p<0.05
For fasting plasma triglycerides, total cholesterol and apoB cholesterol, no significant group effect, time effect, or group by time interaction was observed.

There was a significant group effect (p<0.01) and a significant group by time interaction (p<0.01) for fasting plasma HDL cholesterol. No significant time effect was observed for fasting plasma HDL cholesterol. The two diet groups had significantly different HDL cholesterol levels on days 0 and 7, but not on day 28. The Ensure with Fiber® group showed no significant change in HDL cholesterol over time, whereas the Glucerna® group demonstrated a significant reduction in HDL cholesterol from day 0 to 28.

A significant group by time interaction (p < 0.001) and a significant group effect (p < 0.001) were found for the percentage of oleic acid (18:1n-9) in plasma triglycerides. The Ensure with Fiber® group displayed a significant decrease in the percentage of oleic acid (18:1n-9) in plasma triglycerides from day 0 to 28, while the Glucerna® group displayed a significant increase from day 0 to 7 and day 0 to 28. The two diet groups did not differ in the percentage of oleic acid in plasma triglycerides at the start of the study (day 0), however, as a result of the changes over time, the groups were significantly different in this parameter on days 7 and 28.

There was a significant group effect (p < 0.001), a significant time effect (p < 0.001), and a significant group by time interaction (p < 0.01) found for the percentage of linoleic acid (18:2 n-6) in plasma triglycerides. The Ensure with Fiber® group exhibited a significant increase in this parameter from day 0 to 7 and day 0 to 28, while the Glucerna® Group showed no significant change over time. The two diet groups did not differ in the percentage of linoleic acid in plasma triglycerides at the start of the study
RESULTS

(day 0). However, as a result of the increase in the Ensure with Fiber® Group, the groups were significantly different in this parameter on days 7 and 28.

ILLUSTRATIONS OF GROUP BY TIME INTERACTIONS

The significant group by time interactions found in this study are illustrated in Figures 5 to 8.
RESULTS

FIGURE 5:
Group by Time Interaction for Fasting Plasma Insulin*

* Points represent diet group means on days 0, 7 and 28

FIGURE 6:
Group by Time Interaction for Plasma HDL Cholesterol*

* Points represent diet group means on days 0, 7 and 28
FIGURE 7:
Group by Time Interaction for % Oleic Acid (18:1n-9)
in Plasma Triglycerides*

![Graph showing % Oleic Acid over time for two groups: Ensure with Fiber and Glucerna.](image)

* Points represent diet group means on days 0, 7 and 28

FIGURE 8:
Group by Time Interaction for % Linoleic Acid (18:2n-6)
in Plasma Triglycerides*

![Graph showing % Linoleic Acid over time for two groups: Ensure with Fiber and Glucerna.](image)

* Points represent diet group means on days 0, 7 and 28
RESULTS OF RM ANOVA ANALYSIS OF FASTING PARAMETERS OF CARBOHYDRATE AND LIPID METABOLISM

The p values for the fasting parameters of carbohydrate and lipid metabolism, as assessed by repeated measures ANOVA for unbalanced models, are provided in Table 26.

**TABLE 26:**
P Values for Fasting Parameters of Carbohydrate and Lipid Metabolism

<table>
<thead>
<tr>
<th></th>
<th>Effect of Group</th>
<th>Effect of Time</th>
<th>Group by Time Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>0.284</td>
<td>0.000*</td>
<td>0.951</td>
</tr>
<tr>
<td>Fructosamine</td>
<td>0.093</td>
<td>0.708</td>
<td>0.328</td>
</tr>
<tr>
<td>Insulin</td>
<td>0.344</td>
<td>0.029*</td>
<td>0.028*</td>
</tr>
<tr>
<td>Glucagon</td>
<td>0.241</td>
<td>0.002*</td>
<td>0.345</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.113</td>
<td>0.195</td>
<td>0.780</td>
</tr>
<tr>
<td>Total Cholesterol</td>
<td>0.321</td>
<td>0.420</td>
<td>0.234</td>
</tr>
<tr>
<td>HDL Cholesterol</td>
<td>0.008*</td>
<td>0.860</td>
<td>0.004*</td>
</tr>
<tr>
<td>Apo B Cholesterol</td>
<td>0.540</td>
<td>0.637</td>
<td>0.151</td>
</tr>
<tr>
<td>% Oleic Acid</td>
<td>0.000*</td>
<td>0.539</td>
<td>0.000*</td>
</tr>
<tr>
<td>% Linoleic Acid</td>
<td>0.000*</td>
<td>0.000*</td>
<td>0.002*</td>
</tr>
</tbody>
</table>

* significant at p<0.05
RESULTS

CORRELATIONS OF FATTY ACIDS WITH PLASMA CHOLESTEROL

Correlation coefficients were obtained for the change in oleic (18:1n-9) and linoleic (18:2 n-6) acid in plasma triglycerides with the change in cholesterol levels. The results are shown in Table 27. The only significant finding was an inverse correlation between the change in total cholesterol and the change in linoleic acid from day 7 to day 28 (r = -0.66, p<0.05).

TABLE 27:
Correlation Coefficients of the Change in Fatty Acids in Plasma Triglycerides and the Change in Cholesterol Levels

<table>
<thead>
<tr>
<th>Plasma Measurement</th>
<th>Day 0 to 7</th>
<th>Day 7 to 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oleic acid</td>
<td>0.26</td>
<td>0.51</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>-0.29</td>
<td>-0.66*</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oleic acid</td>
<td>0.02</td>
<td>-0.14</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>-0.28</td>
<td>0.07</td>
</tr>
<tr>
<td>Apo B cholesterol (mmol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oleic acid</td>
<td>-0.29</td>
<td>0.38</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>0.15</td>
<td>-0.50</td>
</tr>
</tbody>
</table>

* p<0.05
CHAPTER FIVE

DISCUSSION

SUMMARY OF PURPOSE

This study was conducted to evaluate the effect of a diet high in monounsaturated fatty acids on glucose and lipid metabolism in patients with NIDDM, as compared to a lower fat, higher carbohydrate diet. Previous studies have suggested that diets high in monounsaturated fat result in better glucose and lipid profiles in NIDDM patients than higher carbohydrate diets. Two nutritional formulas, Ensure with Fiber® (high in carbohydrate) and Glucerna® (high in monounsaturated fat) were used as the major source of energy in the study diets. The main objective in testing these different diets in diabetes is to minimize the risk factors of diabetic complications. These include high day-long and fasting blood glucose levels, which are risk factors for microvascular complications (Diabetes Control and Complications Trial Research Group, 1993), and high plasma triglycerides and VLDL cholesterol and low HDL cholesterol, which have been shown to be powerful predictors of both fatal and nonfatal coronary heart disease events in NIDDM subjects (Laakso et al, 1993). In addition, LDL cholesterol is strongly indicated in the etiology of atherosclerosis (Grundy, 1995).

MAJOR FINDINGS

The major findings of the study were as follows. The Glucerna® diet resulted in lower postprandial increases in blood glucose than the Ensure with Fiber® diet. There was no difference in the fasting serum glucose levels between the two diet groups, however, there was a significant time effect which showed a decrease in fasting serum glucose when all the subjects were
considered together. The diets had no significant effect on serum fructosamine, which is a longer term measure of blood glucose control. The Ensure with Fiber® diet produced a decrease in plasma insulin. When all subjects were considered together, a significant decrease in plasma glucagon over time was observed. As for the lipid parameters, the diets had no significant effect on plasma triglycerides, total cholesterol or apo B cholesterol. There was a significant group by time interaction found for HDL cholesterol, caused by a significant decrease in the Glucerna® group. As expected, the fat content of the diets was reflected in the subjects fatty acid profile of plasma triglycerides. The Ensure with Fiber® group displayed an increase in linoleic acid (18:2n-6) and a decrease in oleic acid (18:1n-9) in their plasma triglycerides, while the Glucerna® group displayed an increase in oleic acid (18:1n-9). A significant inverse correlation was found between the change in the amount of linoleic acid (18:2n-6) in plasma triglycerides and the total cholesterol level, in which an increase in linoleic acid (18:2n-6) was associated with a decrease in total cholesterol.

**SUBJECT CHARACTERISTICS**

The two groups were well matched for the following subject characteristics: age, duration of NIDDM, weight, height, BMI, gender and smoking status. The groups were also well matched for the number of subjects taking oral hypoglycemic medications. Most subjects reported no change in their activity levels throughout the study, and thus activity is not expected to have affected the results of the investigation.
ANTHROPOMETRIC DATA

Anthropometric measurements of the subjects were taken at the beginning and at the end of the study. This was done to obtain descriptive data about the subjects and to assess if any large changes had taken place over the study period. These were not major study variables, and statistical analysis of this information was not done, except for body weight.

Body mass index was calculated at the beginning of the study. Body mass index (BMI, kg/m$^2$) is a measure of relative weight for height. A BMI under 20 indicates underweight, 20-25 indicates a healthy weight, 25-27 may be associated with some health risks for some people, over 27 is associated with increased risk of health problems and a BMI over 30 is considered obese (Gibson, 1990). The mean BMI of the subjects in this study was 26.5 in both of the diet groups, which is slightly above the normal healthy range. Since most NIDDM patients are overweight, it is not surprising that these subjects did not fall in the normal BMI range of 20-25.

