A COMPARATIVE STUDY OF OXIDATIVE STRESS AND TYPE 2 DIABETES MELLITUS IN RURAL AND URBAN COMMUNITIES IN THE ASHANTI REGION, GHANA

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By

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DECLARATION

The research work described in this thesis was carried out at the Department of Molecular Medicine, School of Medical Sciences, KNUST. The work has not been submitted for any other degree.

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ABSTRACT

TITLE: A COMPARATIVE STUDY OF OXIDATIVE STRESS AND TYPE 2 DIABETES MELLITUS IN RURAL AND URBAN COMMUNITIES IN THE ASHANTI REGION, GHANA

BACKGROUND/AIM: A growing area of research is the relationship between oxidative stress and diabetes. Accumulating evidence indicates that oxidative stress, a condition of excessive reactive oxygen species, may play a role in the aetiology of type 2 diabetes mellitus by inducing insulin resistance in the peripheral tissues and impairing insulin secretion from pancreatic beta-cells. However, the link between oxidative stress and the development and progression of diabetes and its complications is still not fully understood. The fast progressive westernization of the Ghanaian society is predisposing increasing numbers of the population to higher rates of oxidative stress and may be the results of the increase in the prevalence of type 2 diabetes and other dysmetabolic conditions. The development of a simple screening tool may help to identify individuals at high risk of development of such dysmetabolic states in the society.

METHODOLOGY: 210 adults were recruited from urban Kumasi and 180 adults were recruited from 3 rural villages in the Ashanti region. Sociodemographic data was collected from the subjects. Anthropometric measurements including blood pressure, weight, and height and waist circumference were determined by qualified nurses. Blood samples were collected after 12 hours of overnight fast for the analysis of glucose, lipids, oxidative stress indices and other biochemical parameters.

RESULTS: In this study, the prevalence of type 2 diabetes mellitus and IFG were considerably higher among the urban subjects (10.5% and 28.0% respectively for urban subjects and 3.8% and 22.1% respectively for rural subjects). Dyslipidaemia and hypertension were also found to be more prevalent in the urban subjects than the rural population. Plasma antioxidant levels were also higher in the rural population than the urban population whiles malondialdehyde levels were found to be higher in the urban population as compared to the rural population.

CONCLUSION: Type 2 diabetes and other dysmetabolic conditions are on the increase in the urban population of the Ashanti. In conclusion, the study hypothesizes that the fast progressive westernization of the Ghanaian culture predisposes individuals to higher rates of systemic oxidative stress as a result of increased exposure to reactive oxidants and this is accelerating the ageing process in the society as evident in the increasing incidence of type 2 diabetes mellitus and other dysmetabolic conditions. Additionally, a simple risk tool like the Ghana Diabetes Risk Score can be used to identify individuals at high risk of development of these dysmetabolic conditions.

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LIST OF ABBREVIATIONS

ADA: American Diabetes Association

AIDS: Acquired Immune Deficiency Syndrome

ADP: Adenosine dinucleotide phosphate

AGE: Advanced glycation end-products

ATP: Adenosine trinucleotide phosphate

4-AAP: 4-aminoantipyrine

ANOVA: Analysis of Variance

BMI: Body mass index

CAT: Catalase

CHER: Cholesterol esterase

CHOD: Cholesterol oxidase

10-YR CHD risk: 10-year coronary heart disease risk

DAP: Dihydroxyacetone phosphate

DTC: 2,4-dinitrophenylhydrazine copper sulphate

D.BP: Diastolic blood pressure

3,5-DHBS: 3-5 dichloro-2-hydroxybenzene

DRS: Ghana Diabetes Risk Score

ETC: Electron transport chain

FFA: Free fatty acid

FPG: Fasting plasma glucose

GLUT2: Glucose transporter 2

GLUT4: Glucose transporter 4

GFAT: glutamine:fructose-6-phosphate amidotransferase

GK: Glycerol kinase

GPx: Glutathione peroxidise

HK: Hexokinase

HDL: High density lipoprotein cholesterol

HPLC: High performance liquid chromatography

HIV: Human Immunodeficiency Virus

IFG: Impaired fasting glycaemia

IGT: Impaired glucose tolerance

IRS: Insulin receptor substrate

IR: Insulin receptor

IKKβ: I$_{\kappa}$B kinase

IDF: International Diabetes Federation

JNK: Jun NH2 Kinase

K^+: Potassium ion

LA: alpha lipoic acid

LDL: Low density lipoprotein cholesterol

MDA: Malondialdehyde

mRNA: messenger ribonucleic acid

mNADH: mitochondria reduced nicotinamide adenine dinucleotide

MAPK: Mitogen-activated protein kinase

NCD: Non-communicable Disease

NGT: Normal glucose tolerance

NAD^+ : Oxidized nicotinamide adenine dinucleotide

NADH: Reduced nicotinamide adenine dinucleotide

NF-κB: Nuclear factor kappa B

PI3K: Phosphatidylinositol 3 kinase

POD: Peroxidase

PEGME: Polyethylene-glycol methyl ether

PVS: Polyvinyl sulfonic acid

PPARγ: Peroxisome proliferator-activated receptor

PKC: Protein kinase C

PTP: Protein tyrosine phosphatase

PUFA: Poly unsaturated fatty acids

RAGE: Receptor of advanced-glycation end-products

ROS: Reactive oxygen species

RNS: Reactive nitrogen species

SAPK: Stress-activated protein kinase

S.BP: Systolic blood pressure

SEM: Standard error of the mean

SIRT1: Sirtuin 1

SOD: Superoxide dismutase

TODB: N,N-Bis(4-sulfobutyl)-3-methylaniline

TG: Hypertriglyceridaemia

TBA: Thiobarbituric acid

TCA: Trichloroacetic acid

TNF-α: Tumor necrosis factor

T2DM: Type 2 diabetes mellitus

VLDL: Very low density lipoprotein cholesterol

WC: waist circumference

WHO: World Health Organization

Chapter 1

INTRODUCTION

1.1 Definition of diabetes mellitus

Diabetes mellitus describes a heterogenous metabolic disorder of multiple aetiology characterized by chronic hyperglycaemia with distortions in carbohydrate, fat and protein metabolism, resulting from defects in insulin secretion, insulin action, or both **[WHO, 1998].**

1.2 Types of diabetes mellitus

There are two main types of diabetes mellitus (type 1 and type 2 diabetes mellitus) though there are other rare forms of diabetes mellitus. Type 1 diabetes (formerly known as insulin-dependent diabetes) is characterized by insulin deficiency resulting from pancreatic beta cell destruction. The aetiology of type 1 diabetes mellitus is either immune mediated, related to either physical destruction of the pancreas (as in pancreatitis or pancreatic cancer) or idiopathic. However, the most prevalent form of diabetes, type 2 diabetes (which accounts for over 90% of all diabetes cases) presents as a spectrum of metabolic abnormalities with prominent insulin resistance and relative insulin deficiency **[WHO, 1998].**

1.3. Diagnosis of Diabetes mellitus

The diagnostic criteria of hyperglycaemia for diabetes mellitus have recently been revised by comprehensive reviews of the world's research findings by the Expert Committees of the American Diabetes Association (ADA) and the World Health Organization (WHO). The reviews have led to a lowering of the fasting glucose diagnostic criteria from ≥7.8 mmol/l to ≥7.0 mmol/l. The 2-hour criterion (≥ 11.0 mmol/L) however was unchanged. Additionally, a new category, impaired fasting glucose (IFG), was established and defined as fasting plasma glucose of 6.1 – 6.9 mmol/l. IFG is a metabolic state considered abnormal but not yet diagnostic of diabetes **[ADA, 1997 and WHO, 1998].**

1.4. Global Prevalence of Diabetes mellitus

In the year 2000, the global number of individuals with diabetes was estimated to be 171 million (2.8% of the world's population), and this figure has been projected to increase in 2030 to 366 million (6.5%), 298 million of whom will be living in developing countries **[WHO, 1998].** Type 2 diabetes mellitus has reached epidemic proportions with explosive increase in incidence worldwide over the past few decades. Although type 2 diabetes mellitus is more prevalent in developed countries, the increase in incidence seems to be more pronounced especially in populations that are experiencing rapid westernization **[Zimmet *et al,* 2002].** Apart from microvascular complications, cardiovascular disease, with its attendant morbidity and mortality, is on the rise in the developing countries. Current evidence suggests that environmental factors are major determinants of the increasing rates of diabetes **[WHO, 1998].** Overweight and obesity are increasing dramatically and contribute to the burden of diabetes mellitus and other chronic health conditions. Indeed, the modern environment promotes behaviors that cause obesity.

