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# **FATTY ACIDS, DIET AND BODY INDICES OF TYPE II DIABETIC CAUCASIANS, AFRICAN AMERICANS AND GHANAIS**

BY

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**MAY, 2001**

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CAUCASIANS, AFRICAN AMERICANS AND GHANAIS**

A thesis submitted to the  
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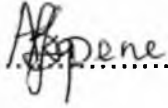
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## DECLARATION

I conducted this research under the supervision of Dr. Anna Lartey of the Department of Nutrition and Food Science, University of Ghana, Legon.



  
.....  
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## DEDICATION

To my brother, Kwaku and sister, Bubu for their love and continuous encouragement.



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## ABSTRACT

Diet, serum lipid profile, metabolic and body indices of Type II diabetics compared to non-diabetic subjects among Caucasian-Americans, African-Americans and Ghanaians were studied in this project work.

Non-insulin dependent diabetes mellitus and non-diabetic Caucasians, African Americans and Ghanaians were recruited. Data collected include food intake and anthropometric measurement. Blood samples were also taken for glucose, non-esterified fatty acids (NEFA) and serum lipid analysis. Serum very low-density lipoproteins, low-density lipoproteins, high-density lipoproteins, total cholesterol and triglyceride levels were determined.

The mean energy intake among Ghanaian non-diabetic controls was 2320 kcal/d, of this 57% of the total energy intake was contributed by carbohydrate. Among the diabetic groups, Caucasians had the highest energy intake of 2383 kcal/d, with carbohydrate contributing 38% of the total energy intake. Ghanaian diabetics recorded the lowest body mass index (BMI:  $26.4 \pm 6.1$  kg/m<sup>2</sup>), percent body fat (20.0  $\pm$  8.7%), and waist to thigh ratio (1.6  $\pm$  0.2) compared to the Caucasians and African American diabetic groups. Low fat intake and high percent body fat levels were observed among the African American diabetics (65g/d and 32.2%) whilst the inverse was observed among the Caucasian diabetics (120g/d and 25.9%).

Caucasian controls (CC) and African American controls (AC) had higher polyunsaturated fatty acid (PUFA) levels but lower saturated fatty acid (SFA) levels (CC- 42% PUFA vs 26% SFA; AC- 48% PUFA vs 27% SFA). Ghanaian controls had high SFA and low levels of PUFA (33% and 31% respectively), similar to their diabetic counterparts (34% and 17%). Caucasian diabetics had high PUFA and low SFA (34% and 25%), and African American diabetics had low PUFA and high SFA (29% and 32%). NEFA ranged between 0.6 and 1.1mEq/L. Total cholesterol, triglycerides and the lipoproteins were within normal range for both diabetics and normal subjects.

The data suggest a rapid turnover in carbohydrate metabolism for the energy needs among Ghanaians and that fat metabolism may differ between Caucasians and African Americans. Also, the data indicate low risk to cardiovascular diseases among the subjects studied.

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## CHAPTER ONE

### INTRODUCTION

#### 1.1 Background

Diabetes Mellitus refers to a group of metabolic disorders characterized by an elevated glucose concentration in the blood due to deficiencies or inefficient insulin action. Insulin, produced in the *B*-cells of the pancreas, acts directly on the liver, muscle cells and adipocytes to increase glycogen synthesis, fatty acid synthesis and to enhance the uptake of glucose by the cells. It also serves as a promoter for the conversion of fats and proteins to energy stores. Impaired insulin action may lead to chronic hyperglycemia, which triggers many of the complications associated with this disease. These complications may also arise from defects in the islet B-cell hormone production and secretion such that there is increased production and release of proinsulin in diabetic subjects compared to non diabetic subjects (Ward *et al*, 1987).

Also, with insulin secretion defects, glucose production by the liver persists longer at mealtimes. Glycogenolysis sensitive to insulin regulation is less controlled in the absence of early insulin response. Gluconeogenesis is also unrestrained due to the high levels of glycerol and non-esterified fatty acids that result from the less efficient inhibition of lipolysis in adipose tissues. These processes result in less efficient uptake of glucose by the muscle and increase the level of postprandial glucose in Type II diabetic subjects.

The symptoms of diabetes mellitus may include fatigue, blurry vision, polydipsia, polyuria, polyphagia, slow healing of wounds and weight loss. Complications arising as a result of diabetes include blindness, kidney failure, amputations, dyslipidemia and cardiovascular diseases. Several factors contribute to the development of diabetes. These factors may be dietary, genetic, environmental or related to endocrine dysfunction.

### 1.1.2 Dietary Factors and NIDDM

#### a) Carbohydrates

The amount, type (Glucose versus fructose) and rate of digestion of dietary carbohydrates are the primary determinants of postprandial glucose and insulin responses (Wolever, 2000). In patients with type II diabetes, insulin's ability to accelerate glucose transport into muscle is reduced, with an apparent impairment of normal translocation of glucose transporters to the cell membrane. Phosphorylation of glucose to glucose-6-phosphate is also decreased in the insulin resistant state, impairing glycolysis and glycogenolysis in muscle and adipose tissues.

#### b) Fatty acids

Fatty acids are esterified and stored as triglycerides in adipose tissues. Fatty acids may either be saturated or unsaturated. Saturated fatty acids have a tendency to increase blood cholesterol levels, although the amount of cholesterol synthesized and metabolized by the body is much greater than that consumed in the diet (Thomas, 1989). Saturated fatty acids are found mainly in animal products such as

butter, cheese and meat, and in vegetable products such as coconut oil and palm oil.

Unsaturated fatty acids may either be mono- or poly- unsaturated. They are mainly obtained from vegetable oils (safflower, soybean, cottonseed, sesame, and sunflower oils), nuts and seeds. Fish is also a good source of unsaturated fatty acids. In the Western diet today, the ratio of n-6 to n-3 fatty acids ranges from approximately 20-30:1 instead of the traditional 1-2:1 (Simopoulos, 1999). Studies indicate that a high intake of n-6 fatty acids increase blood viscosity, vasospasm, vasoconstriction and decrease bleeding time. However, n-3 fatty acids have anti-inflammatory, antithrombotic, antiarrhythmic, hypolipidemic and vasodilatory properties. These beneficial effects of n-3 fatty acids have been shown in the secondary prevention of coronary heart disease, hypertension, Type II diabetes and in some renal diseases.

Non-esterified fatty acids, also known as free fatty acids are released from the adipose tissues under the influence of hormone-sensitive lipoprotein lipase. Increased supply of non-esterified fatty acids to the liver reduce the ability of B-cells to secrete insulin in response to glucose, inhibit glucose uptake and oxidation in muscle cells, and increase insulin resistance.

### 1.1.3 Occurrence of NIDDM

NIDDM mainly occurs when there is insulin resistance and/or insulin secretion dysfunction. In the healthy person, insulin released from the pancreas inhibits

glucose production in the liver and enhances glucose uptake by the muscle and triglyceride synthesis in adipose tissues, thereby maintaining glucose homeostasis. In insulin resistance, this homeostatic condition no longer exists, resulting in increased free fatty acid liberation into the blood.

There may also be insulin secretion dysfunction and as such glucose cannot freely enter the muscle and adipose cells. Increased glucose in the blood results and with the renal threshold of glucose being exceeded glucose is excreted in the urine. In severe cases, proteolysis, ketoacidosis and diabetic coma may result, eventually leading to death.

#### 1.1.4 Incidence of Type II Diabetes

Diabetes mellitus is the seventh leading cause of death in the United States and the fourth leading cause worldwide. One out of every 7 healthcare dollars in the US is spent on diabetes, with \$98 billion as medical expenses and indirect costs attributable to diabetes in 1997 (American Diabetes Association, 1998). Currently, one out of 20 people have Type II diabetes. Type II diabetes accounts for 90-95% of all cases of Diabetes Mellitus. Diabetics are 2 - 4 times more likely to develop cardiovascular diseases or stroke compared to their non-diabetic counterpart.

Race and ethnicity have also been observed to have an impact on type II diabetes. The incidence is also high in Native Americans, Hispanics and poor rural whites. In the US, African Americans have about a threefold higher prevalence of the disease than Caucasians.

In Ghana, the prevalence rate rose from 2% in 1995 to 3.9% in 1997 and increased incidence of the disease is observed in hospitals (Vuvor, 1997). Currently the prevalence rate among adult Ghanaians is about 5% (Amoah, Personal Conversation, Korle-Bu Teaching Hospital, 2001).

## **1.2 Objective**

This research was to find out the differences in the diet, serum lipid profile and body indices of NIDDM subjects compared to non-diabetic subjects among Caucasians, African Americans and Ghanaians. The hypothesis is that the quantity and type of dietary fat intake will differ among races and between diabetic and non-diabetic subjects.

Specific aims were to:

- i. Measure the non-esterified fatty acid present in the serum of each subject using invitro enzymatic colorimetric method,
- ii. Determine the fatty acid composition of serum lipids using a modified Folch procedure (1956) and speciate the fatty acids using gas-liquid chromatography.
- iii. Assess the nutritional status through:
  - a. anthropometric measurement
  - b. a 3-day dietary assessment
  - c. biochemical indices (glucose concentration, total cholesterol, triglycerides, LDL-C, HDL-C and VLDL-C concentrations).

### 1.3 Relevance of Project Work

The prevalence of type II diabetes varies depending on race and ethnicity. In the United States, blacks have about a threefold greater prevalence of the disease than whites. Insulin resistance and obesity are known risk factors for both diabetes and heart disease. Although obesity is more prevalent in African Americans than in Caucasians the difference in obesity rates does not account for the increased risk in the African American population to diabetes (Lovejoy *et al.*, 1996).

There are also controversies regarding the pathogenesis of the disease being a result of insulin secretory dysfunction or insulin resistance, the two major causes of diabetes. Studies have found that in Pima Indians and Caucasians, insulin resistance is believed to be the primary defect in type II diabetes (Matsumoto *et al.*, 2000). However, the presence of insulin-sensitive Type II diabetes has been recognized among Japanese and African Americans. Racial comparative studies in this area are few.

Some studies have been done on insulin concentration and insulin sensitivity among insulin-resistant NIDDM subjects (Osei and Schuster, 1995; Zoratti *et al.*, 2000; Lovejoy *et al.*, 1996; Allen, 2000). However, there have been no known studies done relating anthropometric indices, serum fatty acids, diet and other metabolic indices to diabetes among Caucasians, African Americans and Ghanaians. This work would therefore serve as a pilot study and provide relevant baseline data for future research.

## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1 Diet Intervention Studies

Diabetic aborigines living in the rural area were tested before and after living for seven weeks as hunter – gatherers in their traditional country. There was a rapid weight loss over the period. Despite the high contribution of animal food to the total energy intake (64%), the diet was low in total fat due to the very low fat content of the wild animals. There was also marked improvement in fasting glucose levels, postprandial glucose clearance and insulin response to glucose. A fall in fasting plasma insulin and fasting plasma triglyceride and very low density lipoprotein triglyceride concentration were recorded (O’Dea, 1984)

Researchers developed interest in the applicability of a high carbohydrate diet in native Americans whose traditional diets contained little fat and who recently have been rapidly increasing their intake of dietary saturated fat (Howard and Abbott, 1989; Abbott *et al.*, 1989). In order to investigate the effects of recommended diets on individuals with NIDDM, South Western American Indians were studied. A high carbohydrate diet rich in fiber content was fed to one group and a high fat diet to the other in a longitudinal, crossover study. In both diabetic and non-diabetic patients, the high carbohydrate diet did not result in significant changes in plasma glucose or insulin concentrations. In both diabetic and non-diabetic patients under both study designs, there were consistent significant decreases in total and low-density

lipoprotein cholesterol in subjects on the high carbohydrate diet. In non-diabetic subjects, high-density lipoprotein cholesterol concentrations tended to be lower on the high carbohydrate diet, but in diabetic subjects there were no significant differences in high-density lipoprotein cholesterol among individuals on the two diets.

Grimm (1999) also concluded that a high carbohydrate/low fat diet decreased insulin sensitivity on a short term. However, on a long term, high fat/low carbohydrate diet have a lower satiating power, induce low leptin levels and eventually lead to a higher energy consumption, resulting in obesity and worsening of the insulin resistant state. Grimm therefore recommended moderately high carbohydrate (44-55% of daily caloric intake)/low-fat diet in addition to regular exercise programs.

## **2.2 Abnormal Insulin Action and Impaired Insulin Secretion in Type II Diabetes**

In patients with Type II diabetes Mellitus, insulin action on the liver, adipose tissue and muscle is defective in a variety of ways. In the liver, decreased glucose uptake and unrestrained hepatic glucose production contribute to an increase in both fasting and nonfasting plasma glucose levels. The rate of hepatic glucose production in the diabetics at fasting state is about 17  $\mu\text{mol/kg}$  per minute, approximately 7  $\mu\text{mol/kg}$  per minute higher than in non-diabetic individuals (Kahn, 1996). Also, normal suppression of glucose production after a meal is impaired in the type II diabetic patients. The increased hepatic glucose production in type II

diabetes has been attributed both to stimulation of glycogenolysis and to increases in gluconeogenesis.

The ability of insulin to accelerate glucose transport into muscle is also reduced in type II diabetic individuals. This may be due to impaired translocation of glucose transporters to the cell membrane (DeFronzo, 1997; Zierath *et al.*, 1997). Phosphorylation of glucose-6-phosphate is also decreased and so glycolysis and glycogen synthesis is reduced in muscles.

Impaired insulin secretion may ultimately lead to type II diabetes. This is characterized by several defects of the B-cell hormone including reduced insulin secretion in response to nutrients, reduced rate of insulin production from the pancreas (decreased insulin secretion pulsatility) and impaired processing of proinsulin to insulin.

Proinsulin, the precursor of insulin and a normal secretory product of B-cells, has much less biological activity than insulin. Ward and his colleagues in 1987 found that the concentration of proinsulin is about two times higher in type II diabetics compared to non-diabetic individuals. And so not only is insulin secreted in smaller amounts but also in its less biologically active form.

In non-diabetic individuals, at mealtimes or with intravenous glucose injection, insulin is secreted mainly in two phases, the second phase beginning about 10 minutes after the first phase and continues till glucose homeostasis is maintained.

In type II diabetic and glucose-tolerant individuals, the first phase insulin secretion in response to glucose is lost and as the pancreatic islet dysfunction continues, the second phase insulin secretion gradually diminishes, worsening the hyperglycemic state (Kahn *et al*, 1998). Also, Insulin, normally secreted in a pulsatile fashion, is dampened in the presence of glucose intolerance in type II diabetes.

These insulin secretion defects result in several anomalies. The absence of the early phase insulin secretion results in a longer glucose production by the liver at mealtimes. Glycogenolysis and gluconeogenesis are less restrained and high levels of glycerol and free fatty acids are observed in the blood. The reduced insulin levels also reduce the efficiency of glucose uptake by the muscle. There are therefore higher postmeal glucose excursions in patients with type II diabetes due to defects in insulin secretion.

### **2.3 Lipids and Lipoproteins**

Many studies have shown that diabetes is consistently associated with changes in plasma lipids and lipoproteins (Howard, 1993) and these are of interest because of their possible role in the etiology of cardiovascular disease associated with diabetes. It has become evident that there are ethnic differences in plasma lipoproteins caused both by genetic determinants and by specific cultural and environmental influences unique to the individual ethnic groups.

Fasting blood samples were obtained from Pima Indians in Arizona and cholesterol and triglycerides were measured in very low-density lipoproteins, low density

lipoproteins, high density lipoproteins and its subfractions. Diabetes was consistently associated with elevations in total and very low-density triglycerides in both sexes and in all age groups. Decreases in high-density lipoprotein cholesterol were similar in diabetic men and women and were of much greater magnitude in the less obese (Howard, 1993).

In another study, diabetic Pima Indians had an increased production of very low-density lipoprotein triglyceride compared with weight and age matched non-diabetic control subjects. Diabetic patients, in addition, had decreased fractional catabolic rate for very low-density lipoprotein triglyceride, indicating decreased clearance. Also, the degree of hyperglycemia was significantly correlated with elevations in plasma free fatty acids (Howard *et al*, 1984; Howard *et al*, 1987).

Low-density lipoprotein metabolism was examined among Pima Indians by measuring the production and conversion of very low-density lipoproteins to low-density lipoproteins at the same time through the measurement of <sup>131</sup>I specific activity. In diabetic patients, the fractional catabolic rate for low-density lipoprotein apo B was reduced compared with non-diabetic subjects and a higher proportion of very low density lipoprotein apo B was removed without conversion to low density lipoprotein in diabetic individuals. These alterations may have atherogenic potential and may lead to increased deposition of low-density lipoprotein cholesterol in vessel walls.

Lower concentrations of high-density lipoprotein have been observed among diabetic Pima Indians. Elevation of hepatic lipase levels and lowering of lipoprotein lipase activity have been observed in obese diabetic Pima Indians. Although the mechanism of the control of high density lipoprotein is not well understood, high density lipoprotein compartment increases on transfer of apoprotein and cholesterol from triglyceride-rich lipoproteins during lipolysis (Howard, 1993). Conversely, hepatic lipase activity, by hydrolysis of high-density lipoprotein triglyceride, contributes to the disappearance of high-density lipoprotein from the plasma compartment. Thus changes in lipoprotein and hepatic lipases observed in diabetic patients may act in concert to decrease high-density lipoproteins in diabetic Pima Indians.

#### **2.4 Fatty Acids and NIDDM**

Fatty acid imbalances and subsequent development of NIDDM have been observed to be on rapid rise among Eskimos. Studies on abnormal glucose-tolerant subjects revealed a lower concentration of omega-1 fatty acids (C18: 3 omega 3, C20: 5 omega 3) and some omega 6 fatty acids (C18: 3 omega 6, C20: 3 omega 6, and C22: 4 omega 6). The low concentration of the long chain omega 6 fatty acids in the glucose-impaired state may be related to desaturase activity (Ebbesson *et al.*, 1999). There was also a higher concentration of palmitic (C16: 0) and oleic acid (18: 1 omega 9) among abnormal glucose-tolerant subjects compared to normoglycemic subjects. A similar study by Storm *et al.* (1997) also revealed that diet rich in palmitic acid was not as effective in lowering cholesterol levels compared to stearic acid-rich diets in NIDDM patients

Fatty acid profile and indices of delta 6- and delta 5- desaturase activity were investigated in pre- and post- menopausal normal and type II diabetic women in order to investigate the effect of aging. Comparing dietary intakes serum phospholipids, fatty acid ratios of C18: 3n-6/C18: 2n-6, there were no differences found among pre- and post- menopausal normal and diabetic type II women. However, there was a difference in the C18: 3n-6/C18: 2n-6 diabetic and non-diabetic subjects (Liu *et al*, 2000).

Docosahexaenoic acid (C22: 6n-3), a vital component of the phospholipids of cellular membranes especially in the brain and retina, is necessary for proper functioning of n-3 fatty acids. The n-3 fatty acids favorably affect atherosclerosis, coronary disease, and behavioral disorders (Connor, 2000). These n-3 fatty acids are significant structural components of the phospholipid membranes of tissues throughout the body and are especially rich in the retina, brain and spermatozoa, in which DHA constitutes about 36% of total fatty acid. Another important feature of n-3 fatty acids is their role in the prevention and modulation of certain diseases including coronary heart disease and stroke. Their requirement increases in pregnancy and lactation.

Research is being carried out to investigate the need for the entire spectrum of n-3 fatty acid from alpha linolenic acid (C18: 3n-3) to highly polyunsaturated fatty acid DHA synthesized by alpha linolenic acid. There are also doubts about the appropriate ratio of n-6/n-3 fatty acids. An imbalance can accentuate the n-3 fatty acid deficiency state. The ratio increased by increased consumption of vegetable

oil rich in n-6 fatty acid e.g. linoleic acid (C18: 2n-6) and decreased consumption of foods rich in n-3 fatty acids. The ratio of arachidonic (C20: 4n-6) to DHA may also be important (Connor, 2000).