Body weight was measured at each visit. There was a significant time effect found for body weight, in which the subjects lost a small but significant amount of weight over the four week study period. This change in body weight was not significantly correlated with the change in any of the metabolic parameters of the study. Energy requirements were estimated at the beginning of the study, and based on these calculations a meal plan was given to each subject. At weekly intervals, subjects were weighed and if weight change occurred, energy intake was adjusted to compensate for this. Despite these efforts there was a small, but significant weight loss over time.

The waist to hip ratio was measured at the beginning and end of the study. The waist to hip ratio is an indicator of upper body fatness. This measurement was included because central adiposity (which results in a
large waist to hip ratio) has been associated with insulin insensitivity (Pedersen et al, 1993) and increased risk for coronary heart disease (Oshaug et al, 1995). A waist to hip ratio greater than 1.0 for men and 0.8 for women may be indicative of an increased risk for cardiovascular disease (Gibson, 1990). The mean waist to hip ratio for the men in this study was 0.95 for both the Ensure with Fiber® and Glucerna® groups. This is within the normal healthy range for men. The mean waist to hip ratios for the women in the study were 0.85 and 0.82, respectively, for the Ensure with Fiber® and Glucerna® groups. This is slightly above what is considered healthy.

**STUDY DESIGN**

The present study utilized a randomized controlled design in which each subject followed only one of the specified study diets. The formula, which provided at least 80% of daily calories, was supplied to the subjects and specific recipes and instructions were given concerning extra foods that were allowed. All of the previous studies that have compared a high carbohydrate diet to a high monounsaturated fat diet have utilized a crossover design, in which each subject consumed both diets in random order. Both of these study designs have their own strengths and weaknesses. Since a crossover design utilizes the same subjects in each study group, the between group variation will be very small. Because of this, fewer subjects are required. However, there is usually a chance that a carry over effect may occur from one diet period to the other. A randomized controlled design does not have the problem of carry over effect of the other diet. However, it is much more likely to result in larger between group variation, especially with small sample sizes. Part of the reason for using a randomized design was due to practicality. It was felt for this study, that recruiting subjects to drink
a formula diet for two months instead of one would be very difficult. As it turned out, it took a long time to recruit 16 subjects to participate for only 28 days, and it may have been extremely difficult to find subjects to participate for twice as long. One of the advantages of this study is that it does not have carry over effects that some of the previous studies may have had due to short wash out periods. The length of the present study, being 28 days, allowed comparisons to other studies. Of the previous studies discussed, one study was conducted for 14 days (Campbell et al, 1994), one for 21 days (Garg et al 1992b), three for 28 days (Garg et al 1988; Garg et al 1992; Lerman-Garber et al, 1994) and three for 42 days (Garg et al 1994; Lerman-Garber et al, 1995; Abbruzzese 1993).

**STUDY DIETS**

All of the studies reviewed in this research area have compared the metabolic effects of a high carbohydrate diet to a high monounsaturated fat diet. Although each of these diets in the various studies have been similar, there have been small variations between them.

The high carbohydrate diet (Ensure with Fiber®) in this study contained 55% of energy as carbohydrate (17.6% simple, 37.4% complex), 30.5% fat (5% saturated, 8% monounsaturated, 18% polyunsaturated) and 14.5% protein. A few studies have used diets with even higher amounts of carbohydrate. Four studies had diets with 60% of energy as carbohydrate (Garg et al, 1988; Garg et al 1992b; Lerman-Garber et al, 1994; Lerman-Garber et al, 1995) and one used a diet with 65% of energy as carbohydrate, much of it simple carbohydrate (Garg et al, 1992a). Two studies had the same amount of total carbohydrate as Ensure with Fiber® (Garg et al, 1994; Campbell et al, 1994). As well, the fat compositions of the previous high
carbohydrate diets have tended to be higher in saturated and lower in polyunsaturated fatty acids than Ensure with Fiber®.

The high monounsaturated fat diet in this study (Glucerna®) contained 33.3% of energy as carbohydrate (7.1% simple, 26.4% complex), 50% fat (5% saturated, 32% monounsaturated, and 13% polyunsaturated) and 16.7% protein. Several of the previous studies have utilized diets that have not been as high in total fat. Two studies contained 45% of energy as fat (Garg et al, 1992a; Garg et al, 1994), two utilized a diet with 40% fat (Lerman-Garber et al, 1994; Lerman-Garber et al, 1995) and one with 37% fat (Campbell et al, 1994). Three studies have used diets with the same amount of fat as Glucerna® (Garg et al, 1988; Garg et al, 1992; Rasmussen et al, 1993). The high monounsaturated fat diets in the previous studies have tended to be higher in saturated and lower in monounsaturated or polyunsaturated fatty acids than Glucerna®.

Most of the previous studies have been conducted with solid food diets, either in a metabolic ward setting (Garg et al, 1988; Garg et al, 1992b) or on an outpatient basis in which food was provided by the investigators (Garg et al, 1994) or in which food is prepared by the subjects (Rasmussen et al, 1993; Campbell et al, 1994; Lerman-Garber et al, 1994; Lerman-Garber et al, 1995). Thus, the benefits of a high monounsaturated fat diet have been seen in diets with varying control over dietary compliance. Liquid diets were used in only two other studies (Garg et al, 1992a; Abbruzzese et al, 1993). The former used different formulas than the present study, while the latter used Glucerna®. Thus, there have been previous reports of benefits from a high monounsaturated fat diet in the form of a liquid diet.

The fiber content of the two diets during the present study was similar. Both formulas contained 3.3g of fiber per can. Thus, for a 2000 kcal
diet, consisting of 100% of calories from formula, there would be 25.4g of fiber in the Ensure with Fiber® diet and 27.8g of fiber in the Glucerna® diet. The source of this fiber is soy polysaccharide, which is 90% insoluble and 10% soluble. Similar to the present study, most of the previous studies have compared high carbohydrate and high monounsaturated fat diets with similar amounts of fiber. The amount of fiber between the different studies has varied however, ranging from 0 to 40 g per day. (Garg et al, 1988 Garg et al, 1992a, Garg et al 1992b, Garg et al, 1994, Rasmussen et al, 1993, Campbell et al, 1994). Lerman-Garber et al, (1995) attempted to make the fiber content proportional to the carbohydrate content of the diet by using 42g of fiber in the high carbohydrate diet and 30g in the high monounsaturated fat diet. This may be important because high fiber intakes might prevent the increase in triglycerides induced by a high carbohydrate diet (Anderson et al, 1980). The study by Anderson et al, (1980) that showed this effect of fiber, utilized a diet with a very high amount of 64 g of fiber.

Prior to this study, the subjects in the Ensure with Fiber® group reported consuming more fiber than the subjects in the Glucerna® group. During this study the increased fiber intake in the Glucerna® group may have had some influence on the study results. However, it would be expected that if anything this would improve the performance of Glucerna® compared to Ensure with Fiber®, which was not generally found.

PARAMETERS OF CARBOHYDRATE METABOLISM

Postprandial Blood Glucose

This study found that the high monounsaturated fat diet, Glucerna®, resulted in a lower mean postprandial rise in blood glucose levels than the high carbohydrate diet, Ensure with Fiber®. This finding is consistent with
other studies that have evaluated postprandial blood glucose on a high carbohydrate vs. a high monounsaturated fat diet (Garg et al, 1988; Garg et al 1994; Campbell et al 1994), and more specifically a high carbohydrate formula vs. Glucerna® (Davidson et al, 1989; Peters et al, 1992; Harley et al, 1989; Galkowski et al, 1989). Glucerna® derives 7% of its calories from fructose. Fructose produces less of an acute blood glucose response than sucrose (Crapo et al, 1980; Bantle et al, 1986; Crapo et al, 1986; McAteer et al, 1987). Thus, the lower postprandial blood glucose resulting from the Glucerna® diet is likely due to both the low carbohydrate, high monounsaturated fat content of the diet, and the presence of fructose as the sweetener instead of sucrose. This finding of lower postprandial rises in blood glucose presents a definite advantage of the high monounsaturated fat formula, Glucerna®, since lowering day-long blood glucose levels may help to prevent the chronic microvascular complications of NIDDM (Diabetes Control and Complications Trial Research Group, 1993).

**Fasting Serum Glucose**

The present study indicates that the consumption of the two diets resulted in no significant difference in the pattern of change for fasting blood glucose. This is in agreement with most of the studies reported in the literature showing no significant difference in fasting blood glucose after a high carbohydrate diet or a high monounsaturated fat diet. (Garg et al, 1992a; Garg et al, 1992b; Garg et al, 1994; Campbell et al, 1994; Lerman-Garber et al, 1994; Lerman-Garber et al, 1995). There was a wide range of fasting blood glucose levels represented in these published studies, with group means ranging from 6.6 to 12.2 mmol/L. One study did show a lower fasting blood glucose level after a high monounsaturated fat diet (Rasmussen
et al., 1993), where fasting blood glucose levels were 6.8 and 6.1 mmol/L after the high carbohydrate and high monounsaturated fat diets, respectively. The mean ± SD of fasting serum glucose levels in the present study for the Ensure with Fiber® group were 8.5 ± 1.8, 7.9 ± 1.9, and 7.7 ± 1.8 mmol/L on days 0, 7, and 28, respectively. For the Glucerna® group they were 7.9 ± 1.2, 7.3 ± 1.1, and 6.7 ± 0.9 mmol/L on days 0, 7, and 28, respectively. The normal range for fasting blood glucose is 3.9 to 6.7 mmol/L (Malarkey and McMorrow, 1996). Since these were diabetic subjects it is not unexpected that the glucose levels found in the present study were slightly higher than the normal range. It should be noted, however, that the blood glucose level was not excessively high, indicating that these subjects were in moderate metabolic control.

