CLINICAL, METABOLIC AND IMMUNOLOGICAL CHARACTERISTICS OF GHANAIAN PATIENTS WITH DIABETES MELLITUS

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FELIX-VAL KWAKU TITTY

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KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

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DECLARATION

THE EXPERIMENTAL WORK DESCRIBED IN THIS THESIS WAS CARRIED OUT AT THE DEPARTMENT OF MOLECULAR MEDICINE, SCHOOL OF MEDICAL SCIENCES, KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI, GHANA. THIS WORK HAS NOT BEEN SUBMITTED FOR ANY OTHER DEGREE.

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ABSTRACT

Objectives: This study investigated the clinical characteristics of Ghanaian diabetic patients; prevalence of metabolic syndrome and its components in Ghanaian diabetic patients; and determinants of the metabolic syndrome diagnosed from Ghanaian diabetic patients. Further, association of metabolic syndrome with poor glycaemic control and occurrence and features of autoimmune diabetes and autoantibody-negative type 2 diabetes in Ghanaian diabetic patients were investigated.

Research design and methods: This research was a prospective study covering a period of four years, from August 2004 to June 2008. The study was carried out at the Komfo Anokye Teaching Hospital and the Kwame Nkrumah University of Science and Technology, both in Kumasi on two study populations. For the first population, Ghanaian diabetic patients diagnosed by the WHO criteria were consecutively selected. The sample size was 456. Controls included 120 age- and sex-matched nondiabetic controls. For the second population, recently diagnosed (<1 year) Ghanaian diabetic patients were consecutively selected. The sample size was 120. Controls included 60 age- and sex-matched healthy nondiabetic controls. Socio-demographic and clinical characteristics of subjects were investigated using a standardized questionnaire. Blood pressure and anthropometric measurements (height, weight, waist circumference) of subjects were measured using mercury sphygmomanometer, physician standiometer and scale and a plastic tape respectively. Blood and serum samples from subjects were analysed for relevant biochemical indices using enzymatic methods and ATAC® 8000 Random Access Chemistry Analyzer and its reagent kits. Metabolic syndrome was diagnosed using the National Cholesterol Education Programme Adult Treatment Panel III (NCEP ATP III) criteria. HbA_{1C} was analysed using an inhibition of latex agglutination test, DCA® 2000+ analyzer (Bayer model, USA) and its reagent kits. Insulin and autoantibodies were tested using an Enzyme linked immunosorbent assay (ELISA) technique, DRG International Inc. USA EIA reagent kits, ELISA reader (Tipo model, Italy) and washer (Murex, Great Britain).

Results: The sex distribution of the Ghanaian diabetic patients of the first study population was 30.9% males and 69.1% females. Insulin-requiring diabetics were 24.6% and non-insulin requiring diabetics 75.4%. The mean age was 55.8 ± 12.3 years; 90.6% were ≥40 years of age. The mean age of onset was 49.7 ± 12.5 years; 89.5% had an age of onset ≥35 years or late onset diabetes. The mean diabetes duration was 6.0 ± 5.4 years and 60.5% had diabetes duration 1 – 9 years. Mean preprandial (fasting) glucose level was 9.4 ± 4.5 mmol/L with 62.1% having high preprandial glucose >7.2 mmol/L. The mean BMI was 25.1 ± 4.8 kg/m^2; 44.5% were overweight and obese or had a BMI ≥25.0 kg/m^2. The mean waist circumference was 87.0 ± 13.7 cm; 43.6% had central obesity. Diabetics with confirmed hypertension were 40.1%; hypertensives with inadequate blood pressure control ≥130/80 mmHg were 91.3%. The prevalence of the metabolic syndrome was 55.9% in the first population; prevalence in females (66.0%) was higher than males (33.3%). Low HDL cholesterol was the commonest component (47.4%) of the metabolic syndrome in the first population, followed by hypertension (46.9%). In females central obesity (57.1%) was the commonest component, followed by low HDL cholesterol (53.0%); in males, hypertension (39.7%) was the commonest component, followed by hypertriglyceridaemia (36.2%). Central obesity, hypertension and low HDL cholesterol prevalence was higher in females than males, while hypertriglyceridaemia prevalence was comparable in females and males. Female diabetics individually carried more metabolic syndrome factors than males. The major determinant of the metabolic syndrome diagnosed from Ghanaian diabetics was central obesity (69.4%) followed by hypertension (67.5%). In females the major determinant was central obesity (76.9%), followed by hypertension (67.3%), while in males the major determinant was hypertriglyceridaemia (74.5%), followed by hypertension (68.1%). The most frequent combination of different components was hyperglycaemia, central obesity and hypertension (46.3%). For the second population, the prevalence of the metabolic syndrome in patients with poor glycaemic control was 50.0% and patients with good glycaemic control 33.3%. The prevalence of autoimmunty in the insulin-requiring recently diagnosed diabetic patients was 35.3% and in the non-insulin requiring patients 16.5%. The prevalence of LADA in the non-insulin requiring diabetic patients was

13.5%. The prevalence of LADA and autoimmune type 1 diabetes in the second population were 11.7% and 7.5% respectively, giving total autoimmune diabetes prevalence of 19.2%; single autoantibody positivity was 77.3% and multiple autoantibody positivity 22.7% in the autoimmune diabetic patients. Most of the clinical and metabolic parameters of autoimmune diabetes and type 2 diabetes did not differ. The exceptions were hypertension and central obesity which were more likely in type 2 diabetes than autoimmune diabetes and HbA_{1C} which was higher in autoimmune diabetes than type 2 diabetes.

Conclusion: There were more Ghanaian females with diabetes mellitus than males and more non-insulin requiring than insulin requiring patients. Majority of the diabetic patients were 40 years and above and had late onset diabetes. More than half had diabetes disease duration of 1-9 years and carry clinical modifiable risk factors for microvascular and macrovascular disease. Less than half of the subjects had clinical modifiable risk factors for cardiovascular disease. The metabolic syndrome was frequent in Ghanaian diabetic patients, especially females, and was present at an prevalence slightly less than that in developed countries. Future prevention and control strategies should not overlook the importance of metabolic disease risk factors in Ghana. Interventions that address obesity and hypertension in females and hypertriglyceridaemia and hypertension in males and reduce waist circumference, blood pressure and hypertriglyceridaemia, may reduce the prevalence of the metabolic syndrome, and hence cardiovascular disease in Ghanaian diabetic patients. The major determinants of the metabolic syndrome were not necessarily the commonest metabolic syndrome components of the population under consideration. The metabolic syndrome was associated with poor glycaemic control. However, metabolic syndrome and poor glycaemic control are independent risk factors for cardiovascular disease. Autoimmune diabetes, including autoimmune type 1 diabetes and LADA, occurs in recently diagnosed Ghanaian diabetic patients. Both ICA and GAD autoantibody tests are required to identify autoimmune diabetes, and distinguish it from autoantibody-negative type 2 diabetes. Clinical and metabolic markers cannot be used for this purpose.

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ABBREVIATIONS

AACC:	American Association of Clinical Chemistry
AACE:	American Association of Clinical Endocrinologists
ADA:	American Diabetes Association
AGE(s):	Advanced glycation end products(s)
ATP:	Adenosine triphosphate
BMI:	Body mass index
BUN:	Blood urea nitrogen
cAMP:	Cyclic adenosine monophosphate
CAD:	Coronary artery disease
CHD:	Coronary heart disease
CPT:	Carnitine palmitoyl tranferase
CRP:	C-reactive protein
CVD:	Cardiovascular disease
DCCT:	Diabetes Control and Complications Trial
DKA:	Diabetic ketoacidosis
EDTA:	Ethylenediamine tetraacetic acid (sequestrine)
EGIR:	European group for the Study of Insulin Resistance
EIA:	Enzyme immunoassay
ELISA:	Enzyme-linked immunosorbent assay
ERK:	Extracellular signal regulated kinase
FCPD:	Fibrocalculous pancreatic diabetes

FFA:	Free fatty acid
GABA	gamma-aminobutyric acid
GAD:	Glutamic acid decarboxylase
GDM:	Gestational diabetes mellitus
GDP:	Guanosine diphosphate
GIGT:	Gestational impaired glucose tolerance
GLUT:	Glucose transporter
GRB2	Growth factor receptor binding protein-2
GSK:	Glycogen synthase kinase
GTP:	Guanosine triphosphate
HbA_{1C}/ GHb/A1C:	Glycated haemoglobin
HDL:	High density lipoprotein
HHS:	Hyperosmolar hyperglycaemic state
HLA:	Human leukocyte antigen
IAA:	Insulin autoantibodies
IA-2:	Insulinoma associated antigen
ICA:	Islet cell autoantibodies
IDDM:	Insulin dependent diabetes mellitus
IDL:	Intermediate density lipoprotein
IDF:	International Diabetes Federation
IFG:	Impaired fasting glycaemia
IGT:	Impaired glucose tolerance

IMD: Immune mediated diabetes

IR: Insulin receptor

IRS: Insulin receptor substrate

LADA: Latent autoimmune diabetes of (in) adults

LDL: Low density lipoprotein

MAP: Mitogen-activated protein

MEK: Dual specificity kinase

MetS: Metabolic syndrome

MHC: Major histocompatibility complex

MRDM: Malnutrition related diabetes mellitus

mSOS: Mammalian son of sevenless

mTOR: mammalian target of rapamycin

NCEP: National Cholesterol Education Programme

NCEP ATP III: National Cholesterol Education Programme Adult Treatment Panel III

NIDDM: Non-insulin-dependent diabetes mellitus

OGTT: Oral glucose tolerance test

PAI: Plasminogen activator inhibitor

PDK: PI3-K–dependent kinase

PDPD/PDDM: Protein deficient pancreatic diabetes

PEPCK: Phosphoenol pyruvate carboxykinase

PH : Pleckstrin hmology

PI3-K: Phosphatidylinositol 3-kinase

PIP3:	Phosphatidylinositol 3,4,5-phosphate
PKC:	Protein kinase C
PTB:	Phosphate-tyrosine binding
SMBG:	Self monitoring of blood glucose
USA:	United States of America
Tyr:	Tyrosine
UKPDS:	United Kingdom Prospective Diabetes Study
WHO:	World Health Organization

CHAPTER 1

1. INTRODUCTION

1.1 BACKGROUND OF STUDY

Diabetes mellitus is a metabolic disorder of multiple aetiology, characterized by defects in insulin secretion, insulin action, or both (Alberti and Zimmet, 1998). These defects lead to characteristic deficiency of insulin or its inadequate function, resulting in disturbances in carbohydrate, lipid and protein metabolism, which manifest as chronic hyperglycaemia. Diabetes mellitus may present with characteristic symptoms of marked hyperglycaemia such as glycosuria, osmotic diuresis, polyuria, dehydration, thirst and polydipsia, weight loss, sometimes with polyphagia, and blurred vision. Impairment of growth may also accompany chronic hyperglycaemia. In its most severe form, ketoacidosis or hyperosmolar hyperglycaemic state may develop and lead to stupor, coma and, in the absence of effective treatment, death (Hamblin et al., 1989; Basu et al., 1992). Often symptoms are not severe, or may be absent, and consequently hyperglycaemia sufficient to cause pathological and functional changes may be present for a long time before the diagnosis is made.

The long term effects of diabetes mellitus include progressive development of specific complications of retinopathy with potential blindness, nephropathy that may lead to renal failure and/or peripheral neuropathy with risk of foot ulcers, amputation, Charcot joints; and autonomic neuropathy

(dysfunction) causing gastrointestinal, genitourinary, and cardiovascular symptoms and sexual dysfunction (Stratton et al., 2000; McCance et al., 1994). Diabetic patients are at increased risk of atherosclerotic cardiovascular, peripheral vascular (arterial) and cerebrovascular disease (Fagan and Sowers, 1999; Charles et al., 1996). Hypertension and abnormalities of lipoprotein metabolism are often found in diabetic patients. Diabetes mellitus may be caused by a defect in the immune system, a flaw that may compromise other disease-fighting mechanisms. These effects of diabetes mellitus lead to long-term damage, dysfunction and failure of body systems and various organs, especially the eyes, kidneys, nerves, heart and blood vessels. Thus, diabetes is a heterogenous rather than a single disorder, hence, various specialists are needed to tackle the diabetes mellitus menace.

Type 2 diabetes and cardiovascular disease (CVD) are both characterized and predicted by a number of highly intercorrelated metabolic disorders, including abdominal (central) obesity, insulin resistance, hyperglycaemia, dyslipidaemia and hypertension (Stern, 1995). These cluster of factors have been variably referred to as syndrome X, insulin resistance syndrome and the metabolic syndrome (MetS), with the last term recently adopted by the World Health Organization (WHO) and the National Cholesterol Education Programme (NCEP) Adult Treatment Panel III (ATPIII) (World Health Organization, 1999; NCEP ATPIII, 2001). The metabolic syndrome is also a frequent finding in type 1 diabetes (Thorn et al., 2005). Alone each component of the cluster conveys increased CVD risk, but as a combination they become much more powerful

(Kaplan, 1989). In addition, insulin resistance, a key part of metabolic syndrome, worsens as the number of metabolic syndrome components increases (Bonora et al., 2003). Other disturbances such as microalbuminuria, hyperuricaemia, endothelial dysfunction, abnormalities in fibrinolysis and coagulation, non-alcoholic fatty liver and chronic inflammation have been subsequently linked to the metabolic syndrome (Yudkin, 1999; Steinberg et al., 1996; Groop et al., 1993; Laaksonen et al., 2004).

The prevalence of diabetes mellitus for all age-groups worldwide is estimated to be 2.8% in 2000 and projected to be 4.4% by 2030 (Wild et al., 2004). The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million by 2030 (Wild et al., 2004). This is the estimate of the global prevalence of diabetes mellitus in the year 2000 (as used in the WHO Global Burden of Disease Study) and projections for 2030. Assuming that age-specific prevalence remains constant, the number of people with diabetes mellitus in the world is expected to approximately double between 2000 and 2030, based solely upon demographic changes. The greatest relative increases will occur in the Middle Eastern Crescent, sub-Saharan Africa and India (Wild et al., 2004). The excess global mortality attributable to diabetes mellitus in the year 2000 was estimated to be 2.9 million deaths, equivalent to 5.2% of all deaths (Roglic et al., 2005). Excess mortality attributable to diabetes mellitus accounted for 2─3% of deaths in poor countries and over 8% in the United States of America, Canada and the Middle East. In people 35 – 64 years old, 6 – 27% of deaths were attributable to diabetes mellitus. Globally, diabetes mellitus is likely to be the fifth leading

cause of deaths (Roglic et al., 2005). This shows the magnitude of the growing public health burden of diabetes mellitus worldwide.

A disproportionate amount of the increase in diabetes mellitus is anticipated in low- and middle-income countries, many of which are expected to experience several-fold increase in the number of diabetics (Woodward et al., 2003). Common features of these low-income countries, also called developing or resource-poor countries include, poverty, illiteracy and lack of adequate medical services. Data on diabetes mellitus prevalence in the entire Ghanaian population, which needs a national approach, is unavailable though the prevalence of diabetes mellitus in the Greater Accra Region of Ghana has been found to be 6.3% (Amoah et al., 2002a). Ghana is a developing low-income country, thus from the aforementioned, including the fact that the prevalence rate in the Greater Accra region is higher than the world rate, the implications of diabetes mellitus and its associated diseases should be a major public health concern.

Globally, diabetes mellitus prevalence is similar in men and women but it is slightly higher in men <60 years of age and in women at older ages (King et al., 1998; Wild et al., 2004). The prevalence of diabetes mellitus increases with age for both males and females. In developing countries, the majority of people with diabetes are in the 45 – 64 years age range (King et al., 1998; Wild et al., 2004). In contrast, the majority of people with diabetes mellitus in developed countries are >64 years of age. By 2030, it is estimated that the number of people with diabetes mellitus >64 years of age will be >82 million in developing countries and >48 million in developed countries. Little information is available, using a large

sample size, on the age, sex distribution, duration and other clinical parameters of diabetic patients in Ghana.

The number of people with diabetes mellitus worldwide is increasing due to population growth, aging, urbanization and increasing prevalence of obesity and physical inactivity. In association with increasing diabetes prevalence, this will inevitably result in increasing proportions of deaths from cardiovascular disease as well as increased prevalence and associated consequences of other complications of diabetes mellitus. Diabetes mellitus, with its multiple complications and premature mortality, accounts for at least 10% of total health care expenditure in many countries (International Diabetes Federation, 2003). Diabetes mellitus will be one of the most challenging public health problems of the 21st century.

Although cardiovascular disease and its clustering of metabolic factors known as metabolic syndrome have been a feature of Westernized societies, CVD is now emerging as a major health concern in developing countries including Ghana. The transition from rural to urban life styles is associated with deterioration in the CVD risk profile because of adverse changes in dietary habits and physical activity patterns (Solomons and Gross, 1995). Though the clustering of metabolic factors termed metabolic syndrome has been determined in general populations, type 2 diabetes and overall diabetes populations, the percentage of such risk factors in the overall diabetic population in Ghana and hence its total burden in Ghanaian diabetics has not been worked out. Given the rapid

urbanization in Ghana, the implications of such non-communicable diseases should be a major public health concern.

Current trends in diabetes mellitus research have raised questions on the aetiologic sub-classification of type 1 diabetes using autoantibodies, and differentiating type 1 autoimmune diabetes from idiopathic type 1 diabetes, prevalent in Africans among others. Some type 1 diabetes individuals may masquerade as type 2 diabetes if antibody determinations are not made. In other words, if a search is done for autoantibodies in all new cases of diabetes mellitus, among the non-insulin-requiring diabetic subjects, a significant number may be islet cell antibody-positive (Schiel and Muller, 2000). Zimmet (1995) introduced the term "latent autoimmune diabetes of adults" (LADA) to describe autoimmune late-onset type 1 diabetes. Epidemiological data demonstrate that LADA accounts for 2 – 12% of all cases of diabetes (Turner et al., 1997; Pietropaolo et al., 2000). This general trend was not observed in Nigerian diabetics in whom Oli et al. (1980) found autoantibodies to be rare. This raises questions on the pathogenesis and classification of diabetes mellitus in specific populations around the world, including Ghana. Thus if this trend is not investigated it will lead to incomplete diagnosis and inappropriate management of Ghanaian diabetic patients.

1.2 OBJECTIVES OF STUDY

It is the objective of this study to:

1. Determine the clinical characteristics of Ghanaian diabetic patients.

2. Investigate the prevalence of the metabolic syndrome and its individual components in Ghanaian diabetic patients.
3. Find out the major determinants and clinical characteristics of the metabolic syndrome diagnosed from Ghanaian diabetic patients.
4. Determine whether the metabolic syndrome is associated with poor glycaemic control (HbA_{1C}) in recently diagnosed Ghanaian diabetic patients.
5. Investigate autoimmune diabetes (autoantibody patterns) and autoantibody-negative type 2 diabetes in recently diagnosed Ghanaian diabetic patients.

1.3 JUSTIFICATION FOR THE OBJECTIVES

Clinical features of diabetes mellitus differ from one population to the other. Little information is available on the clinical features, including sex distribution, mean age, age of onset, duration, preprandial (fasting) glucose, body mass index (BMI) and hypertension of a large population of Ghanaian diabetic patients. Thus clinical characterization of diabetes mellitus patients in Ghana is in the right direction for its preventive and epidemiological value. The clinical and metabolic features such as obesity (BMI and waist circumference), hypertension, glycated haemoglobin (HbA_{1C}) and dyslipidaemia in recently diagnosed diabetics need to be investigated to throw more light on the pathogenesis of diabetes mellitus in Ghana. In recently diagnosed diabetics, association of metabolic syndrome with HbA_{1C} needs to be clarified to determine whether they can be used interchangeably as predictors of cardiovascular disease and if so their relative sensitivity worked out.

The problem of early detection of macrovascular or cardiovascular disease (CVD) in Ghanaian diabetic patients using the metabolic syndrome and the determinants of the metabolic syndrome has not been tackled. This is a gap in our preventive measures for macrovascular or cardiovascular diseases, which needs to be investigated, since cardiovascular complications cause about three-fourths of the deaths among diabetic patients. In the area of diabetes mellitus diagnosis, insulin, autoantibodies and other metabolic factors which reveal the underlying pathology and risk factors on which management is based are not performed routinely in Ghana. There is also no information on autoimmunity and autoantibodies in Ghanaian diabetic patients in the literature. Thus, investigating autoimmune diabetes through autoantibody patterns among diabetes mellitus patients in Ghana would clarify the situation.

1.4 EXPECTED BENEFITS

Determination of autoimmune diabetes would not only give insights into the pathogenesis of diabetes mellitus in Ghana, but would lead to appropriate management practices for the individual diabetic patient and the Ghanaian population as a whole. Determination of the metabolic and clinical features of diabetes mellitus in Ghana is also an essential component of the pathogenesis and management practices. The metabolic syndrome with or without hyperglycaemia identifies people with high risk of developing cardiovascular disease. Thus investigating the metabolic syndrome in Ghanaian diabetic patients, including its relationship with HbA_{1C}, especially on a sustainable basis would guide us to early

detection and hence management and prevention of cardiovascular disease, the most common cause of deaths among diabetics worldwide. In other words, epidemiological data would be provided on diabetes mellitus and cardiovascular disease that would support diagnosis, management, prevention and hence planning. All these strategies would reduce the magnitude and burden of diabetes mellitus and its associated cardiovascular disease in Ghana. These strategies ultimately aim at achieving improved health care and hence sound health status for our diabetic patients. This in turn would bring obvious economic gains to the country. Diabetic patients would also benefit from pre-test counseling on the nature of diabetes mellitus and the value of relevant laboratory tests. After this research, diabetic patients would be specifically advised on the nature of their subclass of diabetes, the value of continuous testing, including self monitoring of blood glucose (SMBG), adherence to prescribed dosage of drugs, as well as good life style practices, which would ensure that they live long if they pay heed to advice.

CHAPTER 2

2. LITERATURE REVIEW

2.1 CLASSIFICATION, CLINICAL STAGES AND DIAGNOSIS OF DIABETES MELLITUS

2.1.1 Classification of diabetes mellitus

The first widely accepted classification of diabetes mellitus was published by the World Health Organization (WHO) Expert Committee in 1980 (World Health Organization, 1980) and in modified form, in 1985 by a WHO Study Group (World Health Organization, 1985). The 1980 Expert Committee proposed two major classes of diabetes mellitus and named them, insulin-dependent diabetes mellitus (IDDM) or type 1 and non-insulin-dependent diabetes mellitus (NIDDM) or type 2. In the 1985 Study Group Report, the terms type 1 and type 2 were omitted, but the classes IDDM and NIDDM were retained, and a class, Malnutrition Related Diabetes Mellitus (MRDM) was introduced. In both the 1980 and 1985 reports, other classes of diabetes mellitus included "other types" and impaired glucose tolerance (IGT) as well as gestational diabetes mellitus (GDM).

A revised classification encompassing both clinical stages and aetiological types of diabetes mellitus and other categories of hyperglycaemia has been suggested (Kuzuya and Matsuda, 1997). It has been recommended that the terms

"insulin-dependent diabetes mellitus" and "non-insulin-dependent diabetes mellitus" and their acronyms "IDDM" and "NIDDM", should no longer be used. These terms have been confusing and frequently resulted in patients being classified based on treatment rather than on pathogenesis. The terms type 1 and type 2 were to be reintroduced. The aetiological type named type 1 encompasses those cases attributable to an autoimmune process, as well as those with beta-cell destruction and who are prone to ketoacidosis for which neither the aetiology nor pathogenesis is known (idiopathic). It does not include those forms of beta-cell destruction or failure to which specific causes can be assigned (e.g. cystic fibrosis, mitochondrial defects, etc.). The type named type 2 includes the common major form of diabetes mellitus which results from defect(s) in insulin secretion, almost always with a major contribution from insulin resistance (Kuzuya and Matsuda, 1997).

A subsequent international workshop reviewed the evidence for, and characteristics of, diabetes mellitus seen in undernourished populations (Hoet et al., 1996; Tripathy and Samal, 1997). Whilst it appears that malnutrition may influence the expression of several types of diabetes, the evidence that diabetes mellitus can be caused by malnutrition or protein deficiency per se is not convincing. Therefore it is recommended that the class, "malnutrition-related diabetes mellitus" (MRDM) be deleted. The former subtype of MRDM, protein-deficient pancreatic diabetes (PDPD or PDDM), may be considered as a malnutrition modulated or modified form of diabetes mellitus for which more studies are needed. The other former subtype of MRDM, fibrocalculous

pancreatic diabetes (FCPD), is now classified as a disease of the exocrine pancreas, fibrocalculous pancreatopathy, which may lead to diabetes mellitus (World Health Organization, 1999). The class "impaired glucose tolerance" is now classified as a stage of impaired glucose regulation, since it can be observed in any hyperglycaemic disorder and is itself not diabetes. A clinical stage of impaired fasting glycaemia has been introduced to classify individuals who have fasting glucose values above the normal range but below those diagnostic of diabetes mellitus. Gestational diabetes mellitus is retained but now encompasses the groups formerly classified as gestational impaired glucose tolerance (GIGT) and gestational diabetes mellitus (GDM). Thus the new classification system identifies four major types of diabetes mellitus: type 1, type 2, "other specific types" and gestational diabetes mellitus (World Health Organization, 1999).

Gestational diabetes mellitus is carbohydrate intolerance resulting in hyperglycaemia of variable severity with onset or first recognition during pregnancy. It does not exclude the possibility that the glucose intolerance may antedate pregnancy, but has been previously unrecognized. The definition applies irrespective of whether or not insulin is used for treatment or the condition persists after pregnancy (Metzger, 1991). Individuals at high risk for gestational diabetes include older women, those with previous history of glucose intolerance, those with history of large for gestational age babies, women from certain high risk ethnic groups, and any pregnant woman who has elevated fasting or casual blood glucose levels (Berkowitz et al., 1992). It may be appropriate to screen pregnant women belonging to high-risk populations during the first trimester of

pregnancy in order to detect previously undiagnosed diabetes mellitus. Formal systematic testing for gestational diabetes (Carpenter and Coustan, 1982) is usually done between 24 and 28 weeks of gestation.

Women who become pregnant and who are known to have diabetes mellitus which antedates pregnancy do not have gestational diabetes mellitus but have "diabetes mellitus and pregnancy" and should be treated accordingly before, during and after the pregnancy. In the early part of pregnancy (e.g. first trimester and first half of second trimester) fasting and postprandial glucose concentrations are normally lower than in normal, non-pregnant women. Elevated fasting or postprandial plasma glucose levels at this time in pregnancy may well reflect the presence of diabetes mellitus which has antedated pregnancy, but the criteria for designating abnormally high glucose concentrations at this time have not yet been established.

The aetiological types of diabetes designate defects, disorders or processes which often result in diabetes mellitus. The main aetiological classes of diabetes mellitus are type 1 diabetes mellitus, which in turn is sub-classified into type 1 autoimmune and type 1 idiopathic diabetes mellitus, type 2 diabetes mellitus and "other types" diabetes mellitus. However the major classes of diabetes mellitus are the type 1 and type 2. Type 2 diabetes comprises approximately 90 – 95% of all cases of diagnosed diabetes mellitus, whilst type 1 comprises about 5 – 10% (American Diabetic Association, 2005; World Health Organization, 1999). In both sub-classes of type 1 diabetes mellitus there is absolute deficiency of insulin.

The onset of symptoms is abrupt. Insulin is often required for survival to prevent the development of ketoacidosis, coma and death.

Type 1 autoimmune diabetes mellitus results from an inflammatory autoimmune and T-cells mediated destruction of the insulin-producing beta-cells of the pancreas, usually leading to absolute insulin deficiency. Insulin resistance does not play a major role in its pathogenesis (Atkinson and Maclaren, 1994). Majority of individuals are lean, young and with autoimmune markers associated with diabetes mellitus and most have susceptibility HLA haplotypes (Nepom, 1993; Scott et al., 1997) with linkage to the DQA and DQB genes, and is influenced by the DRB genes. Individuals with this subclass of diabetes mellitus often become dependent on insulin for survival eventually and are at risk for ketoacidosis (Willis et al., 1996). At this stage of the disease there is little or no insulin secretion as manifested by low or undetectable levels of plasma C-peptide (Hother-Nielson et al., 1988). The rate of destruction of beta-cells is quite variable, being rapid in some individuals and slow in others (Zimmet et al., 1994). The rapidly progressive form is commonly observed in children, but also may occur in adults (Humphrey et al., 1998). The slowly progressive form generally occurs in adults and is sometimes referred to as latent autoimmune diabetes in adults (LADA). Some patients, particularly children and adolescents, may present with unprovoked ketoacidosis as the first manifestation of the disease (Japan and Pittsburgh Childhood Diabetes Research Groups, 1985). Others have modest fasting hyperglycaemia that can rapidly change to severe hyperglycaemia and/or ketoacidosis in the presence of infection or other stress. Still others, particularly

adults, may retain residual beta-cell function, sufficient to prevent ketoacidosis, for many years (Zimmet, 1995).

Type 1 idiopathic diabetes mellitus is a subclass of type 1 diabetes mellitus that has no known aetiology, but it is likely related to insulin resistance and transient β-cell dysfunction, perhaps because of glucose desensitization (Banerji et al., 1994; Ramamruthan and Westphal, 2000). Usually these patients have permanent insulinopenia, and they are prone to ketoacidosis, but have no evidence of autoimmunity (McLarty et al., 1990) and it is not HLA associated. Idiopathic type 1 diabetes mellitus has been described mostly in African-Americans (African origin) and Asians as well as other ethnic groups (Tan et al., 2000; Imagawa et al., 2000; Umpierrez et al., 1997). In most patients with idiopathic type 1 diabetes, insulin therapy is better in terms of glycaemic control than either oral hypoglycaemic agents or diet therapy alone and that long-term glycaemic control is better maintained with insulin treatment (Pinero-Pilona et al., 2001).