Fatty acid composition of serum cholesterol esters was investigated in subjects with normal glucose tolerance, impaired glucose tolerance and NIDDM subjects. Palmitic acid (16:0) and palmitoleic acid (16:1) in serum cholesterol esters increased from the normal glucose tolerance group to the impaired glucose tolerance and diabetic groups. In addition, the proportion of linoleic acid (C18: 2) was lower in diabetic subjects than in the subjects with impaired glucose tolerance or normal glucose tolerance. The proportions of gamma-linolenic (C18: 3), dihomo-gamma linoleic (C20: 3) and arachidonic (C20: 4) acids were highest in diabetic subjects and lowest in subjects with normal glucose tolerance. These suggest that subjects with NIDDM or impaired glucose tolerance have had higher dietary intake of saturated fatty acids. Both serum insulin and blood glucose concentrations probably have effect on the elongation and desaturation of fatty acids, but the metabolism of linoleic acid to prostaglandin precursors seems to differ in different types of diabetes, NIDDM patients showing no abnormalities (Solamaa *et al.*, 1990).

The type of fatty acid in the diet may have a negative influence on insulin sensitivity. Cross-sectional studies show significant relationship between serum fatty acids and insulin sensitivity. Insulin resistance is associated with specific fatty acid pattern of the serum lipids with increased proportion of palmitic acid (C16: 0) and palmitoleic acid (C16: 1n-7) and reduced levels of linoleic acid (C18: 0). Linoleic acid

metabolism seem to be disturbed by increased proportions of linolenic acid (C20:3n-6) and a reduced activity of delta 5 desaturase, while the delta 9 and delta 6 desaturases appear to be increased. An increased saturation of the membrane fatty acids and a reduced activity of desaturase have been implicated with insulin resistance (Vessby, 2000; DeFronzo et al, 1981).

## **2.5 Non-Esterified Fatty Acids (NEFA) and NIDDM**

Non-esterified fatty acid is one important link between obesity, insulin resistance and type II diabetes (Boden, 1999). Non-esterified fatty acids in plasma are derived from two sources in normal individuals: release from adipose tissue when the supply of carbohydrate as fuel is low or exhausted, and release from chylomicrons under the influence of lipoprotein lipase. Non-esterified fatty acids, used by the liver and muscle as energy source, interfere with insulin-mediated glucose disposal in the muscle tissues by impairing glucose oxidation and glycogen synthesis, thereby elevating plasma glucose levels in type II diabetic individuals. Impaired glucose oxidation increases lipid oxidation leading to increased insulin resistance (Kahn, 2000). This may be a contributory factor to the positive association between high triglyceride levels and hyperinsulinemia found in insulin resistance syndrome.

Also, increased NEFA concentration could alter glucose tolerance in several ways. Increased NEFA, by inhibiting glucose uptake and oxidation in muscle cells, increases insulin resistance in NIDDM subjects. High NEFA concentrations in the liver act as stimulus to gluconeogenesis. Furthermore, increased NEFA

concentrations prevent normal suppression of hepatic glucose production by insulin and reduce the ability of islet cells to secrete insulin in response to glucose, a defect that can be prevented by inhibitors of fatty acid oxidation. A study among Pima Indians concluded that a high NEFA concentration was a risk marker for NIDDM, independent of percent body fat and fat distribution in the body (Charles *et al.*, 1997).

## **2.6 Esterified Fatty Acids and NIDDM**

A high fat diet coupled with physical inactivity, apart from increasing the risk of obesity, impairs insulin sensitivity and increases the risk of developing NIDDM. Cross-sectional studies show significant relationships between the serum lipid fatty acid composition (which mirrors the quality of fatty acid present in the diet) and insulin sensitivity. Persistent hyperlipidemia, poor dietary habits and low physical activity may alter membrane fluidity and impair receptor functions, ion transport through the membrane, cell energy requirement and cell signaling. Altered membrane lipids can also affect cellular uptake of chylomicrons and other lipoproteins and eventually affect the fat metabolism in individuals. Other factors such as relative concentrations of cholesterol and phospholipid, the length and saturation of component fatty acids all influence membrane fluidity (Freyburger *et al.*, 1989).

A high proportion of saturated fatty acids in the cell membrane may impair insulin action by altering insulin receptor binding and ability to translocate or insert glucose transporters. Also, changes in phospholipid fatty acid interaction with protein kinase

C and reduced ion permeability occur as a result of increased saturated fatty acid in the cell membrane (Vessby, 2000). Studies carried out by Laserre and his colleagues in 1985 revealed that decreased saturated fatty acids and increased unsaturated fatty acids in subjects maintained on isoenergetic diets significantly improved insulin sensitivity. Not only that, variations in the dietary fatty acid composition and cholesterol content were associated with alterations in the intestinal uptake of hexose in control and diabetic rats (Keelan *et al*, 1994).

## **2.7 Metabolic Risk Factors to NIDDM among Races**

Epidemiological studies have demonstrated a higher propensity to develop type II diabetes and its associated chronic complications in certain ethnic groups (Osei and Schuster, 1994). In a study to compare the effects of ethnicity on glucose, insulin, hepatic glucose extraction and insulin sensitivity among healthy Caucasians and African Americans, average glucose levels were found to be identical in both groups but serum insulin levels were about threefold greater among African Americans compared to Caucasians. The mean insulin sensitivity index was found to be 12% lower in the African Americans compared to the Caucasians. These lower insulin sensitivity index values in the African Americans are similar to those of other ethnic groups with a high tendency of developing type II diabetes such as the Pima Indians (Lillioja *et al*, 1987) and Mexican Americans (Haffner *et al*, 1990).

Another study reported comparable upper body obesity, total body fat content and insulin sensitivity index values, with lower plasma glucose concentration among healthy middle-aged obese Caucasians and African Americans (Dowling and Pi-

Sunjer, 1993). In a similar study, Saad et al (1989) measured total glucose disposal in nondiabetic Caucasians and African Americans and found no difference in total glucose disposal rates. These studies therefore suggest a wide variation in the glucose metabolism, insulin secretion and sensitivity in the African American population.

Apart from ethnicity or race, dietary and environmental factors may be major determinants of secretion, sensitivity and metabolism of insulin. These factors may be more important than genetics in determining the glucose in insulin metabolism, and prevalence of type II diabetes in people of African descent. Studies have demonstrated that African Americans are more hyperinsulinaemic and insulin resistant than whites (Freedman et al, 1987; Svec et al, 1992; Rewers et al, 1994). Thus the high prevalence of NIDDM in the African Americans and Afro Caribbean immigrants when compared to native Americans have been partly attributed to the greater rate of obesity with its attendant insulin resistance in these populations. Osei and Schuster in 1994 demonstrated that glucose tolerant African Americans had significantly higher insulin concentration and lower insulin sensitivity than glucose tolerant native Africans residing in their respective countries. Also, evidence suggests that indigenous Africans have higher insulin sensitivity compared to other African migrants living in industrialized countries.

The prevalence and incidence of NIDDM are also increasing in the urban or industrialized areas in Africa where individuals are more sedentary than their counterparts living in rural areas and are still using traditional methods in their

occupational and daily activities (Swai *et al.*, 1990; Mclarty *et al.*, 1990; Banini and Orraca-Tetteh, 1997). This suggests that people of African ancestry are at greater risk for diabetes when exposed to western lifestyle. In a comparative study in which healthy African Americans and Ghanaians who had migrated and stayed over 6 years in the Ohio state in America were observed, Osei and Schuster (1994) found the mean fasting serum glucose, insulin levels and Insulin sensitivity index similar in both groups. There is therefore a probability of rapid adaptation in glucoregulation, beta-cell function and insulin sensitivity among the Ghanaians studied.

## **2.8 Fat Oxidation in Caucasian and African Americans**

African American women gain weight at an earlier age and tend to be heavier than Caucasian women of similar age and economic status (Privette *et al.*, 2000). In addition, African American women lose less weight and lose weight at a slower rate than Caucasian women when placed on the same weight loss regimen. Privette and his colleagues investigated these factors indicating inherent differences in metabolism between the races further. They looked at the capacity of the skeletal muscle, a major site of oxidative lipid disposal, to oxidize fatty acids in the obese groups. This was determined by measuring the rate of carbon dioxide production from the oxidation of palmitate, palmitoyl-CoA and palmitoyl-carnitine. A lower capacity of palmitate oxidation was observed in the African Americans compared to Caucasian. This was attributed to impairment in the reactions involving palmitoyl-CoA synthesis, since activation of palmitate through catalysis by long chain fatty

acyl-CoA synthetase brought about similar oxidative capacity in the African American subjects compared to their Caucasian counterparts.

Also, Hickner and colleagues (2001) investigated differences in fat oxidation during exercise between African American and Caucasian females. They found a 45% lower rate of fat oxidation in African American compared to Caucasian females. Similar patterns were observed among the lean and obese subgroups of this population. These differences observed may be as a result of differences in genetic constitution of the races.

Bower et al (2000) showed that the incorporation of [3-H] oleate into the lipids was higher in adipose tissue from African American than in Caucasian women. To prove that this could be due not to increased activity of lipogenic enzymes but to increased capacity of adipose tissue to take up substrates from circulation, a lipid-loading study in lean and obese African American and Caucasian women was performed. Plasma triglyceride levels were lower in the lean African American than Caucasian women. This suggested an enhanced uptake of substrates from circulation, which may fill the adipocytes with fat faster and result in early obesity in African American than in Caucasian women. There was no difference in plasma triglyceride levels in obese African American and Caucasian women following ingestion of the lipid meal. This may be due to the already enlarged and engorged adipocytes present in the obese subjects.

## CHAPTER THREE

### METHODOLOGY

#### 3.1 Introduction

This collaborative study between North Carolina State University and University of Ghana, Legon, recruited subjects from Raleigh, North Carolina and Accra, Ghana. The study aimed at recruiting 10 subjects (males and females) each from the following categories:

- i. Caucasian NIDDM subjects from Raleigh, North Carolina (CD)
- ii. Caucasian non-diabetic subjects from Raleigh, North Carolina (CC)
- iii. African American NIDDM subjects from Raleigh, North Carolina (AD)
- iv. African American non-diabetic subjects, Raleigh, North Carolina (AC)
- v. Ghanaian NIDDM subjects from Accra, Ghana (GD)
- vi. Ghanaian non-diabetic subjects from Accra, Ghana (GC)

The non-diabetic controls and diabetic subjects were recruited from the same sampling area by word of mouth. Subjects ranged between 23 and 70 years of age, with equal numbers of males and females in each group except for the Caucasian Diabetics (3 males and 2 females). In order to have a baseline for all control subjects, inclusion criteria for control subjects were: glucose and cholesterol readings within normal range (60-95 mg/dl and <200 mg/dl respectively), non-obese

with BMI less than 30 kg/m<sup>2</sup>, and being non-diabetic, having no known diabetic-related conditions.