There was a significant time effect found for fasting serum glucose, with a decrease in blood glucose levels over the 28 day study, when the results for the two groups were combined. This decrease was significant at all time intervals (i.e. from day 0 to 7 from day 7 to 28 and from day 0 to 28). The decrease in blood glucose is likely related to the decrease in glucagon also found after both diets. This finding suggests a benefit of both diets compared to the subjects' regular diets. The mean macronutrient composition of the subjects baseline diets was similar to that of Ensure with Fiber®. Perhaps the lowering of fasting blood glucose can be partially attributed to the consistency of dietary intake, or regular fiber intake, and in the case of the Glucerna® group, less carbohydrate intake. Stress can increase blood glucose levels (Kjeldsen et al., 1996) Perhaps these subjects felt they were doing something "healthy" by participating in a research study and the calming effect of this (reduced stress) may have decreased serum glucose levels. Making food choices may be a stressful event for diabetic
DISCUSSION

persons. During this research study, that source of stress was removed from these subjects. On the other hand, the subjects may have experienced more stress by being on a very restrictive, mostly liquid diet, in which case this could not explain the lowered blood glucose levels. In conclusion, serum glucose levels decreased significantly in this study, however, the effect of the Ensure with Fiber® diet was not different from the effect of the Glucerna® diet.

Fasting Serum Fructosamine

The fructosamine determination, representing average blood glucose levels for the past 1-4 weeks, revealed no significant differences between the two diets in this study. The mean ± SD of fructosamine levels for the Ensure with Fiber® group were 305 ± 33 and 310 ± 61 μmol/L on days 0 and 28. For the Glucerna® group they were 280 ± 26 and 267 ± 35 μmol/L on days 0 and 28. The reference range given by the laboratory that ran the fructosamine analysis was 230 to 300 μmol/L. The test kit itself stated that the high range of normal should be 285 μmol/L. A study which examined fructosamine levels found that the mean ± SD was 227 ± 35 μmol/L for nondiabetic patients, 299 ± 56 μmol/L for individuals with diabetes in good/moderate control and 408 ± 95 μmol/L for individuals with diabetes in poor control (Cefalu, 1991). Thus, the subjects in the present study had fructosamine levels on the high end of normal or just above the normal range, which would be expected for NIDDM patients in moderate metabolic control. As well, they had values very similar to those found previously for diabetic subjects in good/moderate control. There was a trend for a group effect (p=0.093) which suggests that the Ensure with Fiber® group may have been in slightly worse metabolic control than the Glucerna® group. Although a significant
difference in the pattern of change in fructosamine was not found between the two diets, the Ensure with Fiber® diet did produce a slight increase in fructosamine, while the Glucerna® diet produced a slight decrease from baseline. Although these are minor and non-significant effects, they are consistent with the lower postprandial blood glucose responses on the Glucerna® diet. The only other studies of this kind that evaluated serum fructosamine also found no significant difference between a high carbohydrate and a high monounsaturated fat diet (Campbell et al 1994; Lerman-Garber et al, 1994). The fructosamine levels in these studies were similar to those in the present study. In conclusion, the present finding of fructosamine levels shows that the high monounsaturated fat diet, Glucerna®, did not result in significantly better month long average blood glucose control than the high carbohydrate diet, Ensure with Fiber®.

**Fasting Plasma Insulin**

There was a significant group by time interaction found for fasting plasma insulin in this study. The Ensure with Fiber® group started the study with insulin levels of 168 ± 64 pmol/L which dropped to 151 ± 58 pmol/L on day 7 and 148 ± 67 pmol/L at the end of the study. In contrast, the Glucerna® group started the study with lower insulin levels of 129 ± 24 pmol/L which stayed quite consistent over time, being 132 ± 26 and 126 ± 34 pmol/L on days 7 and 28 respectively. The normal range for fasting insulin is 34 to 172 pmol/L (Malarkey and McMorrow, 1996). Thus, the subjects in this study had insulin levels on the high end of normal. Since they were diabetic subjects it is expected that insulin levels might be normal to high. The significant group by time interaction resulted from a significant decrease in plasma insulin in the Ensure with Fiber® group from day 0 to 28. This may
suggest that the subjects following the Ensure with Fiber® diet started in a slight insulin resistant state which showed some improvement over the course of the study. Since both insulin and glucose levels declined over time for this group, it is likely that insulin sensitivity increased. It is not clear why the Ensure with Fiber® diet produced the beneficial effect of lower plasma insulin levels. Perhaps for similar reasons stated above for glucose (i.e. consistency of dietary intake). It might be expected that a high carbohydrate diet would raise insulin levels. However, the Ensure with Fiber® diet contained 55% of energy as carbohydrate and this amount was not any higher in carbohydrate than what the subjects reported eating before the study. A significant time effect was also found for fasting plasma insulin. This was a result of the decrease in the Ensure with Fiber® group, since there was no significant change over time in the Glucerna® group. Two other studies have evaluated fasting plasma insulin after a high carbohydrate and a high monounsaturated fat diet, both of which showed no significant effect of diet. The first included NIDDM subjects with insulin levels similar to those in the present study (Garg et al 1994), while the second included NIDDM subjects with much lower insulin levels (Campbell et al 1994). Significant findings have previously been reported for other parameters of insulin. A high monounsaturated fat diet has been shown to reduce insulin requirements (Garg et al 1988) and reduce day-long insulin levels (Garg et al 1994) compared to a high carbohydrate diet. These parameters of insulin metabolism were not assessed in the present study therefore it is not known what effect Glucerna® and Ensure with Fiber® would have on them. In conclusion, the results of this study showed no benefit of the high monounsaturated fat diet (Glucerna®) on fasting plasma insulin levels. In
fact, the high carbohydrate diet (Ensure with Fiber®) actually decreased fasting plasma insulin levels, while Glucerna® did not.

**Fasting Plasma Glucagon**

The fasting plasma glucagon levels of the subjects in the present study, when considered together, showed a significant decrease in the first week of the study, then remained at that lower level until the completion of the study. There was no difference in response between the two diet groups. The mean ± SD of fasting plasma glucagon in the Ensure with Fiber® group was 298 ± 249 pg/ml on day 0, 210 ± 227 pg/ml on day 7, and 193 ± 168 pg/ml on day 28. The mean ± SD of fasting glucagon in the Glucerna® group was 171 ± 97 pg/ml on day 0, 134 ± 118 pg/ml on day 7, and 102 ± 55 pg/ml on day 28. It should be noted that the standard deviations of the glucagon results are very high, reflecting the large individual variability in this measure. The normal range of fasting glucagon is 50 to 100 pmol/L (Malarkey and McMorrow 1996). The glucagon levels of the present subjects were much higher than the normal range, indicating considerable counterregulatory hormone activity which would tend to elevate blood glucose levels. When glucagon levels are high (as in this study) a decrease in glucagon would be beneficial because it would tend to decrease blood glucose levels. None of the other studies in this area of research have measured fasting glucagon, thus comparisons cannot be made. It has been speculated that persistent hyperglycemia perpetuates insulin resistance and increases glucagon levels in NIDDM patients (Garg et al, 1992). Thus, a high carbohydrate diet, if it increases blood glucose levels, may eventually lead to increased plasma glucagon. Since both diets in this study were found to decrease blood glucose it was not expected that an increase in glucagon through this mechanism
would be observed. The decreased glucagon levels on both of these study diets may have contributed to the decrease in fasting serum glucose found after both diets. It is possible that consistency of dietary intake or possibly reduced stress during the study period may have influenced glucagon levels. In conclusion, both diets in this study were beneficial in bringing the plasma glucagon levels down and did so within the first week of the study, but there was no benefit of one over the other diet in this respect.

PARAMETERS OF LIPID METABOLISM

Fasting Plasma Triglycerides

There was no significant effect of the diets on fasting plasma triglycerides. The mean ± SD of fasting plasma triglycerides in the Ensure with Fiber® group was 2.05 ± 1.28 mmol/L on day 0, 1.35 ± 0.77 mmol/L on day 7, and 2.17 ± 1.61 mmol/L on day 28. The mean ± SD of fasting plasma triglycerides in the Glucerna® group was 1.13 ± 0.88 mmol/L on day 0, 1.00 ± 0.63 mmol/L on day 7, and 1.22 ± 0.88 mmol/L on day 28. The normal range of fasting plasma triglycerides is 0.59 to 3.5 mmol/L (Malarkey and McMorrow, 1996). The mean triglyceride levels of these subjects fell within that normal range. NIDDM subjects as a whole tend to have elevated triglyceride levels, however, since subjects with triglycerides above 4.5 mmol/L were excluded from the study it is not surprising that the plasma triglycerides of these subjects were within the normal range. It was anticipated based on previous studies that the Glucerna® diet might result in lower triglyceride levels than the Ensure with Fiber® diet. A decrease in fasting plasma triglycerides would be beneficial because high triglyceride levels are a risk factor for coronary heart disease in NIDDM patients (Laakso et al, 1993). The mean fasting triglyceride levels of subjects in most of the
studies reported in the literature have been higher than those found in the present study and have resulted in lower plasma triglycerides after a high monounsaturated fat diet than after a high carbohydrate diet (Garg et al 1988; Garg et al 1992a; Garg et al 1992b; Garg et al 1994; Abbruzzese et al, 1995). The above studies have had mean triglyceride levels ranging from 2.19 to 4.45 mmol/L. This lowering effect of plasma triglycerides from a high monounsaturated fat diet was also found in two studies when the initial plasma triglyceride levels was low (Campbell et al, 1994; Lerman-Garber et al, 1994). In Campbell’s study the mean triglyceride level after the high carbohydrate diet was 1.4 and after the high monounsaturated fat diet 1.1 mmol/L. In Lerman-Garber’s study the high monounsaturated fat diet resulted in significantly lower triglycerides compared to baseline (1.75 vs. 1.25 mmol/L - before vs. after), while the high carbohydrate diet did not (1.78 vs. 1.61 mmol/L - before vs. after). Two different studies have found no significant difference in plasma triglycerides after a high carbohydrate and a high monounsaturated fat diet. Rasmussen et al, (1993) reported mean triglyceride levels to be 1.6 mmol/L after both of the study diets. The study by Lerman-Garber et al (1995) found no difference in plasma triglycerides after the two diets, using subjects who were in poor metabolic control and with high plasma triglyceride levels (mean of 3.72 and 3.82 mmol/L after the high carbohydrate and high monounsaturated fat diets respectively), although they found non-significant increases in triglycerides from both diets. The high carbohydrate diet in their study had 42g of fiber/day, while the high monounsaturated fat diet had only 30g of fiber/day. This increase in fiber in the high carbohydrate diet might have contributed to the finding of no difference in triglycerides after the two diets. As well, the fact that the
subjects were in poor metabolic control may have led to a lack of benefit from the high monounsaturated fat diet on plasma triglyceride levels.