1.5. Prevalence of Diabetes mellitus in Ghana

Although diabetes was thought to be rare in sub-Saharan Africa, recent studies from some countries suggests that the disease may now be more common in sub-Saharan Africa than previously thought**[Cooper *et al,* 1997, Mbanya *et al,* 1999, Aspray *et al,* 2000]**. Though epidemiological data on the prevalence of diabetes in Ghana is scanty, evidence suggests that it is on the increase. In the 1950s, the prevalence of diabetes among an outpatient urban population in Accra was estimated at less than 0.5% **[Dodu, 1958].** The impression was therefore created among policy makers that diabetes is rare in Ghanaians. However, a recent study **[Amoah *et al,* 2002];** reported a high prevalence rate of 6.3%. Also, a recent study on the prevalence and sociodemographic aspects of overweight and obesity among residents from rural and urban Accra reported an overall crude prevalence of 23.4% and 14.1%, respectively **[Amoah, 2003].**

1.6. Overview of the development of type 2 diabetes mellitus

Under normal physiological conditions, the plasma glucose concentrations are maintained within a narrow range, despite the wide fluctuations in supply and demand, through a tightly regulated and dynamic interaction between tissue sensitivity to insulin (especially in the liver) and insulin secretion **[DeFronzo, 1997]**. The ingestion of carbohydrates causes a momentary increase in blood glucose concentration, resulting in a rapid release of insulin by the ß-cells within the islets of Langerhans. Insulin then binds to specific receptors in target peripheral tissues to produce its effects. Under normal conditions, insulin promotes the transport of glucose into the skeletal muscle and adipose tissue. In the liver, insulin acts by suppressing glycogenolysis and gluconeogenesis, and in adipose tissue, it inhibits lipolysis. Through the mechanism of decreasing hepatic and adipose glucose production, and by accelerating the uptake of glucose into peripheral tissues, the net effect of insulin's action is to lower blood glucose concentration **[DeFronzo, 1997]**.

In people with type 2 diabetes mellitus (type 2 DM) however; there is a gradual change in glucose homeostasis manifested as glucose intolerance and inefficient uptake of glucose from the blood by the peripheral tissues. The glucose intolerance is caused, in part, by an attenuated biological response to normal concentrations of insulin, a condition known as insulin resistance. In addition, type 2 DM is often associated with a progressive decrease in the sensitivity of the pancreatic ß-cells to glucose stimulation, with a subsequent decrease in insulin secretion. In time, there may be an increased demand for insulin due to worsening of the insulin resistance. Eventually, the combined effects of increased insulin resistance and inadequate insulin secretion in response to a glucose challenge will result in hyperglycaemia, which is a significant and prolonged increase in blood glucose concentration **[Bao *et al,* 1996].** Although the central figure is hyperglycaemia, the effect of diabetes mellitus is not limited to carbohydrate metabolism. Lipid and protein metabolism also play an important role in the progression of the disease. The abnormal glucose metabolism accounts for poorly regulated biochemical processes that glycosylate haemoglobin and other proteins and lipids throughout the body **[Bao *et al,* 1996].** The effects of the dysmetabolism in carbohydrates, lipids, and proteins include long-term damage,

dysfunction and failure of various organs. The symptoms are often not severe, or may be absent, and consequently hyperglycaemia sufficient to cause pathological and functional changes may persist for a long time before the diagnosis is made. The long-term effects of diabetes mellitus include progressive development of the specific microvascular complications including retinopathy with potential blindness, nephropathy that leads to renal failure, and/or neuropathy with risk of foot ulcers and potential amputation and features of autonomic dysfunction, including sexual dysfunction. People with diabetes are at increased risk of macrovascular damage including cardiovascular, peripheral vascular and cerebrovascular disease **[WHO, 1998, Freedman *et al,* 2001, Ford *et al,* 2002].**

Type 2 DM is a heterogeneous syndrome that results from an interaction between *1)* a genetic predisposition and 2*)* environmental factors. In genetically predisposed individuals, the development and progression of type 2 diabetes mellitus appears to be facilitated by factors such as obesity, lack of physical activity, cigarette smoking, high intake of calorie-rich diets and low intake of fruits and vegetables **[WHO, 1998, Halliwell, 2000, Hu *et al,* 2001].**

Type 2 diabetes mellitus has a gradual and insidious onset, and some degree of hyperglycaemia may have been present for several years (10 – 20 years) before the diagnosis is confirmed **[WHO, 1998].** The pathogenesis of diabetes mellitus, regardless of its aetiology, progresses through several clinical stages during its natural history. Moreover, individuals may move from stage to stage in either direction and persons who have, or who are developing diabetes mellitus can be categorized by stage according to the clinical characteristics, even in the absence of information concerning the underlying aetiology **[Bao *et al,* 1996].**

1.7 Pre-diabetes

Impaired glucose metabolism (IGT and IFG) or pre-diabetes refers to a metabolic state intermediate between normal glucose homeostasis and diabetes. IFG and IGT are however not interchangeable and represent different abnormalities of glucose regulation, one in the fasting state (IFG) and the other in the post-prandial state (IGT). IGT is now categorized as a stage in the natural history of disordered carbohydrate

metabolism **[Zimmet *et al,* 2002].** A stage of IFG, just like IGT is also recognized because such subjects, like those with IGT, have increased risks of progressing to diabetes mellitus and macrovascular disease, although prospective data are sparse and early data suggest a lower risk of progression than IGT although a similar CVD risk factor profile has been shown in both IFG and IGT subjects **[Zimmet *et al,* 2002].** Pre-diabetes and type 2 diabetes often cosegregate with hypertension and dyslipidaemia (high triglyceride levels and low serum HDL-cholesterol) as manifestations of the metabolic syndrome which affects 47 million people in the U.S. **[Ford *et al,* 2002].** Additionally, components of the metabolic syndrome can be identified in pre-diabetic subjects several years before the diagnosis of type 2 diabetes mellitus. Epidemiological studies have shown that pre-diabetes confers an increased risk of cardiovascular disease and patients who progress to type 2 diabetes mellitus exhibit additional risk for atherosclerotic disorders, which manifest as a two- to fourfold increase in the prevalence of cardiovascular disease, stroke, and peripheral vascular diseases, compared with non-diabetic subjects **[Eschwege *et al,* 1985, Beckman *et al,* 2002, Ford *et al,* 2002].** The economic and social costs of diabetes are enormous, both for health care delivery and through loss of productivity. In 2007, new research provided revised comprehensive estimates that suggest that the U.S. national economic burden of pre-diabetes and diabetes reached $218 billion. This estimate includes $153 billion in higher medical costs and $65 billion in reduced productivity. The average annual cost per case is $2,864 for undiagnosed diabetes, $9,975 for diagnosed diabetes ($9,677 for type 2 and $14,856 for type 1), and $443 for pre-diabetes (medical costs only). This shows that for each American, regardless of diabetes status, this burden represents a cost of approximately $700 annually **[Dall *et al,* 2010]**. There is therefore an urgency to better understand how prevention and treatment strategies may or may not help reduce costs.