### **3.2 Assessment of Body Size**

To assess the body size of the subjects, their weight, height, skinfold and circumference measurements were made.

#### **3.2.1 Weight**

All subjects for this study were weighed using the weighing scale. The subjects were made to stand comfortably on the scale with minimum clothing and without shoes. The weight was recorded to the nearest 0.5 kg.

#### **3.2.2 Height**

A stadiometer, calibrated to the nearest millimeter, was used. The subjects were made to stand upright with bare feet on the level platform. The sliding headpiece of the stadiometer was moved to touch the crown of the head and the readings taken.

#### **3.2.3 Body Mass Index**

The ratio of the weight in kilograms to the square of the height in meters, known as the Body Mass Index (BMI) was used as an index for body size determination. Subjects were then classified as underweight, normal weight, overweight and obese using the World Health Organization (1985) classification.

### 3.2.4 Skinfold Measurement

Measurement of the subcutaneous fat was made on the left side of the body at selected sites for men: abdomen, chest and thigh and, for women: triceps, supraillium and thigh regions. These critical sites have been shown to correspond to the distribution of body fat in males and females (Durnin and Rahaman, 1967). At each region, the skin with the underlying fat was pinched gently but firmly between the thumb and forefinger and pulled away slightly from the underlying tissues. The Holtain skinfold caliper, calibrated in millimeters, was used for the measurements. The following are the description of the sites of measurements.

#### i. Triceps

The triceps measurement was taken halfway down the upper arm, between the tip of the acromium process of the scapular and the olecranon process of the ulna, at the back of the arm.

#### ii. Abdomen

The horizontal skinfold about 3cm to the left of the umbilicus and about 1cm below the midpoint is measured, with the subject standing erect with body weight evenly distributed on both feet, and abdominal muscles relaxed.

#### iii. Chest

The chest or pectoral skinfold was measured by grasping skinfold with its long axis running from the top of the anterior axillary fold to the nipple.

#### iv. Thigh

The anterior midline of the thigh halfway between the inguinal crease and the proximal border of the patella was measured.

#### v. Suprailiac

This skinfold was measured above the iliac crest at the midaxillary line with the subject standing and relaxed.

#### vi. Percentage body fat

The summation of the chest, abdominal and thigh skinfolds for men and the triceps, suprailia and thigh for women were calculated and the corresponding percentage body fat values were obtained based on equations of Siri (1961). The equation of Siri is as follows:

$$\text{Percent Fat} = (4.95/\text{Density} - 4.50) \times 100\%$$

This compares favorably with tables derived from Durnin and Rahaman (1967) and Jackson and Pollock (1985) giving percentage fat values corresponding to the total values of the skinfolds measured at these sites.

### 3.2.5 Circumference Measurement

#### i. Waist

The waist circumference was measured with a measuring tape (in cm) at the narrowest area below the rib cage and above the umbilicus as viewed from the front.

ii. Hip

The point of maximum circumference around the hips or buttocks was measured with the subject standing.

iii. Thigh

The midpoint of the thigh was measured with the subject standing with his weight on the right thigh and his left thigh relaxed and brought forward.

The ratio of the waist and hip (WHR) measurement served as an index of android (abdominal) fat deposition whilst the calculation of the waist to thigh ratio (WTR) served as an index of the gynoid (lower abdominal) adiposity (Frayn, 2000).

### **3.3 Dietary Assessment**

A 3- day diet record was taken for all the subjects in this study. Caucasian and African American subjects were instructed on how to determine portion sizes. In Ghana a trained interviewer recorded the type and portion sizes of the food taken the previous day upon recruitment of the subject into the study. At the scheduled home visit, the interviewer recorded a 2-day food intake in addition to collection of the fasting blood samples.

The energy value of foods eaten was calculated using the Nutritionist V software for the Caucasians and African American subjects, and Food Composition Tables of Ghana (Eyson and Ankrah, 1973) for the Ghana diet data.

### 3.4 Assessment of Blood Indices

#### 3.4.1 Blood Sample Collection

A drop of blood was obtained from the fingertip for total glucose determination. Also, about 10 ml blood samples were obtained by venipuncture after an overnight fast with vacutainer needles and tubes (Beckton Dickenson, Franklin lakes, NJ). Samples were kept cool until arrival at the laboratory. The clotted samples were then centrifuged (1000xg for 10 mins at 30°C). The recovered serum was divided into 2ml aliquots and stored at -20°C until ready for analysis. For the Ghanaian subjects, the serum samples were frozen, and placed in frozen serum transport tubes. These were then transported in tri-package tubes (Fisher Scientific, PA) and shipped to North Carolina by Federal Express courier services. All laboratory analyses were carried out for all the samples under the same conditions. Serum samples were analysed for fatty acids, triglyceride, total cholesterol and lipoprotein-cholesterols. The protocol was approved by the Institutional Review Board, North Carolina State University, Raleigh, North Carolina.

#### 3.4.2 Fatty acid speciation

##### I. Cleaning procedure

A modified Folch procedure was used (Folch *et al*, 1956). Briefly, 1ml serum sample was added to 20ml chloroform: methanol (2:1) mixture and 5ml of 0.88% potassium chloride solution containing 0.05% BHT (3,5-di-tert-butyl-4-hydroxytoluene) as antioxidant. This was left in the refrigerator overnight for separation. The buffy layer was then aspirated while the lipid sample was dried

under nitrogen. The dried sample was reconstituted in 5ml chloroform and dried down again under nitrogen. The latter process was repeated twice. The dried sample was then reconstituted with 2ml chloroform, overlaid with nitrogen and kept in the freezer for esterification.

ii. Preparation of methyl esters

In the esterification protocol, 100ul of the cleaned-up sample and 25ul of Heneicosanoic acid, C21: 0 (used as internal fatty acid standard) were added to 1ml of Sodium hydroxide/Methanol solution (100ml Methanol, 2g Sodium hydroxide, 0.0025 BHT) and warmed at 80°C for 15 minutes. The solution was then cooled and 2ml Boron trifluoride in methanol (10% w/w) was added for the formation of the fatty acid methyl esters. This was then warmed again (80°C for 15 minutes) and cooled. The lower layer was re-extracted with 1ml hexane and 1ml de-ionised water; 2ml hexane were then added to the solution and shaken vigorously in order to extract the esterified lipids into the hexane layer. The solution was then centrifuged using Annette's bench top centrifuge (1000g for 10mins). The upper three-quarter layer was transferred into a labelled conical tube. The lower layer was re-extracted with 2ml hexane, centrifuged and transferred again.

A spoonful of anhydrous sodium sulfate was added to the solution and shaken with a vortex. This procedure was to remove residual moisture from the sample. The upper three-quarter layer of the solution was then transferred into a hexane-rinsed storage bottle. The lower layer was re-extracted with 1ml hexane, the upper three-

quarter layer transferred into the storage bottle again. The latter step was carried out again till a final volume of 4-5ml was obtained. This was dried under nitrogen, capped tightly and stored in the freezer for the clean-up procedure.

### iii. Clean-up Procedure

This dried-up sample was reconstituted with 5ml 95:5 solution of hexane: ethyl ether and filtered through the florisil column using 95 hexane: 5 ethylether mixture. This was dried down under nitrogen and made up in 25ul isooctane solution in readiness for the gas chromatography analysis.

### iv. The Gas Chromatography (GC) Procedure

The Hewlett Packard 5890 GC (Avondale, PA) attached to the computer (Dell, Round Rock, TX) containing the Chrome-perfect software (Justice Innovations, CA) was used for the analysis of fatty acids present in the serum sample. The conditions set on the GC were the same as those described by Boyd et al, 1999. The absolute response factors and retention times of known fatty acid standards (Nu Check Prep, Inc. Elysian, MN) were used to quantify and identify the fatty acid composition of samples.

#### 3.4.3 Non-esterified fatty acid determination

The Wako NEFA-C kit (Wako chemicals, Richmond, VA) employed the Acyl-CoA synthetase (ACS)- Acyl CoA oxidase (ACOD) method to assess the amount of non-esterified fatty acid present in the serum sample. This enzymatic method relies on a

series of reactions with a resultant purple color formation, which is measured with a spectrophotometer at the absorption wavelength of 550nm. Non-esterified fatty acid was then determined from the optical density measurement of the sample, blank and known concentration of the standard (Wako Chemicals, Richmond, VA, USA). The interference of ascorbic acid (an antioxidant) with the reaction is avoided by the addition of ascorbate oxidase to the reaction mixture.

#### 3.4.4 Measurement of glucose and lipoprotein-cholesterols

The total blood glucose was determined using the Accu-Chek Advantage Blood Glucose Monitor and test strips (Boehringer Mannheim Corporation, Indianapolis) on the field. A drop of blood, obtained from the fingertip with a lancet, was immediately applied to the test strip. This was inserted into the monitor and the blood glucose reading recorded.

The Cholestech L.D.X Lipid Profile Panel –II (Cholestech Corporation, Hayward, CA) was used for the determination of total cholesterol, high-density lipoprotein cholesterol and triglyceride levels in the serum. The Cholestech L.D.X analyzer calculated the Low-density lipoprotein and very low-density lipoprotein cholesterol values. This method applies enzymatic methodology and solid-phase technology to measure the various indices mentioned above. A 6ul serum sample was applied to the Cholestech L.D.X cassette and inserted into the Cholestech L.D.X analyzer. The cassette, impregnated with various reagents and enzymes facilitates the separation and measurement of the lipid components in the serum sample (Allain *et al.*, 1974).

### **3.5 Data Analysis**

Statistical analysis was carried out using SAS version 8.0-computer software (SAS, Cary NC). Analysis of Variance with the General linear model procedure was applied for comparisons of means between diabetics and their corresponding non-diabetic controls, among diabetics and among controls. This was to study the effect of health status (diabetic versus non-diabetic) and race on the parameters measured. The relationships among blood constituents, nutrient intake and body indices were also studied using Pearson correlation procedure. P-values less than 0.05 were considered statistically significant.