The results of the present study do not concur with the majority of the previous work which has found a lowering of plasma triglycerides from a high monounsaturated fat diet as compared to a high carbohydrate diet. As mentioned, most of the other studies (all except three, two that found a significant difference and one that did not) have included subjects with higher plasma triglyceride levels than those in the present study. Thus, the lowering of plasma triglycerides by a high monounsaturated fat diet as compared to a high carbohydrate diet may only be readily apparent when plasma triglyceride levels are high. In fact, in the report by Lerman-Garber et al (1994), it was pointed out that even though plasma triglycerides were lower after a high monounsaturate fat diet, it was the individuals with the higher baseline triglyceride values who obtained large decrements in their triglyceride levels. If this is an important factor, then the low baseline triglycerides in the present study may partly explain why no effect of diet on plasma triglyceride levels was observed.

Another possible (although less likely) contributing factor to this result is the presence of fructose in the Glucerna® formula. Fructose has produced an increase in triglycerides in some studies but not in others. The amount of fructose in Glucerna® accounts for only 7% of energy and at this level it may not be expected that it would have any adverse metabolic effect. However, there are two studies, one in nondiabetic subjects, (Halfrish et al, 1983) and one in NIDDM subjects (Grigoresco et al, 1988) which have found a increase in fasting plasma triglycerides with fructose intakes as low as 7.5-8.6% of energy. Other studies have showed large individual variability in response to dietary fructose. Thus, the subjects in this study may have been sensitive
DISCUSSION

enough to fructose that it counterbalanced the effect of the lower carbohydrate, high monounsaturated diet on triglycerides. The study by Abbruzzese et al (1993) which compared a high carbohydrate formula and Glucerna® in elderly NIDDM patients found apparently lower triglyceride levels after the Glucerna® diet, although it is not known if they were significantly lower. The mean triglyceride levels at baseline, after the high carbohydrate formula and after Glucerna® were 4.41, 4.55 and 3.40 mmol/L respectively. As can be seen, the subjects in the study by Abbruzzese also started with much higher triglyceride levels than the subjects in the present study, which in itself may partly explain the different effects on plasma triglyceride levels. If the presence of fructose in the diet did have an effect on plasma triglycerides in the present study, that effect was not apparent in the study by Abbruzzese.

The decrease in plasma insulin in the Ensure with Fiber® group, and the decrease in serum glucose in both groups, also may have affected plasma triglyceride levels. These results might be indicative of increased insulin sensitivity in both groups, especially in the Ensure with Fiber® group in which insulin levels declined. Since there is some indication that insulin resistance may override the normal suppressive effect of insulin on apo B production (Lewis et al, 1993), an increase in insulin sensitivity may partially restore that suppressive effect, leading to a decrease in apo B production. As well, improved insulin sensitivity might cause greater inhibition of the release of free fatty acids (Krentz et al 1995). This, in addition to the decrease in fasting serum glucose found on both of these study diets, may have reduced the substrate availability for triglyceride synthesis. The way in which high carbohydrate diets have been found to result in higher plasma triglyceride levels as compared to high
monounsaturated fat diets is due to increased triglyceride production (Chen et al, 1995). Thus, the decreased potential for triglyceride production in this study through the mechanisms just described may have contributed to the result of no difference in plasma triglycerides between the two study diets.

In conclusion, this study found no significant effect of the diets on fasting plasma triglycerides and thus no benefit of the high monounsaturated formula, Glucerna® in this respect. This result may have been influenced by the low baseline plasma triglyceride levels, the presence of fructose in the Glucerna® diet and possibly the decreased blood glucose levels and the increased insulin sensitivity especially in the Ensure with Fiber® group where there was a decrease in fasting insulin levels.

**Total Cholesterol**

This study found no significant effect of the diets on total cholesterol. The normal range of total cholesterol is 3.11 to 5.18 mmol/L (Malarkey and McMorrow, 1996). The mean total cholesterol levels in the present study were within this range, although at the upper end of it. The mean total cholesterol levels in the Ensure with Fiber® group were 4.76 ± 0.88 mmol/L on day 0, 4.29 ± 1.42 mmol/L on day 7 and 4.28 ± 1.22 mmol/L on day 28. The mean total cholesterol levels in the Glucerna® group were 5.04 ± 0.90 mmol/L on day 0, 4.92 ± 1.56 mmol/L on day 7 and 5.24 ± 1.62 mmol/L on day 28. As already stated, no significant effects were found. Most of the previously published studies have also found no significant effect of similar diets on total cholesterol levels (Garg et al 1988; Garg et al 1992b, Garg et al 1994, Rasmussen et al 1993; Campbell et al 1994, Lerman-Garber 1994, Lerman-Garber et al 1995). The mean cholesterol levels in these studies ranged from 4.89 to 6.30 mmol/L. Garg and associates (1992a) found total
cholesterol was significantly lower after a high monounsaturated fat diet than a high carbohydrate diet. In their study, the mean baseline total cholesterol was 5.15 mmol/L, after the high carbohydrate diet it was 5.09 mmol/L and after the high monounsaturated fat diet it was 4.44 mmol/L. In conclusion, no significant effects were seen for total cholesterol in the present study, which is consistent with most of the findings of other investigators. However, with regard to the present study it should be noted that there may not have been enough power to detect a difference if it existed. This study had a sample size of only eight subjects in each diet group, and there was considerable interindividual variability. Under these circumstances the possibility of making a type II error, that is accepting the null hypothesis when it is actually false, must be recognized.

HDL Cholesterol

The results of this study show a significant group by time interaction and a significant group effect for plasma HDL cholesterol. Low HDL cholesterol is a risk factor for coronary heart disease in NIDDM (Laakso et al, 1993), and is, therefore, undesirable. The normal range of HDL cholesterol is 1.24 to 1.43 mmol/L (Malarkey and McMorrow 1996). The subjects in the Ensure with Fiber® group started with lower HDL cholesterol levels of 0.98 ± 0.26 mmol/L. The mean HDL cholesterol levels in this group on days 7 and 28 were 0.88 ± 0.23 mmol/L and 1.14 ± 0.35 mmol/L respectively. The subjects in the Glucerna® group started with higher HDL cholesterol levels of 1.45 ± 0.33 mmol/L which were 1.42 ± 0.28 mmol/L and 1.31 ± 0.38 mmol/L on days 7 and 28 respectively. Post hoc analysis revealed that the significant group by time interaction found for this measure was due do a significant decrease in HDL cholesterol in the Glucerna® group from day
DISCUSSION

0 to 28. There was no significant change in the HDL cholesterol levels of the Ensure with Fiber® group over time. A significant group effect was also found for plasma HDL cholesterol. The two diet groups had significantly different HDL cholesterol levels on day 0 and day 7, but not on day 28, indicating that they started out different but gradually became similar in HDL cholesterol levels. The large difference in baseline values between the groups presents a definite limitation in understanding the effects of the diets. It cannot be stated with certainty that if the groups had started with similar HDL cholesterol levels that the same results would occur. It is possible that a part of the reason for this result is a regression towards the mean. This is the only study in which a high monounsaturated fat diet has caused a decrease in HDL. Several studies have shown no significant difference in HDL cholesterol levels after a high carbohydrate or a high monounsaturated fat diet (Garg et al 1992a; Garg et al 1994; Rasmussen et al 1993, Campbell et al 1994; Lerman-Garber et al 1994 Lerman-Garber et al, 1995). These studies had mean HDL cholesterol levels from 0.83-1.04 mmol/L. Two studies have found that a high monounsaturated fat diet resulted in higher HDL levels than a high carbohydrate diet (Garg et al 1988; Garg et al 1992b). In the former of these studies, the baseline mean HDL cholesterol of subjects was 0.83 mmol/L which decreased to 0.78 mmol/L after the high carbohydrate diet and rose to 0.88 mmol/L after the high monounsaturated fat diet. In the latter study the mean baseline HDL cholesterol was 0.75 mmol/L which decreased to 0.68 mmol/L after the high carbohydrate diet and was 0.76 mmol/L after the high monounsaturated fat diet. In the study by Abbruzzese (1993) which used diets consisting of a high carbohydrate formula and Glucerna®, the data indicated that a higher mean HDL cholesterol level was found after the Glucerna diet®, but no statistical
DISCUSSION