1.8 Overview of type 2 diabetes mellitus and oxidative stress

A growing area of research is the relationship between oxidative stress and diabetes mellitus. Several accumulating evidence indicates that oxidative stress, a condition of excessive reactive oxygen species, may play a role in the aetiology of type 2 diabetes mellitus by inducing insulin resistance in the peripheral tissues and impairing insulin

secretion from pancreatic beta-cells **[Evans *et al,* 2003].** However, the link between oxidative stress and the development and progression of type 2 diabetes mellitus and its complications is still not fully understood. A continuing debate is whether oxidative stress from hyperglycaemia possibly working in concert with hyperlipidaemia, is a lead actor or a merely supporting actor in the development of type 2 diabetes mellitus. Diabetics may have a defective antioxidant status as a result of either increased use or reduced intake of antioxidant to fight the excessive free radical production that is associated with diabetes mellitus **[Ahmad *et al,* 2003]**. The sequelae of type 2 diabetes mellitus (hyperglycaemia and hyperlipidaemia) may not only cause elevated peroxidation, but pre-existing high rates of lipid peroxidation may predispose to and accelerate the development of diabetes especially upon exposure to a pro-oxidant challenge **[Laight *et al,* 2000].** Such elevated rates may reflect poor antioxidant intake **[Salonen *et al,* 1995, Ford, 2001]**, but may also involve inherited differences in rates of peroxidation and metabolism of lipid peroxides **[Fachini *et al,* 2000, Halliwell, 2000]**. It may be evident that with the fast progressing westernization of our society that is associated with a high intake of calorie-dense foods coupled with a low physical activity pattern many subjects in high-risk groups may be predisposed to a defective antioxidant status and an increased oxidative stress that may lead to the development of type 2 diabetes mellitus and its complications and increase the economic burden that diabetes puts on healthcare delivery in our country.

To search for a predisposition to oxidative stress, this study will investigate the relationship between levels of antioxidant vitamins and oxidative stress and the risk of development of type 2 diabetes mellitus and other dysmetabolic conditions among individuals in rural and urban communities in the Ashanti region.

The overall aim of the study is to contribute further knowledge in the complex oxidant-antioxidant area by evaluating the relationship between antioxidant status and oxidative stress in the dysmetabolism in type 2 diabetes mellitus and other dysmetabolic states.

Chapter 2

LITERATURE REVIEW

2.1.0. Reactive Oxygen Species (ROS): Historic Background

It is generally accepted that molecular oxygen (O_2) was absent until the appearance of photosynthetic organisms (blue-green algae) about 2.5 billion years ago **[Semenza, 2007]**. The photosynthetic organisms convert carbon dioxide (CO_2) and water to glucose and O_2. In this molecular process, solar energy was transduced into chemical energy of carbon bonds. The water was oxidized to O_2 to generate the reducing power (hydrogen atoms) required for photosynthesis and O_2 was released into the atmosphere as a by-product and the gradual build-up of O_2 in the atmosphere then drove the evolution of aerobic organisms (eukaryotes containing mitochondria) about 1.5 billion years ago **[Semenza, 2007]**. In the mitochondria, glucose is oxidized to CO_2 and water. The reducing equivalents generated from oxidation of glucose pass electrons through the mitochondrial electron transfer chain (ETC) and the build up a proton gradient to drive the phosphorylation of adenosine 5'-diphosphate (ADP) to adenosine 5'-triphosphate (ATP), a process termed "oxidative phosphorylation" **[Semenza, 2007]**. These electrons ultimately react with O_2 to form H_2O (a fully reduced form of O_2). In this molecular process, O_2 serves as a terminal oxidant (receiver of electrons). This process is highly energy-efficient and is superior to respiratory pathways that rely on other terminal oxidants such as fermentation **[Semenza, 2007]**. However, the utilization of O_2 as a terminal substrate in oxidative phsophorylation may have potential dangerous consequences. When electrons pass through the mitochondrial ETC, a fraction of them (0.1-0.5%) escape the ETC and combines with O_2 prematurely, resulting in the generation of the partially reduced product; superoxide **[Imlay, 2003]**. Superoxide and other partially reduced form of O_2 are termed "reactive oxygen species" (ROS) including both the radical and non radical species that participate in the initiation and/or propagation of radical-based chain reactions with biomolecules and interfere with biological processes **[Imlay, 2003, Semenza, 2007]**. Because of their reactivity, the accumulation of ROS beyond the immediate needs of the cell may affect cellular structure and functional integrity, by bringing about oxidative modification (degradation) of critical molecules, such as DNA, proteins, and lipids **[Imlay, 2003, Evans, 2003].**

Research in the field of the "potential toxicity of oxygen" was raised by the publication of Gerschman's free radical theory of oxygen toxicity in 1954, which states that the toxicity of oxygen is due to partially reduced forms of oxygen **[Gerschman *et al,* 1954]**. The toxic effects of oxygen were further supported by Denham Harman, who hypothesized that oxygen radicals may be formed as by-products of enzymic reactions in vivo. In 1956, he described free radicals as a Pandora's box of evils that may account for gross cellular damage, mutagenesis, cancer, and, last but not least, the degenerative process of biological aging **[Harman, 1956].**

The second era of the science of free radicals in living organisms then entered another phase when researchers **[McCord and Fridovich, 1969]** discovered the enzyme superoxide dismutase (SOD) and finally convinced most colleagues that free radicals are important in biology. Numerous researchers were then inspired to investigate oxidative damage inflicted by radicals upon DNA, proteins, lipids, and other components of the cell.

The third era began with the first report describing advantageous biological effects of free radicals when researchers provided further evidence that the superoxide anion, through its derivative, the hydroxyl radical, stimulates the activation of guanylate cyclase and formation of the "second messenger" cyclic guanosine monophosphate cGMP **[Mittal and Murard, 1977]**.

2.1.1.0. Mechanisms of production of ROS

2.1.1.1. Endogenous production of ROS

The endogenous sources of production of ROS include the following:

- Phagocytic cells destroy bacteria or virus infected cells with an oxidative burst of nitric oxide ROS, including nitric oxide, O_2^-, H_20_2, and $OC1^-$ **[Kasai *et al,* 1989].**
- A consequence of normal aerobic respiration (mitochondria consume 0_2, reducing it by sequential steps to produce H_20). Inevitable by-products of this molecular process are O_2^-, H_20_2, and -OH **[Baboir, 2002].**

- Peroxisomes, (organelles responsible for degrading fatty acids and other molecules), produce H_20_2 as a by-product, which is then degraded by catalase. Evidence suggests that, under certain conditions, some of the peroxide escapes degradation, resulting in its release into other compartments of the cell and in increased oxidative DNA damage **[Kasai *et al,* 1989].**

- Cytochrome P450 enzymes in animals constitute one of the primary defense systems against natural toxic chemicals from plants, the major source of dietary toxins. The induction of these enzymes prevents acute toxic effects from foreign chemicals but also results in oxidant by-products that damage DNA [**Kasai *et al*, 1989]**.

2.1.1.2. Exogenous production of ROS

The exogenous sources of production of ROS include the following:

- Natural diets contain plant food with large amounts of natural phenolic compounds, such as chlorogenic and caffeic acid, that may generate oxidants by redox cycling **[Babior, 2000].**

- Cigarette smoking (oxides of nitrogen) in cigarette smoke (about 1000 ppm) cause oxidation of macromolecules and deplete antioxidant levels **[Kiyosawa *et al,* 1990]**.

- Iron (and copper) salts also promote the generation of oxidizing radicals from peroxides through the Haber-Wiess reaction **[Halliwell and Gutteridge, 1990].**

2.2.0. Types of Reactive Oxygen Species

The most common ROS include: Ozone (O_3), singlet oxygen ($1O^2$), the superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), the hydroxyl radical (HO^-), and Peroxyl radicals.