## CHAPTER FOUR

### RESULTS AND DISCUSSION

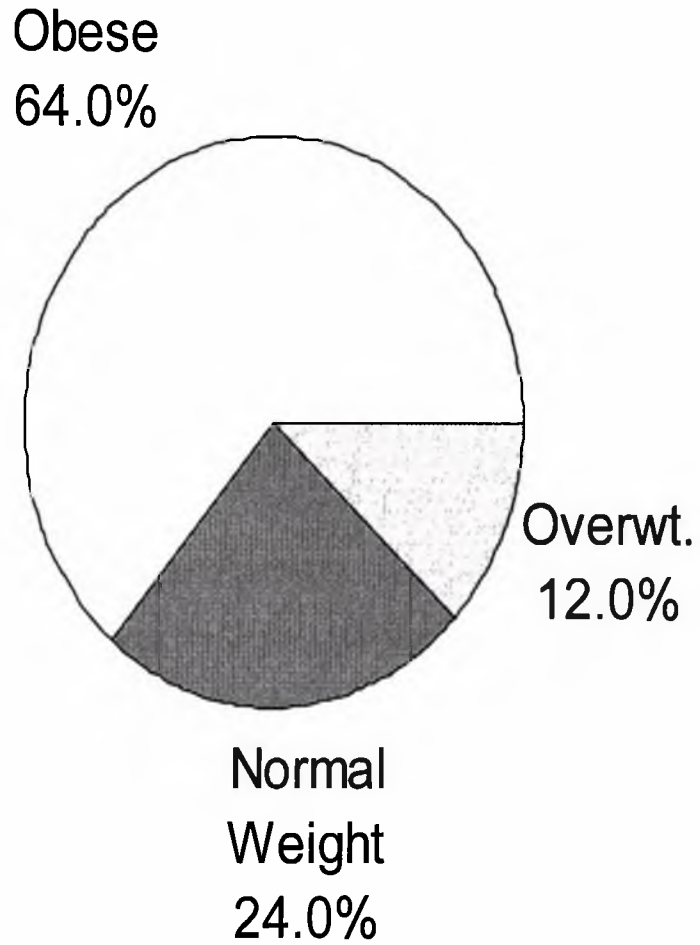
#### 4.1 Background Information

The background information for the subjects in this study is summarized in Table 1. Ten subjects each were recruited for the following groups: Caucasian controls (CC), African American controls (AC), African American diabetics (AD) and Ghanaian diabetics (GD). For the Ghanaian control (GC) subjects recruited, 6 subjects satisfied all the inclusion criteria necessary to be a control subject. An eligible control subject must have glucose and cholesterol reading within the normal range, 60-95 mg/dl and <200 mg/dl, respectively, non-obese with BMI less than 30 kg/m<sup>2</sup>; must be non-diabetic, having no known diabetic related conditions. The number of Caucasian diabetics (CD) successfully recruited was 5. The average ages are as shown in Table 1 and Body Mass Indices ranged between 18.6 and 46.7 kg/m<sup>2</sup>. Among the diabetic subjects studied, about 76% were either overweight or obese (BMI > 26kg/m<sup>2</sup> and 30kg/m<sup>2</sup> respectively) and 24% were of normal weight with BMI <26kg/m<sup>2</sup> (Fig. 1).

	Number of Subjects	Body Mass Index (kg/ m <sup>2</sup> )	% Body Fat	Waist to Hip Ratio	Waist to Thigh Ratio
CC	10	24.9 (7.3) <sup>a</sup>	17.2 (6.5) <sup>a</sup>	0.8 (0.1) <sup>a</sup>	1.5 (0.3) <sup>a</sup>
AC	10	24.3 (4.0) <sup>a</sup>	21.2 (6.3) <sup>a</sup>	0.8 (0.1) <sup>a</sup>	1.4 (0.2) <sup>a</sup>
GC	6	22.1 (2.8) <sup>a</sup>	21.7 (8.3) <sup>a,d</sup>	0.8 (0.1) <sup>a</sup>	1.5 (0.1) <sup>a</sup>
CD	5	36.5 (8.8) <sup>b</sup>	25.9 (8.6) <sup>a</sup>	1.0 (0.0) <sup>b</sup>	2.3 (0.4) <sup>b</sup>
AD	10	33.4 (5.1) <sup>b</sup>	32.2 (3.2) <sup>b</sup>	0.9 (0.1) <sup>c</sup>	1.8 (0.3) <sup>c</sup>
GD	10	26.4 (6.1) <sup>a,c</sup>	20.0 (8.7) <sup>c,d</sup>	0.9 (0.1) <sup>a,c</sup>	1.6 (0.2) <sup>a,c</sup>

Values are means (standard deviation). Values within column with common superscript are not significantly different. P- value < 0.05, for significant differences between Diabetics and their Controls, and among Diabetics. CC = Caucasian Control, AC = African American Control, GC = Ghanaian Control, CD = Caucasian Diabetic, AD = African American Diabetic, GD = Ghanaian Diabetic

Figure 1



## 4.2 Intra- and Inter-Racial Anthropometric Variations

Caucasian and African American Diabetics had significantly higher Body Mass Indices compared to their non-diabetic counterparts ( $p = 0.005$ , Table 1). A borderline significance in BMI was recorded between Ghanaian Controls and Ghanaian Diabetics ( $P = 0.06$ ). This may have been significant with a larger sample size. Comparing the BMI among the diabetic groups, significant differences were observed between African American Diabetics and Ghanaian Diabetics ( $33.4 \pm 5.1 \text{ kg/m}^2$  vs  $26.4 \pm 6.1 \text{ kg/m}^2$ ,  $P < 0.001$ ), and Caucasian Diabetics and Ghanaian Diabetics ( $36.5 \pm 8.8 \text{ kg/m}^2$  vs  $26.4 \pm 6.1 \text{ kg/m}^2$ ,  $P < 0.05$ ). These findings were in contrast to those of Osei and Schuster, 1995; Lovejoy *et al.*, 1996. They found no significant difference in the BMI between Caucasians and African Americans. In all subjects studied, Ghanaians had the lowest BMI among the controls as well as amongst the diabetics.

Table 1 shows the percent body fat estimated from the skinfold measures. African American Diabetics had significantly higher percent Body Fat (BF) compared to the African American Controls while Ghanaian Diabetics had significantly lower body fat values compared to their non-diabetic counterparts. Significant differences were also observed between African American Diabetics and Caucasian Diabetics ( $32.2 \pm 3.2\%$  vs  $25.9 \pm 8.6\%$ ,  $P < 0.05$ ), African American Diabetics and Ghanaian Diabetics ( $32.2 \pm 3.2\%$  vs  $20.0 \pm 8.7\%$ ,  $P < 0.001$ ), and Caucasian Diabetics and Ghanaian Diabetics ( $25.9 \pm 8.6\%$  vs  $20.0 \pm 8.7\%$ ,  $P < 0.05$ ). As expected, there was significant correlation between BMI and percent BF ( $r = 0.6$ ,  $P = 0.0001$ ).

Waist to hip ratio (WHR) is an indicator of android (or abdominal) obesity. This is prevalent among men (Frayn, 2000). It has also been recognized that upper body fat deposition is closely associated with insulin resistance (Frayn, 2000). A WHR above one indicates an increased risk to developing obesity and other related complications such as hypertension, Type II diabetes, atherosclerosis and gout. In Table 1, only the Caucasian diabetic group had a WHR of one. This weak correlation between the Waist to hip ratio and health risk was also observed in other studies (Stevens, 1992; Dowling, 1993). There were however significantly higher waist to hip ratios among the Caucasian Diabetics and African American Diabetics compared to their corresponding control groups. This may be an indication of increased risk of developing obesity and subsequent diabetes later in life.

The waist to thigh ratio (WTR) among the racial groups was calculated in order to assess the level of gynoid (lower abdomen) adiposity present in the diabetic groups compared to their non – diabetic counterparts (Table 1). Significantly higher values were observed between Caucasian Diabetics and African American Diabetics compared to their non-diabetic counterparts, similar to the observations made between the Waist to hip measures. However, the WTR among Ghanaian Diabetics and their non-diabetic controls were not significantly different. Comparing differences among the diabetic racial groups, African American Diabetics had significantly lower WTR than Caucasian Diabetics ( $1.8 \pm 0.3$  vs  $2.3 \pm 0.4$ ,  $P < 0.05$ ) but significantly higher than Ghanaian Diabetics ( $1.8 \pm 0.3$  vs  $1.6 \pm 0.2$ ,  $P < 0.001$ ). Differences were also observed between Ghanaian Diabetics and Caucasian

Diabetics ( $1.6 \pm 0.2$  vs  $2.3 \pm 0.4$ ,  $P < 0.05$ ). Caution must be exercised in interpreting waist to thigh ratios because of body shape variations among racial groups.

### **4.3 Dietary Data**

The average energy intake of the subjects is presented in Table 2. Average food energy intakes ranged between 1614 and 2383 kcal/day. From Table 2, Ghanaian controls recorded higher energy intake compared to other controls though this was not statistically significant. The macronutrient contributing to the high-energy intake was carbohydrate. Ghanaian diabetic subjects also had similar high carbohydrate intakes. Ghanaians had about 100g/d more carbohydrate foods than the African Americans did. However, from the Body Mass Index data in Table 1, Ghanaians had lower Body Mass Index compared to the African Americans. This suggests that the carbohydrate ingested was mainly utilized for energy needs and not stored in adipose tissues. The warm climatic condition prevailing in Ghana may also favor energy utilization rather than deposition.

Fat intake is shown in Table 2. Comparing the control and their diabetic groups, there was a significantly higher fat intake in the Caucasian diabetics compared to the non-diabetic Caucasians ( $P = 0.008$ ). The Caucasian diabetic subjects had almost twice the intake of fat compared to their control subjects. This may be a contributory factor to the high body fat observed among the Caucasian Diabetic subjects.

	Energy (kcal/d)	Protein (g/d)	Carbohydrate (g/d)	Fat (g/d)
CC	1958 (429) <sup>a</sup>	84 (27)	251 (42) <sup>a</sup>	66 (22) <sup>a</sup>
AC	1865 (876) <sup>a</sup>	74 (40)	230 (99) <sup>a,c</sup>	67 (28) <sup>a</sup>
GC	2320 (383) <sup>a</sup>	89 (6)	333 (132) <sup>b</sup>	86 (14) <sup>a</sup>
CD	2383 (1210) <sup>b</sup>	100 (51)	229 (76) <sup>a,c</sup>	120(80) <sup>b</sup>
AD	1614 (464) <sup>c</sup>	83 (22)	175 (58) <sup>c</sup>	65 (27) <sup>a</sup>
GD	2046 (601) <sup>b,c</sup>	81 (27)	317 (117) <sup>a,b</sup>	58 (19) <sup>a</sup>

<sup>1</sup>Values are means (standard deviation). Values within column with different superscript are significantly different. P>0.05, for significant differences between Diabetics and their Controls, among Diabetics and among Controls.