analysis were done. In their study, the mean HDL cholesterol at baseline was 0.81 mmol/L, after the high carbohydrate formula it was 0.76 mmol/L, and after Glucerna® it was 0.90 mmol/L. These studies that have found significantly higher HDL cholesterol after consumption of a high monounsaturated fat diet have had mean HDL cholesterol levels that are lower than those found in the present study, especially for those of the subjects in the Glucerna® group. In fact, for the subjects in the Glucerna® group this applies to the mean HDL cholesterol levels of all the previous studies of this kind. Since the previous studies have been crossover designs, the groups were well matched for initial HDL cholesterol. The randomization of the present study did not produce groups with similar baseline HDL cholesterol levels. Since HDL cholesterol is typically low in NIDDM patients, it is not surprising that the previous studies with NIDDM patients have found low HDL cholesterol levels. In the present study, the subjects on the Ensure with Fiber® diet had values lower than the normal range but similar to those found in other studies. The subjects in the Glucerna® group, however, had unusually high HDL cholesterol levels for NIDDM patients, which were at the upper end of the normal range. The fact that the HDL cholesterol of these subjects fell in spite of the high monounsaturated fat diet may be due to elevated levels of HDL cholesterol prior to the dietary treatment. Perhaps a high monounsaturated fat diet cannot increase HDL cholesterol when it is already high. It should also be noted that although the monounsaturated fat content of the diet increased in the Glucerna® group, the polyunsaturated fat content also increased. The pre-study food records indicated that the Glucerna® subjects consumed a mean of 6% of energy in the form of polyunsaturated fat. During the study that increased to the 13% of energy present in Glucerna®. Polyunsaturated
fatty acids have been shown to decrease HDL cholesterol (Kurishima et al., 1995). Another possible contributing factor to the decrease in HDL cholesterol in the Glucerna® group is the interrelationship of VLDL and HDL cholesterol metabolism. The enzyme CETP transfers cholesterol from HDL to VLDL in exchange for triglycerides. Thus, the finding of decreased HDL cholesterol may possibly reflect an increase in CETP activity. This study measured apo B cholesterol but not VLDL cholesterol. Thus, it cannot be stated with certainty if there was any change in VLDL cholesterol. It would have been interesting to measure CETP activity to see if an increased activity of this enzyme actually occurred. CETP activity has been found to increase during active weight loss (Tall et al., 1993). Perhaps the small weight loss experienced by these subjects, even though it did not significantly correlate with the change in apo B or HDL cholesterol, may have contributed in part to the decrease in HDL cholesterol in the Glucerna® group.

In conclusion, a significant group by time interaction was found for HDL cholesterol which was caused by a significant decrease in the Glucerna® group from day 0 to 28. This finding stands in contrast to that reported in the literature but is confounded by differing baseline values between the two groups and the high HDL cholesterol levels in the Glucerna® group.

**Apo B Cholesterol**

Apo B cholesterol consists of both VLDL cholesterol and LDL cholesterol. These can be measured separately, which has been done in much of the previous work. For simplicity of laboratory technique, the LDL cholesterol is often estimated by precipitating out the apo B particles and measuring the HDL cholesterol in the supernatant. The Friedewald equation (using total cholesterol, HDL cholesterol and triglyceride values) is
then used to calculate the LDL cholesterol (Friedewald et al, 1972). In the present study, the LDL cholesterol was not calculated using this equation because it has been shown to be inaccurate in NIDDM patients due to the altered triglyceride and cholesterol content of VLDL particles (Rubies-Prat et al, 1993). Measurement of HDL cholesterol was done, and the apo B cholesterol was then calculated by subtracting the HDL cholesterol from the total cholesterol. Because it was not measured directly, it should be recognized that the apo B cholesterol values will be influenced by any inaccuracies that may have occurred in either the total or HDL cholesterol assays. Normal ranges of LDL cholesterol are available, however normal ranges of apo B cholesterol are not. Thus, the results can only be compared with previous studies. High VLDL cholesterol levels are a risk factor for coronary heart disease in NIDDM (Laakso et al, 1993), thus a decrease in VLDL cholesterol would be beneficial. High LDL cholesterol has also been associated with coronary heart disease (Grundy, 1995).

The present study revealed no significant effects for Apo B cholesterol. The mean ± SD of apo B cholesterol for the Ensure with Fiber® group was 3.57 ± 0.96 mmol/L on day 0, 3.01 ± 1.45 mmol/L on day 7, and 3.22 ± 1.69 mmol/L on day 28. The mean ± SD of apo B cholesterol for the subjects in the Glucerna® group was 3.59 ± 0.68 mmol/L on day 0, 3.41 ± 3.94 mmol/L on day 7, and 3.94 ± 1.37 mmol/L on day 28.

Most of the previous studies have measured VLDL and LDL cholesterol separately. All of these studies found no effect of the diets on LDL cholesterol (Garg et al 1988, Garg et al 1992a, Garg et al 1992b, Garg et al 1994, Rasmussen et al 1993, Campbell et al 1994, Lerman-Garber et al 1994, Lerman-Garber et al, 1995). When VLDL cholesterol was measured, most of the studies found it to be significantly lower after a high
DISCUSSION

monounsaturated fat diet (Garg et al 1988, Garg et al 1992a, Garg et al 1992b, Garg et al 1994). Only one study found no significant effect of these diets on VLDL cholesterol (Lerman-Garber 1995). Lerman-Garber’s study was done with subjects who were in poor metabolic control, with high lipid levels, neither of which were characteristics of the subjects in the present study. In the above studies, the mean VLDL and LDL cholesterol combined (apo B cholesterol) ranged from 3.97 mmol/L to 5.39 mmol/L. This is slightly higher than the levels found in the present study. Abbruzzese et al, (1993) found an apparent lowering of apo B cholesterol with Glucerna® as compared to a high carbohydrate formula (statistical testing was not available). In this latter study, the mean apo B cholesterol at baseline was 3.45 mmol/L, after the high carbohydrate formula 3.52 mmol/L and after Glucerna® 3.14 mmol/L. Thus, the results from the study in this thesis do not appear to be consistent with the results of Abbruzzese et al, (1993). It is interesting that the levels of apo B cholesterol were very similar in the present study and that of Abbruzzese, indicating that, at least in this comparison, the different response is not due to different starting levels of apo B cholesterol. It is possible that the difference in response is partly due to differing subject characteristics. Other possible contributing factors are described below.

Apo B cholesterol is made up of both LDL and VLDL cholesterol, the majority of which is LDL cholesterol. The results of this study are difficult to compare to other studies because previous findings indicate lower VLDL but not LDL cholesterol after a high monounsaturated fat diet than a high carbohydrate diet. Thus, if the diets in the present study actually affected just VLDL cholesterol, the difference may not be apparent in the apo B cholesterol result, since most plasma apo B is associated with LDL. What can be concluded is that if any difference occurred in either LDL or VLDL

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cholesterol, it was not strong enough to cause a significant difference in the cholesterol content of apo B containing lipoproteins. Based on the previous studies, it might be expected that if there was a large enough decrease in VLDL cholesterol levels from a high monounsaturated fat diet, apo B cholesterol might be lower after the high monounsaturated fat diet. It should be recognized, however, that even if a decrease in VLDL cholesterol occurred, this still may not have enough of an impact of apo B cholesterol to significantly affect it.

The lack of finding lower apo B cholesterol levels after the high monounsaturated fat diet compared to the high carbohydrate diet in the present study may be related to triglyceride levels. In the fasting state, triglycerides are predominately found in the VLDL particles, along with VLDL cholesterol. The way in which high monounsaturated fat diets result in lower triglyceride levels than high carbohydrate diets is by a decreased VLDL-TG production rate (Chen et al, 1995). Decreased VLDL production may also correspond to decreased VLDL cholesterol levels. Thus, the decreased VLDL cholesterol levels after a high monounsaturated fat diet found in previous studies may be interrelated to the decreased triglyceride levels. In fact, these two results have always coexisted in the previously reviewed studies, with either a decrease in both or neither. The two diets in this study had no significant effects on triglyceride levels. Thus, it may be expected that no significant effect on VLDL cholesterol, and thus apo B cholesterol, would be found either.

Another possible, although less likely, explanation for the lack of finding lower apo B cholesterol after the Glucerna® diet is the presence of fructose in the Glucerna® formula. Consumption of fructose as 7% of energy may not be expected to have adverse effects on lipids. However, since studies
have shown large individual variability, some subjects may show the adverse effects of increased triglycerides and VLDL cholesterol with small amounts of fructose intake. In fact, one study done with hyperinsulinemic men consuming 7.5% of energy as fructose found a significant increase in cholesterol (Halfrish et al, 1993). Thus, the effect of fructose may have counterbalanced the usual VLDL cholesterol lowering effect of a high monounsaturated fat diet, thus leading to the finding of no significant difference in apo B cholesterol. An effect of fructose on cholesterol levels was not apparent however, in the previous study done with Glucerna® (Abbruzzese et al, 1993). It is difficult to state with certainty whether fructose influenced the results of this study.