2.2.1. Superoxide anion (O_2^-)

The superoxide is an anionic radical formed by the reduction of molecular oxygen through the acceptance of a single electron. The hydroperoxyl radical, which is unstable at physiological pH could also dissociate to superoxide. In vivo, the O_2^- is mainly produced by the ETC in the mitochondria and microsomes through electron leakage; a phenomenon that increases with an increase in oxygen utilization. Activated phagocytes also possess metabolic pathways for the production of superoxide radicals in response to bacterial infection **[Babior, 2000].**

2.2.2 Hydroxyl radical (HO^-)

The hydroxyl radical may be produced experimentally by various procedures, including exposure to radiation or by decomposition of peroxynitrite **[Beckman *et al,* 2002].** Because of its low half-life, the direct action of the hydroxyl radical is confined to regions immediately in the vicinity of its formation. However, being the most reactive member of the ROS family, it can bring about extensive damage to different types of molecules, including proteins, nucleic acids, and lipids **[Halliwell and Gutteridge, 1990].** In DNA, the HO^- can induce several effects including base and sugar modifications, cross-linking between bases, cross-linking between DNA and protein, strand breaks, and formation of several adducts**.** The action of the hydroxyl radicals on proteins leads to extensive protein–protein cross-linking. This may be further aggravated in metalloproteins and metalloenzymes by transition metals, via the formation of hydroxyl radicals from their precursors, thus resulting in site-specific destruction of the critical regions of the molecule. Extensive studies on the oxidative properties of HO^- radicals have been carried out on membrane lipids in which the polyunsaturated fatty acids (PUFAs) are particularly vulnerable to oxidation **[Halliwell and Gutteridge, 1990, Halliwell, 2000].**

2.2.3 Hydrogen peroxide

Hydrogen peroxide and the superoxide radicals may undergo further transformations in the presence of transition metals (particularly iron and copper) to give rise to the highly reactive hydroxyl radicals, by the Haber–Weiss or Fenton reactions. This special property, combined with the membrane permeability of hydrogen peroxide, gives superoxide and hydrogen peroxide the ability to affect the integrity of distant molecules within the cell **[Halliwell and Gutteridge, 1990, Halliwell, 2000].**

2.2.4 Singlet oxygen

Singlet oxygen ($1O^2$), largely kown to be involved in photochemical reactions, is very reactive, although it does not contain unpaired electrons and therefore is not a free radical. It is formed *in vivo* by enzymatic activation of oxygen through several biological processes **[Halliwell and Gutteridge, 1990]**, for example, through lipooxygenase activity during prostaglandin biosynthesis. It can also be produced by physicochemical reactions, such as energy transfer due to type II photosensitization, thermal decomposition of endoperoxides and dioxetanes, reaction of ozone with human body fluids and interaction between hydrogen peroxide and peroxynitrite. Singlet oxygen is a very reactive ROS and induces various genotoxic, carcinogenic, and mutagenic effects through its action on polyunsaturated fatty acids (PUFAs) and DNA **[Halliwell and Gutteridge, 1990, Sies H, 1993].**

2.2.5 Peroxyl radicals

Several peroxyl radicals are produced primarily during lipid peroxidation, which is initiated by abstraction of a hydrogen atom from unsaturated lipids. Although lipid peroxidation has been found to play a useful role in some biological processes, peroxidation of membrane PUFAs may adversely affect many functionally important processes, such as membrane fluidity, permeability, electrical potential, and controlled transport of metabolites across the membrane **[Halliwell and Gutteridge, 1990, Halliwell, 2000].**

2.3.0 Targets of ROS

Although O_2 is strongly oxidative with respect to its fully reduced form, water, it is a rare stable di-radical because of the kinetic restriction imposed by its two spin-aligned unpaired electrons. Molecular oxygen (O_2) can only react with transition metals or organic radicals with unpaired electron and is a very weak oxidant that cannot efficiently oxidize amino acid or nucleic acid **[Imlay, 2003]**. However, its partially reduced products including superoxide, H_2O_2, and hydroxyl radical (HO·) are more reactive.

The anionic charge of superoxide inhibits its electrophilic activity toward electron-rich molecules and therefore superoxide could only oxidize few biomolecules such as enzymes containing the [4Fe-4S] clusters (aconitase or dehydratase as examples) **[Imlay, 2003]**. The locally positively charged iron atom attracts superoxide electostatically and is therefore particularly susceptible to superoxide damage. H_2O_2 is also a weak oxidant due to the stable oxygen-oxygen bond that limits its reactivity **[Imlay, 2003]**. Although H_2O_2 can oxidize the cysteine (-SH) or methionine residues (-SCH3) of proteins, the reaction is however very slow unless the cysteine residues are rendered more negatively charged by adjacent positively charged residues to form thiolate anion **[Bindoli *et al,* 2008]**. The thiolate form of cysteine residue which is the most nucleophilic amino acid can therefore react readily with H_2O_2. Several protein kinases, phosphatases, and transcription factors with important physiological functions contain thiolate residues that can be oxidized reversibly by H_2O_2. In contrast, the hydroxyl radical reacts readily with most biomolecules including lipids, amino acids, and nucleic acids **[Imlay, 2003]**. Theoretically, the damaging effects of H_2O_2 are mainly due to its conversion to hydroxyl radical by Fenton reaction in the presence of free metals such as copper or iron and the superoxide radical can also react with another poor oxidant nitric oxide (NO·) to generate a very strong oxidant, peroxynitrite ($ONOO^-$), that reacts with most bio-molecules **[Imlay, 2003]**.

The superoxide and hydroxyl radicals have relatively short half-life (estimated intracellular half-life: 10^{-6} and 10^{-9} sec) with very low intracellular concentration (10^{-10} and 10^{-15} M) **[Winterbourn, 2008]**. On the other hand, H_2O_2 is relatively stable (half-life: 10^{-5} sec) with higher intracellular concentration (10^{-5} M) **[Winterbourn, 2008]**.

H_2O_2 is non-polar and can therefore diffuse freely across membranes with a very long diffusion distance (1.5 mm in the presence of 2 mM glutathione) **[Winterbourn, 2008]**. The relative long diffusion distance of H_2O_2 and its ability to reversibly oxidize specific protein residues make it a potent molecule for signal transduction **[D'Autréaux and Toledano, 2007].**

2.4.0 Antioxidants

Antioxidants are described as substances that are able, at relatively low concentrations, to compete with other oxidizable substrates and, thus, to significantly delay or inhibit the oxidation of these substrates **[Halliwell and Gutteridge, 1990, Halliwell, 2000].** This broard definition includes the enzymes SOD, glutathione peroxidase (GPx), and catalase, as well as nonenzymic compounds such as α-tocopherol (vitamin E), β-carotene, ascorbate (vitamin C), and glutathione. They may act possibly by 1) removing or lowering the local concentrations of one or more of the participants in this reaction, such as oxygen, ROS, or metal ions (Fe^{3+}, Cu^{2+}, etc.) which catalyze oxidation, or by 2) interfering with the chain reaction that spreads oxidation to neighbouring molecules. They may also act by enhancing the endogenous antioxidant defenses of the cell. Hence, antioxidants may intervene at any of the three major steps: initiation, propagation, or termination of the oxidative process **[Halliwell and Gutteridge, 1990, Halliwell, 2000].**

2.4.1 Mode of action of Antioxidants

Several critical structures in the cell are protected not only by the availability of several types of antioxidants and may be classified according to their chemical nature and mode of function.