CC = Caucasian Control, AC = African American Control, GC = Ghanaian Control, CD = Caucasian Diabetic, AD = African American Diabetic, GD = Ghanaian Diabetic

Comparing the fat intake to the percent body fat values, Caucasian Diabetics had higher fat consumption and lower percent body fat values while African Americans had lower fat intake but higher percent body fat (Figures 2 and 3). This is indicative that fat metabolism may differ among racial groups as observed by Bower *et al.*, (2000) and Privette *et al.*, (2000). However, in addition to the determination of the total fat consumed, the various fatty acids present in the meal and the amounts consumed must be estimated in order to give a better assessment of the fatty acid composition and to advice patients on the intake of specific fatty acids. The determination of the fatty acids present in Ghanaian foods was not possible in this research since fatty acid composition of Ghanaian diets has not been documented.

#### **4.4 Fatty Acid Speciation**

The serum samples obtained from subjects were analysed for saturated fatty acids (SAT), polyunsaturated fatty acids (PUFA), and monounsaturated fatty acid using the gas chromatography. Fatty acids co-eluting with odd chain fatty acids (OCFA) were also analysed. The average SAT and PUFA values are presented in Table 3. An inverse relation between the SAT and PUFA was observed ( $r = -0.50$ ,  $P = 0.0002$ ). The average PUFA levels revealed significantly lower values among the African American diabetics compared to their non-diabetics and Ghanaian diabetics compared to their non-diabetic counterparts ( $P < 0.05$ ). Inter-racial differences were also observed among the diabetics. In addition, among the control groups, there was a significantly higher serum PUFA level observed for Caucasian Controls compared to Ghanaian Controls, although all the control groups had comparable levels of saturated fatty acid levels

Figure 2:

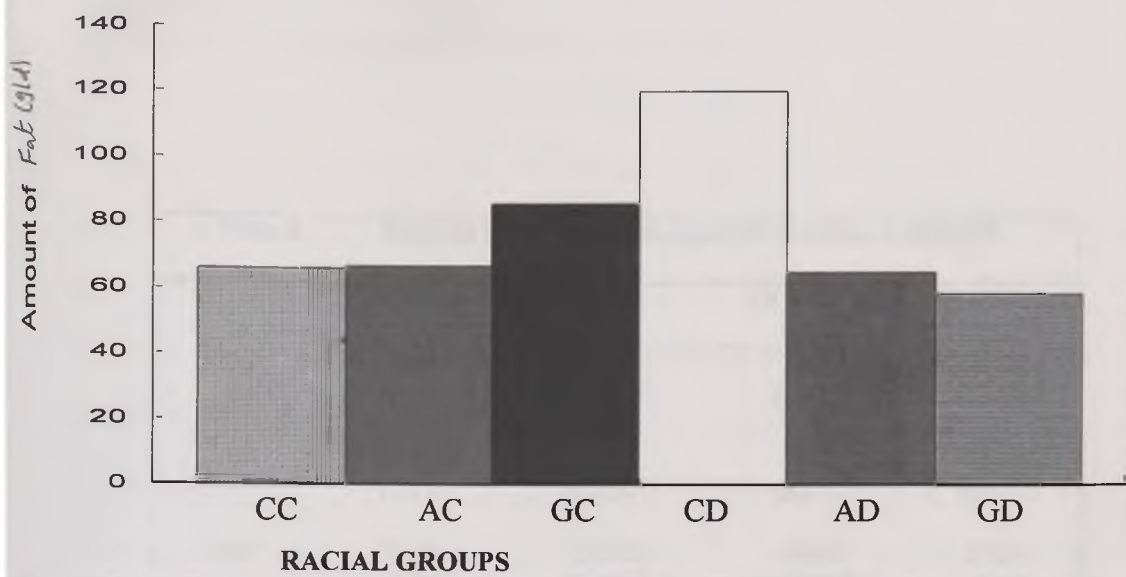
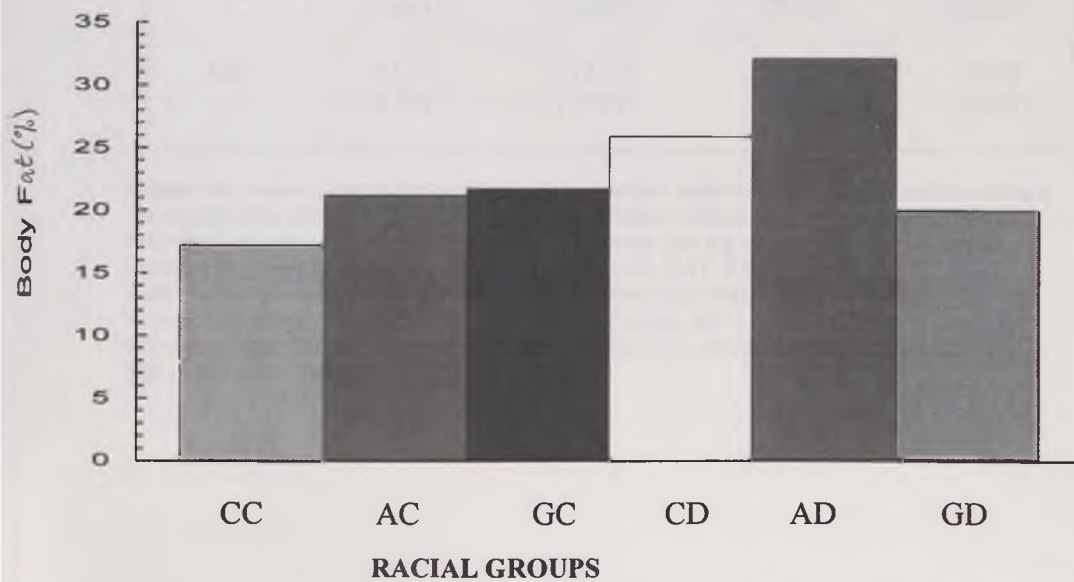


Figure 3:



	SAT. FA (%)	PUFA (%)	OCFA (%)	MUFA (%)
CC	26.06 (8.36)	42.46 (9.46) <sup>a,c</sup>	14.94 (4.94) <sup>a</sup>	29.22 (4.26)
AC	26.56 (7.21)	48.15 (8.05) <sup>a</sup>	18.25 (6.37) <sup>a</sup>	24.10 (6.11) <sup>a</sup>
GC	33.18 (4.50)	30.52 (6.18) <sup>b</sup>	16.95 (6.09) <sup>a</sup>	27.91 (6.51)
CD	24.76 (10.80) <sup>a</sup>	33.90 (13.74) <sup>a</sup>	14.00 (5.52) <sup>a</sup>	28.50 (11.99)
AD	32.10 (6.66) <sup>b</sup>	28.86 (8.49) <sup>b</sup>	25.01 (7.12) <sup>b</sup>	36.04 (5.64) <sup>b</sup>
GD	33.93 (8.71) <sup>b</sup>	17.28 (7.93) <sup>c</sup>	30.20 (14.19) <sup>b</sup>	33.19 (10.91)

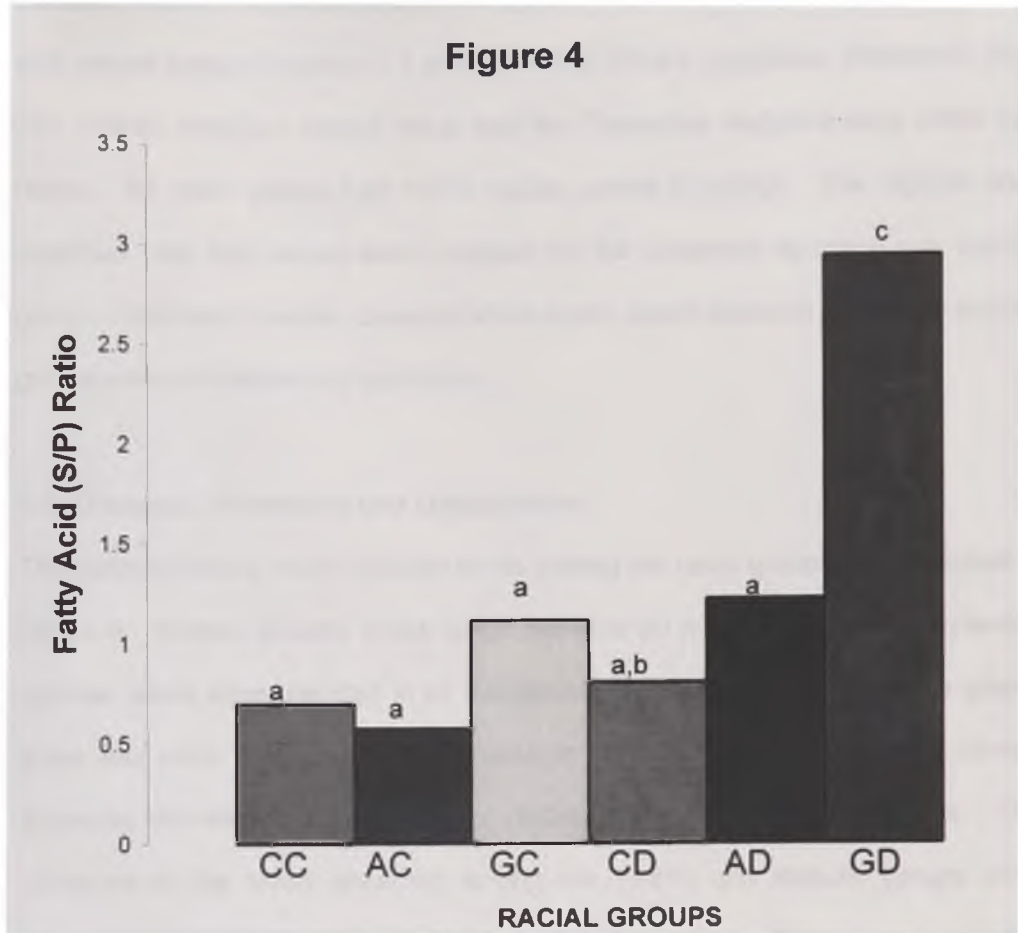
Values are means (standard deviation). Values within column with common superscript are not significantly different. P-value < 0.05, for significant differences between Diabetics and their Controls, among Diabetics and among Controls (No significant difference between Controls and their Diabetics for Saturated fatty acids (SAT. FA)).