Type 2 diabetes mellitus refers to diabetes patients who have relative (rather than absolute) insulin deficiency. Insulin levels may be normal, decreased or increased. There are two identifiable defects in type 2 diabetes mellitus. There is predominantly insulin resistance, that is, decreased ability of insulin to act on peripheral tissues (Defronzo et al., 1992; Lillioja et al., 1993). Thus initially, and usually throughout their lifetime, these individuals do not need insulin treatment for survival. There may also be predominantly impaired insulin secretion due to β-cell defect (dysfunction). However insulin resistance is the primary defect,

preceding the derangement in insulin secretion and clinical diabetes by about 20 years (Khan, 1994., Sacks and McDonald, 1996). The specific aetiologies of this class of diabetes mellitus are unknown, however autoimmune destruction of β-cells of the pancreas does not occur and patients do not have other known specific causes of diabetes mellitus listed under "other specific types" diabetes mellitus.

Majority of type 2 diabetes mellitus patients are obese, and obesity aggravates insulin resistance (Campbell and Carlson, 1993; Bogardus et al., 1985). Those not obese by weight criteria may have increased percentage of body fat distributed predominantly in the abdominal region (Kissebah et al., 1982). Ketoacidosis is not frequent in type 2 diabetes, however if it arises, it results from the stress of another illness such as infection (Banerji et al., 1994; Umpierrez et al., 1995a). The high blood glucose levels in these diabetic patients would be expected to result in higher insulin levels than usually observed had their β-cell function been normal (Polonsky et al., 1996). Thus, insulin secretion is defective and insufficient to compensate for insulin resistance. The risk of developing type 2 diabetes mellitus increases with age, obesity, and lack of physical activity (Zimmet, 1992; Harris et al., 1995). It occurs more frequently in women with prior GDM and in individuals with hypertension or dyslipidaemia. Its frequency varies in different racial/ethnic subgroups (Harris et al., 1995; Zimmet 1992). It is often associated with strong familial, likely genetic, predisposition (Knowler et al., 1993). However, the genetics of this class of diabetes are complex and not clearly defined. Type 2 diabetes is frequently undiagnosed for many years because the hyperglycaemia is often not severe enough to provoke noticeable

symptoms of diabetes mellitus (Mooy et al 1995; Harris, 1993). However, such patients are at increased risk of developing macrovascular and microvascular complications (Mooy et al., 1995; Harris, 1993).

"Other specific types" diabetes mellitus is currently a less common class of diabetes mellitus, but includes those in which the underlying defect or disease process can be identified in a relatively specific manner. They may be due to genetic defects of beta-cell function, genetic defects in insulin action, disease of the exocrine pancreas, endocrinopathies, drug- or chemical-induced and infections (World Health Organization, 1999). Uncommon forms of immune-mediated diabetes and other genetic syndromes sometimes associated with diabetes mellitus also belong to this group.

2.1.2 Clinical stages of diabetes mellitus

The classification of diabetes mellitus encompasses both aetiological types of diabetes mellitus and clinical stages and other categories of hyperglycaemia (Kuzuya and Matsuda, 1997). The clinical stages reflect the various degrees of hyperglycaemia in individual subjects with any of the disease processes which may lead to diabetes mellitus. Diabetes mellitus, regardless of its underlying cause, is sub-divided into insulin requiring for survival and non-insulin requiring. In the first sub-type insulin is required for metabolic control, rather than for survival. In such subjects there may be some endogenous insulin secretion but insufficient to achieve normoglycaemia without added exogenous insulin. The non-insulin requiring sub-type includes those who can be controlled satisfactorily

by diet and drugs other than insulin (World Health Organization, 1999; American Diabetes Association, 2003).

Impaired glucose regulation, that is, impaired glucose tolerance (IGT) and impaired fasting glycaemia (IFG) refer to a metabolic state intermediate between normal glucose homeostasis and diabetes mellitus (Harris, 1989; Charles et al., 1991). However, IFG and IGT are not interchangeable and represent different abnormalities of glucose regulation, one in the fasting state and the other post-prandial, respectively. IFG refers to fasting glucose concentrations which are lower than those required to diagnose diabetes mellitus but higher than the "normal" reference range. IGT refers to fasting 2-hour post glucose concentrations which are lower than those required to diagnose diabetes mellitus but higher than the "normal" reference range. If OGTT is performed, some individuals with IFG will have IGT or diabetes mellitus, but this cannot be determined without an OGTT. If resources allow, it is recommended that all those with IFG have an OGTT to exclude the diagnosis of diabetes mellitus. Individuals who meet criteria for IGT or IFG may be described as having euglycaemia in their daily lives as shown by normal or near-normal glycated haemoglobin levels.

A fasting venous plasma glucose concentration of less than 6.1 mmol/L has been chosen as "normal" glycaemia or normoglycaemia (Brunzell et al., 1976). Although this choice is arbitrary, such values are observed in people with proven normal glucose tolerance, although some may have IGT if an OGTT is performed. Values above this are associated with a progressively greater risk of developing micro- and macrovascular complications (Alberti, 1996; McCance et

al., 1994; Engelgau et al., 1997; Charles et al., 1996). The pathological or aetiological process which often lead to diabetes mellitus begin, and may be recognizable in some subjects who have normal glucose tolerance. Thus the proposed classification includes a state of normoglycaemia in which persons who have evidence of the pathological processes which may lead to diabetes mellitus or in whom a reversal of the hyperglycaemia has occurred, are classified.

2.1.3 Diagnosis of diabetes mellitus

The report of the World Health Organization (WHO) Consultation (Alberti and Zimmet, 1998) summarized the diagnostic interpretations of random (casual) blood glucose homeostasis in non-pregnant subjects. The clinical diagnosis of diabetes mellitus is often prompted by symptoms such as polyuria, polydipsia, recurrent infections, unexplained weight loss, and in severe cases, drowsiness and coma (Umpierrez et al., 1997). High levels of glycosuria are usually present. A single random (casual) blood glucose estimation in excess of the diagnostic values (venous plasma ≥11.1 mmol/L, venous whole blood ≥10.0 mmol/L) establishes the diagnosis in such cases. The report also defines levels of random blood glucose below which a diagnosis of diabetes mellitus is unlikely in non-pregnant individuals (venous plasma <5.5 mmol/L, venous whole blood <4.4 mmol/L). These criteria are as in the 1985 report. For clinical purposes, an OGTT to establish diagnostic status needs only be considered if casual blood glucose values lie in the uncertain range, that is, between the levels that establish or exclude diabetes mellitus (venous plasma ≥5.5 and <11.1 mmol/L, venous whole

blood ≥4.4 and <10.0 mmol/L) and fasting blood glucose levels are below those which establish the diagnosis of diabetes mellitus but above the upper reference limit. If an OGTT is performed, it is sufficient to measure the blood glucose values whilst fasting and at 2 hours after a 75g oral glucose load. For children the oral glucose load is related to body weight: 1.75g per kg. The diagnostic criteria in children are the same as for adults.

The report of the WHO Consultation (Alberti and Zimmet, 1998) summarized the criteria for the diagnosis of diabetes mellitus and impaired glucose homeostasis with respect to fasting and 2-hour post-load glucose concentrations in non-pregnant subjects. Fasting glucose estimation in excess of the diagnostic values (venous plasma ≥7.0 mmol/L, venous whole blood ≥6.1 mmol/L) or 2-hour post glucose load (venous plasma ≥11.1 mmol/L, venous whole blood ≥10.0 mmol/L) or both establish the diagnosis. The values of IFG are a fasting venous plasma glucose concentration of 6.1 mmol/L or greater (venous whole blood 5.6 mmol/L), but less than 7.0 mmol/L (venous whole blood 6.1mmol/L); and if a 2-hour post glucose is measured, a fasting venous plasma glucose concentration of less than 7.8 mmol/L (venous whole blood 6.7 mmol/L). The values for IGT are a fasting venous plasma 2-hour post glucose concentration of 7.8 mmol/L or greater (venous whole blood 6.7 mmol/L), but less than 11.1 mmol/L (venous whole blood 10.0 mmol/L); and if a fasting glucose is measured, a fasting venous plasma glucose concentration of less than 7.0 mmol/L (venous whole blood 6.1 mmol/L).

For clinical purposes, the diagnosis of diabetes mellitus should always be confirmed by repeating the test on another day unless there is unequivocal hyperglycaemia with acute metabolic decompensation or obvious symptoms. Glucose concentrations should not be determined on serum, unless red cells are immediately removed, otherwise glycolysis will result in an unpredictable under estimation of the true concentrations. Glucose preservatives do not totally prevent glycolysis. Thus, if whole blood is used, the sample should be kept at 0 – 4^{o}C, or assayed immediately. If plasma is used, the blood sample should be centrifuged immediately (Alberti and Zimmet, 1998).

An alternative to blood glucose estimation or the OGTT has long been sought to simplify the diagnosis of diabetes mellitus. Glycated haemoglobin (HbA_{1C}) reflecting average glycaemia over the preceding 2─3 months was thought to provide such a test. Although in certain cases it gives equal or almost equal sensitivity and specificity to glucose measurement (McCance et al., 1994), it is not available in many parts of the world and is not well enough standardized for its use to be recommended now. However HbA_{1C} is currently considered the best index of metabolic control for diabetic patients in clinical settings (Nathan et al., 1984; Goldstein, 1984), and participants in epidemiological studies as well as a measure of risk for the development of micro- and macrovascular complications (Moss et al., 1994; Krolewski et al., 1995).

For population studies of glucose intolerance and diabetes, individuals have been classified by their blood glucose concentration measured after an overnight fast and/or 2 hours after a 75g oral glucose load. Since, it may be

difficult to be sure of the fasting state, and because of the strong correlation between fasting and 2-hour values, epidemiological studies or diagnostic screening have in the past been restricted to the 2-hour values only. Whilst this remains the single best choice, if it is not possible to perform the OGTT (e.g. for logistical or economic reasons), the fasting plasma glucose alone may be used for epidemiological purposes (Alberti and Zimmet, 1998).

To determine if gestational diabetes mellitus is present in pregnant women, a standard OGTT should be performed after overnight fasting (12 – 14 hours) by giving 75g anhydrous glucose in 250-300ml water. Plasma glucose is measured fasting and 2 hours after glucose intake. Pregnant women who meet the WHO criteria for diabetes mellitus or IGT are classified as having gestational diabetes mellitus (GDM). After the pregnancy ends, the woman should be re-classified as either having diabetes mellitus, or IGT, or normal glucose tolerance based on the results of a 75g OGTT six weeks or more after delivery (Alberti and Zimmet, 1998).

2.2 AUTOIMMUNE DIABETES AND IMMUNE CELLS IN DIABETES MELLITUS

Prior to clinical onset, autoimmune type 1 diabetes is characterized by lymphocytic infiltration of the islets cells and circulating autoantibodies against a variety of islet cell antigens. The most important predictive autoantibody markers are islet cell autoantibodies (ICA) (Botazzo et al., 1974), autoantibodies to glutamic acid decarboxylase (GADab) (Baekkeskou et al., 1982), insulin

autoantibodies (IAA) (Palmer et al., 1983), and autoantibodies to tyrosine phosphatase (insulinoma associated antigen 2: IA-2 and IA-2β) (Verge, 1996). Islet cell autoantibodies (ICA) can be detected as early as eight years prior to the clinical onset of autoimmune type 1 diabetes (Soeldner, 1985) and thus may serve as an early indicator of the disease or of predisposition to it. Individuals who are ICA-positive may show a progressive loss of the islet cell function as indicated by disruption of the early-phase insulin release. When this early phase insulin release completely stops, clinically overt autoimmune type 1 diabetes develops (Soeldner, 1985).

Autoantibodies to glutamic acid decarboxylase (GADab) were first detected in the serum of patients suffering from the rare neurological disorder, Stiff-Man Syndrome (De Aizpuria, 1992). GAD is the biosynthetic enzyme for the neurotransmitter inhibitor, gamma-aminobutyric acid (GABA) (Erlander and Tobin, 1991). Two forms of GAD, 65Kda and 67Kda, are produced by a single gene and are highly homogenous (Clare-Saltzler et al., 1992), and are both identified in pancreas islet cells and are differentially expressed in human, rat and mouse pancreas (Kim et al., 1993). Insulin autoantibodies (IAA) were first described in 1970 by Hirata and colleagues (1970) in a patient with spontaneous hypoglycaemia. The presence of autoantibodies to insulin, the only beta-cell specific autoantigen, thus far identified, is evidence of ongoing destruction of beta cells. IAA have been characterized and found to be of IgG class similar to insulin antibodies from diabetic patients treated with insulin (Seino et al., 1986). Measurement must be performed before starting insulin treatment, as assays

cannot distinguish between induced antibodies and autoantibodies. Autoantibodies to IA-2, a tyrosine phosphatase-like protein, are found in autoimmune type 1 diabetic patients at and prior to disease onset, are generally more prevalent in younger onset patients, and are associated with rapid progression to overt disease (Christie et al., 1994).

The measurement of these autoantibodies has been shown to be extremely useful in assisting the physician with the prediction, diagnosis, and management of patients with diabetes mellitus. The screening of the general population and high risk individuals such as siblings and families of autoimmune diabetic patients, because of their genetic predisposition to diabetes for all four autoantibodies (ICA, GAD, IAA, IA-2) will help to either prevent or slow down the onset of the disease. A high-risk (asymptomatic) population positive for two or more autoantibodies is vulnerable for developing autoimmune diabetes, usually in the next 5-7 years (Dean et al., 1986).

Insulin deficiency results from autoimmune destruction of the insulin producing pancreatic beta cells (Eisenbarth, 1985; Etzioni, 1987). Such abnormalities (autoimmunity) may be genetically inherited and/or triggered by exposure to toxic chemicals, viral infections and various forms of stress (Rossini et al., 1989). The clinical onset of diabetes does not occur until 80 – 90% of the pancreatic beta cells have been destroyed. This prolonged pre-diabetic phase, that may last up to several years and in which these disease markers are present and measurable, may allow the opportunity to predict and prevent the clinical onset of the disease. During this period, the affected individuals exhibit the diminishing

early-phase release of insulin in response to an intravenous/oral glucose challenge.

Shortly after the original description of islet cell antibodies (ICAs) as a marker for childhood type 1 diabetes, it was realized that some adult-onset patients are also ICA positive (Irvine et al., 1977). Subsequently glutamic acid decarboxylase autoantibodies (GADab) were also discovered as another marker of type 1 diabetes (Baekkeskou et al., 1982). On one hand, studies indicate that as many as 10% of adult patients, initially diagnosed as type 2, eventually become insulin dependent (Niskanen, 1995). These diabetic patients, initially "masquerading" as type 2, are in fact late onset, or slow developing type 1 diabetic patients. Like classical type 1, late onset type 1 diabetes results from the autoimmune destruction of the beta cells. Autoimmune markers, such as autoantibodies to GAD, have been detected in the serum of these diabetic patients many years prior to insulin dependency. Zimmet (1995) introduced the term "latent autoimmune diabetes of adults" (LADA) to describe autoimmune late onset type 1 diabetes. Epidemiological data demonstrate that LADA accounts for 2 – 12% of all cases of diabetes (Turner et al., 1997; Pietropaolo et al., 2000). Typically, patients are positive for GAD autoantibodies, 35 years of age or older, non-obese, and present without ketoacidosis and weight loss.

Although many LADA patients maintain good glycaemic control for several years with sulphonylureas, these patients become "insulin dependent" more rapidly than antibody-negative type 2 diabetic patients (Zimmet, 1995). Unfortunately, the phenotype of these adult-onset diabetic patients, including their

presentation, is extremely variable, resulting in confusion with the nomenclature and classification of these patients. Besides LADA, these patients have been named type 1.5 diabetes, “slowly progressive type 1 diabetes,” “latent type 1 diabetes,” “youth-onset diabetes of maturity,” and even LADA-type 1 and LADA-type 2 (Juneja and Palmer, 1999). While many questions remain, standardizing the nomenclature for the classification of autoimmune diabetes has been proposed (Palmer and Hirch, 2003). Childhood-onset patients with type 1 diabetes, like the adult subjects in the Hosszufalusi et al. (2003) study with the type 1 diabetes, would continue to be classified as having type 1 diabetes. The antibody-positive patients diagnosed over the age of 35 years who do not initially require insulin would continue to be classified as LADA. Importantly, these patients are generally non-obese. On the other hand, the antibody-positive patients with phenotypic type 2 (usually obese and insulin resistant) (Carlsson et al., 2000) would be classified as type 1.5 diabetes as opposed to “obese LADA” or LADA-type 2.

Genetic factors/markers in autoimmune diabetes have also been determined. When self-recognition as part of self-tolerance is in question, the genes of the major histocompatibility complex (MHC) are always involved through their expressed products 羊 HLA proteins. Some haplotypes predispose the immune mediated diabetes (IMD), whereas others protect. HLA class II region on the 6th chromosome is called immune mediated diabetes 1 region (IMD1), which consists of DR and DQ alleles. This HLA gene region plays a role in antigen presentation and initiation of immune responses. Genetically, HLA

DR3 and -4 and their associated DQB1 alleles 0201 and 0302 respectively, predispose to childhood type 1 diabetes and these alleles are also increased in adult patients with autoimmune diabetes. Heterozygote DR3/DR4 confers the highest risk (Thomson et al., 1988). DR2 and DQB1*0602 are strongly protective against childhood type 1 diabetes and consequently are very rarely found in these patients. In contrast, DR2, DQB1 *0602 occurs relatively commonly in LADA (Tuomi et al., 1999); therefore, it is hypothesized that whatever mechanism accounts for HLA, DR2, DQB1*0602 protection against childhood type 1 diabetes is far less effective in protecting against autoimmune diabetes in adults.

In general agreement with the above paradigm, Hosszufalusi et al. (2003) found increased DR3, DQR1*0201 and increased DR4 DQB1*0302 in LADA and adult onset type 1 diabetes compared with control subjects. Unfortunately, data on the protective allele DR2 DQB1 *0602 were not presented. The presence of HLA-DQB1*0302 identifies patients at high risk of requiring insulin treatment (Horton et al., 1999). As far as HLA is concerned, the prevalence of HLA-DQB1 *0201/0302 and HLA-DR3/DR4 seems to be age-related (Buzzetti et al., 1998). Compared with younger patients, individuals older than 20 years at onset of type 1 diabetes are less often heterozygous for HLA-DQB1*0201/0302 (20%) and HLA-DR3/DR4 (12.5羊24%) (Karjalainen et al., 1989). However, in a subsequent study, no difference in genotype frequencies was observed between the young-onset and adult-onset type 1 diabetic patients (Tuomi et al., 1999).

Immune cells have been found to be adversely affected in diabetic patients. Glucose value has been attached to improvement in granulocyte function

(Bagdade et al., 1978) by demonstrating significant improvement in the granulocyte adherence as the mean fasting blood glucose was reduced from 16.3 to 11.0 mmol/L in 10 poorly controlled diabetic patients. Other investigators have demonstrated similar improvements in leukocyte function with treatment of hyperglycaemia (Alexiewicz et al., 1995; MacRury et al., 1989). In vitro trials attempting to define hyperglycaemia thresholds found only rough estimates which showed that a mean glucose greater than 11.1mmol/L causes leukocyte dysfunction (Bagdade et al., 1974; Neilson and Hindson, 1989; Wilson and Reeves, 1986). Normal individuals exposed to transient glucose elevation show rapid reduction in lymphocytes, including all lymphocyte subsets (von Kanel et al., 2001). It has further been reported that individuals with isolated impaired glucose tolerance have high white cell count. In patients with diabetes, hyperglycaemia is similarly associated with reduced T cell populations for both CD-4 and CD-8 subsets. The abnormalities are reversed when glucose is lowered (Bouter et al., 1992). Studies evaluating the effect of hyperglycaemia on the immune system have consistently showed that hyperglycaemia causes immunosuppression. Reduction of glucose by a variety of means reverses the immune function defects.

The association of hyperglycaemia with infection has long been recognized, although the overall magnitude of the problem is still somewhat unclear (Joshi et al., 1999; Wheat, 1980). From a mechanistic point of view, the primary problem has been identified as phagocyte dysfunction. Poorly controlled diabetes mellitus appears to hinder the ability of white blood cells (leukocytes) to

destroy invading microorganisms, making patients more susceptible to infection, both from invading bacteria, fungi, and other foreign organisms, as well as from other benign organisms that usually inhabit the body or environment without causing disease.

2.3 METABOLIC EFFECTS OF INSULIN AND DIABETES MELLITUS

Insulin is the principal hormone responsible for the control of glucose metabolism. It is synthesized by the β-cells of the islets of Langerhans of the pancreas (Orci et al., 1988) as a precursor, pro-insulin, which is processed to form C-peptide and insulin. Both are secreted in equimolar amounts into the portal circulation (Frier et al., 1981). The mature insulin molecule comprises two polypeptide chains, the A chain and the B chain (21 and 30 amino acids respectively). The two chains are linked together by two inter-chain disulphide bridges (A7 to B7 and A20 to B19). There is also an intra-chain disulphide bridge in the A chain (connects residues 6 and 11).

Secretion of insulin is mainly controlled by plasma glucose concentration and the hormone has a number of important metabolic actions. Its first principal function is to control the uptake and utilization of glucose in peripheral tissues via the glucose transporter. This and other hypoglycaemic activities, such as the inhibition of hepatic gluconeogenesis and glycogenolysis are counteracted by the hyperglycaemic hormones including glucagon, epinephrine (adrenaline) growth hormone, thyroxine and cortisol. Insulin concentrations are severely reduced in type 1 diabetes mellitus and some other conditions such as hypopituitarism.

Insulin levels are relatively raised in type 2 diabetes mellitus, obesity, insulinoma and some endocrine dysfunctions such as Cushing's syndrome and acromegaly.

Insulin signaling at the target tissue results in a large array of biological outcomes. These events are essential for normal growth and development and for normal homeostasis of carbohydrate, lipid and protein metabolism. Elucidating the intracellular events after activation of the insulin receptor (IR) has been the primary focus of a large number of investigators for decades, and for excellent reasons. Numerous prospective studies in various populations indicate that insulin resistance and insulin secretory dysfunction predict the development of type 2 diabetes (Weyer et al., 2001; Ferrannini, 1998) and are therefore targets for primary prevention of the disease. Understanding the signalling pathways involved in insulin action could lead to a better understanding of the pathophysiology of insulin resistance associated with type 2 diabetes mellitus and obesity. Identifying associated key molecules and processes could lead to newer and more effective therapeutic agents for treating these common disorders.

Circulating insulin rapidly reaches the target tissue where it interacts with its cognate receptor, the insulin receptor (IR). The IR, which is widely expressed, is a transmembrane tyrosine (Tyr) kinase that is expressed as a tetramer in an $\alpha 2\beta 2$ configuration (Ebina et al., 1985) and joined by disulphide cross-bridges. The α-subunits are extracellular whilst the β-subunits possess an intracellular domain. Insulin binding to specific regions of the α-subunits leads to a rapid configurational change in the receptor which results in autophosphorylation of specific Tyr residues of the intracellular region of the β subunits through a

transphosphorylation mechanism. Autophosphorylation results in activation of the Tyr kinase activity of the receptor (Lee et al., 1997). In the inactive state the catalytic site of the Tyr kinase is occluded by the "activation-loop" preventing access of ATP and various substances. Autophosphorylation of Tyr residues at positons 1,158, 1,162, and 1163 in the activation-loop causes a conformational change that allows ATP and substrates to reach the catalytic site (Hubbard et al., 1994; Hubbard, 1997).

The activated IR kinase phosphorylates substrate proteins on Tyr residues and these phosphorylated Tyr residues serve as docking sites for downstream effectors. Molecules such as Shc, IR substrate (IRS) (Feinstein et al., 1993; Virkamaki et al., 1999), and Gab-1 engage the IR directly and provide a docking interface with down-stream substrates. IRS proteins contain a conserved pleckstrin homology (PH) domain, located at the NH_2-terminus, which serves to localize the IRS proteins in close proximity to the receptor (Haslam et al., 1993; Voliovitch et al., 1995). IRS proteins contain a phosphate-Tyr binding (PTB) domain, COOH-terminal to their PH domain. The PTB domain, present in a number of signaling molecules (Pawson, 1995), shares 75% sequence identity (Sawka-Verhelle et al., 1996) between IRS-1 and IRS-2 and functions as a binding site to the NPXY motif of the juxtamembrane region of the IR to promote IR/IRS-1 interactions (Wolf et al., 1995; Eck et al., 1996). The COOH-terminal region of IRS proteins is poorly conserved. It contains multiple Tyr phosphorylation motifs that serve as docking sites for SH2 domain-containing proteins, like the p85α regulatory subunit of phosphatidylinositol 3-kinase (PI3-

K), growth factor receptor binding protein-2 (Grb2), Nck, Crk, Fyn, SHP-2, and others, all of which mediate the metabolic and growth promoting functions of insulin (White, 1998; Cheatham and Khan, 1995).

Insulin-receptor signaling involves two major pathways: the mitogen-activated protein (MAP) kinase also referred to as Ras/Raf/MAP kinase and the PI3-K. Although these pathways are described in a linear fashion, it should not be forgotton that each pathway could, under certain circumstrances, activate the other. Thus Akt (protein Ser/Thr kinase B) may activate Raf kinase, and conversely, Ras may activate PI3-K. The MAP kinase pathway is activated by the binding of Grb2 to Tyr-phosphorylated Shc or IRS via its SH2 domain. Grb2 is pre-bound to "mammalian Son of Sevenless" (mSOS), a nucleotide exchange protein that catalyzes the exchange of GDP for GTP on Ras (a small GTPase protein); this results in activation of Ras. The prenylated form of Ras binds the inner leaflet of the plasma membrane, and on activation, it binds the NH_2-terminal region of Raf (protein), recruiting Raf to the plasma membrane. Ras-Raf interactions displace the 14-3-3 proteins that are bound to Raf and allows the phosphorylation of Raf by a number of (Ser / Thr) kinases, thus disinhibiting Raf kinase (Roy et al., 1998). Raf-1 activates a dual specificity kinase, MEK1, by phosphorylating two regulatory Ser residues. In turn, MEK1 activates extracellular signal-regulated kinase, ERK-1 and ERK2 by phosphorylating regulatory Tyr and Thr residues (Marshall, 1995). Activated ERKs mediate the growth-promoting effects of insulin by phosphorylating transcription factors such as Elk-1, leading to the induction of genes.

The metabolic response to insulin is primarily mediated via the PI3-K pathway. Following the association of the p85/p110 complex of PI3-K with the IRS moleclules, PI3-K activity results in production of phosphatidylinositol 3,4,5-phosphate (PIP_3). PIP_3 binds to the PH domains of PI3-K-dependent kinase (PDK)-1 and Akt (protein Ser/Thr kinase B). This leads to the activation of PDK1, which in turn phosphorylates and activates Akt. Akt has been implicated in regulating the translocation of GLUT4, an insulin-sensitive glucose transporter expressed by muscle and lipid cells. Interestingly, Akt may not be the only downstream kinase that regulate GLUT4 translocation to the cell surface. Protein kinase C (PKC) isoforms ζ and λ are also activated by PI3-K and PDK1 and regulates GLUT4 translocation (Czech and Corvera, 1999). Indeed, over expression of wild-type PKCζ increases, whereas over expression of a dominant-negative PKCζ decreases basal and insulin-stimulated glucose transport in adipocytes and muscle cells (Etgen et al., 1999; Bandyopadhyay et al., 1997).

Stimulation of glycogen synthesis is another key metabolic effect of insulin. Glycogen synthase kinase-3 (GSK-3) mediates, at least in part, the activation of glycogen synthase in response to insulin. Activation of Akt by insulin results in the phosphorylation and activation of GSK-3 rendering it incapable of inhibiting glycogen synthase activity (Cross et al., 1995). GSK-3 also inactivates the protein synthesis eukaryotic inhibition factor [(eIF)-2B羊 the guanine nucleotide exchange factor] by phosphorylation. Insulin-mediated activation of Akt reverses these processes, thereby enhancing protein synthesis (Welsh and Proud, 1993). Insulin can also activate protein synthesis at the

translational level by phosphorylation of p70S6 kinase and 4E-BP1 via the kinase mammalian Target of Rapamycin (mTOR). In fact, increased 4E-BP1 phosphorylation is controlled by parallel signaling pathway that immediately bifurcates upstream of p70s6k, with the two pathways sharing a common rapamycin-sensitive activator. The phosphorylation of 4E-BP causes it to disassociate from the eIF-4E, thus enhancing its ability to initiate protein synthesis (Lawrence and Abraham., 1997).

2.4 METABOLIC COMPLICATIONS OF DIABETES MELLITUS

2.4.1 Diabetic Ketoacidosis and hyperosmolar hyperglycaemic state

Diabetic ketoacidosis (DKA) and hyperosmolar hyperglycaemic state (HHS) are the two most serious acute metabolic complications of diabetes mellitus, even if managed properly. These disorders can occur in both type 1 and type 2 diabetes. The mortality rate in patients with diabetic ketoacidosis is <5%, whereas the mortality rate of patients with hyperosmolar hyperglycaemic state (HHS) is about 15% (Hamblin et al., 1989; Basu et al., 1992). The prognosis of both conditions is substantially worsened at the extremes of age and in the presence of coma and hypotension (Malone et al., 1992). DKA consists of the biochemical triad of hyperglycaemia, ketonaemia and acidaemia. The degree of hyperglycaemia in DKA is quite variable and may not be a determinant of the severity of DKA. In HHS there is more severe hyperglycaemia and hyperosmolality than DKA. HHS may consist of variable degrees of clinical ketosis as determined by the nitroprusside method and may often present without

coma. Serum osmolality has been shown to correlate significantly with mental status in DKA and HHS (Ennis et al., 1994; Umpierrez, et al., 1997).