As just discussed with regards to HDL cholesterol, it is possible that an increase in CETP activity could have influenced the apo B cholesterol results. If this did occur, it would help to explain the decrease in HDL cholesterol and the lack of the expected decrease in apo B cholesterol from the Glucerna® diet. This is purely speculation, however, and may or may not be a factor in the results. The most likely explanation for the results of apo B cholesterol relate to the low triglyceride levels that were unaffected by the study diets.

In conclusion, the two study diets did not significantly affect apo B cholesterol levels. It is not known if the diets affected VLDL or LDL cholesterol individually. It should also be recognized that although this study found no significant effect of the diets on apo B cholesterol levels, there may have been insufficient power to detect such an effect. One may look at the means of days 0, 7 and 28 and speculate that those changes over time seem clinically relevant. Because this study had only eight subjects in each group, and there was large interindividual variability, the possibility of
making a type II error, that is accepting the null hypothesis when it is actually false, must be recognized.

Fatty Acid Composition of Plasma Triglycerides

The amount of oleic acid in fasting plasma triglycerides, as a percentage of total fatty acids, reflected the fatty acid composition of the diets in this study. Glucerna® contains high amounts of oleic acid, which is the major monounsaturated fatty acid. Ensure with Fiber® on the other hand is low in oleic acid. Prior to the study, the subjects in the Ensure with Fiber® group consumed an estimated mean of 8% of energy from monounsaturated fatty acids, and during the study the formula provided 10% of energy as monounsaturated fatty acids. In contrast, the Glucerna® group subjects consumed an estimated mean of 10% of energy as monounsaturated fatty acids prior to the study, which increased to 32% of energy from monounsaturated fatty acids provided from the formula. A group by time interaction and a group effect were observed. Post hoc analysis revealed that this was due to a combined effect of a significant decrease in oleic acid in the Ensure with Fiber® group from day 0 to 28 and a significant increase in the Glucerna® group from day 0 to 7, and day 0 to 28. At the beginning of the study the groups were similar, however, by day 7 and 28, the two diet groups had significantly different levels of oleic acid in their plasma triglycerides. This difference resulted from the opposite change in this parameter between the two groups. An increase in oleic acid in plasma triglycerides after a high monounsaturated fat diet has also been observed by Garg et al (1988).

The amount of linoleic acid in fasting plasma triglycerides, as a percentage of total fatty acids, also reflected the fatty acid composition of the diets in this study. Linoleic acid is a major polyunsaturated fatty acid and is
the predominant fatty acid in Ensure with Fiber®. Prior to the study, the Ensure with Fiber® group subjects consumed an estimated mean of 5% of energy from polyunsaturated fatty acids, and during the study the formula provided 18% of energy as polyunsaturated fatty acids. In contrast, the Glucerna® group subjects consumed a mean of 6% of energy as polyunsaturated fatty acids prior to the study, which increased to 13% of energy from polyunsaturated fatty acids provided from the formula. A significant group effect, time effect and group by time interaction were found. Post hoc analysis revealed that these effects were due to a significant increase in the Ensure with Fiber® group from day 0 to 7 and from day 0 to 28. The two diet groups started the study with similar percentages of linoleic acid in their plasma triglycerides, but due to the increase in the Ensure with Fiber® group, had significantly different levels on days 7 and 28.

In conclusion, it appears that the Ensure with Fiber® diet resulted in a decrease in oleic and a large increase in linoleic acid as a percentage of total fatty acids in plasma triglycerides. The Glucerna® diet resulted in an increase in oleic acid and no change in linoleic acid as a percentage of total fatty acids in plasma triglycerides. It is interesting to note that these changes happened very quickly. The increase in linoleic acid in the Ensure with Fiber® group and the increase in oleic acid in the Glucerna® group both occurred in the first week of the study, after which they stayed at that increased level throughout the study. Although not recorded here, it is likely that the percentages of saturated fatty acids in both groups may have declined over time, since both study diets were lower in saturated fat than their prestudy intakes. These results demonstrate that dietary fat has been incorporated into fasting plasma triglycerides. As well, they provide evidence that the subjects were actually following the study diets.
Correlations between Fatty Acids and Plasma Cholesterol

The change in the percentage of linoleic acid incorporated into plasma triglycerides was significantly inversely correlated with the change in plasma total cholesterol levels between day 7 and 28 of the study. This finding is consistent with a report which has shown that replacing saturated fat with polyunsaturated fat in the diet results in a decrease in total cholesterol (Kurishima et al, 1995). However, in the present study, the Ensure with Fiber® diet, which is high in linoleic acid, had no significant effect on plasma total cholesterol. No other significant correlations between the change in the percentage of fatty acids in plasma triglycerides and the change in cholesterol levels were found in the present study.

LIMITATIONS OF THE STUDY

This study had several limitations, the most important ones being the small sample size, the large between and within group variation of metabolic parameters, and the missing data points.

The study involved only 16 subjects, 8 in each diet group. It was estimated from other studies using Glucerna® that 16-18 subjects would be required in each group to achieve 80% power at a significance level of 0.05. The study is being continued at another site until this desired sample size is achieved. Thus, the larger study may clarify some of the results.

Many of the study parameters appeared to be different between the groups at baseline, although they were not statistically significantly different. However, these different baseline values may have been clinically relevant. For example the subjects in the Ensure with Fiber® group appeared to be in slightly worse metabolic control at the start of the study than the Glucerna® subjects, as evidenced by higher fasting glucose,
DISCUSSION

fructosamine, insulin and glucagon levels. As well, these subjects had higher plasma triglyceride levels and lower plasma HDL cholesterol levels. The significant group by time interactions for HDL cholesterol and for insulin may have been confounded by the fact that the groups started with clinically different baseline values. It is intended with randomization that subject characteristics will be similar in the different groups. This is generally the case for large sample sizes, however, for small studies this may not happen.

Another limitation of the study was the missing data points. Unfortunately, most of this missing data occurred in the Ensure with Fiber® group. This problem was dealt with by using a statistical procedure that accounts for missing data, which provides an estimate of what the missing value would be based on the behavior of the available data. This is the best technique available, however, it is more desirable to have the actual data for that subject. This is especially true for small sample sizes because the responses of a few subjects may not be typical. Another option was to not use data for subjects with any missing values. This would have decreased the sample size to an unacceptable level, and thus was not done. Since most of the missing data occurred on day 7, another possible solution was to ignore the day 7 data. This analysis was done (although not shown in this report), in addition to the analysis with missing data, and the conclusions drawn were the same. Including the day 7 data provides more information and thus these results are presented. It is important that the same conclusions would be drawn if only the baseline and end of study results were used and also if the subject who dropped out of the study was excluded, resulting in no missing data.

Compliance to the diet was verified by daily food diaries and collection of formula labels from the subjects. These are good indicators of compliance.
but are not absolute assurance of it. As with any study that is conducted on
an outpatient basis, a certain amount of trust is placed in the subjects to be
honest with the investigators about conforming to the dietary regimen.

Most of the previous studies have included subjects who were not
taking medications, whereas, most of the volunteers in this study were on
medications. Although the medications were screened for impact on glucose
or lipid metabolism, it is possible that they may have had some unforeseen
effects on the study results.

**STRENGTHS OF THE STUDY**

This study had some strengths which should be emphasized. A good
relationship was achieved between the investigators and the subjects. From
the information available it appears that the subjects were very compliant
with the study protocol. The changes found in the fatty acid composition of
their plasma triglycerides indicate that the subjects were indeed consuming
the formulas. They reported consuming an average of 90% of calories from
the formula. It is commendable that they were able to do this, and makes it
very likely that the metabolic effects observed were due to the formula diets.
The subjects reported very little variation in their activity level. They put a
tremendous amount of effort into this study, by recording everything they
ate, and performing self blood glucose monitoring before and after each meal,
twice a week.
CONCLUSIONS

The results of this study add to the body of literature evaluating the effects of high monounsaturated fat diets in NIDDM subjects. Much of the previous work has suggested that these diets may be more beneficial to NIDDM subjects than the currently recommended high carbohydrate diets. This is due to the lower postprandial blood glucose levels (which may reduce the risk of microvascular complications) and the lower plasma triglyceride and VLDL cholesterol levels and higher HDL cholesterol levels (which may reduce the risk of macrovascular complications) found in some studies after subjects consumed high monounsaturated fat diets. This is still a new and controversial area of research and more investigation will be required if dietary recommendations for NIDDM subjects are to change. On the positive side for high monounsaturated fat diets, the present study found that the postprandial blood glucose rise was lower after the Glucerna® diet. On the negative side, this study found a significant difference in the pattern of change for HDL cholesterol, in which the high monounsaturated fat diet produced a decrease from high levels. In contrast, other investigators reported either an increase after a high monounsaturated fat diet or no significant effect. However, in the present study, the subjects in the Glucerna® group had high HDL cholesterol levels, and the two groups were very different at baseline. As a result, this finding is difficult to interpret. The usual benefit of a high monounsaturated fat diet in lowering plasma triglycerides was not observed in the present study. It may be that this is related to the low initial plasma triglyceride levels of the subjects, the presence of fructose in Glucerna® and/or the decrease in insulin after the Ensure with Fiber® diet, and the decrease in glucose after both diets.
DISCUSSION

Some time effects were observed in this study, indicating benefits of both study diets over the baseline diets of the study participants. These included mean decreases in fasting serum glucose and fasting plasma glucagon. These findings may be related to the fact that these liquid diets provided a high degree of consistency.