PUFA = Polyunsaturated Fatty Acids, OCFA = Odd-Chain Fatty Acids, MUFA = Monounsaturated Fatty Acids. CC = Caucasian Control, AC = African American Control, GC = Ghanaian Control, CD = Caucasian Diabetic, AD = African American Diabetic, GD = Ghanaian Diabetic.

The Saturated to Polyunsaturated (S/P) ratio among the racial groups is presented in Figure 4. This ratio is an index of membrane fluidity and lower ratios indicate desirable membrane fluidity (decreased saturation). Factors that can influence this ratio include diet, hepatic fatty acid synthesis, desaturase enzymes, hormones and other chemicals (Allen, 2000). Significant differences were observed between African American diabetics and Ghanaian diabetics, and between Caucasian diabetics and Ghanaian diabetics, but not between African American diabetics and Caucasian diabetics. There was a significant increase in the S/P ratio among the Ghanaian Diabetics compared to their non-diabetic controls. The high level found in the Ghanaian diabetics may be a result of dietary intake and adaptation to saturated fat diet since majority of the oil products produced and consumed in Ghana (e.g. palm and coconut products) are naturally saturated. The high levels may also imply ignorance about the fatty acid composition, which may lead to wrong food choices. It also suggests that the Ghanaian diabetics had their condition poorly controlled compared to the Caucasian and African American diabetic groups.

#### **4.5 Non-Esterified Fatty Acids**

The presence of high levels of non-esterified fatty acids in the blood during fasting indicates breakdown of triglycerides in adipose tissues for energy needs of the body (Charles *et al.*, 1997). This process may lead to impairment of glucose oxidation and glycogen synthesis.

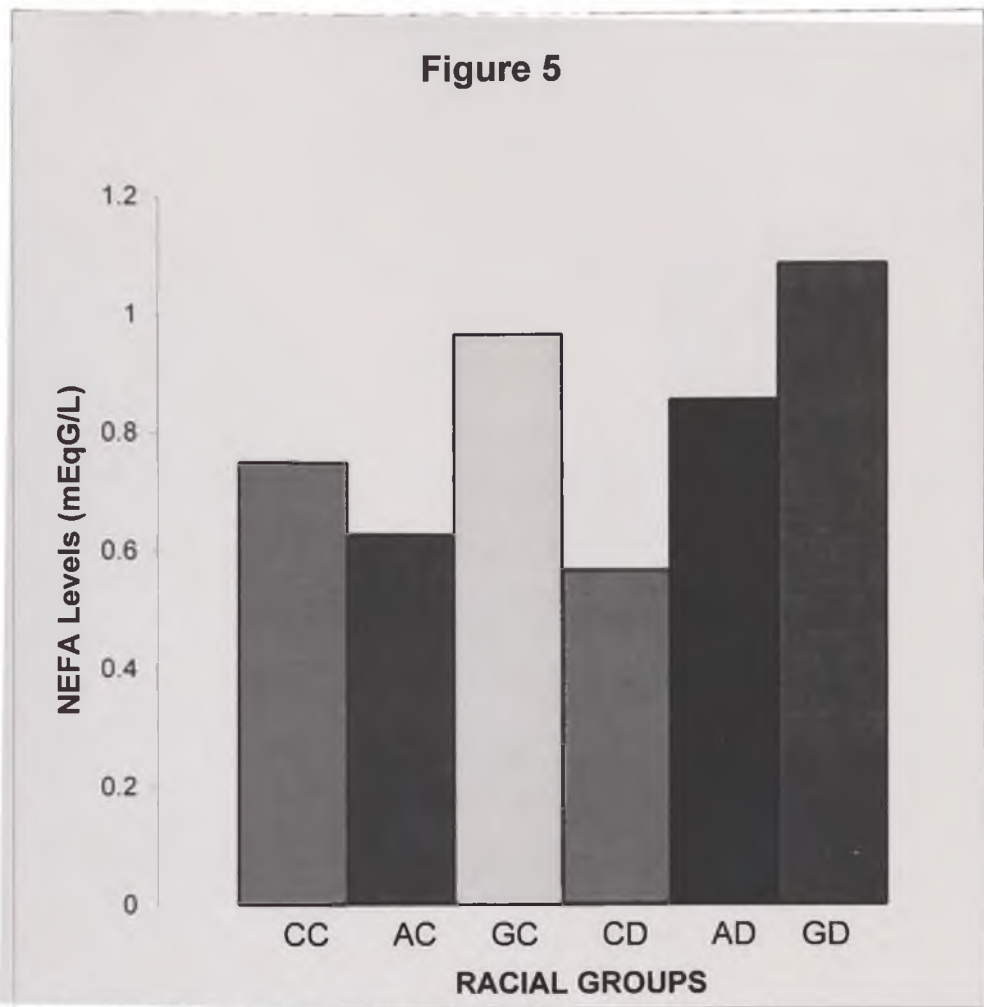
**Figure 4**

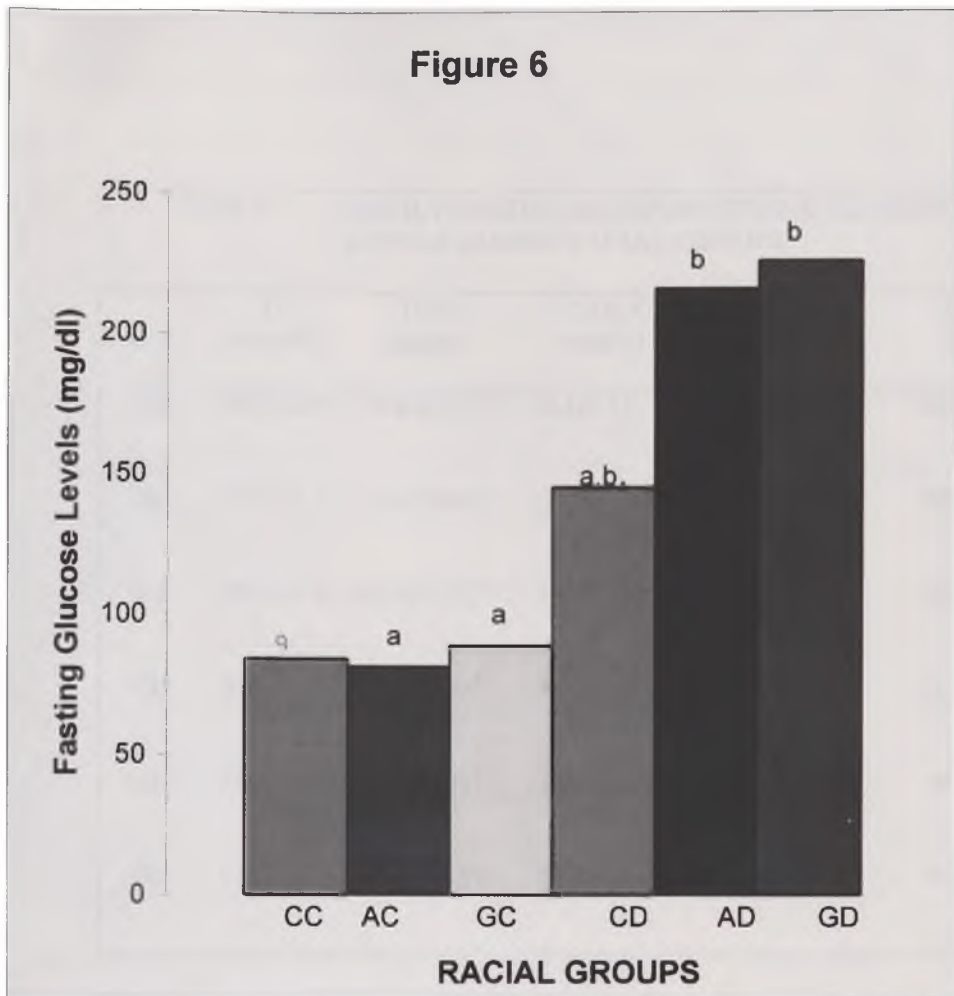
Results of serum NEFA are shown in Figure 5. Recommended non-esterified fatty acid values ranged between 0.1 and 0.6 mEq/l (Wako chemicals, Richmond, VA). The African American control group and the Caucasian diabetics were within this range. All other groups had NEFA values above 0.6mEq/l. The highest non-esterified fatty acid values were obtained for the Ghanaian diabetics and control group. Variations however observed within races, within diabetics and within control groups were not statistically significant.

#### **4.6 Glucose, Cholesterol and Lipoproteins**

The average fasting serum glucose levels among the racial groups are presented in Figure 6. Normal glucose levels range between 60 and 95 mg/dl. As expected, glucose levels were elevated in all the diabetic subjects studied. From the graph, there was more than a two-fold increase in the serum glucose levels in African American diabetics and the Ghanaian diabetics compared to their controls. The variations in the levels observed among the control and diabetic groups were statistically significant between the African American and the Ghanaian subjects but not among the Caucasians.

The total serum cholesterol and triglycerides levels are shown in Table 4. Normal values for these indices are below 200 mg/dl (Cholestech Corporation, Hayward, CA). Both control and diabetics of all groups were within the normal range. Intra- and inter- racial variations in the cholesterol levels were not significant. There were however higher triglyceride levels among the Caucasian Diabetics and African American Diabetics compared to their non-diabetic controls.