Although the pathogenesis of DKA is better understood than that of HHS, the basic underlying mechanism for both disorders is a reduction in the net effective concentration of circulating insulin (Polonsky et al., 1994), coupled with concomitant elevation of counterregulatory stress hormones, such as glucagon, epinephrine (adrenaline), growth hormone, thyroxine and cortisol. Thus DKA and HHS are extreme manifestations of impaired carbohydrate regulation that can occur in diabetes mellitus (Umpierrez et al., 1997). Although many patients manifest overlapping metabolic clinical pictures, each condition can also occur in relatively pure form. In patients with DKA, the deficiency in insulin can be absolute, or it can be insufficient relative to an excess of counterregulatory hormones. In HHS, there is a residual amount of insulin secretion that minimizes ketosis but does not control hyperglycaemia. This leads to severe dehydration and impaired renal function leading to decreased excretion of glucose (Ennis et al., 1994). These factors coupled with the presence of a stressful condition result in more severe hyperglycaemia than that seen in DKA. In addition, inadequate fluid intake contributes to severe hyperosmolality, the hallmark of HHS. These pathogenic topics will be outlined under various subheadings.

The most common precipitating factor in the development of DKA or HHS is infection (Ellemann et al., 1984). The most common types of infections are pneumonia and urinary tract infections, accounting for 30–50% of cases. Other acute medical illnesses which are precipitating causes include alcohol

abuse, trauma, pulmonary embolism, myocardial infarction, and pancreatitis, which can occur both in type 1 and type 2 diabetes (Petzold et al., 1971). Various drugs that alter carbohydrate metabolism, such as corticosteroids, pentamidine, sympathomimetic agents, and α- and β-adrenergic blockers, and excessive use of diuretics in the elderly may also precipitate the development of DKA and HHS. Psychological factors and poor compliance, leading to omission of insulin therapy, are important precipitating factors for recurrent ketoacidosis. In young female patients with type 1 diabetes, psychological problems complicated by eating disorders may be contributing factors in up to 20% of cases of recurrent ketoacidosis (Polonsky et al., 1994; Rydall et al., 1997). Factors that may lead to insulin omission in younger patients include fear of weight gain with good metabolic control, fear of hypoglycaemia, rebellion against authority and stress related to chronic disease (Polonsky et al., 1994). Noncompliance with insulin therapy has been found to be the leading precipitating cause for DKA in urban African-Americans and medically indigent patients (Musey et al., 1995; Umpierrez et al., 1997). Elderly individuals with new onset diabetes or individuals with known diabetes who become hyperglycaemic and are unaware of it or are unable to take fluids when necessary are at risk for HHS.

2.4.2 Carbohydrate metabolism

When insulin is deficient (absolute or relative), hyperglycemia develops as a result of three processes: increased gluconeogenesis, accelerated glycogenolysis, and impaired glucose utilization by peripheral tissues (Luzi et al., 1988; Vaag et

al., 1992). Increased hepatic glucose production results from the high availability of gluconeogenic precursors (Felig and Wahren, 1971), such as the amino acids alanine and glutamine (as a result of accelerated proteolysis and decreased protein synthesis), lactate (as a result of increased muscle glycogenolysis) and glycerol (as a result of increased lipolysis), and from the increased activity of gluconeogenic enzymes. These include pyruvate carboxylase, phosphoenolpyruvic carboxykinase (PEPCK), fructose-1, 6-bisphosphatase and glucose-6-phosphatase, which are further stimulated by increased levels of stress hormones in DKA and HHS (Foster and McGarry, 1983; Siperstein, 1992; Exton, 1987; Hue, 1987). From a quantitative standpoint, increased glucose production by the liver and kidney represents the major pathogenic disturbance responsible for hyperglycaemia in these patients, and gluconeogenesis plays a greater metabolic role than glycogenolysis (Foster and McGarry, 1983; Siperstein, 1992; Exton, 1987; Hue, 1987).

Although the detailed biochemical mechanisms for gluconeogenesis are well established, the molecular basis and the role of counterregulatory hormones in DKA are the subject of debate. Very few studies have attempted to establish a temporal relationship between the increase in the level of counterregulatory hormones and the metabolic alterations in DKA (Schade and Eaton, 1980). However, studies of insulin withdrawal in previously controlled patients with type 1 diabetes indicate that a combination of increased catecholamines and glucagon (and a decreased level of free insulin) in a well-hydrated individual may be the initial event (Luzi et al., 1988; Alberti, et al., 1975; Kitabchi, 1989). Furthermore,

in the absence of dehydration, vomiting or other stress situations, ketosis is usually mild, while glucose levels increase with simultaneous increases in serum potassium (Kitabchi, 1989).

Animal studies have shown that catecholamines stimulate glycogen phosphorylase via β-receptor stimulation and subsequent production of cAMP-dependent protein kinase. Decreased insulin in the presence of an ambient level of glucagon, which is usually higher in diabetic than in nondiabetic individuals, leads to a high glucagon-to-insulin ratio, which inhibits production of an important metabolic regulator fructose-2,6-bisphosphate. A high glucagon-to-insulin ratio leads to phosphorylation and activation of fructose-2,6-bisphosphatase, decreasing the amount of fructose-2,6-bisphosphate. Reduction of this intermediate stimulates the activity of fructose-1,6-bisphosphatase (an enzyme that catalyzes the hydrolysis of fructose-1,6-bisphosphate to fructose-6-phosphate) and inhibits phosphofructokinase, the rate-limiting enzyme in the glycolytic pathway (Pilkis et al., 1990). Gluconeogenesis is further enhanced through stimulation of phosphoenolpyruvic carboxykinase (PEPCK) by the increased ratio of glucagon to insulin in the presence of increased cortisol in DKA (Pilkis et al., 1990; Granner and Pilkis 1990). In addition, the rapid decrease in the level of available insulin also leads to decreased glycogen synthase. These interactions can be summarized as follows:

↑glucagon/insulin + ↑catecholamines →

↑cAMP →↑cAMP-dependent protein

kinase →↓fructose-2,6-biphosphate→

↓glycolysis and ↑gluconeogenesis and

↓glycogen synthase

The final step in glucose production occurs by hydrolysis of glucose-6-phoshate to glucose, which is catalyzed by another rate-limiting enzyme of gluconeogenesis, hepatic glucose-6-phosphatase, which is stimulated by increased catabolic hormones and decreased insulin levels. Major substrates for gluconeogenesis are lactate, glycerol, alanine (in the liver) and glutamine (in the kidney). Alanine and glutamine are provided by the process of excess proteolysis and decreased protein synthesis which occurs as a result of increased catabolic hormones and decreased insulin (Felig and Wahren, 1971; Wasserman and Vranic, 1986). In DKA and HHS, hyperglycaemia leads to glycosuria which causes an osmotic diuresis, polyuria, dehydration, thirst and polydipsia, resulting in loss of water and electrolytes, hypovolemia and decreased glomerular filtration rate, which further increase the severity of hyperglycaemia. Although increased hepatic gluconeogenesis is the main mechanism of hyperglycaemia in severe ketoacidosis, recent studies have shown that a significant portion of gluconeogenesis may be accomplished via the kidney (Meyer et al., 1998). Decreased insulin availability and partial resistance which exist in DKA and HHS by different mechanisms also contribute to decreased peripheral glucose utilization and add to the overall hyperglycaemic state in both conditions. It is to be noted that lipolysis occurs mainly in adipose tissue whilst other events occur primarily in the liver (except some gluconeogenesis in the kidney).

2.4.3 Lipid and ketone metabolism

The increased production of ketones in DKA is the result of a combination of insulin deficiency and increased concentrations of counterregulatory hormones, particularly epinephrine, which lead to the phosphorylation and activation of hormone-sensitive lipase in adipose tissue (Jensen et al., 1989; McGarry, 1979; Nurhan et al., 1992). The increased activity of tissue lipase causes a breakdown of triglyceride into glycerol and free fatty acids (FFAs). Although glycerol is used as a substrate for gluconeogenesis in the liver and the kidney, the massive release of FFAs assumes pathophysiological predominance in the liver, where the FFAs serve as precursors of the ketoacids in DKA (DeFronzo et al., 1994; McGarry, 1979). In the liver, FFAs are oxidized to ketone bodies, a process predominantly stimulated by glucagon. Increased concentration of glucagon in DKA reduces the hepatic levels of malonyl-CoA by blocking the metabolism of pyruvate to acetyl-CoA through inhibition of acetyl-CoA carboxylase, the first rate-limiting enzyme in de novo fatty acid synthesis (McGarry, 1979; Nurjhan et al., 1992; Gerich et al., 1976). Malonyl-CoA inhibits carnitine acyl transferase I (CAT-I), the rate-limiting enzyme for transesterification of fatty acyl-CoA to fatty acyl-carnitine, regulating oxidation of fatty acids to ketone bodies. CAT-I is required for movement of FFA into the mitochondria, where fatty acid oxidation takes place. The increased fatty acyl-CoA and CAT-I activity in DKA, lead to increased ketogenesis in DKA (McGarry et al., 1989; Zammit, 1994). In addition to increased production of ketone bodies, there is evidence that clearance of ketones

is decreased in patients with DKA (Reichard et al., 1986; Balasse and Fery, 1989). This decrease may be due to low insulin concentration, increased glucocorticoid level and decreased glucose utilization by peripheral tissues (Nosadini et al., 1989).

The role of individual counterregulatory hormones in the process of ketogenesis is reviewed below. Some of the first studies demonstrating net ketogenesis by the human liver in patients with DKA were done about 56 years ago (Bondy et al., 1949). By combining measurements of arterial and hepatic venous ketone concentrations and estimation of splanchnic blood flow in patients with DKA, the liver was demonstrated to produce large amounts of ketones. However, insulin treatment was demonstrated to reduce ketone production promptly. These findings were subsequently confirmed and extended with improved analytical techniques (Owen et al., 1977). Rates of ketogenesis have not been measured in hyperosmolar hyperglycaemic patients using either organ balance or isotopic methods. Subsequent work using tracer methods (Miles et al., 1983) has demonstrated that even brief withdrawal of insulin from type 1 diabetic patients results in prompt development of ketosis. Insulin withdrawal from diabetic patients, however, leads to complex changes in circulating concentrations of many stress hormones. As a result, it is difficult to dissect the relative contributions of insulin deficiency and stress hormone excess in the regulation of ketogenesis. This is well illustrated in studies examining glucagon action. Numerous in vitro and some in vivo studies have demonstrated a potent role for glucagon in the stimulation of ketogenesis. However, some of these studies have

used very high glucagon concentrations and their physiological significance has been questioned. In a study in which blood glucose concentrations were carefully controlled (to eliminate suppressive effects of hyperglycaemia on lipolysis), a lipolytic effect of glucagon was demonstrated (Carlson et al., 1993). Another human study (Beylot et al., 1991) demonstrated modest increases in ketogenesis when plasma glucagon was increased in insulin-deficient subjects. In contrast with the somewhat equivocal actions of physiological or near-physiological concentrations of glucagon, cortisol appears to have a more predictable stimulatory action on ketogenesis (Goldstein et al., 1994). This may result from both effects on peripheral lipolysis and increased supply of FFAs, as well as from direct hepatic effects.

Growth hormone may also play a prominent role in ketogenesis. Even modest physiological doses of growth hormone can markedly increase circulating levels of FFAs and ketone bodies (Moeller et al., 1992). Since these changes with growth hormone administration are observed within 60 minutes, increased ketogenesis appears to be the result of the action of growth hormone itself rather than locally generated insulin-like growth factor I (IGF-I/somatomedin C). It has been reported that in patients with type 1 diabetes, the administration of growth hormone leads to significant increases in FFAs, ketone bodies and glucose concentration (Press et al., 1984). Adrenergic stimulation can also increase lipolysis and hepatic ketogenesis. Epinephrine secretion by the adrenal medulla is markedly enhanced in DKA. In vitro, epinephrine has a marked effect to increase lipolysis in adipocytes. In vivo, epinephrine can increase plasma concentrations of

FFAs, at least when insulin deficiency is present. In addition, epinephrine facilitates hepatic ketogenesis directly (Avagaro et al., 1993). Norepinephine at concentrations that approximate those seen in the synaptic cleft stimulates lipolysis by adipocytes and enhances ketogenesis (Keller et al., 1984).

In addition to the individual effects of stress hormones, infusion of combinations of counterregulatory hormones has been observed to have synergistic effects when compared with those seen with single hormone infusions (Shamoon et al., 1981). Indeed, in the setting of fixed levels of insulin, infusing mixtures of stress hormones to reach high physiological/severe stress levels can precipitate marked increases in lipolysis and ketogenesis (DeFronzo et al., 1994; McGarry et al., 1989). Spontaneous DKA is characterized by simultaneous elevations of multiple insulin-antagonizing (counterregulatory) hormones (Schade and Eaton, 1979) in the face of reduced insulin, which brings about the altered metabolic profiles seen in DKA. Thus, spontaneous DKA is analogous to a fasting state, where ketosis is accompanied by elevations of counterregulatory hormones and reduction of insulin but to a lesser degree than in DKA. The condition in DKA has been referred to as a "superfasted" state (Cahill, 1970).

Having suggested that stress hormones either singly or in combination are major contributors to ketogenesis and the development of the acidotic state in DKA, the question arises whether HHS differs from DKA with regard to stress hormone secretion. There are surprisingly few data regarding this issue. Reduced concentrations of FFAs, cortisol, and growth hormone (Gerich et al., 1971) and reduced levels of glucagon have been demonstrated in HHS relative to DKA

(Lindsey et al., 1974). In another study, 12 HHS and 22 DKA patients showed no differences with regard to FFAs, cortisol, or glucagon (Chupin et al., 1981). This work is of special interest because it demonstrated that in HHS, both basal and stimulated C-peptide levels were five- to sevenfold higher than those in the DKA group.

The scarcity of data available on HHS prevents firm conclusions as to whether or not differences in stress hormone profiles contribute to the less prominent ketosis in that setting. Available data are consistent with multiple contributing factors, with the most consistent differences being lower growth hormone and higher insulin in HHS than in DKA (Gerich et al., 1971; Chupin et al., 1981). The higher insulin levels (demonstrated by high basal and stimulated C-peptide) in HHS provide enough insulin to inhibit lipolysis in HHS (since it takes less insulin for antilipolysis than for peripheral glucose uptake) but not enough for optimal carbohydrate metabolism (McGarry et al., 1989; Schade and Eaton, 1977). Although plasma shows similar levels of FFAs in HHS and DKA, plasma FFAs may not be reflective of portal vein FFA levels, which in turn regulate ketogenesis. It is important to emphasize that studies performed before 1980 which showed similar blood levels of insulin in DKA and HHS (Vinik et al., 1970) used assays that were not free from interference from proinsulin. Since patients with DKA and HHS present with an overlapping syndrome, the differences between DKA and HHS become matters of degree, not fundamental pathogenetic differences. However, it is important to remember that hyperosmolality of severe DKA, which occurs in about one-third of DKA patients

(Schade and Eaton, 1983) is secondary to fluid losses due to osmotic diuresis and to variable degrees of impaired fluid intake due to nausea and vomiting; the hyperosmolality in HHS patients is due to more prolonged osmotic diuresis and to inability to take fluid. This can be secondary either to mental retardation (in certain cases in children) or to chronic debilitation in elderly patients who are unaware of or unable to take adequate fluid (Golden et al., 1985).

2.4.4 Water and electrolyte metabolism

The development of dehydration and sodium depletion in DKA and HHS is the result of increased urinary output and electrolyte losses (DeFronzo et al., 1975). Hyperglycaemia leads to osmotic diuresis in both DKA and HHS. In DKA, urinary ketoanion excretion on a molar basis is generally less than half that of glucose. Ketoanion excretion, which obligates urinary cation excretion as sodium, potassium and ammonium salts, also contributes to solute diuresis. The extent of dehydration, however, is typically greater in HHS than in DKA. At first, this seems paradoxical because patients with DKA experience the dual osmotic load of ketones and glucose. The more severe dehydration in HHS, despite the lack of severe ketonuria, may be attributable to the more gradual onset and longer duration of metabolic decompensation (Wachtel et al., 1987) and partially to the fact that patients presenting with HHS typically have an impaired fluid intake. Other factors that may contribute to excessive volume losses include diuretic use, fever, diarrhoea, nausea and vomiting. The more severe dehydration, together with the older average age of patients with HHS and the presence of other

associated diseases, almost certainly accounts for the higher mortality of HHS (Wachtel et al., 1987). In addition, osmotic diuresis promotes the net loss of multiple minerals and electrolytes (Na, K, Ca, Mg, Cl, and PO_4). Although some of these can be replaced rapidly during treatment (Na, K, and Cl), others require days or weeks to restore losses and achieve balance (DeFronzo et al., 1975).

The severe derangement of water and electrolytes in DKA and HHS is the result of insulin deficiency, hyperglycaemia and hyperketonemia (in DKA). In DKA and HHS, insulin deficiency per se may also contribute to renal losses of water and electrolytes because insulin stimulates salt and water reabsorption in the proximal and distal nephron and phosphate reabsorption in the proximal tubule (DeFronzo et al., 1975; DeFronzo et al., 1976). During severe hyperglycaemia the renal thereshold of glucose (10 mmol/L) and ketones is exceeded. Urinary excretion of glucose in DKA and HHS may be as much as 200g/day and urinary excretion of ketones in DKA may be about 20–30 g/day, with total osmolar load of about 2,000 mOsm (DeFronzo et al., 1976). The osmotic effects of glycosuria result in impairment of NaCl and H_2O reabsorption in the proximal tubule and loop of Henle. The ketoacids formed during DKA (β-hydroxybutyric and acetoacetic) are strong acids that fully dissociate at physiological pH. Thus, ketonuria obligates excretion of positively charged cations (Na^+, K^+, NH_4^+). The hydrogen ions are buffered by plasma bicarbonate, resulting in metabolic acidosis. The retention of ketoanions leads to an increase in the plasma anion gap.

During HHS and DKA, intracellular dehydration occurs as hyperglycemia and water loss lead to increased plasma tonicity, leading to a shift of water out of cells. This shift of water is also associated with a shift of potassium out of cells into the extracellular space. Potassium shifts are further enhanced by the presence of acidosis and the breakdown of intracellular protein secondary to insulin deficiency (Castellino et al., 1987). Furthermore, entry of potassium into cells is impaired in the presence of insulinopenia. Marked renal potassium losses occur as a result of osmotic diuresis and ketonuria. Progressive volume depletion leads to decreased glomerular filtration rate and greater retention of glucose and ketoanions in plasma. Thus, patients with a better history of food, salt, and fluid intake prior to and during DKA have better preservation of kidney function, greater ketonuria, lower ketonemia, and lower anion gap and are less hyperosmolar. These patients may therefore, present with greater degrees of hyperchloremic metabolic acidosis (Adrogue et al., 1982). On the other hand, diabetic patients with a history of diminished fluid and solute intake during the development of acute metabolic decompensation, plus loss of fluid through nausea and vomiting typically present with greater degrees of volume depletion, increased hyperosmolality, and impaired renal function and greater retention of glucose and ketoanions in plasma. The greater retention of plasma ketoanions is reflected in a greater increment in the plasma anion gap. Such patients may present with greater alteration of sensoria, which is more commonly found in HHS than DKA (Wachtel et al., 1987). However, in HHS, as mentioned above, the inability to take fluid (often in elderly patients) plus other pathogenic

mechanisms lead to greater hyperosmolality. During treatment of DKA with insulin, hydrogen ions are consumed, as ketoanion metabolism is facilitated. This contributes to regeneration of bicarbonate, correction of metabolic acidosis, and decrease in plasma anion gap. The urinary loss of ketoanions as sodium and potassium salts, therefore represents the loss of potential bicarbonate (Halperin and Cheema-Dhadli, 1989), which is gradually recovered within a few days or weeks (Sacks, et al., 1981).

2.4.5 Diagnosis of diabetic ketoacidosis and hyperosmolar hyperglycaemic state

The easiest and most urgent laboratory tests after a prompt history and physical examination are determination of blood glucose by finger prick and urinalysis with reagent strips to assess qualitative amounts of glucose, ketones, nitrite and leukocytes in urine. The initial laboratory evaluation of a patient with suspected DKA or HHS should include immediate determination of arterial blood gases, blood glucose and blood urea nitrogen (BUN); determination of serum electrolytes, osmolality, creatinine, ketones, urinalysis and a complete blood count with differential. Bacterial cultures of urine, blood and other tissues should be obtained.

The most widely used diagnostic criteria for DKA are blood glucose >13.9 mmol/L (>250 mg/dl), arterial pH <7.3, serum bicarbonate <15 mEq/L and moderate degree of ketonaemia and/or ketonuria. Accumulation of ketoacids usually results in an increased anion gap metabolic acidosis. The plasma anion gap is calculated by subtracting the major measured anions (chloride and

bicarbonate) from the major measured cation (sodium). Since potassium concentration may be altered by acid-base disturbances and by total body stores, it is not routinely used in the calculation of anion gap (DeFronzo et al., 1994; Umpierrez et al., 1996). The normal anion gap has been historically reported to be 12 mEq/L, and values >14–15 mEq/L have been considered to indicate the presence of an increased anion gap metabolic acidosis (DeFronzo et al., 1994; Umpierrez et al, 1996). Most laboratories, however, currently measure sodium and chloride concentrations using ion-specific electrodes. The plasma chloride concentration typically measures 2–6 mEq/L higher with ion-specific electrodes than with prior methods; thus, the normal anion gap using the current methodology has been reported to be in the range of 7–9 mEq/L (Winter et al., 1990; Sadjadi., 1995). Using these values an anion gap of >10–12 mEq/L would indicate the presence of increased anion gap acidosis (Winter et al., 1990; Sadjadi; 1995).

Although these criteria for DKA have served well for research purposes, they may be somewhat restrictive for clinical practice. For example, the majority of patients admitted with the diagnosis of DKA present with mild metabolic acidosis; however, they show elevations of both serum glucose and β-hydroxybutyrate concentration (Umpierrez et al., 1997). Patients with severe ketoacidosis typically present with a bicarbonte level <10 mEq/L and/or a pH <7.0, have total serum osmolality >330 mOsm/kg, usually present with mental obtundation (Kitabchi and Fisher, 1981) and are more likely to develop complications than those patients with mild or moderate forms of ketoacidosis.

Assessment of ketonuria and ketonaemia, the key diagnostic features of ketoacidosis, is usually performed by nitroprusside reaction. However, nitroprusside reaction provides a semiquantitative estimation of acetoacetate and acetone levels. This assay underestimates the severity of ketoacidosis because it does not recognize the presence of β-hydroxybutyric acid, which is the main ketoacid in DKA (Stephens et al., 1971). Therefore, if possible, direct measurement of β-hydroxybutyrate, which is now available in many hospital settings, is preferable in establishing the diagnosis of ketoacidosis (Koch and Feldbruegge, 1987; Umpierrez et al., 1995b).

In some cases, the diagnosis of DKA can be confounded by the coexistence of other acid-base disorders. Arterial pH may be normal or even increased, depending on the degree of respiratory compensation and the presence of metabolic alkalosis from frequent vomiting or diuretic use (Paulson and Godallah, 1993). Similarly, blood glucose concentration may be normal or only minimally elevated in 15% of patients with DKA (<300mg/dl), such as in alcoholic subjects or patients receiving insulin. In addition, wide variability in the type of metabolic acidosis has been reported. It has been reported that 46% of patients admitted for DKA had high anion gap acidosis, 43% had mixed anion gap acidosis and hyperchloremic metabolic acidosis and 11% had only hyperchloremic metabolic acidosis (Adrogue et al., 1982).

In assessment of blood glucose and electrolytes in DKA, certain precautions need to be taken in interpreting results. Severe hyperlipidaemia, which is occasionally seen in DKA, could reduce serum glucose and sodium

(Kaminska and Pourmotabbed, 1993) levels, factitiously leading to pseudohypo- or normoglycemia and pseudohyponatraemia, respectively, in laboratories still using volumetric testing or dilution of samples with ion-specific electrodes. This should be rectified by clearing lipaemic blood before measuring glucose or sodium or by using undiluted samples with ion-specific electrodes. Creatinine, which is measured by a colorimetric method, may be falsely elevated as a result of acetoacetate interference with the method (Assadi et al., 1985). Hyperamylasaemia, which is frequently seen in DKA, may be the result of extrapancreatic secretion (Vinicor et al., 1979) and should be interpreted cautiously as a sign of pancreatitis. The usefulness of urinalysis is only in the initial diagnosis for glycosuria and ketonuria and detection of urinary tract infection. For quantitative assessment of glucose or ketones, the urine test is unreliable because urine glucose concentration has poor correlation with blood glucose levels (Morris et al., 1981; Malone et al., 1976) and the major urine ketone β-hydroxybutyrate, cannot be measured by the standard nitroprusside method (Stephens et al., 1971).

Diagnostic criteria for HHS include plasma glucose concentration >33.3 mmol/L (>600 mg/dl), serum total osmolality >330 mOsm/kg and absence of severe ketoacidosis. However, the laboratory profiles of HHS in previous series have shown higher mean values of glucose (55.4 mmol/L i.e. 998 mg/dl) and osmolality (363 mOsm/L), with blood urea nitrogen (BUN) 65 mg/dl, HCO_3 21.6 mEq/L sodium 143 mEq/L, creatinine 2.9 mg/dl, and anion gap 23.4 mg/dl (Howard et al., 1992; Matz, 1997). By definition, patients with HHS have a serum

pH ≥7.3, a serum bicarbonate >18 mEq/L and mild ketonaemia and ketonuria. Approximately 50% of the patients with HHS have an increased anion gap metabolic acidosis as the result of concomitant ketoacidosis and/or increase in serum lactate levels (Matz, 1997).

The majority of patients with hyperglycaemic emergencies present with leukocytosis. On admission, serum sodium concentration is usually low in DKA because of the osmotic flux of water from the intracellular to the extracellular space in the presence of hyperglycaemia. To assess the severity of sodium and water deficits, serum sodium may be corrected by adding 1.6 mEq to the measured serum sodium for each 5.55 mmol/L (100 mg/dl) of glucose above 5.55 mmol/L (100 mg/dl) (Katz, 1973). On admission, serum potassium concentration is usually elevated because of a shift of potassium from the intracellular to the extracellular space caused by acidaemia, insulin deficiency, and hypertonicity. On the other hand, in HHS, the measured serum sodium concentration is usually normal or elevated because of severe dehydration. In this setting, the corrected serum sodium concentration would be very high. On admission, serum phosphate level in DKA may be elevated despite total body phosphate depletion.

2.5 GLYCATED HAEMOGLOBIN

Epidemiological studies have confirmed that hyperglycaemia is the most important factor in onset and progress of diabetes complications, both in type 1 and type 2 diabetes mellitus. Mechanisms connecting hyperglycaemia with long term complications of diabetes have been investigated. Among others, a large number of useful proofs indicated the involvement of nonenzymatic glycation

processes (Lyons and Jenkins, 1997). Nonenzymatic glycation is the process by which glucose is chemically bound to amino groups of amino acids of proteins, without the involvement of enzymes. It occurs by a series of chemical reactions described by a chemist Maillard (1912).

Maillard reactions are complex and multilayer and can be analyzed in three degrees. The first reaction is a classical covalent reaction in which, by means of N-glycoside bonding, a sugar-protein complex is formed. (Amadori rearrangement). It is an early product of nonenzymatic glycation, an intermediate which is a precursor of all later compounds. The second degree includes the formation of numerous intermediary products among which some are very reactive and further continue with glycation reactions. The third, final phase, consists of a complex polymerization reaction of the second stage products, in the process of which heterogeneous structures called advanced glycation endproducts (AGEs) are formed (Singh et al., 2001, Vlassara et al., 1994). It was believed that the primary mechanism in Maillard reactions was exclusively the pathway that originated from high glucose concentration. However, recent data show that, in spite of the fact that sugars are the main precursors of AGE compounds, numerous intermediary metabolites, i.e. α-oxoaldehydes also creatively participate in nonenzymatic glycation reactions. Such intermediary products are generated during glycolysis (methylglyoxal) or lipid peroxidation (Lyons and Jenkins, 1997) and they can also be formed by autooxidation of carbohydrates (glyoxal). Another route is the polyolic pathway by which glucose is metabolized through sorbitol, then fructose to α-oxoaldehydes. Alpha-oxoaldehydes modify

AGEs surprisingly fast, in contrast to classical Maillard reactions which are very slow.

A classical example of nonenzymatic glycation is the formation of glycated haemoglobin (GHb), also commonly referred to as glycosylated haemoglobin, glycohaemoglobin, HbA_{1C}, HbA1, or A1C. Glycated haemoglobin is a term used to describe a series of stable minor haemoglobin components formed slowly and nonenzymatically from haemoglobin and glucose. HbA_{1C} has been the first studied glycated protein, but it was soon discovered that other structural and regulatory proteins, are also subject to nonenzymatic glycation, forming glycation endproducts. The initial step in the reaction is the condensation of a free primary amine on haemoglobin with the carbony1 of the glucose, resulting in the formation of a Schiff base, that is, early Maillard reaction (1912). This Schiff base is not stable and may either dissociate or undergo an Amadori rearrangement to form a stable ketoamine. There is now considerable evidence for an Amadori-type rearrangement of the adduct glucose with the NH_2-terminal valine of the β-chain (HbA_{1C}) as well as the NH_2-terminal valine of the α-chain and for ε-amino groups of certain lysine residues on α- and β-chains. Since haemoglobin circulates in each erythrocyte for about 120 days, there is some opportunity in this cell for late Maillard reactions or nonenzymatic reactions to occur (the products of these reactions are referred to as advanced glycation end products [AGEs]), and the extent of these changes appears to correlate with GHb values (Makita et al., 1992). In the formation of AGEs, the Amadori product is degraded into deoxyglucosones, which react again with free amino groups to form

other products (Angyal, 1979). The rate of formation of GHb is directly proportional to the ambient glucose concentration.

Glycation has both physiological and pathophysiological significance in tissues that are longer lived (connective tissue, vascular endothelium, etc.). In physiological conditions glycation can be detected in the ageing process (Vlassara et al., 1994), and the reactions are significantly faster and more intensive with frequently increased glucose concentrations. In diabetology the importance of these processes is manifest in two essential issues: 1. effect of protein glycation on changes in their structure and function and 2. use of glycated protein levels as a parameter of integrated glycaemia (Brownlee, 2000; Bucala and Cerami, 1992). Since erythrocytes are freely permeable to glucose, the level of GHb in a blood sample provides a glycaemic history of the previous 120 days, the average erythrocyte lifespan. GHb most accurately reflects the previous 2–3 months of glycaemic control. However, recent (i.e. 3 – 4 weeks earlier) plasma glucose levels contribute considerably more (50%) to the level of HbA_{1C} (Tahara, 1993) than do long-past (i.e., 3 – 4 months earlier) plasma glucose levels (10%).