NIDDM patients sometimes require nutritional formulas, either as a supplement or as complete enteral feeding. In these circumstances, the dietitian or clinician in charge of the patient’s care must decide which formula is the most appropriate choice. That choice should be made based on the individual patient and his or her specific needs. Glucerna® may be beneficial if blood glucose excursions need to be kept low. Glucerna® feeding, however, may not result in the beneficial effects of lowered plasma triglycerides and VLDL cholesterol and raised HDL cholesterol, as expected from previous studies, especially if plasma triglycerides are already low. If postprandial blood glucose is not a major concern to the patient and triglycerides are already low, Ensure with Fiber® may be just as good a choice as Glucerna®.
RECOMMENDATIONS FOR FUTURE RESEARCH

Further research needs to be done to establish whether high monounsaturated fat diets are beneficial for NIDDM patients, and if so under what circumstances. The following is a list of areas that need further investigation.

1. The effect of fructose in Glucerna® on parameters of carbohydrate and lipid metabolism should be further investigated.

2. The effect of differing patient characteristics on metabolic responses to high carbohydrate and high monounsaturated fat diets should be investigated further to determine for which patients and under what circumstances these diets may be beneficial. This study could be repeated in NIDDM patients with high triglyceride levels.

3. It is possible that fiber may have an effect on blood glucose and lipid levels. In uncontrolled situations, diets high in carbohydrate have the potential to provide more fiber than diets high in fat. Most of the studies (including the present one) comparing high carbohydrate and high monounsaturated fat diets in NIDDM have used diets with similar amounts of fiber. Thus, it is possible that the benefits of high monounsaturated fat diets found in the previous studies may not be seen if they were compared to high carbohydrate, high fiber diets. The effect of fiber in these diets should therefore be assessed.
4. The effect of a high monounsaturated fat diet on additional aspects of lipid metabolism (i.e. lipid peroxidation or plasma free fatty acids) should be investigated.

5. A concern may arise regarding the possibility of weight gain from a high fat diet. This would be undesirable since most NIDDM patients would benefit from weight loss. Therefore, the long term effect of diets high in monounsaturated fat should be assessed to determine if they lead to weight gain in NIDDM subjects.

6. If further studies verify the beneficial effects of high monounsaturated fat diets on plasma glucose and lipid levels in NIDDM, long term assessment of these diets should be made on the incidence of diabetic complications.
REFERENCES


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APPENDIX A
RECRUITMENT POSTER
APPENDIX C

NEWSPAPER ADVERTISEMENT
DIABETES STUDY

Volunteers required who have
Type II Diabetes:
• Ages 19-70 years
• Stable blood glucose levels
• Normal blood lipid levels

Required to consume a liquid nutritional supplement for 28 days. Honorarium paid.

Call 822-6869
APPENDIX D
CONSENT FORM
PATIENT CONSENT FORM

Research Project: Effects of Feeding Enteral Nutritional Products Differing in Fat and Carbohydrate Content on Plasma Glucose, Lipid and Lipoprotein Levels of Patients with Non-Insulin Dependent Diabetes Mellitus (NIDDM).

The purpose of this study is to assess the effects of short term (7 day) and long term (28 day) feeding with GLUCERNA, a high fat, low carbohydrate complete nutritional formula or ENSURE with FIBRE, a high carbohydrate, lower fat complete nutritional formula, on blood glucose and blood fat levels [cholesterol, triglycerides, lipoproteins (fat carriers) and lipid peroxides (oxygen altered fats)] of individuals with NIDDM.

GLUCERNA is a specially designed nutritional formula for patients with diabetes mellitus. It provides 33% of calories from carbohydrate, 50% from fat, and contains a source of dietary fibre. This formula has been shown to be beneficial for blood glucose control, because of the lower amount of carbohydrate, and also the type of carbohydrate (complex sources that are more slowly absorbed ie. hydrolyzed cornstarch, fructose and soy polysaccharide). It is not known whether the high fat content has any impact on blood fat levels. The fat sources in GLUCERNA have large amounts of monounsaturated fats, which are believed to influence blood fats in a positive way, however this issue has not been clearly tested.

ENSURE with FIBRE provides 55% of calories from carbohydrate, 30.5% from fat and contains a source of dietary fibre. It, like GLUCERNA, also contains complex carbohydrates (soy polysaccharide), but has somewhat different fat sources (more polyunsaturates). Thus the main differences between the two formulas are the amount and type of fat and the amount of carbohydrate.

When you agree to participate in the study, you will be given four cans of one formula to consume at home over an eight hour period. This will then be repeated with the other formula on another day. If both formulas are well-tolerated and the taste is acceptable, you will then begin the study.

As a participant in this study, you will be randomly assigned to receive one of these formulas for 28 days. During the study period you will be asked to take 100% of your nutrient intake as formula. However, you may choose to take a minimum of 80% of your nutrient intake as formula and a maximum of 20% of your nutrient intake as "mini meals". "Mini meals" will consist of specific foods to be eaten in specific amounts. These foods will have the same fat, carbohydrate and protein content as your assigned formula. You will be provided with all of the formula that you need over the study period. A Registered Dietitian will provide assistance with planning formula and meal intake as required.

Protocol #BG28 (C93-008)
Subject (Initials)_____
Subject (Number)_____

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After the initial screening visit and formula trial, you will be required to visit the study site at UBC (School of Family and Nutritional Sciences) 5 times (Days 1, 8, 15, 22 and 29) over the study period. Blood samples will be taken after an overnight fast on days 1, 8 and 29 of the study. Blood will be drawn from a vein in the arm. The amount of blood taken on Day 1 and 29 will be approximately 35 mL (slightly more than 2 tablespoons) and on Day 8 it will be approximately 10 mL (2 teaspoons). Body weight will be measured on days 1, 8, 15, 22 and 29. Skinfold and girth measurements will be done on days 1 and 29.

Throughout the study period you will be asked to keep a daily diary of the amount of formula and food consumed. You will be asked to record your own weight and monitor your blood glucose at least 2 days per week. Finger prick blood glucose measurements should be taken before and after breakfast, lunch and supper on these two days. At each weekly visit (days 8, 15, 22, 29), you will be asked to return empty formula cans and have your diary reviewed. Adjustments will be made to your formula and food requirements as necessary.

Side effects of nutritional formulas are minimal. In most cases, the formulas are a beneficial and nutritious alternative to regular food. During initial consumption of a formula, patients sometimes experience bloating, diarrhea or mild cramping until they get used to it. Side effects of having blood samples taken include mild pain at the site and possible minor bruising.

Any information resulting from this research study will be kept strictly confidential. Your information will all be recorded by a code number, known only by the research coordinator. Your medical information, related to the study only, may be inspected by a representative of Abbott Laboratories (manufacturers of GLUCERNA and ENSURE with FIBRE) in the presence of the Principal Investigator or Project Co-ordinator. Copies of the relevant data which identify you only by code number may be required by Abbott Laboratories, but you will not be identified by name.

The time required of you beyond that needed to consume the formula, will be a total of 6-8 hours for visits to the Study Center at the School of Family and Nutritional Sciences, UBC (about 1 1/2 hours per week), and a total of 3-4 hours for monitoring weight, food intake and blood glucose levels at home.

When you make your visits to the Study Center, at the School of Family and Nutritional Sciences, UBC, you will be reimbursed for your travel expenses, parking, and meals on the test days. If the study protocol is followed precisely, as outlined by the investigators, you will receive an honorarium of $300 for your participation. Fifty dollars ($50) will be given to you after the blood test on Day 8, and $250 will be given to you at completion of the study.

If you have any questions or concerns regarding the research study, please call Elaine Eriks (Study Co-ordinator) or Dr. Linda McCargar (Principal Investigator) at the numbers listed on the next page. Investigators will be available at both UBC and St. Paul's Hospital sites for consultation.

Please be aware that you may decline to enter the study or you may withdraw from the study at any time without any consequences to continuing medical care.

Protocol #BG28 (C93-008)
Subject (Initials) ______
Subject (Number) ______

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APPENDIX E

FOOD RECORD FORM
GUIDELINES FOR KEEPING A FOOD RECORD

A food record is a detailed description of each food or beverage item taken over a day. Providing as much detail as possible about foods that you eat will help to assess your diet accurately.

PLEASE READ THESE GUIDELINES BEFORE YOU START YOUR FOOD RECORD.

KEEP A RECORD OF EVERYTHING THAT YOU EAT OR DRINK FOR 4 DAYS IN A ROW ON THE ATTACHED FORMS. INCLUDE 1 WEEKEND DAY IN THE RECORDING PERIOD.

 RECORD the PORTION SIZE-Don’t guess if you can measure!

It may be helpful to measure how much your regular glasses, cups and bowls contain before you start. The attached food pictures are provided to help you with portion sizes.

For example, you might record:

Volume:
1 cup or 8 oz or 250 mL of 2% milk
1 tablespoon or 15 mL of peanut butter or cream cheese
1 teaspoon or 5 mL of margarine

Size:
1 "2 inch by 3/4 inch by 3/4 inch" piece of cheddar cheese
1 medium egg, poached
1 small apple

Weight:
2 ounces or 60 grams of lean hamburger meat or chicken
(use labels on packages to help you)
Be SPECIFIC about the TYPE of food, BRAND name and the CONTENT of mixed dishes

For example:

Cookies: TYPE: (chocolate chip or digestives)  
BRAND: (Dare or homemade)  
SIZE: 2 inch diameter

Milk: TYPE: (skim, or 2%)  
AMOUNT: (4 ounces or 125 mL)

DESCRIBE mixed foods as if you were WRITING A RECIPE

Everyone has their own way of making everyday foods!