**Figure 6**

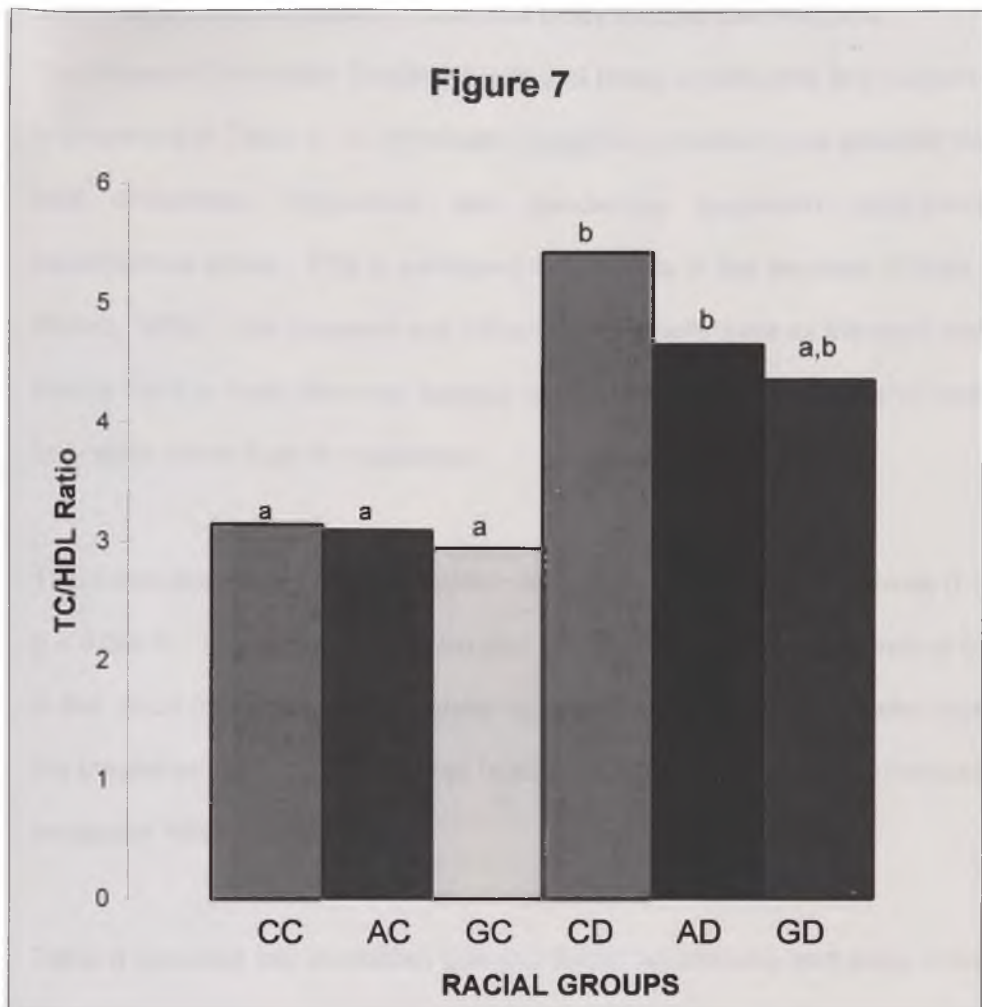
	TC (mg/dl)	TRIG (mg/dl)	VLDL-C (mg/dl)	LDL-C (mg/dl)	HDL-C (mg/dl)
CC	184 (16.9)	90.6 (42.2) <sup>a</sup>	18.1(8.4) <sup>a</sup>	104.0 (17.4)	62.1 (13.9) <sup>a</sup>
AC	185 (13.1)	83.4 (34.0) <sup>a</sup>	16.8 (6.8) <sup>a</sup>	108.0 (13.2)	62.2 (10.4)
GC	176 (38.2)	82.8(44.2) <sup>a,c</sup>	16.7 (5.4) <sup>a,c</sup>	94.4 (27.2)	62.4 (22.3)
CD	207 (40.2)	154(36.3) <sup>b,c</sup>	30.9 (7.4) <sup>b,c</sup>	135.0 (35.5)	41.7 (11.0) <sup>b</sup>
AD	215 (67.6)	163 (88) <sup>b</sup>	32.5(17.4) <sup>b</sup>	136.0 (63.1)	46.3 (6.2)
GD	178 (74.9)	105 (34.5) <sup>c</sup>	21.4 (7.1) <sup>a,c</sup>	118.0(57.1)	47.1 (20.9)

Values are means (standard deviation). Values within column with common superscript are not significantly different. P-value < 0.05, for significant differences between Diabetics and their Controls, and among Diabetics.

TC = Total Cholesterol, TRIG = Triglyceride, VLDL-C= Very Low Density Lipoprotein Cholesterol, LDL-C = Low Density Lipoprotein Cholesterol, HDL-C = High Density Lipoprotein Cholesterol. CC = Caucasian Control, AC = African American Control, GC = Ghanaian Control, CD = Caucasian Diabetic, AD = African American Diabetic, GD = Ghanaian Diabetic

Also, among the diabetics, a significant difference between triglyceride levels of African American Diabetics and Ghanaian Diabetics was observed ( $P < 0.01$ ). One of the principal determinants of the rate of triglyceride synthesis is the supply of NEFA to the liver. Therefore with increased NEFA levels, high rate of triglyceride synthesis in the blood must be observed. This was not observed with the triglyceride and NEFA levels recorded between the Ghanaian Diabetic and control group. Low triglyceride levels were recorded, although NEFA levels were elevated. This may be explained by the variation in body fat distribution (Zoratti *et al.*, 2000). Zoratti and his colleagues (2000) found that hepatic triglyceride synthesis was affected by release of NEFA from centrally located (abdominal) body fat depots. Perhaps the Ghanaian subjects secreted higher levels of NEFA from fat depots other than the lower abdominal fat depot in comparison to the other racial groups.

Average serum low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) concentrations are presented in Table 4. LDL-C between 90 and 130 mg/dl is recommended (Cholestech Corporation, Hayward, CA). All groups were within normal range. The differences within race, controls and diabetics were not significant. High-density lipoprotein cholesterol levels below 35 mg/dl indicate risk to cardiovascular diseases. All the subjects studied showed no indication of risk to cardiovascular diseases. The ratio of total cholesterol to high-density lipoprotein cholesterol is also used to assess the risk of developing cardiovascular diseases. A TC/HDL ratio above 5 indicates increased risk (Cholestech Corporation, Hayward, CA). From Figure 7, the Caucasian Diabetics therefore showed increased risk to developing cardiovascular diseases.



#### 4.7 Macronutrient, Lipid Profile and Body Indices Correlations

The Pearson Correlation Coefficient between blood constituents and nutrient intake is presented in Table 5. A significantly negative correlation was obtained between total cholesterol, triglyceride, and low-density lipoprotein cholesterol and carbohydrate intake. This is consistent with reports in the literature (O'Dea, 1984; Abbott, 1989). The presence and utilization of carbohydrate as the main source of energy for the body decrease lipolysis and so favor the synthesis and storage of fatty acids rather than its breakdown.

There was also a significant correlation between NEFA and glucose levels ( $r = 0.48$ ,  $p = 0.0004$ ). This is indicative of the fact that the presence of high levels of glucose in the blood (or its decreased uptake by glucose transporters into cells) increases the breakdown of triglyceride stored in adipose tissues and therefore increasing the amount of NEFA in the blood.

Table 6 presents the correlation between blood constituents and body indices. A positive correlation was observed between body indices and blood constituents, with the exception of high-density lipoprotein cholesterol, which recorded a negative correlation. This observation is in consonance with previous studies (Zoratti *et al.*, 2000), which observed that plasma triglyceride levels, which, in turn is affected by VLDL and NEFA levels regulate high-density lipoprotein cholesterol level. All these relate to the synthesis and storage of energy in the body.

	Glucose	Total Cholest.	Trig.	VLDL	LDL	HDL
Energy	-0.26	-0.27	-0.24	-0.22	-0.31*	0.15
Carbohydrate	-0.18	-0.33*	-0.31*	-0.28	-0.32*	-0.05
Protein	-0.20	-0.24	-0.10	-0.09	-0.28	-0.01
Fat	-0.23	-0.12	-0.12	-0.11	-0.19	0.18
Saturated FA	0.10	0.15	0.04	0.06	0.12	0.07
MUFA	0.26	-0.19	0.22	0.24	-0.17	-0.32*
PUFA	-0.35*	0.03	-0.10	-0.11	-0.02	0.09
OCFA	0.20	-0.03	0.04	0.05	0.08	-0.18
NEFA	0.48*	0.25	0.16	0.18	0.32*	-0.19

\*P&lt;0.05

	Glucose	Total Cholest.	Trig.	VLDL	LDL	HDL
BMI	0.16	0.11	0.57*	0.57*	0.15	-0.52*
% BF	0.08	0.29*	0.38*	0.39*	0.23	-0.06
WHR	0.14	0.06	0.45*	0.45*	0.17	-0.59*
WTR	0.21	0.14	0.51*	0.50*	0.21	-0.48*

\*P&lt;0.05

VLDL = Very Low Density Lipoproteins, LDL = Low Density Lipoproteins  
HDL = High Density Lipoproteins, MUFA = Monounsaturated Fatty Acid  
PUFA = Polyunsaturated Fatty Acid, OCFA = Odd Chain Fatty Acid  
NEFA = Non-esterified Fatty Acid, BMI=Body Mass Index, BF=Body Fat,  
WHR=Waist to Hip Ratio, WTR=Waist to Thigh Ratio

## CHAPTER FIVE

### CONCLUSION AND RECOMMENDATION

#### 5.0 Conclusion

Not all the diabetic subjects were obese. This implied that obesity is not a necessary condition for the development of diabetes.

The dietary data indicated high carbohydrate diets with low Body Mass Index among Ghanaian subjects compared to the other racial groups. This may imply that there is a rapid energy turnover in carbohydrate utilization among the Ghanaian subjects. Warm climatic conditions in Ghana may also contribute to the low Body Mass Index observed.

High levels of saturated fatty acids were observed among the Ghanaian subjects compared to the other racial groups. This may be due to ignorance of the fatty acid composition in the meals consumed and intake of food products containing high levels of saturated fatty acids. Also, comparing the percent body fat and the fat consumption values, fat metabolism among Caucasians and African Americans may occur at different rates, with African Americans absorbing and releasing stored fat at a slower rate compared to Caucasians.

Finally, Total Cholesterol, triglycerides and the lipoproteins were within normal range for the study population. All subjects studied indicated low risk to developing cardiovascular diseases.

### **5.1 Recommendation**

Analysis of the fatty acid composition of Ghanaian diets is recommended. This will serve as a guide in planning meals with known amounts of the various fatty acids present.

A longitudinal study on diet, fatty acids, NEFA and insulin levels among races on a larger population is also recommended. This will enable an in-depth study of the interactions among these indices.

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