Measurements of glycated proteins, primarily haemoglobin and serum proteins, have added a new dimension to the assessment of glycaemia. Blood and urine glucose and urine ketone tests cannot provide the patient and health care team with an objective measure of glycaemia over an extended period of time. However, with a single measurement, glycated proteins can quantify average glycaemia over weeks and months, thereby complementing day-to-day testing (Singer et al., 1989) of blood and urine glucose and urine ketones. It also provides

an additional advantage because GHb values are free of day-to-day glucose fluctuations and are unaffected by exercise or recent food ingestion. HbA_{1C} is currently considered the best index of metabolic control for diabetic patients in clinical settings (Nathan et al., 1984; Goldstein, 1984) and participants in epidemiological studies.

Routine use of GHb testing in all patients with diabetes mellitus is recommended by the American Diabetes Association (2004), first to document the degree of glycaemic control at initial assessment, then as part of continuing care. GHb is also used as a measure of risk for the development of micro- and macrovascular diabetic complications (Moss, et al., 1994; Krolewski, et al., 1995). The test is also being used increasingly by quality assurance programmes including the American Diabetes Association to assess the quality of diabetes care (Davidson, 1998). Elevated C-reactive protein concentrations increased with increasing HbA_{1C} levels. This suggests an association between glycaemic control and systemic inflammation in diabetics (King et al., 2003). HbA_{1C} concentration is also related to prevalent coronary disease or carotid intimal thickening in nondiabetic individuals (Vitelli et al., 1997).

HbA_{1C} has been suggested as a diagnostic and screening tool for diabetes mellitus in the general population (Rohlfing et al., 2000). In acutely ill patients with random hyperglycaemia at hospital admission, an HbA_{1C} level >6.0% reliably diagnoses diabetes mellitus, and an HbA_{1C} level <5.2% reliably excludes it (Greci et al., 2003). It has been suggested that, in diabetic patients, management plan should be adjusted to achieve normal or near normal glycaemia with an A1C

goal of <7% (Lawson et al., 1999; Stratton et al., 2000). More stringent goals (i.e., a normal A1C <6%) can be considered in individual patients and in pregnancy. Less stringent treatment goals may be appropriate for patients with a history of severe hypoglycaemia, patients with limited life expectancies, very young children or older adults and individuals who have disease conditions associated with diabetes mellitus.

2.6 METABOLIC SYNDROME AND DIABETES MELLITUS

2.6.1 Features and epidemiology of the metabolic syndrome

Reaven (1988) noted that several risk factors (e.g. dyslipidaemia, hypertension, hyperglycaemia) commonly cluster together. This clustering he called syndrome X and he recognized it as a multiplex risk factor for CVD. The syndrome is, however, much older, having been already observed by Kylin (1923), who described the clustering of hypertension, hyperglycaemia and gout as a syndrome. Reaven (1988) and subsequently others (Zimmet, 1992) postulated that insulin resistance underlies syndrome X, hence the commonly used term insulin resistance syndrome. Other researchers use the term metabolic syndrome (Zimmet, 1992), for this clustering of metabolic risk factors. Alone each component of the cluster conveys increased CVD risk, but as a combination they become much more powerful (Kaplan, 1989). In addition, insulin resistance, itself a key part of the metabolic syndrome, worsens as the number of metabolic syndrome components increases (Bonora et al., 2003). Subsequently, several

other abnormalities, including microalbuminuria, hyperuricaemia, endothelial dysfunction, abnormalities in fibrinolysis and coagulation, nonalcoholic fatty liver and elevated levels of chronic inflammation have been linked to the metabolic syndrome (Yudkin, 1999; Steiberg et al., 1996; Groop et al., 1993; Laaksonen et al., 2004). Recently metabolic syndrome (MetS) has been adopted by the World Health Organization (WHO) and the National Cholesterol Education Programme Adult Treatment Panel III (NCEP ATP III) (WHO, 1999; NCEP ATP III, 2001). This latter term avoids the implication that insulin resistance is the primary or only cause of associated risk factors.

Although NCEP ATPIII identified CVD as the primary clinical outcome of the metabolic syndrome, most people with this syndrome have insulin resistance, which confers increased risk for type 2 diabetes. When diabetes becomes clinically apparent, CVD risk rises sharply. Thus, the major consequences of the metabolic syndrome are type 2 diabetes and increased cardiovascular risk (Alexander, 2003). The risks associated with the metabolic syndrome, as is currently conceived is 30-50% for diabetes, 12-17% for cardiovascular disease and about 6-7% for all cause mortality (Ford, 2005). Beyond CVD and type 2 diabetes, individuals with metabolic syndrome seemingly are susceptible to other conditions, notably polycystic ovary syndrome, fatty liver, cholesterol gallstones, asthma, sleep disturbances and some forms of cancer.

NCEP ATPIII (2002) identified 6 components of the metabolic syndrome that relate to CVD: Central (abdominal) obesity, atherogenic dyslipidaemia,

raised blood pressure, insulin resistance and glucose intolerance, proinflammatory state and prothrombotic state. Abdominal obesity is the form of obesity most strongly associated with the metabolic syndrome. It presents clinically as increased waist circumference. Atherogenic dyslipidaemia manifests in routine lipoprotein analysis as raised triglycerides and low concentrations of HDL cholesterol (Rubins, 2000). A more detailed analysis usually reveals other lipoprotein abnormalities, e.g. increased remnant lipoproteins, elevated apolipoprotein B, small LDL particles, and small HDL particles. All of these abnormalities have been implicated as being independently atherogenic. Elevated blood pressure strongly associates with obesity and commonly occurs in insulin-resistant persons. Hypertension thus commonly is listed among metabolic risk factors. However, some investigators believe that hypertension is less "metabolic" than other metabolic syndrome components. Certainly, hypertension is multifactorial in origin. For example, increasing arterial stiffness contributes significantly to systolic hypertension in the elderly. Even so, most conference participants favoured inclusion of elevated blood pressure as one component of the metabolic syndrome (NCEP ATPIII, 2002).

Insulin resistance is present in the majority of people with the metabolic syndrome. It strongly associates with other metabolic risk factors and correlates unvariably with CVD risk. These associations, combined with belief in its priority, account for the term insulin resistance syndrome. Even so, mechanisms underlying the link to CVD risk factors are uncertain, hence the ATP III's classification of insulin resistance as an emerging risk factor. Patients with

longstanding insulin resistance frequently manifest glucose intolerance, another emerging risk factor. When glucose intolerance evolves into diabetes-level hyperglycemia, elevated glucose constitutes a major, independent risk factor for CVD (Grundy et al., 2004). A proinflammatory state, recognized clinically by elevations of C-reactive protein (CRP), is commonly present in persons with metabolic syndrome. Multiple mechanisms seemingly underlie elevations of CRP. One cause is obesity, because excess adipose tissue releases inflammatory cytokines that may elicit higher CRP levels. A prothrombotic state, characterized by increased plasma plasminogen activator inhibitor (PAI)-1 and fibrinogen, also associated with the metabolic syndrome. Fibrinogen, an acute-phase reactant like CRP, rises in response to a high-cytokine state. Thus, prothrombotic and proinflammatory states may be metabolically interconnected (NCEP ATP III, 2002).

The most frequent combination of the symptoms of the syndrome is central obesity with high blood pressure or dyslipidaemia. However, features of the metabolic syndrome may also occur in individuals with insulin resistance but who have normal blood glucose levels, and who may never develop type 2 diabetes (Haffner et al., 1990). Implications of having the syndrome include a three-fold increase in risk for coronary heart disease and stroke compared to individuals with normal glucose tolerance. It is well documented that the features of the metabolic syndrome can be present for up to 10 years before detection of the glycaemic disorders (Mykkanen et al., 1993). This is important in relation to the aetiology of the hyperglycaemia and the associated cardiovascular disease

(CVD) risk, and the potential to prevent CVD and its morbidity and mortality in persons with glucose intolerance. The metabolic syndrome with normal glucose tolerance identifies the subject as a member of a group at very high risk of future diabetes mellitus. The metabolic syndrome efficiently identifies subjects likely to have IGT on OGTT and thus be eligible for diabetes prevention interventions (Meigs et al., 2004).

Epidemiological studies confirm that this syndrome occurs commonly in a wide variety of ethnic groups including Caucasians, Afro-Americans, Mexican-Americans, Asian, Indians, Chinese, Australian Aborigines, Polynesians and Micronesians (Zimmet, 1992). Globally, the incidence of this syndrome is rising at an alarming rate. However, the true prevalence of the disease is unknown. This is partly due to the lack of an accepted definition for the metabolic syndrome. In the United States, it is estimated that 47 million (25% of residents) have the metabolic syndrome, a figure which is expected to increase in parallel with the rising epidemic of obesity (Ford et al., 2002). In the United Kingdom, it has been suggested that as many as 25% of the population show clear signs of the metabolic syndrome (Tonkin, 2003). Incidence is higher in certain ethnic groups like Asian and Afro-Caribbean groups, women with polycystic ovary disease, and in patients with schizophrenia and non-alcoholic fatty liver disease.

Most studies comparing criteria for the metabolic syndrome have focused on the WHO and NCEP ATPIII definitions. In patients with type 2 diabetes the prevalence of the metabolic syndrome by the WHO proposal has been found to be 81% and by the NCEP ATPIII criteria 78% (Marchesini et al., 2004). Ford and

Giles (2003) classified 25% of U.S.A participants as having the metabolic syndrome using the WHO definition and 23.9% using the NCEP ATPIII definition; suggesting that the definitions identify a similar group of people with concordant classification of 86.2% and discordance in a substantial minority (i.e. 15 – 20%). Specific racial/ethnic/sex groups (e.g. African-American men) had more discordance than the overall population. The prevalence of the metabolic syndrome in Arab Americans was 23% by the NCEP ATPIII definition and 28% by the WHO definition (Jaber et al., 2004). Although the prevalence increases significantly with age and BMI in both sexes by both definitions, differences in estimates were noted. With NCEP ATP III, the age specific rates were similar for men and women aged 20–49 years but were significantly higher for women aged ≥50 years. With the WHO, rates were higher for men than women aged 20–49 years and similar for those aged ≥50 years. Prevalence of the metabolic syndrome in older individuals has been found to be 28.1% by the NCEP ATPIII criteria and 21.0% by the WHO criteria (Scuteri et al., 2005). The two sets of criteria provided concordant classification for 80.6% of participants. The prevalence of the metabolic syndrome in Omani adults was 21.0% by the NCEP ATPIII criteria (Al-Lawati et al., 2003). The prevalence was 19.5% and 23% in men and women respectively.

2.6.2 Criteria for clinical diagnosis of the metabolic syndrome

There are four different definitions of the metabolic syndrome as suggested by the World Health Organization (WHO, 1998, revised in 1999),

National Cholesterol Education Programme Adult Treatment Panel III (NCEP ATPIII, 2001) the European Group for the Study of Insulin Resistance (Balkau et al., 2002), and the American Association of Clinical Endocrinologists (Bloomgarden, 2003). A simpler measurement for the metabolic syndrome relies on waist circumference and blood triglyceride levels only. This has been called a "hypertriglyceridaemic waist" and is diagnosed as a triglyceride level greater than 2.0 mmol/L and a waist circumference greater than 90.0 cm (Lemieux et al., 2000). The four main criteria are similar in many aspects, but they also reveal fundamental differences in positioning of the predominant causes of the syndrome. Each will be reviewed briefly.

In 1998, a World Health Organization (WHO) consultation group outlined a provisional classification of diabetes that includes a working definition for the metabolic syndrome (Alberti and Zimmet, 1998). This report was finalized in 1999. This report states that a person has the metabolic syndrome if he or she has diabetes or impaired glucose tolerance or insulin resistance and two or more of the following abnormalities: (1) dyslipidaemia – triglyceride ≥1.7 mmol/L and/or HDL cholesterol <0.9 mmol/L in males and <1.0 mmol/L in females. (2) Hypertension – blood pressure ≥140/90 mmHg and/or on antihypertensive medication. (3) Central obesity – waist-to-hip ratio >0.90 in males or >0.85 in females and/or BMI >30 kg/m^2. (4) Microalbuminuria – urinary albumin excretion rate ≥20 ug/min or an albumin: creatinine ratio ≥20 mg/g. The guide group also recognized CVD as the primary outcome of the metabolic syndrome. However, it viewed insulin resistance as a required component for diagnosis.

Insulin resistance was defined as one of the following: type 2 diabetes, impaired fasting glucose (IFG), impaired glucose tolerance (IGT), or for those with normal fasting glucose (<6.1 mmol/L) a glucose uptake below the lowest quartile for background population under hyperinsulinaemic euglycaemic conditions. In addition to insulin resistance, two other risk factors are sufficient for a diagnosis of metabolic syndrome. A higher blood pressure was required than in ATP III. BMI (or increased waist: hip ratio) was used instead of waist circumference, and microalbuminuria was listed as one criterion. The requirement of objective evidence of insulin resistance should give more power to predict diabetes than does ATP III, but like ATP III, the presence of type 2 diabetes does not exclude a diagnosis of metabolic syndrome. A potential disadvantage of the WHO criteria is that special testing of glucose status beyond routine clinical assessment may be necessary to diagnose metabolic syndrome.

Subsequently, the National Institute of Health: Third Report of the National Cholesterol Education Programme Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in adults (NCEP ATPIII, 2001) provided a new working definition for the metabolic syndrome. A person has the metabolic syndrome if he or she has three or more of the following: (1) Central obesity – waist circumference >102 cm in males and >88 cm in females. (2) Hypertriglyceridaemia – triglyceride ≥1.70 mmol/L. (3) Low HDL cholesterol <1.0 mmol/L in males and <1.3 mmol/L in females. (4) Hypertension – blood pressure ≥130/85 mmHg and/or on antihypertensive medication. (5) Fasting plasma glucose ≥6.1mmol/L. When 3 to 5 of the listed characteristics are present,

a diagnosis of metabolic syndrome can be made. The primary clinical outcome of metabolic syndrome was identified as CHD/CVD. Abdominal obesity, recognized by increased waist circumference, is the first criterion listed. Its inclusion reflects the priority given to abdominal obesity as a contributor to metabolic syndrome. Also listed are raised triglycerides, reduced HDL cholesterol, elevated blood pressure, and raised plasma glucose. Cutpoints for several of these are less stringent than usually required to identify a categorical risk factor, because multiple marginal risk factors can impart significantly increased risk for CVD. Explicit demonstration of insulin resistance is not required for diagnosis; however, most persons meeting ATP III criteria will be insulin resistant. Finally, the presence of type 2 diabetes does not exclude a diagnosis of metabolic syndrome.

According to the European Group for the Study of Insulin Resistance (Balkau et al., 2002), a person has the metabolic syndrome if he or she has insulin resistance or hyperinsulinaemia (non-diabetic subjects only) and two or more of the following: 1. Fasting plasma glucose >6.1 mmol/L. 2. Dyslipidaemia – triglyceride >2.0 mmol/L and/or HDL cholesterol <1.0 mmol/L or treated for dyslipidaemia. 3. Hypertension – blood pressure >140/90mmHg and/or on anti-hypertensive medication. 4. Central obesity – waist circumference >94 cm in males and >80 cm in females. The European Group for the Study of Insulin Resistance (Balkau et al., 2002) clinical criteria for the metabolic syndrome is a hybrid of those of NCEP ATPIII and WHO. It has higher triglyceride values (2.0 mmol/L) but lower waist circumference values (>94 cm in males and >80 cm in

females) (Grundy et al., 2004). Similarly, the American Association of Clinical Endocrinologist (AACE) (Bloomgarden, 2003) proposed a third set of clinical criteria for the insulin resistance syndrome. These criteria appear to be a hybrid of those of NCEP ATP III and WHO metabolic syndrome. However, no defined number of risk factors is specified; diagnosis is left to clinical judgment based on the risk factors. The main risk factor components and their cutpoints for abnormality include: Overweight/obesity with BMI $\geq 25 kg/m^2$, elevated triglyceride >1.69 mmol/L, low HDL cholesterol <1.04 mmol/L for males and <1.29 mmol/L for females and elevated blood pressure ≥130/85mmHg.

2.7 DIABETES MELLITUS RESEARCH IN GHANA

Data on diabetes mellitus prevalence in the entire Ghanaian population are unavailable. However the crude prevalence of diabetes mellitus in the Greater Accra region of Ghana has been found to be 6.3% (Amoah et al., 2002a). In this same research, the age-adjusted prevalence of diabetes mellitus, IFG and IGT, were 6.4, 6.0 and 10.7% respectively. Diabetes was also found to be more common in males than females (7.7 vs. 5.5%), and worsening of glycaemic status tended to be associated with increase in age, body mass index, systolic and diastolic blood pressures. Fasting glucose and oral glucose tolerance test (OGTT) criteria for glucose homeostasis were compared in a community study in Accra, Ghana, using the ADA and WHO diagnostic criteria. The prevalence of undiagnosed diabetes mellitus by fasting (3.2%) and OGTT (3.1%) criteria were similar (Amoah, 2002), and both giving an overall prevalence of undiagnosed

diabetes mellitus of 4.5%. In this same study, the prevalence of impaired glucose tolerance (IGT) (15.8%) was higher than that of impaired fasting glucose (IFG) 10.7%). Thus 42% subjects with diabetes by OGTT had normal or impaired fasting glucose, showing poor agreement between the two diagnostic criteria.

Pathogenic mechanisms of type 2 diabetes mellitus in indigenous Ghanaians were found to be characterized by severe beta-cell dysfunction and moderate reduction in insulin sensitivity (Amoah et al., 2002b). The metabolic and anthropometric risk factors for cardiovascular disease (CVD) in native Ghanaians with varying degrees of glucose tolerance were also characterized using the ADA criteria. It was found that both IFG and diabetes mellitus were associated with beta cell dysfunction, insulin resistance (Amoah et al., 2002b) and elevated serum triglyceride. However, the well established cardiovascular risk factors such as body mass index, body fat distribution and blood pressure did not track with the increasing glucose intolerance in native Ghanaians at the time of diagnosis.

In another study, the levels of cholesterol, high density lipoprotein cholesterol (HDL-C) and triglyceride in Ghanaian diabetics were found to be similar to their age-matched healthy controls (Nyarko et al., 1997). In a study of total antioxidant status in Ghanaian type 2 diabetic patients, Dosoo et al. (2001) found a significantly reduced total antioxidant status in these patients. This suggests the existence of lower antioxidant defence in uncontrolled type 2 diabetes mellitus and hence use of antioxidant therapy in such patients. Further research in Ghana revealed that, aqueous extract of Ocimum canum decreased

levels of fasting blood glucose, free radicals, serum total cholesterol, low density lipoprotein cholesterol (LDL-C) and body weight, while serum high density lipoprotein cholesterol (HDL-C) increased in diabetic mice (Nyarko et al., 2002). These findings justify the use of Ocimum canum extract as antidiabetic folk medicine.

Facilities and resources for diabetic care were found to be inadequate in regional health facilities in Southern Ghana (Amoah et al., 1998). Thus it was suggested that training of health care personnel in diabetes mellitus management and education may enhance diabetes mellitus care. Additional findings suggest that the best immediate solutions lie in addressing availability and cost of biomedical care and quality food, with concomitant attention to community education and self-help groups as negotiators of psychosocial and economic support (De-Graft, 2004). To tackle the diabetes epidemic a national diabetes care and education programme has been developed (Amoah et al., 2000). The approach is by way of trained diabetes teams consisting of physicians, dietitians and nurse educators at two tertiary institutional levels (teaching hospitals) who in turn trained teams consisting of physicians, dietitians or diettherapy nurses, nurse educators and pharmacists at regional and district/sub-regional levels to offer care and education to patients and the community. This review shows that diabetes research in Ghana cuts across prevalence, diagnosis, pathogenesis and complications as well as management and therapy. The efforts of these researchers including Amoah, A.G., Owusu, S.K. and Nyarko A. is highly

commended, for providing the features of diabetes mellitus in Ghana despite our poor and inadequate medical facilities.

CHAPTER 3

3. METHODOLOGY

3.1 RESEARCH DESIGN

Two study populations (groups of subjects), labeled A and B below, were selected for this research.

A. Ghanaian diabetic outpatients with diabetes more than one year on insulin and/or diet with oral hypoglycaemic drugs. Controls included Ghanaian subjects with normal glucose tolerance.

B. Recently diagnosed (<1 year) Ghanaian diabetic outpatients on insulin or diet with oral hypoglycaemic drugs, but not both. Controls included apparently healthy Ghanaian subjects with normal glucose tolerance and clinical characteristics.

3.1.1 Study design

A and B − This research was a prospective study covering a period of four years, from August 2004 to June 2008.

3.1.2 Study site (area)

A and B − This study was conducted at the diabetic outpatients department and the Clinical Biochemistry laboratory of the Komfo Anokye Teaching Hospital (KATH) and the Kwame Nkrumah University of Science and

Technology (KNUST), all in Kumasi, Ghana, West Africa. Kumasi is situated in the middle belt of Ghana, about 260 km north of Accra. It is the second major city in Ghana and has a population of 1.2 million (Ghana Demographic Health Survey, 2003).

3.1.3 Study population

A – Ghanaian diabetic patients who attended the diabetic out-patient's clinic of the hospital as well as age and sex-matched Ghanaian nondiabetic controls, all living in Kumasi. Consecutive diabetic patients with diabetes more than one year who met the inclusion criteria were selected for the study. The sample size was 456. Similarly, Ghanaian nondiabetic age- and sex-matched controls who met the inclusion criteria were selected. The sample size was 120 (38 males and 82 females).

B − Recently (<1 year) diagnosed Ghanaian diabetic patients who attended the diabetic out-patient's clinic of the hospital as well as Ghanaian healthy age- and sex-matched nondiabetic controls, all living in Kumasi. Consecutive diabetic patients who met the inclusion criteria were selected. The sample size was 120. Similarly, Ghanaian age- and sex-matched healthy nondiabetic subjects who met the inclusion criteria were selected. The sample size was 60 (15 males and 45 females). The two study populations were to ensure early markers and established markers of diabetes mellitus.

3.1.4 Inclusion criteria

A – Ghanaian diabetics on medication, that is, on insulin and/or diet with oral hypoglycaemic drugs, diagnosed using the WHO criteria, who consented to participate in the study. The controls included Ghanaian subjects with normal glucose tolerance, assessed using WHO criteria, which involved 75g oral glucose tolerance test, with an absence of diabetes mellitus within first-degree relatives.

B − Ghanaian diabetic patients, recently diagnosed and being treated for diabetes mellitus, diagnosed using WHO criteria. They were either on insulin or diet with oral hypoglycaemic agents, that is, prompt insulin-requiring or non-insulin requiring (apparent type 2 diabetes), but not both. The controls included Ghanaian apparent healthy subjects with normal glucose tolerance, assessed using WHO criteria, which involved 75g oral glucose tolerance test. They also had normal clinical characteristics and absence of diabetes mellitus within first-degree relatives. All patients and controls consented to take part in this study. No qualified patient, control or healthy control subject declined to participate in the study.

3.1.5 Exclusion criteria

A – Non-Ghanaian diabetic patients, diabetic pregnant women who were not physiologically normal, acutely ill diabetic patients, too ill to be interviewed, or those with severe medical conditions were excluded from the study. Non-Ghanaian control subjects, nondiabetic control subjects with diabetes within first degree relatives and abnormal glucose tolerance were excluded from the study.

B − Non-Ghanaian diabetic patients, diabetic patients who had diabetes mellitus duration of one year and above, patients who were on both insulin and diet with oral hypoglycaemic drugs, pregnant women who were not physiologically normal, acutely ill patients, too ill to be interviewed, or those with severe medical conditions were excluded from this study. Non-Ghanaian control subjects, healthy control subjects who were obese, had allergic diseases, history of recurrent infections, metabolic or other diseases, were on pharmacologically active agents, had physiological states such as pregnancy, stress or excessive exercise and diabetes within first degree relatives were excluded from the study. Apparently healthy subjects with abnormal haemoglobin, white cell counts, BMI, waist circumference, blood pressure and glucose tolerance, assessed using WHO criteria, which involved 75g oral glucose tolerance test, were excluded from the study.

3.1.6 Participants recruitment

A − Recruitment was based on previous and current symptoms and test results as well as medication profile. Control recruitment was based on normal glucose tolerance and absence of diabetes within first-degree relatives.

B − Patient recruitment was based on previous and current symptoms and test results, medication profile and duration of diabetes. Healthy control recruitment was based on apparent health, absence of disease and physiological states, normal glucose tolerance, normal haemoglobin, total and differential white cell count and absence of diabetes within first-degree relatives.

3.1.7 Data Collection

A and B − A standard questionnaire was used to collect information on socio-demographic and patient's profile such as age, sex, tribe, duration of diabetes, presence of other metabolic and infectious diseases and family history of common metabolic diseases. Others were current and previous medication, intake of pharmacological agents, such as drugs including contraceptives, tobacco and alcohol, and specific physiological states such as pregnancy, stress and excessive exercise. An additional profile for control or healthy control subjects included presence of diabetes within first-degree relatives. For population B patients the medication profiles of diabetics whose disease duration was less than six months, were reviewed at six months of diabetes mellitus duration, to ascertain any changes in drug use.

3.1.8 Physical measurements

A and B − For both diabetics and controls, body weight and height were measured using a standard physician's scale and standiometer, to the nearest 0.1 kg and 0.5 cm respectively, with subjects in lightweight clothing without shoes. BMI was calculated as weight/height^2 (kg/m^2). Waist circumference was measured with a plastic anthropometric tape on bare skin of standing subjects during midrespiration at the narrowest indentation midway between the lowest rib and the iliac crest and at the level of the umbilicus. It was measured to the nearest 0.1 cm. Duplicate measures were made and averages were used in the analysis. Systolic and diastolic blood pressures were obtained with a mercury

sphygmomanometer and auscultory methods. Two blood pressure recordings were obtained from the right arm of each patient in a sitting position after 30 min of rest, at 5 min intervals, and their mean values calculated.

3.1.9 Biochemical measurements

A and B − Venous Blood samples were taken from subjects after a 12- to 14-hour overnight fast. Venous blood was collected into fluoride-oxalate bottles, fluoride inhibits the enolase reaction in glycolysis by forming magnesium fluorophosphate complex, vacutainer tubes (Becton Dickinson, Rutherford, N.J.) and sequestrene (EDTA) bottles (for population B). The blood samples in the fluoride bottles were immediately centrifuged at 1,000g for 5 minutes to prevent glycolysis from occurring and analyzed for glucose levels. The samples in the vacutainer tubes were also centrifuged after 30 minutes at 1,000g for 15 minutes at room temperature. Serum was separated into plain sample containers and frozen at –20^{o}C for 2 weeks to 1 month and analyzed for triglyceride and high density lipoprotein cholesterol (HDL-C).

Additionally, for serum samples from population B, total cholesterol was measured and low density lipoprotein (LDL) cholesterol derived from Friedewald's formula (Friedewald et al., 1972) using the autoanalyzer. Insulin, islet cell autoantibodies (ICA), autoantibodies to glutamic acid decarboxylase (GADab) and insulin autoantibodies (IAA) were determined on samples from population B using the serum. The samples in the EDTA bottles were also analyzed for HbA_{1C} levels for population B patients, to ascertain the levels in

autoimmne diabetes and type 2 diabetes, haemoglobin, total and differential white blood cell counts for population B controls.

3.1.10 Diagnostic criteria for the metabolic syndrome

A and B − Metabolic syndrome was diagnosed using the criteria recommended by the NCEP ATPIII, that is, the presence of three or more of the following risk factors: 1. Central obesity i.e. waist circumference in males >102 cm and females >88 cm, 2. Hypertriglyceridaemia i.e. triglyceride ≥1.70 mmol/L, 3. Low HDL cholesterol i.e. HDL cholesterol in males <1.00 mmol/L and in females <1.30 mmol/L, 4. Hypertension i.e. blood pressure ≥130/85 mmHg and/or on antihypertensive medication, and 5. Hyperglycaemia i.e. a fasting glucose ≥6.1mmol/L. All patients in this study were coded as positive for hyperglycaemia (i.e. glucose ≥6.1mmol/L).

3.1.11 Ethical considerations

A and B − The research protocol was reviewed and approved by the Committee for Human Research, Publications and Ethics of KNUST and KATH. The objectives and benefits of the study were explained to the diabetic patients, control or healthy control subjects at the time of initial data collection, and verbal and written consent were obtained from them.

3.1.12 Statistical analysis

A and B − Statistical analysis were performed using the statistical package for social sciences (SPSS) for windows programme version 11.0. Means and standard deviations (SD) were determined for quantitative data. χ^2 test was used for comparing proportions to determine the statistical significance of differences in proportions. A p value of less than 0.05 was considered significant.

3.2 ASSAY METHODS

3.2.1 Determination of glucose, total cholesterol, triglyceride and HDL cholesterol using ATAC® 8000 Random Access Chemistry System and its reagent kits

Glucose, total cholesterol, triglycerides and HDL cholesterol were measured using enzymatic methods and the procedures of ATAC PAK® glucose reagent kits (product no. 532-018), ATAC PAK® cholesterol reagent kits (product no. 516-018), ATAC PAK® triglyceride reagent kits (product no. 589-018), ATAC PAK® HDL cholesterol reagent kits (product no. 541-004) and ATAC 8000 Random Access Chemistry System (autoanalyzer élan diagnostics, A4-001-1198). The procedures are described by the manufacturer for glucose and cholesterol (ATAC® 8000 Random Access Chemistry System Operator's manual, Fifth Printing. Clinical Data, Smithfield RI. 8/1998), and triglyceride and HDL cholesterol (ATAC 8000 Random Access Chemistry System Operator's manual, Clinical Data/Elan Part No. 170-001, Fifth Printing. Clinical Data, Smithfield RI. 9/1999). Basically, working reagents were prepared from ATAC PAK® reagents

as described by the manufacturer and a split boat cap was inserted into the opening of the reagent boat. The preparation date was written on the boat and the boat inserted into the designated position on the reagent tray. Reagent boats were allowed to equilibrate on the tray for 30 minutes before use. Programming, calibration (after loading a new reagent) using provided standards, and running of normal and abnormal controls to ensure that the controls lie within acceptable ranges were done. The instrument automatically compared calibration factors against preprogrammed acceptance criteria. Assay of samples were then carried out as directed in the operator's manual. The standards were calibrated against international WHO approved reference material NIBSC 66/304. LDL cholesterol was derived from Friedewald's formula [LDL cholesterol (mmol/L) = total cholesterol (mmol/L) – HDL cholesterol (mmol/L) – triglyceride (mmol/L)/2.2] using the autoanalyzer (Friedewald et al., 1972).