For example:

How do you make a cheese sandwich?  
Do you use margarine or butter or mayonnaise?  
Do you add lettuce or tomato slices?  
What type of cheese and bread do you use?  
How much of each item do you use?

Attaching recipes for items such as casserole dishes or labels from prepackaged foods such as frozen dinners is helpful.

RECORD IMMEDIATELY after EACH MEAL and SNACK

Keep track of foods eaten as you eat them...take your food record with you if you eat away from home.
TIME AND PLACE | FOOD AND BEVERAGE ITEMS CONSUMED | QUANTITY EATEN
--- | --- | ---
12:30 pm Home | Macaroni and Cheese |
- cooked macaroni | 1 cup | 
- home made cheese sauce (made with butter, flour, cheddar cheese and 2% milk) | 1/2 cup | 
- Tomato juice | 4 oz glass | 
Whole wheat dinner roll | 1 1/2" diameter | 
margarine (Becel) | 2 tsp | 

Answer the following once you have finished your food record ...

1. Does the record represent a usual diet for you?

   YES    NO

If NO, I would usually eat:

Did you take any vitamin and mineral supplements? YES  NO

if YES, supplements taken

FOOD RECORD FORM
FOOD RECORD RECORDING FORM

Initials: 
Study Number: 

DATE: 

<table>
<thead>
<tr>
<th>TIME AND PLACE</th>
<th>FOOD AND BEVERAGES CONSUMED</th>
<th>QUANTITY EATEN</th>
</tr>
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FOOD RECORD FORM
APPENDIX G

HEALTH CONDITIONS OF SUBJECTS
## HEALTH CONDITIONS OTHER THAN NIDDDM

Ensure with Fiber® Group

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<thead>
<tr>
<th>SUBJECT</th>
<th>CONDITION</th>
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<tbody>
<tr>
<td>1</td>
<td>History of hiatus hernia</td>
</tr>
</tbody>
</table>
| 3       | Mild neuropathy and myopathy  
Hyperthyroidism |
| 7       | Bipolar affective disorder  
Cataracts starting to harden  
History of spastic irritable colon  
Arthritis |
| 8       | High blood pressure  
Mild peripheral neuropathy and ataxia |
| 10      | History of gout |
| 11      | Hypothyroidism  
Hay fever |
<p>| 202     | None |
| 203     | None |</p>
<table>
<thead>
<tr>
<th>SUBJECT</th>
<th>CONDITION</th>
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<tbody>
<tr>
<td>2</td>
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<td>4</td>
<td>Gastrointestinal ulcer</td>
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| 5       | Hypertension  
          Occasional diarrhea  
          Arthritis |
| 6       | Angina  
          Occasional heartburn |
| 9       | Arthritis |
| 12      | Lupus (in remission)  
          Multiple Sclerosis (in remission)  
          Hay fever |
| 13      | Mild asthma  
          Hypertension (controlled with medication)  
          Mild colitis, diverticulitis  
          Hypothyroidism (controlled with medication) |
| 201     | None      |
## REGULAR MEDICATIONS
Ensure with Fiber® Group

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<thead>
<tr>
<th>SUBJECT</th>
<th>DRUG NAME</th>
<th>REASON FOR USE</th>
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<tbody>
<tr>
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</tr>
<tr>
<td>3</td>
<td>Synthroid</td>
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<td>7</td>
<td>Ferrous gluconate</td>
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<tr>
<td></td>
<td>Vitamin C</td>
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<td></td>
<td>Carbamezepine</td>
<td>mood stabilizer</td>
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<td>Misoprostal</td>
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<td>Doxipine</td>
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<td>hypertension</td>
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<td>Enalapril</td>
<td>hypertension</td>
</tr>
<tr>
<td></td>
<td>Glyburide</td>
<td>NIDDM</td>
</tr>
<tr>
<td>10</td>
<td>Glyburide</td>
<td>NIDDM</td>
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APPENDIX I

CHANGES AND EVENTS OCCURRING DURING THE STUDY
CHANGES AND EVENTS OCCURRING DURING THE STUDY

ENSURE WITH FIBER® GROUP

Subject #1
1. Subject consumed less than 80% of calories from formula. During week one he consumed 76%, week two 76%, week three 77%, and week four 77%.

Subject #7
1. At the follow up visit on day 15 subject reported feeling bloated.
2. On day 22 subject decided to withdraw from the study for personal reasons.

Subject #8
1. On day 4 subject ate a small order of french fries and forfeited 2 cans of ensure with fiber.
2. Subject reported loose stools on days 8 and 9.

Subject #10
1. During the first week the subject was consuming only formula. At the first follow up visit the subject reported not sleeping well at night. He thought it may be because he was hungry. It was suggested he consume a half can minimeal as a bedtime snack.
2. At the first follow up visit the subject reported more fluctuations in blood glucose than normal. It was suggested that he consume a minimeal as a morning snack.
3. Activity level of the subject increased during the second week. He was walking more and doing more housework.
4. On day 28 the subject reported increased stains on his teeth. He thought that this may be due to a lack of chewing.

Subject #11
1. On day 15 subject reported mucous in his throat. He also reported feeling hungry.
2. On day 28 subject reported darkening of the teeth and a dry throat.

Subject #202
1. The pre-study food record was not collected because there was not enough time.
2. On day 8 it was noted that the subject sometimes forgets to take his oral hypoglycemic medication in the evening. When this occurs he takes them the next morning with his usual morning medication. It was
emphasized to him that he should try to remember to take his medication at the proper times.

3. Subject experienced bruising from the first blood draw. This was still present on day 8, thus the second blood draw was not done. The bruising was resolved by day 15.

Subject #203
1. The pre-study food record was not collected because there was not enough time.
2. Subject experienced bruising from the first blood draw. This was still present on day 8, thus the second blood draw was not done. The bruising was resolved by day 15.
3. During week three subject consumed only 79% of calories from formula.

GLUCERNA® GROUP

Subject #2
1. During the fourth week the subject consumed only 78% of calories from formula.

Subject #4
1. Subject had two hypoglycemic reactions during the first week which he treated with dextrose tablets. This is not an unusual occurrence for him.
2. From days 21 to 25 the subject had a cough. He took cough syrup as needed during these days.
3. On day 26 the subject drank six dealcoholized beer (near beer) while at a social engagement.

Subject #5
1. On day 24 subject had a headache and took one regular strength Tylenol.

Subject #6
1. On days 12 to 18 subject experienced cold and flu symptoms. On days 13 and 14 his intake of formula and minimeals decreased slightly. Amoxillan Novo (an antibiotic) was taken on days 13 to 15 and three Tylenols were taken on day 14.
2. On days 21 to 24 subject did not take his regular oral hypoglycemic medication because his fasting blood glucose was low.

Subject #9
1. On day 2 subject took two regular anacins for a headache.
2. On days 26 and 27 and 28 the subject had a headache. On each of these days he took 2, 3 and 1 Tylenol 3 tablets respectively.

Subject #12
1. On days 2 to 8 subject had a flu. Her appetite and dietary intake was decreased during that time. During that time she took 2 extra strength Tylenol sinus to help her symptoms. On day 2, two tsp. of bismuth was taken for an upset stomach. On day 1 four Halls cough drops were taken for a sore throat. During that week she was also less active than usual.
2. At the second follow up visit on day 15 the subject reported some constipation. On days 10 and 13 a suppository had been taken for this. She thought that the constipation may have been a result of the Tylenol she had taken the week before.
3. Patient reported an increase in asthma symptoms due to a change in the weather on days 10 to 23. Ventelin and Beclovent, both of which are inhalant asthma medications were taken during this time as needed to allow easier breathing.
4. On day 26 subject was at a social engagement. She has a salad with dressing, a bun, turkey and a 4 oz glass of white wine.
5. On days 27 and 28 two Bayer aspirin were taken to ease arthritic symptoms.

Subject 13
1. On day 5 subject experienced mild diarrhea and nausea.
2. During the third week the subject’s activity level increased slightly.
3. On days 12 and 14 the subject experienced rhinitis. Rhinocort inhaler was taken as needed on these days to control symptoms.

Subject 201
1. The pre-study food record was not collected because there was not enough time.
2. The subject indicated he only takes in oral hypoglycemic medication on days when his fasting blood glucose is >9mmol/L. Subject took his pills on two days during the first week. No pills were taken for the remainder of the study because fasting blood glucose was never greater than 9mmol/L.
SERUM GLUCOSE

Ensure with Fiber

Glucerna

TIME (days)
SERUM GLUCOSE (mmol/L)
SERUM FRUCTOSAMINE

Ensure with Fiber

Glucerna

TIME (days)

SERUM FRUCTOSAMINE (pmol/L)

166
PLASMA INSULIN

Ensure with Fiber

Glucerna

TIME (days)

PLASMA INSULIN (pmol/L)
PLASMA TRIGLYCERIDES

Ensure with Fiber

Glucerna
PLASMA TOTAL CHOLESTEROL

Ensure with Fiber

Plasma Total Cholesterol (mmol/L)

TIME (days)

Glucerna

Plasma Total Cholesterol (mmol/L)

TIME (days)
PLASMA HDL CHOLESTEROL

Ensure with Fiber

Glucerna

TIME (days)
OLEIC ACID (18:1) AS A PERCENTAGE OF TOTAL FATTY ACIDS IN PLASMA TRIGLYCERIDES

**Ensure with Fiber**

**Glucerna**