2.4.1.1 Naturally-occurring enzyme antioxidants

These antioxidants act on specific ROS after they are formed and degrade them to less harmful products. Examples of these antioxidants include superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). SODs convert the superoxide radical to hydrogen peroxide, which although not a free radical by itself, exhibits its reactivity via its precursor, the hydroxyl radical **[Halliwell and Gutteridge, 1990, Halliwell, 2000]**. Catalase, SODs, and GPx constitute the major intracellular enzymic antioxidants, while the extracellular antioxidants are mainly of

the preventive and scavenging types **[Halliwell and Gutteridge, 1990, Halliwell, 2000].**

2.4.1.2 Preventive antioxidants

Preventive antioxidants act by binding to and sequestering oxidation-enhancers and transition metal ions, such as iron and copper, which contain unpaired electrons and strongly accelerate free radical formation **[Frei *et al,* 1988, Halliwell and Gutteridge, 1990, Halliwell, 2000]**. The preventive antioxidants include transferrin and lactoferrin (which bind ferric ions), ceruloplasmin (which binds copper), haptoglobins (which bind hemoglobin), hemopexin (which binds heme), and albumin (which binds copper and heme) **[Frei *et al,* 1988, Halliwell and Gutteridge, 1990, Halliwell, 2000].**

2.4.1.3 Scavenging or chain-breaking antioxidants

These antioxidants act by presenting themselves for oxidation at an early stage in the free radical chain reaction, producing low-energy molecules that are unable to propagate the chain further. Lipid-soluble and water-soluble antioxidant scavengers act in cellular environments that are either hydrophobic or hydrophilic, respectively. The major lipid-soluble scavengers are vitamin E (alpha-tocopherol), beta-carotene, and coenzyme Q (CoQ) while ascorbic acid, various thiols, uric acid, and bilirubin exhibit their functions in the aqueous medium [**Frei *et al,* 1988, Halliwell and Gutteridge, 1990, Halliwell, 2000].**

2.5.0. Overview of Oxidative Stress

The formation of reactive oxygen-containing molecules is a normal consequence of a variety of biochemical reactions. Therefore, there is the need to maintain a critical balance between the generation of these ROS and antioxidant defense. “Oxidative stress” occurs when this balance between pro-oxidants and antioxidants is upset. Oxidative stress is essentially an imbalance between the production of various reactive species and the ability of the organism's natural protective mechanisms to cope with these reactive compounds and prevent their deleterious effects. Because of their reactivity, the accumulation of ROS beyond the immediate needs of the cell may affect

both normal metabolism and physiology of cellular structure and functional integrity, by bringing about oxidative degradation of critical molecules, such as DNA, proteins, and lipids **[Sies, 1984 and Ames *et al,* 1993].** Living cells and tissues have several mechanisms for reestablishing the original redox state after a temporary exposure to increased ROS concentrations. Cells or tissues are in a stable state if the rates of ROS generation and scavenging capacity are essentially constant and in balance. Redox signaling mechanism requires that this balance can be disturbed, either by an increase in ROS concentrations or a decrease in the activity of one or more antioxidant systems. In higher organisms, the activation of endogenous ROS-generating systems may induce such an oxidative event. However, similar responses may be induced by oxidative stress conditions generated by several other environmental factors **[Sies,1984 and Frei *et al* 1988]**. If the initial increase in the level of ROS is relatively small, the antioxidative response may be sufficient to compensate for the increase in ROS and to reset the original balance between ROS production and ROS-scavenging capacity. Under certain conditions, however, the rate of ROS production is increased more strongly and persistently, and the antioxidative response may not be sufficient to reset the system to the original redox homeostasis. In such cases, the system may still reach equilibrium, though the resultant quasi-stable state may now be associated with higher ROS concentrations. **[Sies, 1984 and Frei *et al* 1988]**.

2.5.1.0. The Role of Free Radicals in Normal Cellular Physiology

2.5.1.1. Maintenance of Redox Homeostasis

The term "redox signaling" is essentially used to describe a regulatory process in which the cellular signal is delivered through oxidation-reduction chemical processes **[Forman 2009]**. This redox signaling is used by a wide variety of organisms, including bacteria, to induce protective responses against oxidative damage and to reset the original state of "redox homeostasis" after a temporary exposure to ROS **[Forman 2009].** Oxygen radicals and other ROS cause modifications of proteins which play a critical role in the maintenance of redox homeostasis **[Grune *et al,* 1999**]. These oxidative modifications may lead to changes in protein function, or increased susceptibility to proteolytic attack by proteasomes **[Stamler, 1992, Grune, 1999]**.

There are differences in susceptibility of proteins to oxidative damage. For example, the redox-sensitive amino acids of bovine serum albumin have been shown to be oxidized about twice as fast as those of glutamine synthase. Also, intact proteins are less sensitive to oxidation than misfolded protein **[Levine, *et al,* 1996]**.

There exist several mechanisms for reestablishing the original redox state after a temporary exposure to increased ROS concentrations in living cells and tissues. The production of nitric oxide, for example, is subject to a direct feedback inhibition of nitric oxide synthase (NOS) activity by nitric oxide **[Buga, 1993, Abu-Soud, 1995]**. The elevated ROS concentrations can induce in many cells the expression of genes whose products exhibit antioxidative activity **[Levine *et al,* 1996]**. A major mechanism of redox homeostasis is based on the ROS-mediated induction of redox-sensitive signal cascades that lead to increased expression of antioxidative enzymes or an increase in the cystine transport system, which, in turn, facilitates in certain cell types the increase in intracellular glutathione which plays a critical role in redox homeostasis **[Levine *et al,* 1996]**. This shows that physiological manifestations of redox regulation involve typically a temporary increase and/or a temporary shift of the intracellular thiol/disulfide redox state toward more oxidative conditions. In the long run, these mechanisms tend to maintain a stable redox state **[Forman 2009]**. Additionally, changes in the intracellular thiol/disulfide redox state have been shown to trigger the same redox-responsive signaling proteins and pathways as those triggered by hydrogen peroxide **[Breitkreutz *et al*, 2000]**.

Additionally, because proteins generally provide less ROS scavenging activity than the equivalent amount of the free amino acids contained in them, it suggests that oxidative enhancement of proteolysis also contributes, at least to some extent, to the maintenance of redox homeostasis **[Levine *et al,* 1996]**.

2.5.1.2. ROS production by phagocytic NADPH oxidase

Neutrophils and activated macrophages can produce large amounts of superoxide and its derivatives via the phagocytic isoform of NADPH oxidase (a heme-containing protein complex) **[Nathan and Root, 1977, Keisari, 1983]** In an inflammatory environment hydrogen peroxide is produced by activated macrophages at an estimated rate of $2–6 \times 10^{-14}$mol·h^{-1}·$cell^{-1}$ and this may reach concentrations of 10–100 μM in the vicinity of these cells **[Nathan and Root, 1977, Keisari, 1983]**. The massive production of antimicrobial and tumoricidal ROS in an inflammatory environment is termed the "oxidative burst" and plays a critical role as a first line of defense against environmental pathogens. This physiological relevance of this NADPH oxidase system as a primary defense agent is suggested by the observation that mice lacking the NADPH oxidase components $gp91^{phox}$ or p47 exhibit reduced resistance to infection **[Dinauer *et al*, 1997]**. Additionally, activated neutrophils and macrophages may generate singlet oxygen by reactions that involve either myeloperoxidase or NADPH oxidase **[Steinbeck *et al*, 1993]**. The combined activities of NADPH oxidase and myeloperoxidase systems in phagocytes also lead to the production of hypochlorous acid (HClO), one of the strongest physiological oxidants and a powerful antimicrobial agent **[Hampton *et al*, 1998]**.

2.5.1.3 Redox-Mediated Amplification of Immune Responses

The lymphocytes are carriers of immunological specificity and, therefore, play an important role in the defense against environmental pathogens. A complex combination of regulatory mechanisms ensures that even minute amounts of pathogen activate highly aggressive responses without causing major damage to the host tissue. The immune response essentially involves the lymphocyte receptor for antigen, receptors for costimulatory signals, and various types of cytokines **[Shapiro *et al*, 1997]**. The immune response is also subject to regulation by redox processes. The functional activation of T lymphocytes is strongly enhanced by ROS and/or by a disturbance in the intracellular glutathione redox state **[Breitkreutz *et al*, 2000]**. Superoxide and/or physiologically relevant concentrations of hydrogen peroxide have been shown to augment the production of interleukin-2 by antigenically or mitogenically stimulated T cells in various experimental systems **[Roth and Droge,**

1987 and Los *et al,* 1995]. Low micromolar concentrations of hydrogen peroxide have also been shown to induce the expression of the interleukin-2 receptor in a mouse T-cell lymphoma line **[Los *et al,* 1995]**.