3.2.1.1 Principle of glucose determination using ATAC PAK® glucose reagent kit

Early methods for glucose analysis were based on the ability of glucose to reduce cupric ions or alkaline ferricyanide and on the binding of o-toluidine dye. These methods have been replaced by enzymatic methods largely due to the improvement in both specificity and precision. Of the common enzymatic assays used for glucose, the hexokinase method linked to the production of NADH is both the most popular and the most specific for glucose, and is outlined as follows:

Glucose + ATP $\xrightarrow{HK}$ Glucose –6-phosphate +ADP

Glucose-6-phosphate + NAD^+ $\xrightarrow{\text{G-6-PDH}}$ 6-Phosphogluconate + NADH

Hexokinase (HK) catalyzes the phosphorylation of glucose by ATP to produce glucose-6-phosphate, which is then oxidized by NAD^+ in a reaction catalyzed by glucose-6-phosphate dehydrogenase (G-6-PDH) to 6-phosphogluconate with the simultaneous reduction of NAD to NADH. The intensity of the colour developed measured as increase in absorbance at 340 nm is directly related to the concentration of glucose in the sample.

3.2.1.2 **Principle of total cholesterol determination using ATAC PAK® cholesterol reagent kit**

Hundreds of methods for cholesterol determination have been published. A single step, fully enzymatic, procedure was first described by Allain (1974). This method is an adaptation of that procedure, and it is stated as follows:

Cholesterol Esters + H_2O $\xrightarrow{\text{CE}}$ Cholesterol+ Fatty Acids

Cholesterol + O_2 $\xrightarrow{\text{CO}}$ Cholest-4-ene-3-one + H_2O_2

$2H_2O_2$ + 4-Aminoantipyrine + Phenol $\xrightarrow{\text{POD}}$ Quinoneimine + $4H_2O$

Free cholesterol and cholesterol produced from the enzymatic hydrolysis of cholesterol esters are oxidized in the presence of cholesterol oxidase. The resulting hydrogen peroxide combines with phenol and 4-aminoantipyrine to produce a red quinoneimine dye in the presence of peroxidase. The intensity of the colour developed measured as increase in absorbance at 510 nm is proportional to the concentration of total cholesterol in the sample.

3.2.1.3 Principle of triglyceride determination using ATAC PAK® triglyceride reagent kit

Early methods for estimating triglycerides involved a saponification step to liberate glycerol from the triglycerides, which was then determined by chemical means. Bucolo and David (1973) introduced a method to liberate and measure the glycerol through coupled enzyme reactions. Spayd et al. (1978) later described a method that utilized glycerol phosphate oxidase. This reagent is a modification of that method and is outlined as follows:

Triglyceride + 3 H_2O $\xrightarrow{\text{Lipase}}$ Glycerol + 3 Fatty Acids

Glycerol + ATP $\xrightarrow{\text{GK}}$ Glycerol-3-Phosphate + ADP

Glycerol-3- Phosphate + O_2 $\xrightarrow{\text{GPO}}$ Dihydroxyacetone phosphate + H_2O_2

$2H_2O_2$ + 4-Chlorophenol + 4-AAP $\xrightarrow{\text{POD}}$

Quinoneimine + HCl + $4H_2O$

Glycerol produced from the enzymatic hydrolysis of triglyceride as well as the endogenous glycerol in the sample is metabolized to glycerol-3-phosphate by adenosine triphosphate (ATP) in a reaction catalyzed by glycerol kinase (GK). The resulting glycerol-3-phosphate is oxidized by O_2 in the presence of glycerol phosphate oxidase (GPO) to produce hydrogen peroxide and dihydroxyacetone phosphate. The hydrogen peroxide reacts with 4-chlorophenol and 4-aminoantipyrine (4-AAP) in the reagent to produce quinoneimine, a red dye and hydrochloric acid and water. The intensity of the colour developed measured as increase in absorbance at 510nm is proportional to triglyceride concentration in the sample.

3.2.1.4 Principle of HDL cholesterol determination using ATAC PAK® direct HDL reagent kit

Step 1

LDL, VLDL, Chylomicrons $\xrightarrow{\text{Polyanion}}$ Stable Complexes

Step 2

HDL-Cholesterol $\xrightarrow{\text{Detergent, CE}}$ Cholesterol + Fatty Acids

Cholesterol + O_2 $\xrightarrow{\text{CO}}$ Cholest-4-ene-3-one + H_2O_2

$2H_2O_2$ + 4-Aminoantipyrine + DSBmT $\xrightarrow{\text{POD}}$ Quinoneimine + $4H_2O$

A polyanion contained in the first reagent selectively binds LDL, VLDL and chylomicron lipoproteins, preventing them from participating in the subsequent reactions. When the second reagent is added, a unique detergent solubilizes only the HDL lipoprotein particles, thus releasing the HDL cholesterol to react with cholesterol esterase (CE) and cholesterol oxidase (CO) as above. The released hydrogen peroxide reacts with 4-aminoantipyrine and disodium N,N-bis (4-sulphobutyl)-m-toluidine (DSBmT) in the presence of peroxidase to form a coloured complex. The intensity of this complex measured as an increase in absorbance at 510 nm is directly proportional to the amount of HDL cholesterol in the sample.

3.2.2 Determination of islet cell autoantibodies, autoantibodies to glutamic acid decarboxylase, insulin autoantibodies and insulin using enzyme linked immunosorbent assay technique and DRG® International EIA reagent kits

Islet cell autoantibodies (ICA), autoantibodies to glutamic acid decarboxylase (GADab), insulin autoantibodies (IAA) and insulin were determined by enzyme linked immunosorbent assay (ELISA) technique using DRG International Inc., USA EIA ICA reagent kits (product no. EIA-1594), EIA GADab reagent kits (product no. EIA-1910), EIA IAA reagent kits (product no. EIA-1593), EIA insulin reagent kits (product no. EIA-2935) an ELISA reader (Tipo model N Matricola Cotruito manufactured, Italy) and ELISA washer (Murex, Dynatech Med Prod. Ltd., Guensey Channe Islands Great Britain). Reagents and materials were supplied by the manufacturer in the kit. Additional materials required but not supplied were specified and acquired. Working reagents were prepared from kit reagents according to the manufacturer's specifications in the user's manual. The procedures are described by the manufacturer in the user's manual for ICA (EIA-1594), GADab (EIA-1910), IAA (EIA-1593) and insulin (EIA-2935) and summarized under the principle of the procedure. The standards were calibrated against international WHO approved reference material NIBSC 66/304.

3.2.2.1 Principle of ICA, GADab and IAA determination using enzyme linked immunosorbent assay technique and DRG® International EIA reagent kits

The test kit contained 12 microwell strips of 96 wells precoated with purified islet cell antigen (for ICA determination), GAD antigen (for GADab determination) or human insulin (for IAA determination). Standards (3 calibrators for GAD determination only) positive control, negative control, and dilutions of patient serum (100uL of 10uL serum in 1 ml distilled water) were added in duplicate to the appropriate microwells. During an incubation period, antibodies (ICA specific IgG antibodies, GAD specific IgG antibodies, human IgG specific antibodies to insulin) present in the serum sample were allowed to react at room temperature with antigen molecules on the microwells. The excess/unbound serum proteins from serum were washed-off from the microwells. An enzyme (alkaline phosphatase) labeled goat-antibody, specific to human IgG was added to the antigen-antibody complex in each well and a second incubation done. During the second incubation step alkaline phosphatase enzyme complex was bound to the antigen-antibody complex. After washing off excess unreacted enzyme complex (conjugate) from the microwells, a substrate, p-nitrophenyl phosphate (PNPP) was added, then a third incubation was done and finally hydroxide (1M NaOH) was added to stop the reaction. The colour generated was measured spectrophotometrically at 405 nm and absorbance (OD) values recorded. The intensity of the colour developed is directly proportional to the concentration of autoantibodies (ICA, GADab, IAA) in the test serum sample. ICA positive and

negative controls, GAD positive and negative controls and IAA positive and negative controls served as internal quality controls to ensure valid results.

3.2.2.2 Reagents – contents of the kit

1. Microwell strips (with the holder) 12 strips
2. IgG enzyme conjugate (concentrate) 2 x 1.0 ml
3. Sample diluent (concentrate) 1 x 25.0 ml
4. Conjugate diluent 1 x 10.0 ml
5. Positive control (human serum) 1 x 1.5 ml
6. Negativecontrol (human serum) 1 x 1.5 ml
7. Substrate solution (PNPP) 1 x 15.0 ml
8. Washing buffer (concentrate) 1 x 20.0 ml
9. Stopping solution (IM NaOH) 1 x 6.0 ml

3.2.2.3 **Materials - acquired**

1. Distilled or deionized water.
2. Absorbent paper towels to blot dry the strips after washing and parafilm/plastic wraps to cover strips during incubations.
3. Suitable sized glass tubes for serum dilution.
4. Micropipette with disposable tips to deliver 10 µl, 50 µl and 100 µl.
5. A microtiter plate washer.
6. 5ml pipettes for conjugate diluent delivery.
7. A 500 ml graduated cylinder.

8. Microtiter plate reader with 405 nm absorbance capability.
9. Plastic label tape, to tape unused wells before assay.
10. A microtiter plate washer

3.2.2.4 Reagent preparation and storage

1. ICA-IgG enzyme conjugate reconstitution: Accurately transfer 5ml of the conjugate diluent into one bottle containing the IgG enzyme conjugate (concentrate). Close the bottle and mix thoroughly by inversions. Store the diluted conjugate at 2─8^{o}C when not in use. Record the date of reconstitution on the label. This diluted reagent expires 30 day after reconstitution. Two bottles containing the conjugate concentrate are provided. Each bottle contains enough conjugate for 6 strips. Reconstitute as needed.

2. ICA-sample diluent buffer: Transfer the entire contents (25ml) into 100ml of distilled/deionized water in a suitable container. Mix thoroughly; label the container as ICA-sample diluent, and store at 2─8^{o}C. The diluted reagent is stable until the expiration shown on the vial.

3. ICA-wash solution: Transfer the entire contents into 480ml of distilled/deionized water in a 500ml container. Mix thoroughly to dissolve all crystals; label the container as ICA-wash, and store at 2─8^{o}C. The diluted reagent is stable until the expiration shown on the vial.

4. Serum Sample Preparation: Accrurately pipette 10µl (0.010ml) of serum sample into 1.0ml of the working sample diluent into an already labeled glass tube. Mix thoroughly.

3.2.2.5 Assay procedure

The test kit contains 12 microwell strips coated with purified islet cell antigens. The number of microwell strips used in each assay depends upon the number of serum samples to be tested. If 12 microwell stips are used, a total of 45 sample sera can be tested in duplicate with this kit.

Important note: Bring all the reagents, including serum samples, to room temperature (25^{o}C) before starting the assay. Incubation temperatures varying by greater than $\pm$ 1^{o}C can definitely affect results.

1. Assemble the number of microwell strips needed for the test in the holder provided. The microwell strip must be snapped in place, or it will fall out and break.

1. Familiarize yourself with the indexing system of wells, e.g. well # A1, B1, C1, D1, etc.
2. Dispense 100 µl of negative control into microwells C1 and D1.
3. Dispense 100 µl of positive control into microwells E1 and F1.
4. Add 100 µl of diluted sample serum (see #4, reagent preparation) to microwells G1 and H1. For more patient samples, use additional strips and add other diluted patient samples to microwells in duplicate. There should be 100 µl of solution in each microwell to be assayed except A1 and B1, which are empty at this point and and will be used later.
5. Any strips not used should be properly stored with desiccant in the ziplock bag provided for the next run. Any wells not used on the strip should be properly covered and saved for the next run.

6. Cover the plate with a parafilm/plastic wrap (to prevent contamination) and leave for 1 hour at room temperature (25^{o} + 1^{o}C).
7. After incubation, discard the solution into sink by quick decantation and blot the plate dry by tapping gently onto a paper towel. Wash each well 3 times with 300 µl (0.3ml) of the ICA wash solution with an automatic plate washer. Avoid air bubbles in the well during washing. Repeat the procedure two more times and blot the plate dry with a paper towel.
8. Add 100 µl of IgG enzyme conjugate reagent (see #1, reagent preparation) to all microwells except A1 and B1
9. Cover the plate with a parafilm/plastic wrap and let it stand at room temperature (25^{o} + 1^{o}C) for one hour.
10. After incubation, repeat the washing step (step #8) and blot the microwells dry.
11. Add 0.1 ml (100 µl) of substrate solution to all microwells including wells A1 and B1. Be sure to dispense the substrate reagent at a rapid steady pace without any interruption.
12. Cover the plate and leave it in the dark for 30 minutes at room temperature (25^{o} + 1^{o}C).
13. After 30 minutes, promptly add 50 µl of the stopping solution into each well at a rapid steady pace without any interruption.
14. Set up the microtiter plate reader to read the absorbance at 405 nm according to the manufacture's instructions, and blank the plate reader with well A1 or B1.

15. Calculate the data (see below).

3.2.2.6 Calculation of data

ICA and IAA were calculated as follows: The average absorbance (OD) reading of a sample or control done in duplicate was calculated. The average reading of the negative control was designated N, positive control P and sample S. The cut-off point (X) of each run was calculated using manufacturer's formula: Cutoff Point (X) = N x 2.5. Either (+) or (-) autoantibodies in the sample was recorded by comparing the average sample (S) O.D. value with the calculated cut-off point value. A positive value was greater than the cutoff point and a negative value less or equal to the cutoff point.

GAD autoantibodies were calculated as follows: on linear graph paper, each calibrator value (as indicated on the calibrator vial label) was plotted on the X-axis and its corresponding absorbance value on the Y-axis. A best-fit straight line was drawn between the three points. The GAD value of each patient's serum was determined using its absorbance value and extrapolating from the X-axis. The GAD value of each sample was interpreted as follows: <1.00 was negative, >1.05 was positive and values between 1.00 - 1.05 indeterminate. A positive result (>1.05) indicated the presence of GAD autoantibodies in the patient's serum sample. A negative result (<1.00) indicated the absence of GAD autoantibodies or below the limit or resolution of the test. An indeterminate (borderline) value (1.00 - 1.05) was interpreted as absence of GAD autoantibodies for the purpose of this research.

3.2.2.7 Principle of insulin determination using enzyme linked immunosorbent assay technique and DRG® International EIA reagent kits

The DRG Insulin ELISA is a solid phase two-site enzyme immunoassay. It is based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the insulin molecule. The test kit contained 12 microwell strips of 96 wells precoated with purified anti-insulin monoclonal antibodies. Six standards, positive control, negative control, and patient serum (25uL) were added in duplicate to the appropriate microwells. A conjugate, biotin conjugated mouse monoclonal anti-insulin antibodies was dispensed into each well. During incubation at room temperature without covering the plate, insulin in the sample reacted with biotin-conjugated anti-insulin antibodies and anti-insulin antibodies bound to the microtitration well. Unbound biotin labeled antibody from serum was removed by washing. An enzyme complex (horse radish peroxidase) was added to each well and a second incubation done. During the second incubation step streptavidin horse radish peroxidase enzyme complex was bound to the biotin anti-insulin antibody. After washing off excess unreacted enzyme complex from the microwells, a substrate, 3,3′,5,5′-tetramethylbenzidine (TMB) was added, then a third incubation was done and finally acid (0.5M H_2SO_4) was added to stop the reaction. The colour generated was measured spectrophotometrically at 450 nm and absorbance (OD) values recorded. The intensity of the colour developed was directly proportional to the concentration of insulin in the test serum sample. Insulin controls at both

normal and pathological levels served as internal quality controls to ensure valid results.

3.2.2.8 Reagents ─ contents of the kit

1. PLA IAA	= IAA-Microwell strips (with the holder)	12 strips
2. CONJ ENZ 5X	= IAA-Anti-human IgG enzyme conjugate (conc.)	2x1.0ml
3. DIL SPE 5X	= IAA-Sample diluent (concentrate)	1x25.0ml
4. CONJ ENZ DIL	= Isletest conjugate diluent	1x10.0ml
5. CTRL +IAA	= IAA-Positive control (human serum)	1x1.5ml
6. CTRL + IAA	= IAA-Negative control	1x1.5ml
7. SUBS PNPP	= Isletest substrate solution	1x15.0ml
8. DUF WASH 25X	= Isletest washing buffer (concentrate)	1x20.0ml
9. SOLN STP	= Isletest stopping solution (IM NaOH)	1x6.0ml

3.2.2.9 Materials - acquired

1. Distilled or deionized water
2. Absorbent paper towels, to blot dry the strips alter washing steps, and parafilm plastic wraps to cover strips during incubations.
3. Suitable sized glass tubes for serum dilution.
1. Micropipette with disposable tips to deliver 10µl, 50µl and 100µl.
2. A microtiter plate washer.
3. 5ml pipettes for substrate buffer and conjugate diluent delivery.
4. A 500ml graduate cylinder.
5. Microtiter plate reader with 405 nm absorbance capabilities.

6. Plastic label tape, to tape-up unused wells before assay.

3.2.2.10 Reagent preparation and storage

1. IAA-IgG enzyme conjugate reconstitution:

   Accurately transfer 5ml of the IAA-conjugate diluent into one dropper bottle containing IAA-IgG enzyme conjugate (concentrate). Close the bottle and mix thoroughly by inversions. Store the diluted conjugate at 2─8^{o}C at all times. Record the date reconstitution on the label. This diluted reagent expires 30 days after reconstitution. Each of the two conjugate (concentrate) bottles is sufficient for 6 strips. Reconstitute as needed.

2. IAA-Sample diluent buffer:

   Transfer the entire contents (25ml) into 100 ml of distilled/deionized water in a suitable container. Mix thoroughly; label the container as IAA-Sample diluent, and store at 2─8^{o}C. The diluted reagent is stable until the expiration shown on the vial.

3. Isletest wash solution:

   Transfer the entire contents into 480 ml of distilled/deionized water in 500ml container. Mix thoroughly; label the container as Isletest wash, and store at 2-8^{o}C. The diluted reagent is stable until the expiration shown on the vial.

4. Serum Sample Preparation

   Accurately pipette 10µl (0.010ml) of serum sample into 1.0 ml of the working sample diluent into an already labeled glass tube. Mix thoroughly.

3.2.2.11 Assay procedure

The test kit contains 12 microwell strips coated with human insulin. The number of microwell strips used in each assay depends upon the number of serum samples to be tested. If 12 microwell strips are used, a total 45 patient sera can be tested in duplicate with this kit.

1. Assemble the number of strips needed for a test run in the holder provided. The microwell strip must be snapped in place firmly or it will fall out and break.

2. Familiarize yourself with the indexing system of wells, e.g. #A1, B1, C1, D1, etc. and label the strips used with a marking pen.

3. Dispense 100 µl of IAA-negative control into microwells C1 and D1.

4. Dispense 100µl of IAA-positive control into microwells E1 and F1.

5. Add 100 µl of diluted patient serum to microwells G1 and H1. For more patient samples, use additional strips and add diluted samples to microwells in duplicate. There should be 100µl of solution in each microwell to be assayed except A1 and B1, which are empty at this point and will be used later.

6. Any wells not used on the strip should be properly covered and saved for the next run. Any well strips not used should be stored with the desiccant in the ziplock bag provided at 2─8^{o}C for the next run.

7. Cover the plate with a parafilm/plastic wrap (to prevent contamination) and leave at 2─8^{o}C overnight (12─16 hours).

8. The next morning, decant the solution into sink by quick decantation. Blot the plate dry by tapping gently on a paper towel. Wash each well 3 times with 300 µl

of the IAA-wash solution with an automatic plate washer. Repeat the procedure two more times and blot the plate dry with a paper towel.

9. Add 100µl of IAA-IgG enzyme conjugate reagent to all microwells except wells A1 and B1.

7. Cover the plate with a parafilm/plastic wrap and let it stand at 25^{o} + 1^{o}C for one hour.
8. After incubation, repeat the washing step (step #8) and blot dry the microwells.
9. Add 0.1 ml (100µl) of substrate solution to all microwells including wells A1 and B1. Be sure to dispense the substrate solution at a rapid steady pace without any interruption.
10. Cover the plate and leave it in the dark for 30 minutes at 25^{o} + I^{o}C.
11. After 30 minutes promptly add 50ml of the stopping solution into each well at a rapid steady pace without any interruption.
12. Set up microplate reader to read the absorbance at 405nm according to manufacturing instructions, and blank the plate reader with well A1 or B1.
13. Calculate the data.

3.2.2.12 Calculation of data

The average absorbance values for each set of standards, controls and patient samples were calculated. A standard curve was constructed by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X)

axis. Using the mean absorbance value for each sample, the corresponding concentration from the standard curve was determined.

3.2.3 Determination of haemoglobin A_{1C} using DCA® 2000+ analyzer and its reagent kits

Haemoglobin A_{1C} (HbA_{1C}) was measured using the procedures of DCA 2,000 Haemoglobin A_{1C} reagent kit (product no. 5035B, 10's), DCA 2,000 normal and abnormal control kit (product no. 5068) and DCA 2,000+ analyser (Bayer model 5031, USA) as described by the manufacturer (Eaton et al., 1997). Basically, the analyzer was calibrated. Normal and abnormal controls, and patient samples were prepared according to the manufacturer's instructions. The controls and reagent test cartridges were loaded into the DCA 2,000+ analyzer. After that, the test results were displayed in six minutes. The DCA 2,000+ analyzer was calibrated by the manufacturer. Values of calibration parameters were based on Diabetes Control and Complications Trial (DCCT) Research Group (1993) reference method. The values for the calibration parameters were encoded into the calibration card provided by the manufacturer with each lot of reagent cartridges. All DCA 2,000 control results were within the acceptable range specified in the control package inserted.

3.2.3.1 Principle of haemoglobin A_{1C} determination using DCA® 2000+ reagent kits

The assay was based on a latex immunoagglutination inhibition methodology (Craine, 1987). Both the concentration of haemoglobin A_{1C} specifically and the concentration of total haemoglobin were measured and the ratio reported as percent haemoglobin A_{1C} (Knowles et al., 1986). All the reagents required for performing both reactions were contained in the DCA 2,000 Haemoglobin A_{1C} (HbA_{1C}) reagent cartridge. For the measurement of total haemoglobin, potassium ferricyanide was used to oxidize haemoglobin in the sample to methaemoglobin. The methaemoglobin then complexed with thiocyanate to form thiocyan-methaemoglobin, the coloured species, which was measured. The extent of colour development at 531 nm was proportional to the concentration of total haemoglobin in the sample. For the measurement of specific HbA_{1C}, an inhibition of latex agglutination assay was used. An agglutinator (synthetic polymer containing multiple copies of the immunoreactive portion of HbA_{1C}) caused agglutination of latex coated with HbA_{1C} specific mouse monoclonal antibody. This agglutination reaction caused increased scattering of light which was measured as an increase in absorbance at 531 nm. HbA_{1C} in whole blood specimens competed for the limited number of antibody-latex binding sites causing an inhibition of agglutination and a decreased scattering of light. The decreased scattering was measured as a decrease in absorbance at 531nm. The HbA_{1C} concentration was then quantified using a calibration curve of absorbance versus HbA_{1C} concentration.

The percent HbA_{1C} in the sample is then calculated as follows:

$$\% HbA_{1C} = \frac{[HbA_{1C}]}{[\text{Total Haemoglobin}]} \times 100$$

All measurements and calculations were performed automatically by the DCA 2000+ analyzer, and the screen displayed percent HbA_{1C} at the end of the assay.

3.2.4 Determination of haemoglobin and white blood cell counts using ATAC Haemacount 16 cell counter and its reagents

Haemoglobin, total and differential white blood cell counts were measured using the procedures of ATAC Haemacount 16 CD counter (cell-dyn CD 1800. Abbott laboratories, USA) and its PAC reagents. Reagents and standards (calibrators) were supplied by the manufacturer. The procedures including instrument operation are described by the manufacturer in the operator's manual (CD 1800 Operators manual 9140390C-March, 2004) and summarized under the principle of procedure.

3.2.4.1 Reagents and their functions

Diluent: The ATAC-PAC diluent is an azide-free, isotonic liquid for diluting whole blood specimens. This diluent is formulated to produce a white blood cell differential analysis when used with the ATAC PAC lyse.

Lyse: The ATAC-PAC lyse is an azide-free lysing reagent that rapidly breaks down the red blood cell walls, releases the haemoglobin from the cell and reduces the size of cellular debris to a level that does not interfere with white blood cell

analysis. Additionally the reagent contains cyanide radical that complexes with the free haemoglobin to form cyanmethaemoglobin, the absorbance of which is directly proportional to the haemoglobin concentration over the clinically useful range. This lyse must be used with the ATAC PAC diluent to produce the white blood cell differential analysis. This reagent is stable for 30 days after opening.

3.24.2 Controls and calibrators

Whole blood products (calibrators and controls) are used to calibrate and verify operation of ATAC HemaCount 16 analysers. Biochemistry laboratory systems and haematology calibrators are commercially prepared whole blood products with assigned values traceable to reference methods. Calibrators are stable for 30 days and are appropriate for calibrating the ATAC HemaCount 16 when used with the ATAC PAC reagent system. To operate the ATAC HemaCount 16, the ATAC-PAC of reagents is needed along with three levels of Biochemistry laboratory systems control material.

3.2.4.3 Principle of the determination of haemoglobin and white blood cell count using ATAC HaemaCount 16 cell counter and its reagents

The ATAC HemaCount 16 determines values for the white blood cell count, red blood cell count and platelet count through refinements of the well-established impedance method of counting and sizing cells. An electric field is created through an aperture through which blood cells suspended in isotonic diluent pass. As individual cells pass through the aperture, they cause an increase

in impedance of electric current directly proportional to their size. The instrument counts individual cells and sorts them by size, thus providing both count and cell size distribution analyses. Diluent can have a significant effect on cell size determination. It is important, therefore, to assume the integrity of the diluent by controlling the osmolality and protecting it from contamination by bacteria, molds and debris including dust. White blood cell counting requires the rapid destruction of red blood cells while leaving the white blood cell nuclei intact. A lysing agent causes haemolysis of the red blood cells rendering their cell stroma electrically invisible and therefore uncounted.

The ATAC HemaCount 16 electronically measures white blood cells, red blood cells and platelets. The contrast between electrical conductivity of cells and diluent provides the mechanism used for sensing cells. Blood is diluted in a buffered diluent solution. A volume of this dilution is drawn through a 78-micron aperture in the RBC/PLT cuvette and a 100-micron aperture – the WBC cuvette, while an electric current is applied through momentarily increasing resistance to the electric current. This temporary impedance change is proportional to the size of the cell. The instrument amplifies these electric impulse effects and counts them to measure WBCs and RBCs. For cell volume parameters HCT, MCV and MPV, the instrument measures the amplitude of the electric impulse produced by each cell passing through the aperture-sensing zone. The computer then calculates a volumetric average of the cells for MCV and multiplies by the RBC to calculate HCT.

Dual closed systems automatically dilute, mix and analyse white cell related parameters and red cell related parameters separately and simultaneously. The WBC system counts white cells and groups them by size to provide a 3-part differential. The differential results include the absolute numbers and percent values for the groups: lymphocytes, Mids and granulocytes. The WBC cuvette is equipped with electro optics for determining the haemoglobin concentration using the cyanmethaemoglobin method. The RBC system counts and groups red cells and platelets by size to provide size distribution histograms for each printed report. In the DIRECT MODE, the ATAC HemaCount 16 aspirates, automatically dilutes and analyses whole blood (EDTA treated) samples. In the SAMPLE SAVER MODE, the unit makes the analysis on less than 60µl of sample. The ATAC Hemacount 16 is designed to be used with the ATAC-PAC reagents. Each pack contains enough diluent, lyse cleaner for about 200 tests.

CHAPTER FOUR

4. RESULTS

4.1 CLINICAL CHARACTERISTICS OF GHANAIAN DIABETIC PATIENTS

The total number of diabetic patients studied was 456 and consisted of 141 (30.9%) males and 315 (69.1%) females. The selected diabetic population included 250 (54.8%) Twi-speaking people, 30 (6.6%) Fantes, 38 (8.3%) Guans, 40 (8.8%) Ga-Adangbes, 66 (14.5%) Northern peoples and 23 (5.0%) Ewes. It also included 9 (2.0%) who belonged to other minor tribes.

The ages of the patients ranged from 13 to 90 years with a mean of 55.8 ± 12.3 S.D. It was observed from the age distribution of the diabetic patients (Table 1) that, 43 (9.4%) were <40 years, 241 (52.9%) 40 – 59 years and 172 (37.7%) ≥60 years. The age of males ranged from 15 to 85 years with a mean of 55.4 ± 12.2 S.D. The females ranged in age from 13 to 90 years with a mean of 56.0 ± 12.4 S. D. The range of the age of onset of diabetes in the study diabetic population was 10 to 89 years with a mean of 49.7 ± 12.5 S.D. The age of onset distribution (Table 1) shows that 6 (1.3%) had an age of onset <20 years or early onset, 42 (9.2%) 20 – 34 years or intermediate age onset, and 408 (89.5%) ≥35years or late onset. The age of onset for males ranged from 14.3 to 76.0 years with a mean of 49.6 ± 12.2. That of females ranged from 10 to 89 years, with a mean of 49.7 ± 12.6.

The duration of diabetes mellitus ranged from 0.25 (3 months) to 30.0 ≥years, with a mean of 6.0±5.4 S.D. The duration of diabetes mellitus in the study population revealed that, 86 (18.9%) had a duration of <1 year, 276 (60.5%) a duration of 1 – 9 years, 76 (16.7%) a duration of 10 –19 years and 18 (3.9%) aduration of ≥20 years (Table 1). The duration in males ranged from 0.25 (3 months) to 26 years, with a mean value of 5.8 ± 4.9 S.D. In females the duration ranged from 0.25 (3 months) to 30.0 years, with a mean value of 6.1± 5.6 S.D.

Preprandial (fasting) glucose levels ranged from 2.9 to 30.2 mmol/L, with a mean value of 9.4±4.5. Sixty-eight (14.9%) had low preprandial glucose levels of <5.0 mmol/L (Table 1), 105 (23.0%) had good preprandial glucose level of 5.0 to 7.2 mmol/L, whilst 283 (62.1%) had high or abnormal preprandial glucose level of >7.2 mmol/L, according to the American Diabetes Association (2004). In males preprandial glucose levels ranged from 3.1 to 30.2 mmol/L with a mean of 9.7±5.2 S.D., whilst in females it ranged from 2.9 to 27.3 mmol/L, with a mean of 9.2±4.2 S.D.

Body Mass Index (BMI) values ranged from 16.5 kg/m^2 to 42.0 kg/m^2, with a mean of 25.1± 4.8 S.D. The recommendations of the United States National Institute of Health were followed to classify patients as normal weight, overweight or obese. It was observed that, 3 (0.7%) diabetic patients were underweight or had a BMI of <18.5 kg/m^2, 250 (54.8%) had normal BMI of 18.5 – 24.9 kg/m^2, 106 (23.2%) were overweight with a BMI of 25.0 – 29.9 kg/m^2 and 97 (21.3%) were obese with BMI of ≥30 kg/m^2 (Table 1). In males BMI ranged

from 16.5 to 42.0 kg/m^2, with a mean of 24.1± 4.8 S.D. Body Mass Index values ranged from 16.0 to 39.8 kg/m^2 in females with a mean of 25.6 ± 4.7.

Diabetics with confirmed hypertension on antihypertensive medication (Table 1) were 183 (40.1%), the remaining 273 (59.9%) were without confirmed hypertension. Furthermore, diabetics with hypertension on antihypertensive medication, whose blood pressures were <130/80 or had controlled blood pressure (Table 1) were 16 (8.7%), whilst those with blood pressure ≥130/80 or inadequate blood pressure control were 167 (91.3%). Forty-three (30.5%) males and 140 (44.4%) females were diabetics with confirmed hypertension on antihypertensive medication. Ninety-eight (69.5%) males and 175 (55.6%) females were not hypertensive. Insulin-requiring diabetic patients were 112 (24.6 %) and included 52 (11.4%) who were on insulin only and 60 (13.2%) who were on both insulin and oral hypoglycaemic drugs. Non-insulin requiring diabetic patients (Table 1) were 344 (75.4%).

Table 1
Clinical characteristics of 456 Ghanaian diabetic patients

Sex		
	Male	30.9%
	Female	69.1%
Age (yrs)		
	Mean	55.8±12.3
	<40	9.4%
	40 − 59	52.9%
	≥60	37.7%
Age of onset (yrs)		
	Mean	49.7±12.5
	<20	1.3%
	20 − 34	9.2%
	≥35	89.5%
Duration of diabetes (yrs)		
	Mean	6.0±5.4
	<1	18.9%
	1 − 9	60.5%
	10 − 19	16.7%
	≥20	3.9%
Preprandial glucose (mmol/L)		
	Mean	9.4±4.5
	<5	14.9%
	5.0 − 7.2	23.0%
	>7.2	62.1%
Body mass index (Kg/m^2)		
	Mean	25.1±4.8
	<18.5	0.7%
	18.5 − 24.9	54.8%
	25.0 − 29.9	23.2%
	≥30	21.3%
Hypertension		
	Confirmed, on medication	40.1%
	With controlled blood pressure (<130/80 mmHg)	8.7%
	With inadequate blood pressure control (≥130/80 mmHg)	91.3%
Insulin requiring status		
	Insulin requiring	24.6%
	Non-insulin requiring	75.4%

4.2 PREVALENCE OF THE METABOLIC SYNDROME AND ITS INDIVIDUAL COMPONENTS IN GHANAIAN DIABETIC PATIENTS

The number of diabetic patients with the metabolic syndrome (Table 2) was 255 (55.9%), meaning that 201 (44.1%) were without the metabolic syndrome. Male diabetics with the metabolic syndrome were 47 (33.3%) and those without 94 (66.7%). In females 208 (66.0%) were having the metabolic syndrome (Table 2), whilst 107 (34.0%) were without the syndrome. Metabolic syndrome was significantly more prevalent in females than males. The number of the nondiabetic control population of 120 with the metabolic syndrome was 11 (9.2%). Metabolic score (1-5) calculation was based on the number of metabolic syndrome factors each patient fulfilled. Twenty-six (5.7%) diabetic patients had a metabolic score of 5 (Table 3); those who had 4 out of the 5 metabolic syndrome factors were 99 (21.7%); those with 3 factors 131 (28.7%); those with 2 factors 134 (29.4%); and those who had only 1 metabolic syndrome factor were 66 (14.5%). It was observed that 2 (1.4%) males and 24 (7.6%) females had a metabolic score of 5 (Table 3). Sixteen (11.3%) males and 83 (26.3%) females had a metabolic score of 4; males and females with metabolic score of 3 were 29 (20.6%) and 102 (32.4%) respectively; males and females with metabolic score of 2 were 58 (41.1%) and 76 (24.1%) respectively; males and females with metabolic score of 1 were 36 (25.5%) and 30 (9.5%) respectively (Table 3). It is those with metabolic scores of 3 and above who were classified as having the metabolic syndrome.

Waist circumference values for the total diabetic population ranged from 50.0 to 134.0 cm, with a mean value of 87.0 ± 13.7 S.D. Waist circumference in males ranged from 60.0 to 120.0 cm, with a mean of 85.6 ± 11.4 S.D. In females, waist circumference ranged from 50.0 to 134.0 cm, with a mean of 88.4 ± 14.5 S.D. One hundred and ninety-nine (43.6%) diabetic patients had central obesity (that is, waist circumference >102 cm in males and >88.0 cm in females) (Table 2). Central obesity was observed in 19 (13.5%) males and 180 (57.1%) females. Triglyceride values in the total diabetes mellitus population ranged from 0.25 to 9.4 mmol/L, with a mean value of 1.70 ± 1.02. In males triglyceride levels ranged from 0.50 to 7.10 mmol/L with a mean value of 1.65 ± 1.00. Female triglyceride levels, ranged from 0.25 to 9.40 mmol/L, with a mean value of 1.72 ± 1.04. It is depicted (Table 2) that 171 (37.5%) diabetic patients had hypertriglyceridaemia (that is, triglyceride levels ≥1.7 mmol/L). Fifty-one (36.2%) males and 120 (38.1%) females had hypertriglyceridaemia.

HDL cholesterol values for the entire diabetic population ranged from 0.29 to 4.49 mmol/L, with a mean value of 1.26 ± 0.51 S.D. Among males, HDL cholesterol values ranged from 0.29 to 4.49 mmol/L, with a mean value of 1.20 ± 0.54 S.D. Female HDL cholesterol levels ranged from 0.36 to 3.70 mmol/L, with a mean value of 1.29 ± 0.50 S.D. Two hundred and sixteen (47.4%) diabetic patients had low HDL cholesterol values of <1.00 mmol/L in males and <1.30 mmol/L in females (Table 2). Low HDL cholesterol was present in 49 (34.8%) males and 167 (53.0%) females. Diabetic patients who had blood pressure ≥130/85 mmHg and/or were on antihypertensive medication were 214 (46.9%).

Fifty-six (39.7%) males and 158 (50.2%) females had blood pressures ≥130/85 mmHg and/or were on antihypertensive medication.

In addition to hyperglycaemia, low HDL cholesterol was the commonest component (47.4%) of the metabolic syndrome among Ghanaian diabetic patients, followed by hypertension (46.9%). In females central obesity (57.1%) was the commonest component of the metabolic syndrome, followed by low HDL cholesterol (53.0%). In males, hypertension (39.7%) was the commonest component of the metabolic syndrome, followed by hypertriglyceridaemia (36.2%). Central obesity, low HDL cholesterol and hypertension were significantly (P = 0.0001, 0.001, 0.001 respectively) more prevalent in female diabetics than males. However the prevalence of hypertriglycaeridaemia was not significantly (P = 0.18) different in female and male diabetics.

Table 2
Prevalence of metabolic syndrome and its components in Ghanaian diabetic patients

Metabolic syndrome and its components	Diabetic patients N=456		Males N=141		Females N =315		P value
	No.	%	No.	%	No.	%	
Metabolic syndrome	255	55.9	47	33.3	208	66.0	0.001
Central Obesity	199	43.6	19	13.5	180	57.1	0.0001
Hypertriglyceri-daemia	171	37.5	51	36.2	120	38.1	0.18
Low HDL Cholesterol	216	47.4	49	34.8	167	53.0	0.001
Blood Pressure ≥ 130/85 mmHg and/or on medication	214	46.9	56	39.7	158	50.2	0.001

Table 3
Metabolic score distribution of Ghanaian diabetic patients

Metabolic score	Diabetic patients N=456		Males N=141		Females N =315	
	No.	%	No.	%	No.	%
5	26	5.7	2	1.4	24	7.6
4	99	21.7	16	11.3	83	26.3
3	131	28.7	29	20.6	102	32.4
2	134	29.4	58	41.1	76	24.1
1	66	14.5	36	25.5	30	9.5

4.3 DETERMINANTS AND CLINICAL CHARACTERISTICS OF THE METABOLIC SYNDROME DIAGNOSED FROM GHANAIAN DIABETIC PATIENTS

One hundred and seventy-seven (69.4%) diabetics with the metabolic syndrome had central obesity (Table 4). Seventeen (36.2%) males and 160 (76.9%) females had central obesity. Hypertriglyceridaemia was observed in 146 (57.3%) of the metabolic syndrome group of diabetics. Hypertriglyceridaemia was observed in 35 (74.5%) males and 111 (53.4%) females. Low HDL cholesterol was observed in 163 (63.9%) of the diabetics with the metabolic syndrome. Twenty-nine (61.7%) males and 134 (64.4%) females had low HDL cholesterol. The metabolic syndrome diabetics also had 172 (67.5%) individuals with blood pressure ≥130/85 mmHg and/or were on antihypertensive medication. Male and female patients with blood pressure ≥130/85 mmHg and/or were on antihypertensive medication were 32 (68.1%) and 140 (67.3%) respectively.

Apart from hyperglycaemia (which was common to all patients), central obesity was the major determinant (69.4%) of the metabolic syndrome among Ghanaian diabetics with the metabolic syndrome, followed by hypertension (67.5%). In females central obesity was the major determinant (76.9%) of the metabolic syndrome, followed by hypertension (67.3%). A different pattern was observed in males in whom the major determinant was hypertriglyceridaemia (74.5%), followed by hypertension (68.1%). Central obesity was significantly (P = 0.001) more prevalent in female metabolic syndrome diabetics than males whilst hypertriglyceridaemia was significantly (P = 0.001) more prevalent in male

metabolic syndrome diabetics than females. However low HDL cholesterol and hypertension were not significantly (P = 0.16, 0.236 respectively) different in the metabolic syndrome female and male diabetics.

Among diabetics with the metabolic syndrome, the most frequent combination of the different components was hyperglycaemia, central obesity and hypertension, which was found in 118 (46.3%) diabetics with the metabolic syndrome (Table 5). Twelve (24.5%) males and 106 (51.5%) females had hyperglycaemia, central obesity and hypertension. Other major combinations included hyperglycaemia, central obesity and low HDL cholesterol which was present in 102 (40.0%) metabolic syndrome patients. It was present in 6 (12.2%) males and 96 (46.6%) females. Hyperglycaemia, hypertension and low HDL cholesterol was found in 94 (36.9%) metabolic syndrome patients. It was observed in 17 (34.7%) males and 77 (37.4%) females. Hyperglycaemia, central obesity and hypertriglyceridaemia was present in 89 (34.9%) metabolic syndrome patients. It was present in 11 (22.4%) males and 78 (37.9%) females. Hyperglycaemia, hypertension and hypertriglyceridaemia was observed in 88 (34.5%) metabolic syndrome patients. This combination of components was observed in 21 (42.8%) males and 67 (32.5%) females. Hyperglycaemia, low HDL cholesterol and hypertriglyceridaemia was present in 87 (34.1%) metabolic syndrome patients. Twenty (40.8%) males and 67 (32.5%) females had this combination of different components.

Hyperglycaemia, central obesity and hypertension (46.3%) was the most frequent combination of different components among diabetics with the metabolic

syndrome, followed by hyperglycaemia, central obesity and low HDL cholesterol (40.0%). Similarly, in females, hyperglycaemia, central obesity and hypertension (51.5%) was the most frequent combination of different components among diabetics with the metabolic syndrome, followed by hyperglycaemia, central obesity and low HDL cholesterol (46.6%). A different pattern was observed in male diabetics with the metabolic syndrome in whom, hyperglycaemia, hypertriglyceridaemia and hypertension was the most frequent combination of different components (42.8%). This was closely followed by hyperglycaemia, hypertriglyceridaemia and low HDL cholesterol (40.0%).

The ages of diabetics with the metabolic syndrome ranged from 27.0 to 90.0 years, with a mean value of 57.8 ± 11.1 S.D. Twelve (4.7%) were <40 years, 138 (54.1%) 40 – 59 years and 105 (41.2%) ≥60 years (Table 6). In males, the age of the metabolic syndrome group of diabetics ranged from 38 to 75 years, with a mean value of 57.1 ± 9.4. In females it ranged from 27 to 90 years, with a mean value of 57.9 ± 11.5 S.D. The metabolic syndrome group of diabetics had an age of onset that ranged from 20 – 89 years, with a mean of 51.3 ± 11.7 S.D. Only 1 (0.4%) of the diabetics with the metabolic syndrome had an age of onset <20 years, 15 (5.9%) 20 – 34 years and 239 (93.7%) ≥35 years (Table 6). In males the age of onset of the metabolic syndrome patients ranged from 28.0 to 72.5 years, with a mean of 50.9 ± 10.7 S.D. Among females it ranged from 20 to 89 years, with a mean of 51.4±12.0 S.D.

Diabetes duration of the diabetics with the metabolic syndrome ranged from 0.25 to 30 years, with a mean of 6.3 ± 5.9 S.D. Forty-eight (18.8%) had a

duration of <1 year, 149 (58.5%) a duration of 1–9 years, 44 (17.2%) a duration of 10 – 19 years and 14 (5.5%) a duration of ≥20 years (Table 6). Male diabetics with the metabolic syndrome had diabetes duration which ranged from 0.25 to 26.0 years, with a mean of 6.2± 5.5 S.D; in females diabetes duration ranged from 0.25 to 30 years, with a mean of 6.3 ± 5.9 S.D.

In diabetics with the metabolic syndrome, preprandial (fasting) glucose levels ranged from 2.9 to 30.2 mmol/L, with a mean value of 9.3 ± 4.4 S.D. Thirty-eight (14.9%) had preprandial glucose levels <5.0 mmol/L, 49 (19.2%) preprandial glucose 5.0 – 7.2 mmol/L and 168 (65.9%) preprandial glucose levels >7.2 mmol/L (Table 6). In males preprandial glucose levels ranged from 3.3 to 30.2 mmol/L, with a mean of 9.9 ± 5.6 S.D. In females it ranged from 2.9 to 27.3 mmolL, with a mean of 9.2 ± 4.1 S.D.

Body mass index (BMI) values in diabetics with the metabolic syndrome ranged from 18.6 to 42.0 kg/m^2, with a mean value of 26.9 ± 4.8 S.D. This means no diabetic with the metabolic syndrome was underweight or had a BMI of <18.5 kg /m^2 (Table 6). However 101 (39.6%) had a normal BMI of 18.5 to 24.9 kg/m^2, 69 (27.1%) were overweight with a BMI of 25.0 – 29.9 kg/m^2 and 85 (33.3%) were obese, with a BMI of ≥30 kg/m^2. In males BMI values ranged from 19.4 to 42.0 kg/m^2, with a mean value of 26.7 ± 5.7 S.D., and in females it ranged from 18.6 to 39.8 kg/m^2, with a mean value of 27.0 ± 4.6 kg/m^2.

Diabetics with the metabolic syndrome who had confirmed hypertension and were on antihypertensive medication (Table 6) were 153 (60.0%). Those who were without confirmed hypertension were 102 (40.0%). Among diabetics with

the metabolic syndrome and hypertension, there were 16 (10.5%) with controlled blood pressure of <130/80 and 137 (89.5%) with inadequate blood pressure control of ≥130/80 (Table 6). Insulin-requiring diabetics with metabolic syndrome were 50 (19.6%), including 20 (7.8%) who were on insulin only, and 30 (11.8%) who were on both insulin and oral hypoglycaemic drugs/diet. Non-insulin-requiring diabetics with the metabolic syndrome (Table 6) were 205 (80.4%).

Table 4

Determinants of the metabolic syndrome diagnosed from Ghanaian diabetic patients

Metabolic syndrome component	Diabetic patients N=255		Males N=47		Females N =208		P value
	No.	%	No.	%	No.	%	
Central Obesity	177	69.4	17	36.2	160	76.9	0.001
Hypertriglyceridaemia	146	57.3	35	74.5	111	53.4	0.001
Low HDL Cholesterol	163	63.9	29	61.7	134	64.4	0.16
Blood Pressure ≥ 130/85mmHg and/or on medication	172	67.5	32	68.1	140	67.3	0.236

Table 5

Distribution of major combinations of metabolic syndrome components

Frequent combinations of metabolic syndrome components	Diabetic patients N=255		Males N = 49		Females N=206	
	No.	%	No.	%	No.	%
Hyperglycaemia, central obesity and hypertension	118	46.3	12	24.5	106	51.5
Hyperglycaemia, central obesity and low HDL cholesterol	102	40.0	6	12.2	96	46.6
Hyperglycaemia, hypertension and low HDL cholesterol	94	36.9	17	34.7	77	37.4
Hyperglycaemia, central obesity and hypertriglyceridaemia	89	34.9	11	22.4	78	37.9
Hyperglycaemia, hypertension and hypertriglyceridaemia	88	34.5	21	42.8	67	32.5
Hyperglycaemia, low HDL cholesterol and hypertriglyceridaemia	87	34.1	20	40.8	67	32.5

Table 6
Clinical characteristics of 255 Ghanaian diabetic patients with metabolic syndrome

Sex		
	Male	33.3%
	Female	66.0%
Age (yrs)		
	Mean	57.8±11.1
	<40	4.7%
	40 − 59	54.1%
	≥60	41.2%
Age of onset (yrs)		
	Mean	51.3±11.7
	<20	0.4%
	20 − 34	5.9%
	≥35	93.7%
Duration of diabetes (yrs)		
	Mean	6.3±5.9
	<1	18.8%
	1 − 9	58.5%
	10 − 19	17.2%
	≥20	5.5%
Preprandial glucose (mmol/L)		
	Mean	9.3±4.4
	<5	14.9%
	5.0 − 7.2	19.2%
	>7.2	65.9%
Body mass index (Kg/m^2)		
	Mean	26.9±4.8
	<18.5	0.0%
	18.5 − 24.9	39.6%
	25.0 − 29.9	27.1%
	≥30	33.3%
Hypertension		
	Confirmed, on medication	60.0%
	With controlled blood pressure (<130/80 mmHg)	10.5%
	With inadequate blood pressure control (≥130/80 mmHg)	89.5%
Insulin requiring status		
	Insulin requiring	19.6.6%
	Non-insulin requiring	80.4%

4.4 ASSOCIATION OF METABOLIC SYNDROME WITH GLYCAEMIC CONTROL IN RECENTLY DIAGNOSED GHANAIAN DIABETIC PATIENTS

Forty-eight (40.0%) of the recently diagnosed diabetic patients had good glycaemic control (HbA_{1C} <7.5), whereas 72 (60.0%) had poor glycaemic control (HbA_{1C} ≥7.5%). Fifty-two (43.3%) of the recently diagnosed diabetes patients had metabolic syndrome. Metabolic syndrome was present in 6 (19.4%) males and 46 (51.7%) females. Sixteen (33.3%) of the patients with good glycaemic control had metabolic syndrome and 36 (50.0%) of those with poor glycaemic control had metabolic syndrome. Metabolic syndrome was significantly (P = 0.01) higher in patients with poor glycaemic control than those with good glycaemic control.

4.5 AUTOIMMUNE DIABETES (AUTOANTIBODY PATTERNS) AND AUTOANTIBODY-NEGATIVE TYPE 2 DIABETES IN RECENTLY DIAGNOSED GHANAIAN DIABETIC PATIENTS

A total of 120 recently diagnosed (<1 year) Ghanaian diabetic patients were involved in this part of the study, including 31 (25.8%) males and 89 (74.2%) females. They included 69 (57.5%) Twi-speaking people, 7 (5.8%) Fantes, 11 (9.2%) Guans, 9 (7.5%) Ga-Adangbes, 15 (12.5%) Northern peoples and 6 (5.0%) Ewes. It also included 3 (2.5%), who belonged to other minor Ghanaian tribes. The mean age of this group of diabetic patients was 48.2±13.4

years, with a range of 12.0 – 80.0 years. The mean age of onset of the study population was 47.9±13.4 years with a range of 11.5 – 79.7 years. The mean BMI was 25.7±5.4 kg/m^2 (range 15.1 to 45.0 kg/m^2).

Out of a healthy non-diabetic control population of 60, 2 (3.4%) had autoantibodies to ICA or GAD. This included 1(1.7%) subject with autoantibodies to ICA and another 1 (1.7%) with autoantibodies to GAD. No healthy nondiabetic control subject had autoantibodies to IAA. Among the Ghanaian population of recently diagnosed diabetic patients, 17 (14.2%) were insulin-requiring, whilst the remaining 103 (85.8%) were non-insulin requiring. Among the insulin-requiring diabetic patients, 6 (35.3%) had autoantibodies to ICA and/or GAD. These included 2 (11.8%) who had both ICA and GADab, 5 (29.4%) who had ICA and 3 (17.6%) who were positive for GAD. Since all the insulin-requiring diabetic patients in this study have been treated with insulin, IAA was not measured in them.

Seventeen (16.5%) of the non-insulin requiring patients were positive for ICA and/or GAD and/or IAA. This consisted of 1 (0.9%) who was positive for all three autoantibodies, ICA, GAD and IAA, 2 (1.9%) who were positive for ICA and GAD, 2 (1.9%) who were positive for ICA and IAA and 1 (0.9%) who was positive for both GAD and IAA. Furthermore, 10 (9.7%) of the subjects were positive for ICA, 9 (8.7%) for GAD and 3 (2.9%) for IAA. The 3 (2.9%) patients who were positive for IAA were also positive for ICA and/or GAD. Thus the autoantibody-negative non-insulin requiring recently diagnosed Ghanaian diabetic patients, also referred to as type 2 diabetes were 86 (71.7%) and the unclassified

patients, who were insulin-requiring diabetic patients without autoantibodies, 11 (9.1%).

Fourteen (13.5%) of the recently diagnosed non-insulin requiring diabetics were considered as having latent autoimmune diabetes of adult onset (LADA), since these patients were positive for one or more of ICA, GAD or IAA, age of onset of diabetes >35 years, and insulin therapy not indicated in the first 6 months after diagnosis. Thus, 14 (11.7%) patients among the total recently diagnosed Ghanaian diabetic population had LADA. It was realized that 11 (78.6%) of the LADA group of patients had single autoantibody positivity and 3 (21.4%) multiple (two or more) autoantibody positivity. It was further noted that, 3 (2.9%) of the recently diagnosed non-insulin requiring diabetics had autoimmune diabetes, but not LADA, that is, these patients were positive for one or more of ICA, GAD or IAA, age of onset of diabetes ≤35 years, and insulin therapy not indicated in the first 6 months after diagnosis.

Autoimmune diabetes, excluding LADA, in the non-insulin requiring group and autoimmune diabetes in the insulin-requiring group together gave a total of 9 (7.5%). This represented the number of autoimmune type 1 diabetes in the total recently diagnosed diabetic population. Six (66.7%) of the autoimmune type 1 diabetes patients had single autoantibody positivity, whilst 3 (33.3%) had multiple autoantibody positivity. Six (35.3%) insulin-requiring diabetic patients had autoantibodies or autoimmune diabetes (Figure 1), the non-insulin requiring group 17 (16.5%) and the nondiabetic healthy controls 2 (3.4%). Thus, 23 (19.2%) autoimmune diabetes patients were present among the total recently

diagnosed diabetes population, comprising 4 (3.3%) who wese positive for both ICA and GAD, 15 (12.5%) who were positive for ICA and 12 (10.0%) who were positive for GAD. Finally, 17 (73.9%) of the total autoimmune diabetes patients had single autoantibody positivity and 6 (26.1%) multiple autoantibody positivity.

Twenty-three (19.2%) autoimmune diabetic patients and 86 (71.7%) autoantibody-negative type 2 diabetic patients were present among the 120 recently diagnosed (<1 year) diabetic patients. The mean age of the recently diagnosed autoimmune diabetic patients was 45.4±15.5 years, and it varied from 13.0 to 70.0 years; that of the autoantibody-negative recently diagnosed type 2 diabetic patients was 50.4±11.7 years, with a range of 25.0 to 80.0 years (Table 7). The difference in the mean age between the two groups of patients was not statistically significant (P = 0.09). The mean age of onset of the recently diagnosed autoimmune diabetic patients was 44.8±15.6 years, and it ranged from 12.5 to 69.6 years; the mean age of onset for the type 2 diabetic patients was 50.2±11.6 years, with a range of 24.4 to 79.8 years. There was no statistically significant (P = 0.06) difference in the mean age of onset between the autoimmune diabetic patients and the autoantibody-negative type 2 diabetic patients.

The mean preprandial (fasting) glucose value of the recently diagnosed autoimmune diabetic patients was 9.0±5.9 mmol/L, and it varied from 3.5 to 27.3 mmol/L; that of the type 2 diabetic patients was 7.5±4.0 mmol/L, with a range of 3.3 to 22.0 mmol/L (Table 7). There was no statistically significant (P= 0.16) difference in the mean preprandial glucose between the two groups of patients.

The mean HbA_{1C} value of the recently diagnosed Ghanaian autoimmune diabetic patients was 8.2±3.0%, and it ranged from 4.4 to 14.0%. For the type 2 diabetic patients the mean HbA_{1C} was 7.7±2.2%, with a range of 4.6 to 14.0% (Table 7). Mean HbA_{1C} showed statistically significant (P = 0.01) difference between the autoimmune diabetic patients and the type 2 diabetic patients. The mean value of BMI of the recently diagnosed autoimmune diabetic patients was 25.2±4.5 kg/m^2, and it varied from 18.7 to 33.2 kg/m^2; that of the recently diagnosed type 2 diabetic patients was 26.2±5.3 kg/m^2, with a range of 15.1 to 45.0 kg/m^2 (Table 7). Mean BMI did not show any statistically significant (P= 0.26) difference between the two groups of patients. The recently diagnosed autoimmune diabetic patients with confirmed hypertension on medication were 4 (17.0%) and the type 2 diabetic patients with confirmed hypertension on medication, 23 (26.0%). Confirmed hypertension was significantly (P = 0.001) higher in the type 2 diabetic patients than the autoimmune diabetic patients.

The recently diagnosed autoimmune diabetic patients had a mean waist circumference value of 87.9±11.5 cm, and it ranged from 65.0 to 104.0 cm. Eight (34.0%) were classified as having central obesity (that is, waist circumference >102 cm in males and >88 cm in females). The recently diagnosed type 2 diabetic patients had a mean waist circumference value of 89.9±10.4cm, and it ranged from 66.0 to 131.0 cm. Thirty-three (43.0%) were classified as having central obesity (Table 7). Mean waist circumference was not significantly (P = 0.43) different in the two groups of patients. However central obesity was significantly (P = 0.04) more prevalent in the type 2 diabetic patients than the autoimmune

diabetic patients. The mean total cholesterol value of the recently diagnosed autoimmune diabetic patients was 4.3±1.1 mmol/L and it ranged from 2.2 to 6.5 mmol/L; that of the recently diagnosed type 2 diabetic patients was 4.7±1.3 mmol/L, with a range of 1.5 to 7.4 mmol/L. There was no statistically significant (P= 0.28) difference in the mean cholesterol between the two groups of patients. The recently diagnosed autoimmune diabetic patients mean triglyceride value was 1.3±0.50 mmol/L, and it varied from 0.60 to 2.65 mmol/L. For the type 2 diabetic patients the mean triglyceride value was 1.3±0.6 mmol/L and it ranged from 0.20 to 3.98 mmol/L (Table 7). Mean triglyceride did not show any statistically significant (P= 0.85) difference between the type 2 diabetic patients and the autoimmune diabetic patients.

Mean HDL cholesterol level of the recently diagnosed autoimmune diabetic patients was 1.3±0.50 mmol/L and it varied from 0.39 to 2.16 mmol/L; that of the recently diagnosed type 2 diabetic patients was 1.4±0.50 mmol/L, with a range of 0.43 to 2.80 mmol/L (Table 7). There was no statistically significant (P= 0.40) difference in the mean HDL cholesterol between the two groups of patients. The mean LDL cholesterol value of the recently diagnosed autoimmune diabetic patients was 2.4±0.90 mmol/L and it ranged from 0.40 to 4.75 mmol/L. The mean level for the type 2 diabetic patients was 2.7±1.1 mmol/L and it ranged from 0.07 to 5.03 mmol/L. Mean LDL cholesterol did not show any statistically significant (P= 0.35) difference between the type 2 diabetic patients and the autoimmune diabetic patients. The mean fasting insulin level of the recently diagnosed autoimmune diabetic patients was 7.8±3.1μIU/ml and it ranged from

1.5 to 16.5μIU/ml; that of the recently diagnosed Ghanaian type 2 diabetic patients was 8.5±4.8 μIU/ml, with a range of 1.5 to 19.5 μIU/ml (Table 7). There was no statistically significant (P= 0.49) difference in the mean fasting insulin level between the two groups of patients.