The exposure of T lymphocytes to physiologically relevant concentrations of environmental ROS or other moderate oxidative stress inducers does not bypass the requirement for signaling cascades initiated by specific cell membrane receptors, but such exposure can amplify signaling cascades after relatively weak receptor stimulation **[Breitkreutz *et al,* 2000]**. The signaling cascades from various cell membrane receptors are differentially regulated by ROS. For example, transcription from the interleukin-2 promoter is strongly enhanced in Jurkat T cells by exposure to 50 μM hydrogen peroxide in combination with, but not without anti-CD28 ligands, indicating that the redox effect can enhance the stimulatory signal from the antigen receptor but cannot replace the signal from the CD28 costimulatory receptor **[Breitkreutz *et al,* 2000]**.

2.5.1.4. ROS and Control of Respiratory Ventilation

Oxygen homeostasis in higher organisms is maintained by a tight regulation of the red blood cell mass and respiratory ventilation **[Acker, 1994]**. Carotid bodies are sensory organs that detect changes in arterial blood oxygen. They are essentially composed of glomus type I chemoreceptor cells that release neurotransmitters in response to hypoxia. The changes in the level of electrical activity in the efferent fibers of the carotid sinus nerve, relays the sensory information to the brain stem neurons that regulate breathing **[Acker, 1994]**. Evidence indicates that changes in oxygen concentration are sensed independently by several different ROS-producing proteins including a b-type cytochrome with properties similar to those of the cytochromeb_{558} in the NADPH oxidase system in neutrophils **[Acker, 1994]**. Some studies also suggest that changes in the rate of mitochondrial ROS production may play a major role in oxygen-sensing activity by the carotid bodies **[Bunn and Poyton, 1996]**.

2.5.1.5. Regulation of Vascular Tone and Platelet Adhesion

Guanylate cyclase, is an enzyme which belongs to the family of heterodimeric heme proteins and catalyzes the formation of cGMP, which is then utilized as an intracellular amplifier and second messenger in several physiological responses **[Ignarro and Kadowitz, 1985]**. Nitric oxide binds to the heme moiety of guanylate cyclase leading to a disruption in the planar form of the heme iron and this resultant conformational change then activates the enzyme. The resultant product cGMP modulates the function of protein kinases, phosphodiesterases, ion channels, and other physiologically important targets. Important examples include the regulation of smooth muscle tone **[Ignarro and Kadowitz, 1985]** and the inhibition of platelet adhesion **[Radomski *et al,* 1987]**. Superoxide and hydrogen peroxide may also play a role in the activation of guanylate cyclase **[Mittal and Murard, 1977]** possibly via a NOS-mediated pathway. However, other studies suggest that compound I, a form of catalase, may play a critical role in the activation of guanylate cyclase by hydrogen peroxide **[Burke and Wolin, 1987]**. After interacting with hydrogen peroxide, catalase is converted into compound I (an oxidized heme intermediate) which is normally converted back into its reduced form by a second molecule of hydrogen peroxide **[Burke and Wolin, 1987].**

2.5.2.0. Mechanisms of generation of ROS in Type 2 Diabetes Mellitus

The proportion of molecular oxygen reduced to superoxide rather than water is increased if *1)* the proton gradient at the mitochondrial matrix is high and *2)* the proper flux of electrons through the ETC is energetically less favored **[Boveris and Chance, 1973].** Because the proton gradient is coupled to the conversion of ADP into ATP, the mitochondrial ROS generation is essentially strong if the availability of ADP is low **[Boveris and Chance, 1973]**. Under these conditions the components of the ETC are largely in the reduced state. Since ATP consumption and APD availability are particularly low during periods of sleep when the muscular activity is low, the mitochondrial oxidative stress may be particulary high at night. Additionally, a study on the gastrocnemius muscle in mice revealed that the activity of complex IV decreases by 90% from 10 to 26 months of age **[Desai *et al,* 1996]**. This process is

likely to typically impair the proper flux of electrons through the ETC and to consequently enhance the mitochondrial production of superoxide.

The influx of electrons into the ETC is also determined by the availability of the electron donors NADH (complex I) and succinate (complex II) **[Boveris and Chance, 1973].** The availability of NADH is determined by the bioavailability of mitochondrial energy substrates such as acetyl CoA. Therefore an excess of substrates is normally prevented through a tight regulation of key enzymes in the glycolytic pathway **[DeFronzo, 1997]**. Phosphofructokinase (PFK), one of the early enzymes in this pathway, is inhibited by ATP and citrate and thereby coupled to the generation of pyruvate and acetyl CoA to the cellular energy demand. This feedback control of the glycolytic pathway can be overridden, however, by excess fructose-6-phosphate, the PFK substrate derived from glucose via glucose-6-phosphate **[DeFronzo, 1997]**.

The disproportionate amount of the excess glucose taken up by muscle tissue in patients with type 2 diabetes mellitus in the postabsorptive state due to plasma hyperglycemia is converted glycolytically into lactate. In one study **[Nishikawa *et al,* 2000]**, pathologically relevant elevation of the extracellular glucose concentration in cultured aorta endothelial cells was shown to cause a major increase in ROS production by the mitochondrial ETC.

Chronic hyperglycaemia can activate several well-characterized biochemical pathways that play a significant role in the development of diabetic complications. In each of these cases, activation of these pathways appears to be linked to a hyperglycaemia-mediated rise in ROS production and consequent increase in oxidative stress [**Esposito *et al,* 1989].**

2.5.2.1 AGE/RAGE pathway

Advanced glycated end-products (AGEs) describe a heterogeneous group of combined carbohydrates, proteins, lipids, and nucleic acids that are formed non-enzymatically **[Brownlee, 1995]**. AGEs formation have been shown to be enhanced in the presence of hyperglycaemia and oxidative stress **[Makita *et al,* 1992]**.

The AGEs bind to their cognate cell-surface receptors, RAGE, resulting in the activation of several post-receptor signaling and the activation of gene expression with a consequential generation of intracellular oxygen free radicals **[Esposito *et al,* 1989, Schmidt and Stern, 2000]**.

2.5.2.2. Polyol pathway

When intracellular glucose rises, the aldose reductase activity is stimulated and catalyzes the formation of sorbitol, which can be oxidized to fructose by sorbitol dehydrogenase **[Stevens *et al,* 2000]**. Intracellular accumulation of sorbitol may not only cause cell damage but can activate several stress-sensitive signaling pathways including p38, MAPK and JNK and generation of ROS. The significant role of the activation of the polyol pathway as a cause of diabetic complications has been demonstrated in transgenic mice that overexpress the aldose reductase gene, and by the observations that inhibitors of this enzyme prevent the development of neuropathy, nephropathy, retinopathy, and cataract formation in these animals **[Lee *et al,* 1995, Yamaoka *et al,* 1995]**.

2.5.2.3. Hexosamine pathway

The fact that excessive influx of glucose or FFA results in the activation of the hexosamine biosynthetic pathway have been established in a variety of cell types **[Herbert *et al,* 1996]** and it has been proposed that the activation of this pathway leads to insulin resistance and the development of late complications of diabetes **[Hawkins *et al,* 1997, Herbert *et al,* 1996]**. Mice that overexpress glutamine:fructose-6-phosphate amidotransferase (GFAT), the rate-limiting enzyme of hexosamine biosynthesis, have been shown to be insulin resistant **[Herbert *et al,* 1996]**. Overexpression of GFAT in the liver of transgenic mice drives their phenotype toward energy storage, resulting in hyperlipidemia and obesity **[Herbert *et al,* 1996]**. Recent studies have implicated the activation of the hexosamine pathway by hyperglycemia-induced increase in ROS formation. Additionally, the hexosamine pathway can also function as a cellular "sensor" of energy availability and mediates the effects of glucose on the expression of several gene products like leptin **[Wang *et al,* 1998, Rossetti, 2000]**.