Figure 1
Prevalence of autoantibodies in insulin-requiring and non-insulin requiring recently diagnosed Ghanaian diabetic patients and healthy nondiabetic controls

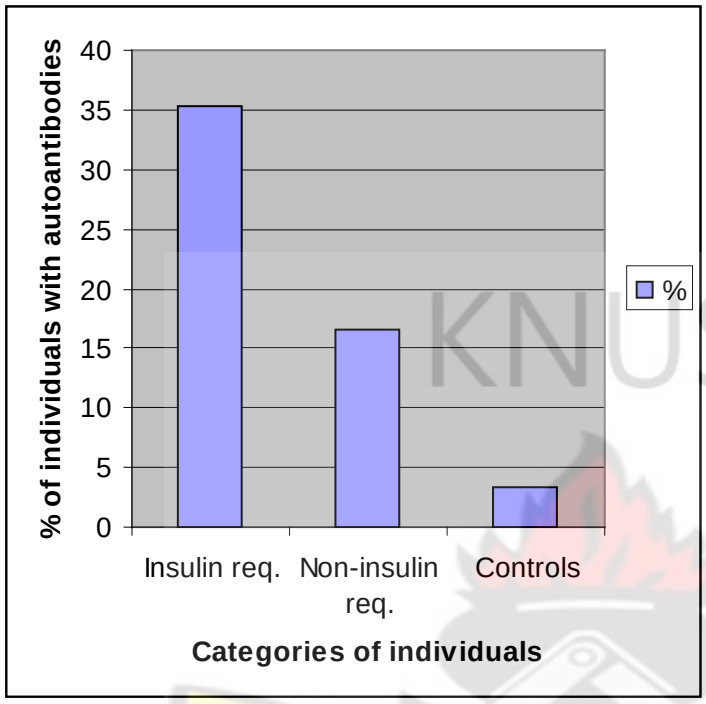

Table 7
Clinical and metabolic characteristics of autoimmune diabetes and autoantibody-negative type 2 diabetes in recently diagnosed Ghanaian diabetic patients

Clinical and metabolic parameters	Autoimmune diabetes (N=23)	Type 2 diabetes (N=86)	P values
Mean age (years)	45.4±15.5	50.4±11.7	0.09
Mean age of onset (years)	44.8±15.6	50.2±11.6	0.06
Mean preprandial glucose (mmol/L)	9.0±5.9	7.5±4.0	0.16
Mean HbA_{1C} (%)	8.2±3.0	7.7±2.2	0.01
Mean BMI (Kg/m^2)	25.2±4.5	26.2±5.3	0.26
Hypertension (no./%)	4 (17.0%)	23 (26.0%)	0.001
Mean waist circumference (cm)	87.9±11.5	89.9±10.4	0.43
Central obesity (no./%)	8 (34.0%)	33 (43.0%)	0.04
Mean total cholesterol (mmol/L)	4.3±1.1	4.7±1.3	0.28
Mean triglycerides (mmol/L)	1.3±0.5	1.3±0.6	0.85
Mean HDL Cholesterol (mmol/L)	1.3±0.5	1.4±0.5	0.40
Mean LDL Cholesterol (mmol/L)	2.4±0.9	2.7±1.1	0.35
Mean insulin (μIU/ml)	7.8±3.1	8.5±4.8	0.49

CHAPTER FIVE

5. DISCUSSION

5.1 CLINICAL CHARACTERISTICS OF GHANAIAN DIABETIC PATIENTS

The total number of people with diabetes mellitus worldwide among adults ≥20 years of age is projected to rise from 171 million in 2000 to 366 million in 2030 (Wild et al., 2004). The "top three" countries estimated to have the highest numbers of people with diabetes mellitus in 2000 and 2030 are India, China and United States of America (King et al., 1998; Wild et al., 2004). About 2.5 million people are living with diabetes mellitus in Africa. Globally, diabetes mellitus prevalence is similar in men and women but it is slightly higher in men <60 years of age and in women at older ages. The prevalence of diabetes mellitus increases with age for both males and females (King et al., 1998; Wild et al., 2004). Most studies in diabetes have been done on type 1 diabetes as a class, and then type 2 diabetes, as a separate class. Therefore, the clinical indices of the total diabetes population in this study would be roughly compared with type 1 and type 2 diabetes.

The ethnic distribution of the diabetic patients in this study was representative of the Ghanaian population. Out of a population of 456 diabetic patients, the percentage of diabetics that were females (69.1%) was significantly (P = 0.001) higher than the percentage that were males (30.9%), giving a male to female ratio of 1:2.2. Therefore there were more Ghanaian females with diabetes

mellitus than males. This is consistent with the observation made by Wild et al. (2004) that, there were more women with diabetes mellitus than men. The combined effect of a greater number of elderly women than men in most populations, and the increasing prevalence of diabetes mellitus with age is the most likely explanation for this observation (Wild et al., 2004). Furthermore, Europeans have a moderate to low prevalence of diabetes mellitus than most racial and ethnic groups worldwide, but higher than the Bantu in Tanzania and the Chinese in Da Qing (King and Rewers, 1993). About half of the male European populations had higher and half had lower prevalences of diabetes than United States non-Hispanic white men, whereas United States non-Hispanic white women had higher prevalences than most of their female counterparts in all age-groups.

The mean age of the diabetics was 55.8 ± 12.3 years. The mean age of females (56.0 ± 12.4 years) was not significantly (P = 0.62) different from that of males (55.4 ± 12.2 years). The mean age was similar to a mean age of 55.2±13.5 found among type 2 diabetes patients from Mexico (Aguilar-Salinas et al., 2003) but different from a mean age of 68.1±11.1 reported from Sweden (Carlsson et al., 2007) and a value of 48.7±16.2 reported for type 1 diabetes (Carlsson et al., 2007). The largest percentage (52.9%) of diabetic patients in this study were found in the age group, 40 – 59 years. However, a sizeable percentage (37.7%), were ≥60 years. Only a low percentage (9.4%) were below 40 years, a value that is comparable to a value of 13.0% obtained for type 2 diabetic patients <40 years (Aguilar-Salinas et al., 2003). This pattern has confirmed that the prevalence of

diabetes mellitus increases with age for both males and females; further in developing countries, the majority of people with diabetes mellitus are in the 45 – 64 years range (King et al., 1998; Wild et al., 2004).

Type 1 diabetes is usually defined as an onset of diabetes before the age of 35 years (Thorn et al., 2005), but may occur after this age. The age of onset of usual type 2 diabetes is ≥45 years (Hillier and Kathryn, 2001), but it may occur in younger people. Thus a clear distinction of diabetes using age of onset is impossible. The mean ages of onset of the total diabetic population, diabetic males and females were 49.7 ± 12.5, 49.6 ± 12.2 and 49.7 ± 12.6 respectively, showing that the mean ages of onset for males and females were not significantly different (P = 0.94). Majority (89.5%) of the diabetes mellitus patients had an age of onset of ≥35 years, that is, late onset, a feature which is more largely associated with type 2 diabetes mellitus. The mean age of onset of type 1 diabetes was found to be 39.7±3.5 in Brazil (Rosario et al., 2005), a value slightly lower than that observed in this study.

The mean duration of diabetes mellitus for the total diabetes population in Brazil was 6.0 ± 5.4 years. There was no statistically significant (P = 0.60) difference in the mean duration of diabetes between females (6.1 ± 5.6 years) and males (5.8 ± 4.9). The mean duration of Saudi type 2 diabetic patients (11±6 years) was higher (Akbar, 2002) than the value obtained for the total diabetic population in this study. A small percentage (18.9%) of the diabetic patients had disease duration of <1 year. A greater percentage (60.5%) had diabetes duration of 1 – 9 years. This fell to 16.7% for the duration of 10 – 19 years, and fell

further to 3.9% for the duration of ≥20 years. Only 9.0% of type 2 diabetic patients had disease duration >10 years (Aguilar-Salinas et al., 2003) in Mexico. The implications are that more than half of the Ghanaian diabetics have diabetes disease duration 1-9 years.

Mean preprandial (fasting) glucose and percentage of populations with abnormal preprandial glucose are rare in the literature. This is because HbA_{1C} values are the standard indicators for glycaemic control. However, preprandial glucose is the more widely used parameter in hospitals, clinics and home self-monitoring of blood glucose in both developed and developing countries. Mean HbA_{1C} (8.5%) in type 2 diabetic patients were found to be high, and 72.1% of the patients had high (>7.0%) HbA_{1C} levels (Miller et al., 2003). Similar to the aforementioned HbA_{1C} values, mean preprandial glucose (9.4 ± 4.5 mmol/L) for the study diabetic population was high. Mean preprandial glucose level did not differ significantly (P = 0.32) in male diabetics (9.7 ± 5.2mmol/L) and females (9.2 ± 4.2). A sizeable percentage, 23.0%, had good preprandial glucose levels of 5.0 – 7.2 mmol/L, whilst a few (14.9%) had low preprandial glucose level of <5.0 mmol/L. Similar to the aforementioned HbA_{1C} values, more than half (62.1%) of the Ghanaian diabetics had a high preprandial glucose level of >7.2 mmol/L, which is outside the near-normal levels (5.0 – 7.2 mmol/L) recommended by American Diabetic Association (2004) for diabetic patients. This calls for more vigorous measures to be put in place in the management of diabetes mellitus at Komfo Anokye Teaching Hospital in Ghana to maintain near-normal glucose levels. This is because maintaining near-normal glucose levels of 5.0 – 7.2

mmol/L minimizes the risks for both hyper- and hypoglycaemic episodes, and for a long time minimizes the risk for microvascular and macrovascular diabetic complications (Goldstein et al., 2004).

Body Mass Index (BMI) mean value of the total diabetes mellitus population, 25.1 ± 4.8 kg/m^2, was different from a value of 29.2±5.7 kg/m^2 and 29.6±4.8 reported for type 2 diabetic patients (Aguilar-Salinas et al., 2003; Carlsson et al., 2007), and a value of 23.1±2.9 observed in type 1 diabetes (Rosario et al., 2005). It was revealed that 21.3% of the diabetic patients were obese, with a BMI ≥30 kg/m^2 whilst 23.2% were overweight with a BMI 25.0 – 29.9 kg/m^2. Thus, 44.5% were overweight or obese or had a BMI ≥25.0 kg/m^2, which is lower than a value of 75.0% overweight or obese type 2 diabetic patients reported in Mexico (Aguilar-Salinas et al., 2003). The mean BMI of males (24.1 ± 4.8 kg/m^2) indicate a normal BMI whilst that of females (25.6 ± 4.7 kg/m^2) indicate overweight, values which indicate that females have a significantly higher BMI (P = 0.029) than males. Similarly, the percentage of female diabetics who were obese (23.5%) and overweight (26.0%) were significantly (P = 0.001; P = 0.001) higher than the corresponding values of 16.3% and 17.0% for male diabetics. This is consistent with earlier results by Akbar (2002) that indicated that obesity was more common in females than males in type 2 diabetic patients. Obesity characterized by excess body fat is probably the most notable risk factor for the development of type 2 diabetes (Edelstein et al., 1997). It is estimated that the risk for type 2 diabetes attributable to obesity is as much as 75.0% (Manson and Spelsberg, 1994). Thus, a higher percentage of Ghanaian female diabetics

(69.1%) than males (30.9%) as a result of obesity and type 2 diabetes were evident.

The concordance of hypertension and diabetes mellitus is increased in populations; hypertension is disproportionately higher in diabetics (Sowers and Haffner, 2002), while persons with elevated blood pressure are 2.5 times more likely to develop diabetes mellitus within 5 years (Sowers and Bakris, 2000) than persons without either condition. In type 1 diabetes, hypertension is often the result of underlying nephropathy. In type 2 diabetes hypertension may be present as part of the metabolic syndrome. The percentage of diabetic patients with confirmed hypertension in this study was 40.1%, a value that supports earlier findings (Sowers and Haffner, 2002). The percentage of diabetics with hypertension, whose blood pressures were not adequately controlled or ≥130/80 was high (91.3%). The percentage of female diabetics (44.4%) with hypertension was significantly (P = 0.001) higher than in males (30.5%).

Geis et al. (2002) reported that 71.0% of all United States of America adults with diabetes were hypertensive, a rate that is exceedingly higher than the Ghanaian value (40.1%). Nevertheless, a rate of 40.1% hypertension in diabetics, with 91.3% of them having inadequate blood pressure control at Komfo Anokye Teaching Hospital is worrying. This is because the coexistence of hypertension and diabetes mellitus is particularly pernicious because of strong linkage of the two conditions with cardiovascular disease (Fagan and Sowers, 1999), stroke (Davis et al., 1999), progression of renal disease (Maki et al., 1995) and diabetic retinopathy. Thus, extra care must be put in place to control blood pressure in

diabetics with hypertension at Komfo Anokye Teaching Hospital. Achieving more stringent blood pressure goals will require increased attention. It has been observed that adequate blood pressure control improves cardiovascular disease outcomes, especially stroke, when aggressive blood pressure targets are achieved (American Diabetes Association, 2003). The United Kingdom Prospective Diabetes Study (UKPDS) (Adler et al., 2000) has demonstrated that 10 mmHg decrease in systolic blood pressure was associated with average reductions in rates of diabetes-related mortality of about 15.0%; and microvascular complications of retinopathy or nephropathy of about 13.0%.

Insulin-requiring diabetic patients were 24.6%, whilst non-insulin requiring diabetic patients 75.4%, giving a ratio of 1:3 respectively. Type 1 diabetes often, but not always, requires insulin treatment, whilst type 2 diabetes usually, but not always, does not require insulin treatment. Nevertheless, insulin dependency is the major clinical criterion for distinguishing between type 1 and 2 diabetes. Thus the ratio of apparent type 1 to type 2 diabetes among Ghanaian diabetic patients was 1: 3. Considering a world percentage of 5 – 10% and 90 – 95% for type 1 and 2 diabetes respectively (ADA, 2005; WHO, 1999), it is likely that, more type 1 diabetics and less type 2 diabetics are present in Ghanaian diabetic patients as compared to other major populations, especially in the developed world.

5.2 PREVALENCE OF THE METABOLIC SYNDROME AND ITS INDIVIDUAL COMPONENTS IN GHANAIAN DIABETIC PATIENTS

Metabolic syndrome has attracted much attention as a risk cluster for cardiovascular disease (CVD) in type 2 diabetes (Isomaa et al., 2001), nondiabetic subjects (Lakka et al., 2002) and recently in type 1 diabetes (Thorn et al., 2005). The NCEP ATP III criteria for the metabolic syndrome were chosen to determine the prevalence of the metabolic syndrome. The NCEP ATP III definition for the metabolic syndrome was chosen rather than the World Health Organization or European Group for the Study of Insulin Resistance criteria because it relies on variables that are easily measurable by physicians and thus is convenient operationally both in clinical practice and in epidemiological studies (Domanski and Proschan, 2004). The latter two definitions include insulin resistance and microalbuminuria (WHO, 1999) which are not easily measurable. In addition, the NCEP ATP III proposal, but not the WHO criteria, more clearly identify the burden of coronary heart or cerebrovascular disease associated with the metabolic syndrome. The metabolic syndrome is associated with a 38.0% increased risk of coronary heart disease (Marchesini et al., 2004; Scuteri et al., 2005). The results in this study are specific for the Ghanaian population because it has been shown that metabolic syndrome varies substantially with ethnicity, even after adjustments for BMI, age, socioeconomic status and other predictor variables (Park et al., 2003; Ford et al., 2002; Meigs et al., 2004). As much as possible, care was taken to compare the results in this study only with results from the

literature that were produced according to the NCEP ATP III criteria because some discrepancies have been observed depending on the definition chosen.

The prevalence of the metabolic syndrome defined according to the NCEP ATP III criteria for Ghanaian diabetic patients (55.9%) was significantly (0.0001) higher than that in age- and sex-matched Ghanaian nondiabetic controls (9.2%). Thus a low value of the metabolic syndrome in Ghanaian nondiabetics shows that in the absence of diabetes the prevalence of the metabolic syndrome is minimal. The prevalence of the metabolic syndrome in female diabetics (66.0%) was significantly (P = 0.001) higher than in males (33.3%). Individuals with the metabolic syndrome are at increased risk for CHD (Lakka et al., 2002). In Framingham, the metabolic syndrome alone predicted about 25% of all new-onset CVD (Wilson et al., 1998). In the absence of diabetes, the metabolic syndrome generally did not raise 10-year risk for CHD to >20%; this is the threshold for NCEP ATP III's CHD risk equivalent. Mortality and morbidity studies in diabetes have revealed that two-thirds to three-quarters of patients with diabetes mellitus will eventually die of CVD (Bloomgarden, 2003). Thus, once detected, vigorous and early management of the metabolic syndrome, may have a significant impact on the prevention of CVD (Eriksson and Lindegarde, 1991).

Metabolic syndrome prevalence rates have been mostly determined separately for type 1 and type 2 diabetes and for general populations. However, recently, and after commencement of this study, the prevalence of the metabolic syndrome has been found to be 68.5% (Loizou et al., 2006) among diabetic patients in the Republic of Cyprus by the NCEP ATP III criteria, a value that is

slightly higher than the value of 55.9% obtained among Ghanaian diabetics in this study. This could be explained by the difference in the prevalence of the individual components of the metabolic syndrome between different ethnic groups. In the same study, the prevalence of the metabolic syndrome was 86.1% among subjects with IFG, 43.6% among those with IGT, 22.2% in the overall population and 12.3% among subjects with normal glucose tolerance.

The prevalence value of 55.9% for the total diabetes population arrived at in this study is higher than a value of 39.0% obtained for Finnish type 1 diabetes patients (Thorn et al., 2005); but lower than a value of 78.0% obtained for Caucasian type 2 diabetes patients (Marchesini et al., 2004), all by the NCEP ATP III criteria. The prevalence rate of the metabolic syndrome of 55.9% among Ghanaian diabetic patients is understandable because the prevalence of the metabolic syndrome in type 2 diabetes is higher than type 1 diabetes, therefore logically the prevalence rate of the total diabetes mellitus population (mainly type 1 and 2 diabetes) should lie between the two values. The prevalence rate of 55.9% of the metabolic syndrome obtained for Ghanaian diabetics is higher than values of 24.0% and 23.0% (Meigs et al., 2003) obtained for Caucasian general population and a value of 23.9% (Ford et al., 2002) obtained for American adults, all by the NCEP ATP III definition.

The prevalence of the metabolic syndrome in a cohort of patients suffering from atherosclerotic CVD was 46%, and in coronary artery disease patients 41% (Goter et al., 2004), values which are lower than that of the Ghanaian diabetic patients. This is because, although microvascular disease is relatively specific for

diabetes mellitus, macrovascular disease appears as an acceleration of the illness seen in persons without diabetes. This group of patients therefore contributed to the large percentage of CVD patients without metabolic syndrome. It is also understandable why metabolic syndrome should be determined in total diabetes mellitus populations, since it allows direct comparison, not only with atherosclerosis, but other disease states such as hypertension and obesity.

It was observed that 5.7% and 21.7% of the Ghanaian diabetic patients had metabolic scores of five and four respectively. These 27.4% (5.7+21.7%) of the diabetic patients are said to have severe metabolic syndrome. In other words they carry higher risk for cardiovascular disease. It was revealed that 28.0% of the diabetic patients had a metabolic score of three, that is, they satisfy the minimum requirements of the metabolic syndrome and hence carry cardiovascular risk. Further, 29.4% and another 14.5% had metabolic scores of two and one respectively, and did not have metabolic syndrome. Nevertheless, the management of diabetic patients with metabolic score of one and two should focus on strategies for reduction of these minimal CVD risk factors. This is because each component of the cluster alone conveys increased CVD risk, but as a combination, they become much more powerful (Kaplan, 1989). A greater percentage of female diabetics had metabolic scores of five (7.6%) and four (26.3%) than their male counterparts, where the same values were 1.4 and 11.3% respectively. On the other hand a greater percentage of males had metabolic scores of two (41.1%) and one (25.5%) than female diabetics where the same values were 24.1% and 9.5% respectively. These indicate that female diabetics

individually carry more metabolic syndrome factors or risk for CVD than males. Four of the five diagnostic criteria of the metabolic syndrome were observed in 14.0% males and 13.0% of female type 1 diabetes patients (Thorn et al., 2005). This is slightly higher than the value of 11.3% obtained for Ghanaian male diabetics but lower than the value of 26.3% obtained for Ghanaian female diabetics.

Type 2 diabetes is associated with devastating complications usually classified as microvascular (retinopathy, nephropathy, neuropathy) and macrovascular (coronary vascular disease, stroke, peripheral vascular disease). Cardiovascular disease (CVD) complications are far more common than microvascular complications and are the leading cause of death and disability in type 2 diabetes (Shantaram, 1999). Multiple risk factors are associated with CVD in type 2 diabetic patients, including hypertension, hyperlipidaemia and obesity (Haffner, 2000). These risk factors are also the main features of the metabolic syndrome. These same components have been observed in type 1 diabetes (Thorn et al., 2005), and potentially contribute to increased cardiovascular risk. Since type 1, like type 2 diabetes patients, carry cardiovascular risk factors, it was imperative to consider the prevalence of these factors in the overall diabetic population which is composed mostly of type 1 and 2 diabetes for comparative, clinical and epidemiological purposes.

Low HDL cholesterol was the commonest component of the metabolic syndrome in the Ghanaian diabetic patients, central obesity the commonest in female diabetics and hypertension the commonest in males. The prevalence of

the metabolic syndrome components in the Ghanaian diabetic population, male diabetics and female diabetics were different. Knowing the commonest risk factor in different populations will provide a guide to prevention and treatment. These results are in fair agreement with Al-Lawati et al (2003), who, using the NCEP ATPIII criteria found low HDL cholesterol to be the commonest component, though followed by abdominal (central) obesity. The percentage of female diabetics (53.0%) with low HDL cholesterol was significantly (P = 0.001) higher than in males (34.8%). Similarly, a significantly (P = 0.0001) larger proportion of female diabetics (57.1%) than males (13.5%) had central obesity, a result that is consistent with Al-Lawati et al (2003). Additionally, a significantly (P = 0.001) greater proportion of female diabetics (50.2%) than males (39.7%) were hypertensive. However, the percentage of female diabetics (38.1%) with hypertriglyceridaemia was not significantly (P = 0.18) different from their male (36.2%) counterparts. In comparing type 1 and 2 diabetes with overall diabetic population, hypertension was the most frequent component in type 1 diabetes (Thorn et al., 2005), dyslipidaemia the commonest in type 2 diabetes (Isomaa et al., 2001) and low HDL cholesterol the commonest in Ghanaian diabetic patients, all by the NCEP ATP III criteria.

The reductions in HDL associated with type 2 diabetes and insulin resistance are multifactorial, but a major factor appears to be increased transfer of cholesterol esters from HDL to apo B_{100}-containing triglyceride-rich lipoproteins (VLDL, IDL), with reciprocal transfer of triglyceride to HDL. Triglyceride-rich HDL particles are hydrolyzed by hepatic lipase and, as a result, are rapidly

catabolized and cleared from plasma (Hopkins and Barter, 1986). Typically, the reduced HDL levels in plasma of patients with type 2 diabetes are manifest as reductions in the HDL_{2b} subspecies and relative or absolute increases in smaller denser HDL_{3b} and HDL_{3C}. It is well documented that reduced HDL cholesterol levels are associated with an increased risk of coronary heart disease (CHD) (Gordon et al., 1989). A number of functions of HDL particles may contribute to direct cardioprotective effects, including promotion of cellular cholesterol efflux and direct antioxidative and anti-inflammatory properties. Moreover, low HDL cholesterol levels are often accompanied by elevated triglyceride levels (Larmache et al., 1996), and the combination has been strongly associated with an increase risk of CHD (Assmann and Schulte, 1992; Manninen et al., 1992). Increased levels of both HDL_2 and HDL_3 particles may have cardioprotective effects (Lamarche et al., 1997; Rubins et al., 1999). Individuals with type 2 diabetes and CHD tend to have small HDL particles (Syvanne et al., 1995). In addition hypertriglyceridaemia is independently associated with low levels of HDL_2 and small HDL particle size.

The evidence for a relationship between plasma triglyceride levels and the risk of coronary artery disease (CAD) is largely based on epidemiologic studies. A meta-analysis of 17 population-based prospective studies found that for each 1 mmol/L increase in plasma triglyceride, there is a 32.0% increase in coronary disease risk for men and a 76% increase in risk for women (Hokanson and Austin, 1996). Adjustment for the effects of HDL cholesterol and other risk factors attenuated the risk to 14.0% in men and 37.0% in women, but these values

remained statistically significant. Direct atherogenic effects of triglyceride-rich particles, especially intermediate density lipoprotein (IDL) and remnant lipoproteins, may account for this independent contribution of plasma triglyceride levels to coronary diasease risk (Krauss, 1998).

5.3 DETERMINANTS AND CLINICAL FEATURES OF THE METABOLIC SYNDROME DIAGNOSED FROM GHANAIAN DIABETIC PATIENTS

Today, there is a growing interest in a cluster of synergistically interacting cardiovascular risk factors called metabolic syndrome. The syndrome is increasingly recognized as a risk factor for diabetes mellitus, CVD (Ford et al., 2002; Isomaa et al., 2001) and cardiovascular mortality (Trevisan et al., 1998). The major determinant of the metabolic syndrome in different populations when determined would serve as targets for interventions that address metabolic syndrome and hence cardiovascular disease in diabetic patients. Central obesity was the major determinant (69.4%) of the metabolic syndrome among Ghanaian diabetics with the metabolic syndrome, followed by hypertension (67.5%). Similarly, in females central obesity was the major determinant (76.9%) of the metabolic syndrome, followed by hypertension (67.3%). A different pattern was observed in males in whom the major determinant was hypertriglyceridaemia (74.5%), followed by hypertension (68.1%). Central obesity was significantly (P = 0.001) more prevalent in female metabolic syndrome diabetics than males while hypertriglycaeridaemia was significantly (P = 0.001) more prevalent in male metabolic syndrome diabetics than females. However low HDL cholesterol and

hypertension were not significantly (P = 0.16, 0.236 respectively) different in the metabolic syndrome female and male diabetics. Knowing the major determinant of metabolic syndrome would provide a better guide to early prevention and treatment than commonest metabolic syndrome factors. Though not exactly the same, Palaniappan et al. (2004) found central obesity to be the dominant predictor, followed by low HDL cholesterol in nondiabetic adults by the NCEP ATPIII criteria.

The same idea of the major determinant of the metabolic syndrome was revealed by the fact that hyperglycaemia, central obesity and hypertension (46.3%) was the most frequent combination of different components among diabetics with the metabolic syndrome. Similarly, in females, hyperglycaemia, central obesity and hypertension (51.5%) was the most frequent combination of different components among diabetics with the metabolic syndrome. A different pattern was observed in male diabetics with the metabolic syndrome in whom, hyperglycaemia, hypertriglyceridaemia and hypertension was the most frequent combination of different components (42.8%). In Finnish type 1 diabetic patients, hyperglycaemia, low HDL cholesterol and hypertension was the most frequent combination of different components (Thorn et al., 2005). Thus the most frequent combination of different components of the metabolic syndrome in Ghanaian diabetics with the metabolic syndrome is different from that observed in Finish type 1 diabetic patients.

These results suggest that obesity is the dominant factor of metabolic syndrome in Ghanaian diabetics with the metabolic syndrome. Thus interventions

that address obesity and reduce waist circumference may reduce the prevalence of the metabolic syndrome in Ghanaian diabetics. Obesity is a well-established risk factor for CHD in the general population (Rimm et al., 1995); Willett et al., 1995). It otherwise associates with higher CVD risk (Grundy et al., 2004). Abdominal (central) obesity especially correlates with metabolic risk factors. NCEP ATPIII considered "obesity epidemic'' as mainly responsible for the rising prevalence of the metabolic syndrome. Obesity contributes to hypertension, high serum total cholesterol and triglyceride, low HDL cholesterol, hyperglycaemia and increases oxidative stress (Serrano, 1998). The strong association between abdominal obesity and metabolic risk factors led NCEP ATPIII to define the metabolic syndrome essentially as a clustering of metabolic complications of obesity.

Obesity also decreases fibrinolysis, increases erythrocyte aggregation, and induces endothelial dysfunction (Perticone et al., 2001). Acute-phase C-reactive protein, a marker of systemic inflammation and a significant predictor of CHD, was increased in overweight individuals (Visser et al., 1999). Among those with diabetes, obesity may aggravate glucose intolerance, hyperinsulinaemia and insulin resistance (Perriello et al., 1995). Thus several studies have reported that moderate weight loss improves cardiovascular risk profiles such as glycaemic control, insulin sensitivity, blood pressure and blood cholesterol in obese patients with type 2 diabetes (Lean et al., 1990; Goldstein, 1992; Torjesen et al., 1997).

The major determinant of the metabolic syndrome was central obesity whilst the commonest metabolic syndrome factor in the total diabetes mellitus

population was low HDL cholesterol. In male diabetics the major determinant of the metabolic syndrome was hypertriglyceridaemia whilst the commonest metabolic syndrome factor was hypertension. In female diabetics central obesity was the major determinant of the metabolic syndrome as well as the commonest metabolic syndrome factor. This pattern clearly shows that the major determinant of the metabolic syndrome may not necessarily be the most prevalent metabolic syndrome component of the population under consideration. This is partially explained by the fact that the most prevalent metabolic syndrome factor of a population may not be necessarily involved in the clustering of factors that determine the metabolic syndrome. Thus, we need to determine the major determinant of the metabolic syndrome rather than the most prevalent metabolic syndrome factors in a population under consideration. However if both are determined we need always to distinguish between the major determinant of the metabolic syndrome and the commonest metabolic syndrome factor of the population involved.