2.5.2.4. PKC pathway

Evidence shows that in tissues in which diabetic complications develop, the concentration of diacylglycerol, an allosteric activator of PKC, is increased **[Koya and King, 1998]**. Several isoforms of PKC are activated as a consequence of the increase in diacylglycerol. PKC-ß for example is the major isoform that is induced in the vasculature, kidney, and retina **[Koya and King, 1998]**. Chronic hyperglycaemia causes an increase in PKC activity and is associated with many processes involved in the pathology of diabetic complications including the regulation of vascular permeability, blood flow, and neovascularisation **[Ishii *et al,* 1996]**. The significant role of the activation of the PKC pathway as a major cause of diabetic complications is strongly supported by the ability of a specific synthetic inhibitor of PKC-ß to ameliorate abnormal retina and renal haemodynamics in diabetic rats **[Ishii *et al,* 1996]**. Additionally, activation of the PKC pathway by hyperglycaemia synergizes with other stress-kinase pathways. For example, hyperglycaemia led to a PKC-dependent enhancement of the activation of MAPK by the vasoactive peptide endothelial-1 in mesangial cells [**Glogowski *et al,* 1999]**.

2.5.3.0. Oxidative stress and Insulin resistance

Although oxidative stress is associated with the macro- and microvascular complications of diabetes mellitus **[Brownlee, 2001]**, it has also been linked to insulin resistance *in vivo* (defined as a subnormal response to a given amount of insulin) **[Ceriello, 2000]**. Several clinical trials have demonstrated improved insulin sensitivity in previously insulin-resistant and/or diabetic patients treated with the antioxidants vitamin C, vitamin E, alpha-lipoic acid (LA), and glutathione **[Hirai *et al,* 2000, Hirashima *et al,* 2000].** In patients with type 2 diabetes, both acute and chronic administration of LA also improves insulin resistance **[Jacob *et al,* 1995, Jacob *et al,* 1996].**

2.5.3.1. Oxidative stress, IRS phosphorylation, Activation of stress-kinases, and insulin resistance

The insulin receptors carry intrinsic tyrosine kinase activities. Insulin binding to insulin receptor triggers an auto-phosphorylation of the receptor. The phosphorylated receptor next phosphorylates the insulin receptor substrate proteins (IRS) on tyrosine residues. This tyrosine phosphorylation activity is a distinguished feature of insulin receptor since tyrosine phosphorylation is infrequent (< 0.03% of total amino acid phosphorylation) in mammalian cells. Oxidative stress has been shown to result in the activation of multiple serine kinase cascades **[Kyriakis and Avruch, 1996]**. There exist a number of potential targets of these kinases in the insulin signaling pathway, including the insulin receptor (IR) and the insulin receptor substrate (IRS) family of proteins. Increased phosphorylation of the IR or IRS on discrete serine or threonine sites inhibits the extent of their tyrosine phosphorylation, with a consequential impairment of insulin action **[Li *et al,* 1999, Qiao *et al,* 1999]**. The serine/threonine phosphorylated forms of IRS molecules are less able to associate with the IR and other downstream target molecules, especially phosphatidylinositol 3-kinase **[Paz *et al,* 1996]**, resulting in impaired insulin action including protein kinase B activation, and glucose transport **[Birnbaum, 2001]**.

For example, induction of oxidative stress in 3T3-L1 adipocytes with H_2O_2 inhibits insulin-stimulated glucose transport **[Tirosh *et al,* 1999]** and this effect was found to be selective for insulin-stimulated signaling compared with platelet-derived growth factor-stimulated signaling and was reversed by preincubation with the antioxidant alpha-Lipoic acid **[Tirosh *et al,* 1999].** Recent evidence also shows that IKKß, which activates NF-κB, is increased in insulin-resistant muscle from a variety of sources. Activation of IKKß inhibits insulin action; salicylates and ligands for PPARγ, both of which inhibit IKKß activity can restore insulin sensitivity both *in vitro* and *in vivo* **[Yuan *et al,* 2001, Kim *et al,* 2001].** Treatment with aspirin and other salicylates alters the phosphorylation patterns of the IRS proteins, resulting in decreased serine phosphorylation and increased tyrosine phosphorylation **[Yuan *et al,* 2001, Kim *et al,* 2001]**. Ouchi *et al,* (2000) have also shown evidence that suggests that the potent insulin sensitizing activity of adiponectin, the circulating protein secreted from adipocytes may also be associated with inhibition of NF-κB activation

Evidence for the support for the importance of IKKß in insulin resistance *in vivo* is provided by results of recent gene knockout experiments in mice. IKKß (+/-) heterozygotes were more insulin sensitive as judged by increased glucose infusion rate during hyperinsulinemic-euglycaemic clamp) compared with their normal (+/+) littermates **[Yuan *et al,* 2001, Kim *et al,* 2001]**. The significant improvement in insulin sensitivity was even more expressed when IKKß (+/-) mice were crossbred with insulin-resistant *ob/ob* mice.

Treatment of patients with type 2 diabetes mellitus for 2 wk with high-dose aspirin (7g/day) resulted in reduced hepatic glucose production and fasting hyperglycaemia and increased insulin sensitivity **[Hundal *et al,* 2001]**. All these data support a role for activation of IKKß in the pathogenesis of insulin resistance and type 2 diabetesmellitus and suggest that it might be an attractive pharmacological target to increase insulin sensitivity.

Additional evidence derived from cellular models, transgenic animals, and humans demonstrates the importance of serine/threonine phosphorylation of IRS proteins in the regulation of ß-cell function **[Aspinwall *et al*, 2000]**. Accordingly, enhanced serine/threonine phosphorylation on the IR or its substrates due to increased stress-sensitive kinase activity [*e.g.* NF-κB-activating kinases, p38 MAPK, JNK/SAPK, PKC, IKKß] or other serine/threonine kinases could provide a mechanistic explanation to link activation of the stress pathways to multiple cellular pathologies.

2.5.3.2. Oxidative stress, protein tyrosine phosphatases, and Insulin resistance

Alteration of the intracellular redox balance can also result in the oxidation and inactivation of protein tyrosine phosphatases (PTPases) **[Heffetz *et al* 1990].** This class of enzymes, along with dual-function phosphatases, plays a major role in regulating a wide variety of signaling pathways including the stress-activated pathways **[Goldstein *et al* 1998]**. It has been known that phosphotyrosyl turnover is essential for insulin-stimulated glucose transport in adipocytes and muscle **[Frost and Lane, 1985]**. Although the reversible inhibition of certain PTPases such as PTP-1B improves insulin action and is antidiabetogenic **[Mahadev *et al,* 2001]** oxidation of the

cysteine residues required for catalytic activity inactivates PTPases and can result in insulin resistance *in vitro* **[[Frost and Lane, 1985]**.