Out of a population of 255 diabetic patients with the metabolic syndrome, the percentage of females (66.0%) was significantly (P = 0.001) higher than the percentage of males (33.3%), giving a male to female ratio of 1:2.0. Therefore there were more Ghanaian female diabetics with the metabolic syndrome (66.0%) than males (33.3%). It was revealed that more than half (54.1%) of the metabolic syndrome group of diabetic patients were 40 – 59 years, whilst an appreciable percentage (41.2%) were ≥60 years. Only a small percentage (4.7%) was below 40 years. Thus the metabolic syndrome is prevalent in Ghanaian diabetics who

are 40 years and above. This is of concern because metabolic syndrome has been found to be associated with a significant CVD risk particularly in men aged ≥45 years and women aged ≥55 years (Lorenzo et al., 2007), values that are consistent with that in this study. Advancing age probably affects all levels of pathogenesis, which likely explains why prevalence of the metabolic syndrome rises with advancing age (Ford et al., 2002).

Another indicator of the metabolic syndrome in Ghanaian diabetic patients is the fact that a very large percentage (93.7%) had late onset (≥ 35 years) diabetes mellitus. More than half (58.5%) of the metabolic syndrome group of diabetic patients had diabetes duration of 1 – 9 years; low percentages of 18.8%, 17.2%, 5.5% were observed for the duration <1 year, 10 – 19 years and ≥20 years respectively. Thus metabolic syndrome is mostly observed during 1 – 9 years of diabetes disease duration and should be the target group for monitoring.

A high percentage (65.9%) of metabolic syndrome group of diabetics had high preprandial glucose levels of >7.2 mmol/L. Diabetes or preprandial glucose levels of ≥6.1 is an indicator of the metabolic syndrome, more so high preprandial glucose levels of >7.2 mmol/L. The diabetics with metabolic syndrome had 27.1% overweight (BMI of 25.0 – 29.9 kg/m^2) and 33.3% obese (BMI of ≥30 kg/m^2) patients, giving a total percentage of obese and overweight patients as 60.4%. BMI is correlated with the metabolic syndrome in both men and women (Park et al., 2003). In addition, due to the fact that abdominal obesity is also correlated with the metabolic syndrome, NCEP ATP III uses it rather than BMI. This becomes important in overweight individuals with a BMI of 25.0 to 29.9

kg/m^2 and large waist circumference (>102 cm in men and >88cm in women) who may have metabolic syndrome despite not being obese (Park et al., 2003). This is confirmed by a substantial percentage of 27.1% who were overweight and had metabolic syndrome in this study.

It was observed that 19.6% of the diabetics with the metabolic syndrome were insulin-requiring, whilst 80.4% were non-insulin requiring. The ratio of insulin-requiring to non-insulin requiring metabolic syndrome diabetics is 1:4. In other words the ratio of apparent type 1 diabetes to apparent type 2 diabetes among diabetics with metabolic syndrome in Ghana was 1:4; showing a preponderance of apparent type 2 diabetes in the Ghanaian metabolic syndrome diabetic population. It is clear that type 1 diabetes, like type 2 diabetes, is associated with an increased risk for CHD and that this risk is evident at a young age. Underlying this enhanced risk is a wide range of modifiable risk indicators such as standard CHD risk factors (blood pressure, lipids, and smoking), as well as specific elements such as renal disease (Orchard et al., 2006).

5.4 ASSOCIATION OF METABOLIC SYNDROME WITH GLYCAEMIC CONTROL IN RECENTLY DIAGNOSED GHANAIAN DIABETIC PATIENTS

CVD is the leading cause of death in type 2 diabetes (De Marko et al., 1999). Poor glycaemic control and metabolic syndrome are all risk factors for CVD (Grundy et al., 2004; Isomaa et al., 2001). The distribution of glycaemic control among recently diagnosed diabetic patients was 40.0% with good

glycaemic control (HbA_{1C} <7.5%), that is, controlled diabetes and 60.0% with poor glycaemic control (HbA_{1C} ≥7.5%), that is, poorly controlled diabetes. Thus, showing that, 60.0% of the patients may be associated with CVD by HbA_{1C} value. The prevalence of the metabolic syndrome among the recently diagnosed diabetic patients was 43.3%. This implies that 43.3% of the patients carry risk for CVD by metabolic syndrome prevalence value. Poor glycaemic control (60.0%) was the more sensitive indicator of CVD than metabolic syndrome (43.3%) by this study. The prevalence of the metabolic syndrome in patients with poor glycaemic control (50.0%) was significantly (P =0.001) higher than that in patients with good glycaemic control (33.3%). Thus the metabolic syndrome was associated with worsening glycaemic control. This confirms earlier reports by Thorn et al. (2005) that metabolic syndrome is correlated with poor glycaemic control.

5.5 AUTOIMMUNE DIABETES (AUTOANTIBODY PATTERNS) AND AUTOANTIBODY-NEGATIVE TYPE 2 DIABETES IN RECENTLY DIAGNOSED GHANAIAN DIABETIC PATIENTS

For a clinician, the distinction between type 1 and type 2 diabetes is not always straightforward. The presence or absence of islet autoantibodies is one of the more direct ways to distinguish between type 1 and type 2 diabetic patients. It is now believed that among the non-insulin requiring diabetic subjects at diagnosis, a significant minority are islet cell antibody-positive (Schiel and Muller, 2000). These patients who clinically are difficult to distinguish from type

2 diabetic subjects test positive for those markers that characterize patients with type 1 diabetes. The term latent autoimmune diabetes in adults (LADA) was introduced to define adult diabetic patients initially non-insulin requiring but with immune markers of type 1 diabetes that, in a number of cases, progress to insulin dependency (Tuomi et al., 1993). Autoantibodies against islet antigens allow us to clearly distinguish autoimmune diabetes in adults from autoantibody-negative type 2 diabetes and provide the strongest evidence that autoimmune diabetes are autoimmune disorders.

A wide variation in the prevalence of autoantibodies in both European and non-European populations has been described. This is due to the differences in the markers or autoantibodies chosen to define the condition, the characteristics of the patients (e.g. newly, or recently, diagnosed) and the population under consideration (e.g. type 1, type 2, insulin-requiring, non-insulin requiring etc.). Others include study design and inclusion criteria (e.g. age) and specificity and sensitivity of the antibody assay methods. The lack of standardized approach to the estimation of autoantibody prevalence prevents the direct comparison of existing data between studies or different countries or populations. Such a comparison could be extremely useful for establishing the size of the autoimmune diabetes, including LADA population and to see whether the prevalence of autoimmune diabetes incuding LADA differs in different countries. Thus only rough comparisons can be made.

A total of 120 recently diagnosed (< 1 year) Ghanaian diabetic patients were involved in this aspect of the study, with a male to female ratio of 1:3. The

tribal distribution of the diabetic patients in this study was representative of the Ghanaian population. The prevalence of ICA and GAD autoantibodies in the healthy population has been reported elsewhere to be 0.1– 0.5% (Riley and MacLaren, 1984) and 1– 2% (Niskanen et al., 1995). Other studies found the prevalence of GAD to be 0.6% and 1.7% in healthy subjects (Takeda et al., 2002; Motola et al., 1999). In this study each of ICA and GAD autoantibody was found to have an incidence of 1.7% among 60 Ghanaian healthy nondiabetic control adults. The prevalence of these autoantibodies observed in this study among the Ghanaian healthy nondiabetic subjects, as well as the reported values, were low.

The comparison of studies measuring GAD autoantibodies in non-European populations has shown that these autoantibodies are rare in Filipinos and patients of African origin (Medici et al., 1999). Similarly, Oli et al. (1980) found that ICA was rare in Nigerians. In contrast, among the insulin-requiring group (apparent type 1 diabetes) of the recently diagnosed diabetics in this study, 35.3% of the patients were positive for ICA and GAD. This means they were autoimmune diabetes patients. This was made up of 29.4% who were positive for ICA and 17.6% who were positive for GAD autoantibodies. It has been documented that ICA and autoantibodies to GAD are characteristic of the immune-mediated form of type 1 diabetes and are present in 70.0 – 80.0% of Caucacian patients with new-onset type 1 diabetes (Atkinson and Maclaren, 1993; Kawasaki and Eisenbarth, 1996). ICA has been reported to be present in 70.0% of patients with recent onset IDDM (Irvine et al., 1977; Landrum et al., 1976). Studies in America by Neufield et al. (1980) have shown that in White Caucasian

type 1 diabetes patients, ICA was detected in 45% after 1 year of diagnosis. This latter value is roughly higher than the value of 29.4% obtained in this study. Autoantibodies to GAD have been found in 70.0 – 90.0% of type 1 diabetic patients and been shown to be the most sensitive single marker for identifying persons at risk for developing diabetes (Vandewalle, 1995). A positive GAD autoantibody was found in 31.8% of South African Black and Indian subjects with type 1 diabetes (Motola et. el., 1999), a value which is roughly higher than the value of 17.6% obtained in this study. This roughly indicates that, the prevalence of GAD and ICA autoantibodies in Ghanaian recently diagnosed insulin-requiring diabetic patients or apparent type 1 diabetes is lower than that reported for Caucacian type 1 diabetic patients.

Among the non-insulin requiring recently diagnosed (<1year) diabetic patients 16.5% were positive for ICA and/or GAD and/or IAA, a value that lies within the percentage range of 10.0 – 20.0% reported for the world population (Niskanen et al., 1995; Leslie and Pozzilli, 1994). This included 9.7% who were positive for ICA, 8.7% for GAD and 2.9% for IAA. This shows that IAA is rare in both Ghanaian healthy control individuals and non-insulin requiring recently diagnosed Ghanaian diabetics. The very few (2.9%) non-insulin requiring patients who were positive for IAA were also positive for ICA and/or GAD. Therefore measuring IAA does not provide any additional information and hence it is not so beneficial. The presence or absence of autoantibodies is one of the most direct ways to distinguish between type 1 and 2 diabetic patients. Thus the autoantibody-negative non-insulin requiring recently diagnosed diabetic patients,

also referred to as type 2 diabetes, was found to be 71.7% and the unclassified patients, who were insulin-requiring diabetic patients without autoantibodies, 9.1%. It has been documented that the presence of ICA and/or GAD autoantibodies is the best predictor of early insulin requirement (Turner et al., 1997). Of the patients with both ICA and GAD, 94.0% required insulin therapy versus only 14.0% of those without either autoantibody.

Autoantibodies to GAD has been found to be a useful predictive marker for the development of insulin dependency in type 2 diabetes (Tuomi et al., 1999; Turner et al., 1997; Zimmet et al., 1994). The autoantibody to GAD positivity of 8.7% obtained in this study for recently diagnosed non-insulin requiring diabetic patients lies within the range of 6 – 10% obtained for Caucasian non-insulin dependent diabetic patients (Niskanen et al., 1995; Tuomi et al., 1999). In a Japanese study, 10.0% of non-insulin treated diabetic patients with duration of disease <5 years had GAD autoantibodies (Kasuga et al., 1996). Gottsater et al. (1995) found that at onset, 24.0% of Swedish non-insulin treated patients had GAD autoantibodies. Another Swedish study reported that the prevalence of GAD was 3.2% in apparent type 2 diabetic patients (Wroblewski et al., 1998). Up to 16.0% of Chinese apparent type 2 diabetic patients were found to be GAD autoantibody-positive (Thai et al., 1997). The United Kingdom Prospective Diabetes Study (UKPDS) reported that, among patients with apparent type 2 diabetes, the frequency of autoantibodies to GAD, ranged from 34.0% in those aged 25 – 35 years at diagnosis to 7.0% in those aged 55 – 65 years at diagnosis

(Turner et al., 1997). These prevalence rates are similar, lower or higher than the Ghanaian value.

The term latent autoimmune diabetes in adults (LADA) was introduced to define adult diabetic patients initially non-insulin requiring but with immune markers of type 1 diabetes that, in a number of cases, progress to insulin dependency (Tuomi et al., 1993). The concept of latency indicates patients of adult age who do not require insulin at least for some time after diagnosis and who possess immunological and genetic features typical of type 1 diabetes. LADA patients have several features of classic type 1 diabetes in addition to islet cell and GAD autoantibody positivity, including high rates of HLA-DR3 and DR4 (Niskanen et al., 1995; Caillat-Zucman et al., 1992). Adults with non-insulin requiring diabetes who are positive for GAD and/or ICA require insulin treatment significantly earlier after diagnosis than GAD^- and ICA^- patients (Leslie and Pozzilli, 1994). Epidemiological data demonstrate that LADA accounts for 2.0 – 12.0% of all cases of diabetes (Turner et al., 1997). The recently diagnosed (<1 year) non-insulin requiring diabetic patients had 13.5% of the patients with LADA. Thus the total recently diagnosed Ghanaian diabetes population had 11.7% patients with LADA, a value that is consistent with the aforementioned values reported by Turner et al. (1997). The prevalence of single autoantibody positivity in the LADA group of diabetics was 78.6% and multiple (two or more) autoantibody positivity 21.4%.

Measurement of autoantibodies is also of significant value for the diagnosis and management of diabetes. The prevalence of autoimmune type 1

diabetes in the recently diagnosed diabetic population was found to be 7.5%. Thus, providing evidence that autoimmune type 1 diabetes occurs in the Ghanaian diabetic population. The prevalence of single autoantibody positivity in the Ghanaian recently diagnosed autoimmune type 1 diabetes patients was 66.7% whilst multiple autoantibody positivity was 33.3%. The prevalence of autoimmune diabetes in the Ghanaian recently diagnosed diabetes population was found to be 19.2%. This included 12.5% who were positive for ICA and 10.0% who were positive for GAD. Among the recently diagnosed autoimmune diabetes patients the prevalence of single autoantibody positivity was 77.3% and multiple autoantibody positivity 22.7%.

In the population-based Cremona study in Italy, autoantibodies to GAD were found in 2.8% of those with diabetes (Bosi et al., 1999), whereas, a higher prevalence of GAD positivity (>20%) was observed in Northern Italy (Bruno et al., 1999). A study in Papua New Guinea found an absence of GAD autoantibodies, indicating low autoimmune diabetes in this ethnic group (Dowse et al., 1994). In contrast, a Swedish study (Wroblewski et al., 1998) reported that the prevalence of GAD autoantibodies was 8.0% overall in newly diagnosed diabetic patients. A Dutch population-based study (Ruige et al., 1997) found GAD autoantibody positivity in 3.5% of patients already known to have diabetes. These prevalence rates cannot be directly compared with the value obtained in this study due to differences in inclusion criteria and assay methods. However, it is apparent from these existing data that, autoimmune diabetes including LADA could represent a sizeable proportion of diabetes. Accurate estimates of prevalence have

important connotations, not only for the correct classification of diabetes, but also for the potential of developing early intervention strategies and estimating the magnitude of the public health problem that this subtype of diabetes presents.

Single autoantibody positivity was significantly (P = 0.02) higher in LADA (78.6%) than autoimmune type 1 diabetes (66.7). Conversely, multiple autoantibody positivity was significantly (P = 0.01) higher in autoimmune type 1 diabetes (33.3) than LADA (21.4). Similarly, single autoantibody positivity was found to be significantly (P = 0.0001) higher in LADA (59%) than in adult-onset type 1 diabetes (23%) among patients in Hungary (Hosszufalusi et al., 2003). In the same study, the titres of ICA and GAD were not different among LADA, adult-onset type 1 diabetes and type 2 diabetes. Based on this observation, the authors concluded that the presence of single autoantibody positivity rather than low titre indicates the less aggressive destruction of islet cells. Single autoantibody positivity (ICA or GAD) was mainly responsible for both LADA and autoimmune type 1 diabetes rather than multiple autoantibody positivity in recently diagnosed Ghanaian diabetic patients. This implies that both ICA and GAD tests should be used to identify autoimmune diabetes among Ghanaian diabetic patients. Though, ICA has higher prevalence than GAD in this study and earlier studies (Kobayashi et al., 1993), ICA in type 2 diabetes has been found to be low in titer and sometimes fluctuate or disappear (Kobayashi et al., 1987; Christie and Delovitch, 1990; Hosszufalusi et al., 2003). In addition, it has been reported that, at diagnosis, both ICA and GAD autoantibodies were shown to be predictors of insulin dependency, but GAD autoantibodies persisted for a longer

time and had higher sensitivity as predictors than ICA (Tuomi et al., 1999). Therefore combined measurement of ICA and GAD autoantibodies provide more useful information. Autoimmune diabetes among the recently diagnosed diabetes patients was mainly due to the presence of ICA and GAD autoantibodies. Seissler et al. (1998) confirmed that ICA and GAD autoantibodies indicate slow disease progression or less aggressive destruction of islet cells; hence autoimmune diabetes among Ghanaian diabetic patients would be expected to progress slowly rather than have an acute onset.

It has been documented (Hosszufalusi et al., 2003) that, there were no differences between LADA and adult type 1 diabetes with respect to clinical and metabolic indicators. The recently diagnosed Ghanaian autoimmune diabetes patients, who were adult autoimmune type 1 and LADA patients were therefore conveniently grouped together and compared with the recently diagnosed Ghanaian autoantibody-negative type 2 diabetic patients. Age and age of onset were all not significantly (P = 0.09, and 0.06 respectively) different in type 2 diabetes and autoimmune diabetes. Similarly, preprandial glucose level was not significantly (P = 0.16) different in autoimmune diabetes and type 2 diabetes. These observations are in conformity with the already known characteristics of autoimmune type 1 as compared to type 2 diabetic patients. (Hosszufalusi et al., 2003; Tuomi et al., 1999; Isomaa et al., 1999). HbA_{1C} levels were significantly (P = 0.01) higher in autoimmune diabetes than type 2 diabetic patients. This was in contrast to earlier reports which found no difference in the levels of HbA_{1C} in

LADA, adult-onset type 1 diabetes and type 2 diabetes (Hosszufalusi et al., 2003; Tuomi et al., 1999; Isomaa et al., 1999).

The type 2 diabetes patients in this study had a higher prevalence of hypertension (P = 0.001) and central obesity (P = 0.04) than the autoimmune diabetic patients. Similarly, Hosszufalusi et al. (2003) found the prevalence of hypertension to be higher in type 2 diabetics than LADA and autoimmune type 1 diabetes. Further, in this study, values for BMI, waist circumference, total cholesterol, triglycerides, HDL cholesterol, and LDL cholesterol were statistically not significant (P = 0.26, 0.43, 0.28, 0.85, 0.40, 0.35 respectively) in the two groups of patients. In contrast, higher values for BMI, total cholesterol and triglyceride but lower levels of HDL cholesterol in type 2 diabetes as compared to type 1 diabetes and LADA has been reported (Hosszufalusi et al., 2003; Tuomi et al., 1999; Isomaa et al., 1999). In this study, insulin levels were found to be comparable (P = 0.49) in autoimmune diabetes and type 2 diabetes. A similar parameter C-peptide was found to be lower in type 1 diabetes and LADA than type 2 diabetes at diagnosis, but the difference disappeared with progression of disease.

Clinical and metabolic parameters that were not significantly different (P = ns) in Ghanaian autoimmune diabetic and autoantibody-negative type 2 diabetic patients included age, age of onset, preprandial glucose, HbA_{1C}, BMI and waist circumference. Others were total cholesterol, triglyceride, HDL cholesterol, LDL cholesterol and insulin. The exceptions were hypertension and central obesity, which were significantly (P = 0.001, P =0.01 repectively) higher in autoantibody-

negative type 2 diabetes than autoimmune diabetes and HbA_{1C} which was significantly (P = 0.01) higher in autoimmune diabetes than type 2 diabetes. Therefore, generally, clinical and metabolic markers cannot be used to distinguish autoimmune diabetes from type 2 diabetes in Ghanaian diabetic patients. It has been reported that only diabetic patients with multiple autoantibody positivity had lower BMI and lower frequency of hypertension compared with autoantibody-negative type 2 diabetic patients (Lohmann et al., 2001). Patients with single autoantibody positivity had the same clinical and metabolic markers as autoantibody-negative type 2 diabetic patients (Lohmann et al., 2001). The autoimmune diabetic patients in this study were mostly single autoantibody-positive and this therefore explains why there was no significant difference in most of the clinical and metabolic markers.

CHAPTER SIX

6. CONCLUSION AND RECOMMENDATIONS

6.1 CLINICAL CHARACTERISTICS OF GHANAIAN DIABETIC PATIENTS

There were more Ghanaian females with diabetes mellitus than males and more non-insulin requiring than insulin requiring patients. Majority of the Ghanaian diabetic patients were 40 years and above and had late onset diabetes. More than half of the Ghanaian diabetic patients had diabetes disease duration of 1-9 years. Similarly, more than half had high preprandial glucose levels, that is, carried modifiable factors for microvascular and macrovascular disease. Less than half of the Ghanaian diabetic patients were obese and overweight or had central obesity, with values in females higher than males. Smilarly, less than half of the patients had confirmed hypertension, and almost all of these hypertensives had inadequate blood pressure control. Confirmed hypertension was more common in female diabetic patients than males. The latter two indicators show that less than half of the patients carried modifiable factors for cardiovascular disease. More stringent measures need to be put in place in the management of diabetes mellitus at Komfo Anokye Teaching Hospital to prevent microvascular and cardiovascular disease.

6.2 PREVALENCE OF THE METABOLIC SYNDROME AND ITS COMPONENTS IN GHANAIAN DIABETIC PATIENTS

More than half of the Ghanaian diabetic patients had metabolic syndrome. Low HDL cholesterol was the commonest component of the metabolic syndrome, followed by hypertension, in the study population. In females central obesity was the commonest component of the syndrome, followed by low HDL cholesterol. In males, hypertension was the commonest component, followed by hypertriglyceridaemia. Female Ghanaian diabetic patients had higher prevalence of the metabolic syndrome, central obesity, hypertension, low HDL cholesterol and individually carried more metabolic syndrome risk factors than male diabetics. Thus, females are more prone to cardiovascular disease than their male counterparts. Once detected, vigorous and early management of the metabolic syndrome, would have a significant impact on the prevention of CVD.

6.3 DETERMINANTS AND CLINICAL FEATURES OF THE METABOLIC SYNDROME DIAGNOSED FROM GHANAIAN DIABETIC PATIENTS

The major determinant of the metabolic syndrome was central obesity, followed by hypertension in the Ghanaian diabetic patients; central obesity, followed by hypertension in female diabetics; and hypertriglyceridaemia, followed by hypertension in male diabetic patients. The same idea of the major determinant of the metabolic syndrome was revealed by the fact that

hyperglycaemia, central obesity and hypertension was the most frequent combination of different components in diabetics with the metabolic syndrome. These factors should serve as targets for metabolic disease control in Ghanaian diabetic patients. The major determinant of the metabolic syndrome may not necessarily be the commonest metabolic syndrome component of the population under consideration. This is partially explained by the fact that the commonest metabolic syndrome factor of a population may not be necessarily involved in the clustering of factors that determine the metabolic syndrome.

There were more Ghanaian female diabetics with the metabolic syndrome than males and more non-insulin requiring than insulin requiring metabolic syndrome patients. The metabolic syndrome was mostly present in Ghanaian diabetics with the metabolic syndrome who were 40 years and above and had late onset diabetes. More than half of the metabolic syndrome patients had diabetes disease duration 1 – 9 years and high preprandial glucose. Similarly, more than half of the Ghanaian diabetics with the metabolic syndrome were obese and overweight and had confirmed hypertension. Almost all these hypertensives had inadequqte blood pressure control. More stringent measures need to be put in place in the management of metabolic syndrome in diabetic patients at Komfo Anokye Teaching Hospital to prevent cardiovascular disease.

6.4 ASSOCIATION OF METABOLIC SYNDROME WITH GLYCAEMIC CONTROL IN RECENTLY DIAGNOSED GHANAIAN DIABETIC PATIENTS

Poor glycaemic control was the more sensitive indicator of cardiovascular disease than metabolic syndrome. Metabolic syndrome was associated with poor glycaemic control ($HbA_{1C} \geq 7.5\%$), that is, poorly controlled diabetes. However there were significant percentages of metabolic syndrome (33.3%) in patients with good glycaemic control. Therefore poor glycaemic control and metabolic syndrome are independent risk factors for cardiovascular disease. Hence poor glycaemic control (HbA_{1C}) cannot replace metabolic syndrome as an indicator of cardiovascular disease.

6.5 AUTOIMMUNE DIABETES (AUTOANTIBODY PATTERNS) AND AUTOANTIBODY-NEGATIVE TYPE 2 DIABETES IN RECENTLY DIAGNOSED GHANAIAN DIABETIC PATIENTS

The findings in this study confirm the presence of autoimmune diabetes in insulin-requiring recently diagnosed Ghanaian diabetic patients, but at an prevalence that might be lower than Caucasian type 1 diabetic patients. The prevalence of autoimmune diabetes in the non-insulin requiring recently diagnosed Ghanaian diabetic patients is comparable to that reported for the world population. Similarly, the prevalence of LADA in the non-insulin requiring and the overall recently diagnosed diabetes population was consistent with values reported for Caucasians. Autoimmune diabetes, including autoimmune type 1 diabetes and LADA, occurs in recently diagnosed Ghanaian diabetic patients. These findings are of value in developing early intervention strategies, correct classification of diabetes and for public health purposes. Single autoantibody

positivity (ICA or GADab) was mainly responsible for autoimmune diabetes, including LADA and autoimmune type 1 diabetes, rather than multiple autoantibody positivity. Clinical and metabolic parameters in autoimmune diabetes and autoantibody-negative type 2 diabetic patients did not differ. The exceptions were hypertension and central obesity, which were more likely in autoantibody-negative type 2 diabetes than autoimmune diabetes and HbA_{1C} which was higher in the latter than in the former.

6.6 RECOMMENDATIONS

Suggestions to deal with adverse effects of clinical, metabolic and immunological aspects of Ghanaian diabetic patients

1. Individuals aged 35 years and above, especially females and those obese, overweight and hypertensive, should be screened for diabetes mellitus for early detection and management.

2. Management of diabetes mellitus patients should be intensified from the first to the ninth year of disease duration to help control the disease.

3. More vigorous measures should be put in place in the management of diabetes mellitus at Komfo Anokye Teaching Hospital in Ghana to maintain near-normal glucose levels. This is because maintaining near-normal

glucose levels of 5.0 – 7.2 mmol/L minimizes the risks for both hyper- and hypoglycaemic episodes, and for a long time minimizes the risk for microvascular diabetic complications (Goldstein et al., 2004).

4. Determination of the metabolic syndrome should be incorporated early into routine diagnostic schemes for diabetics. This is because once detected, vigorous and early management of the metabolic syndrome, would have a significant impact on the prevention of CVD (Eriksson and Lindegarde, 1991).

5. Autoantibody (ICA, GAD) determinations should be incorporated early into the routine differential diagnostic schemes of Ghanaian diabetic patients to effectively differentiate between autoimmune type 1 diabetes, LADA and autoantibody-negative type 2 diabetes. This is because clinical indicators alone cannot distinguish between these classes of diabetes correctly or classify diabetes mellitus. It is also of value for developing early intervention strategies during management of diabetic patients.

Suggestions for further research

6. Metabolic syndrome should be determined in Ghanaian type 1 diabetic patients as a group and in type 2 diabetic patients as a separate group.
7. Metabolic syndrome should be determined in the general Ghanaian population.

8. The causes of obesity and how it relates to diabetes mellitus and metabolic syndrome in Ghanaian patients should be investigated.

9. Autoantibody determinations should be carried out in association with C-peptide levels to determine the incidence rate of type 1 and 2 diabetes in Ghanaian diabetic patients.

10. Autoantibodies should be determined in newly diagnosed Ghanaian diabetic patients.

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APPENDIX

APPENDIX 1

CLINICAL, METABOLIC AND IMMUNOLOGICAL CHARACTERISTICS OF PATIENTS WITH DIABETES MELLITUS IN GHANA

STUDY QUESTIONNAIRE FOR DIABETIC PATIENTS

RELEVANT SOCIO-DEMOGRAPHIC

1. Name:
2. Serial No.:
3. Pathological No.:
4. Age:
5. Sex: M/F
6. Tribe: Twi-speaking, Fante, Guan, Ga-Adangbe, Northern peoples, Ewes, Others-

RELEVANT INTERVIEW

7. Family history of common metabolic diseases: Nil, Diabetes, Hypertension, Obesity, Others -
8. Diagnosed as Diabetic: No./Yes - How long?
9. Diabetes drug profile: Nil/Insulin/Oral hypoglycaemic drugs and diet/Both, Others -
10. Any current disease: Nil/Hypertension/Obesity/Recurrent infections/Others -
11. Intake of other pharmacological agents: Nil/Tobacco/Alcohol/ Others –
12. Specific physiological states: Nil/Pregnancy/Stress/Excessive exercise/Others -
13. Physical activity: Nil/Office work/Sports related/Gardening/Farming/Others -

CLINICAL, METABOLIC AND IMMUNOLOGICAL CHARACTERISTICS OF PATIENTS WITH DIABETES MELLITUS IN GHANA

STUDY QUESTIONNAIRE FOR CONTROL/HEALTHY INDIVIDUALS

RELEVANT SOCIO-DEMOGRAPHIC

1. Name:
2. Serial No.:
3. Pathological No.:
4. Age:
5. Sex: M/F
6. Tribe: Twi-speaking, Fante, Guan, Ga-Adangbe, Northern peoples, Ewes, Others-

EXCLUSION CRITERIA BASED ON INTERVIEW

7. Family history of common metabolic diseases: Nil, Diabetes, Hypertension, Obesity, Others -
8. Presence of diabetes in first degree relatives: Nil./Yes
9. Disease risk: Nil/Diabetes/Hypertension/Obesity/Asthma/Occupational disease/ Recurrent infections/Others -
10. Intake of pharmacological agents: Nil/Tobacco/Alcohol/Contraceptives/Others –
11. Specific physiological states: Nil/Pregnancy/Stress/Excessive exercise/Others -
12. Physical activity: Nil/Office work/Sports related/Gardening/Farming/Others -