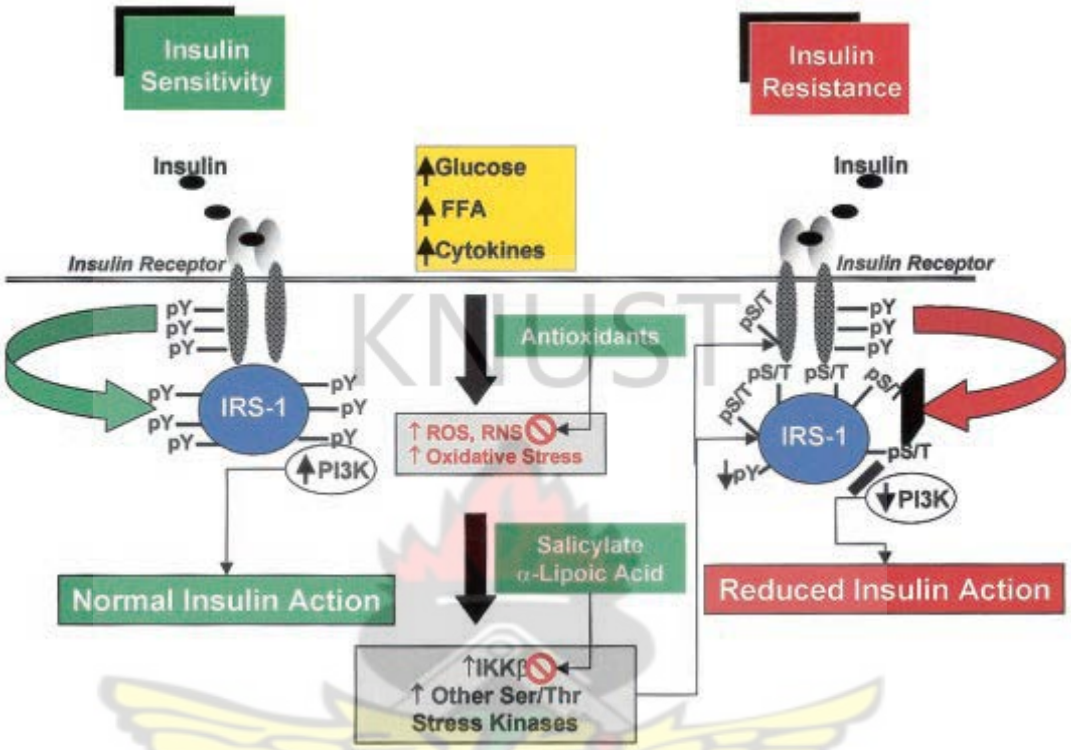


Figure 2.1. The role of serine kinase activation in oxidative stress_induced insulin resistance.

A variety of stimuli, including hyperglycemia, elevated FFA levels, cytokines, and others, increase ROS (and RNS) production and oxidative stress. This results in the activation of multiple stress-sensitive serine/threonine (Ser/Thr) kinase signaling cascades such as IKKβ and others. Once activated, these kinases are able to phosphorylate multiple targets, such as the IR and IRS proteins (including IRS-1 and IRS-2). Increased phosphorylation of IR or IRS proteins on discrete serine or threonine sites (pS/T) decreases the extent of insulin-stimulated tyrosine phosphorylation (pY). Consequently, the association and/or activities of downstream signaling molecules (e.g., phosphatidylinositol 3-kinase [PI3K]) are decreased, resulting in reduced insulin action (insulin resistance). The protective effects of antioxidants (e.g., LA) on oxidative stress_induced insulin resistance could relate to their ability to preserve the intracellular redox balance (neutralizing ROS) or, analogous to pharmacological agents (e.g. salicylates, p38 MAPK inhibitors), to block the activation of stress-sensitive kinases. **(Diagram courtesy Evans *et al,* 2003).**

2.5.3.3. Obesity, free fatty acids and Insulin resistance

The insulin resistance in obesity is evident before the development of chronic hyperglycaemia **[Defronzo, 1997]**. Therefore, it seems unlikely that insulin resistance, at the prediabetic stage, results from oxidative stress triggered by hyperglycaemia *per se*. However, the strong association of obesity and insulin resistance **[Ferraninni *et al,* 1997]** tends to suggest that a major mediator of oxidative stress-induced insulin resistance at the prediabetic stage might be a circulating factor secreted by the adipocytes **[Evans *et al,* 2003]**. In view of this fact, several possible candidate molecules have been suggested including TNF-α **[Moller, 2000]**, leptin **[Cohen *et al,* 1996]**, FFA **[Muller *et al,* 1997]**, and resistin **[Steppan *et al,* 2001]**. However, the evidence strongly suggests that FFA are the most likely link between obesity and insulin resistance **[Evans *et al,* 2003]**.

The plasma FFA content is increased in many states of insulin resistance including obesity and type 2 diabetes mellitus **[McGarry, 1999]**. There is an inverse relationship between fasting plasma FFA concentrations and the accumulation of intramyocellular triglyceride and insulin resistance **[Krssak *et al*, 1999**]. Malonyl-CoA, the first committed intermediate molecule in fatty acid biosynthesis and an inhibitor of carnitine palmitoyl transferase 1, plays a major role in regulating fatty acid synthesis and oxidation **[Ruderman *et al,* 1999]**. This suggests that any dysregulation of malonyl-CoA production that leads to sustained increase in intracellular concentrations of malonyl-CoA and FFA would result in a reduced capacity to oxidize fat, leading to increased tissue stores, and could play a key role in the pathogenesis of insulin resistance and impaired ß-cell function **[Evans *et al,* 2003]**. These data therefore implicate FFA as a causative link between obesity, insulin resistance, and development of type 2 diabetes mellitus**.**

2.5.3.4. Fatty acids, stress-activated pathways, oxidative stress and insulin resistance

In addition to the ability of FFA and their metabolites to impair insulin action by stimulating inhibitory protein kinase activity, FFA could also impair insulin action by increasing the level of oxidative stress. Indeed, this increased oxidative stress might

provide a mechanistic basis for the observed FFA (and/or metabolite)-induced increase in serine kinase activity as aleady discussed above **[Kyriakis and Avruch, 1996].**

In support of this idea, *in vitro* evidence indicates that elevated FFA has numerous adverse effects on mitochondrial function through the uncoupling of oxidative phosphorylation, and the generation of reactive oxygen species **[Morino *et al,* 2006]**. Free fatty acids not only induce a state of oxidative stress, but also disrupt the endogenous antioxidant defenses by decreasing intracellular glutathione [**Henning *et al,* 2001]**. As a consequence of their ability to increase ROS formation and deplete glutathione, FFA are able to activate NF-κB possibly via FFA-mediated activation of PKC **[Dichtl *et al,* 1999]**, which has the unique ability to activate NF-κB. As discussed above, activation of this stress-sensitive pathway results in the expression of genes known to be associated with impaired insulin action along with the complications of diabetes. Henning *et al,* (2001) have shown that FFA-induced activation of NF-κB can be prevented by pretreatment with vitamin E and other antioxidants. It should also be noted that FFAs and many of their derivatives interact directly with transcription factors to regulate gene expression **[Evans *et al,* 2003, Morino *et al,* 2006]** and all these have implications on insulin action.

Further evidence of the significance of FFA in insulin resistance is supported by the findings of Paolissa *et al,* (1996) that showed a significant inverse correlation between fasting plasma FFA concentration and the ratio of reduced/oxidized glutathione in patients with type 2 diabetes mellitus. Additionally, in healthy subjects, infusion of FFA (as 10% Intralipid) causes increased oxidative stress measured by increased malondialdehyde levels and a decline in the plasma reduced/oxidized glutathione ratio and restoration of redox balance by infusing glutathione improves insulin sensitivity along with ß-cell function **[Paolisso *et al,* 1996, Slatter *et al,* 2000]**.

Taken together, these studies suggest that activation of stress-signalling pathways play a role in FFA-induced insulin resistance and since these same signalling pathways also play a role in diabetic complications, these studies suggest a unifying hypothesis of FFA- and hyperglycaemia-induced oxidative stress causing both insulin resistance and late diabetic complications. Moreover, the induction of insulin resistance by FFA-

induced oxidative stress may serve as an early marker of late diabetic complications **[Evans *et al,* 2003, Morino *et al,* 2006]**.

2.5.3.5. Oxidative stress and Beta-cell function

The ß-cell is particularly susceptible to the damages inflicted by oxidative stress because it is not under insulin-stimulated glucose transport **[Evans *et al,* 2003]**. Through the concerted efforts of GLUT2 (the high K_m glucose transporter), glucokinase (the glucose sensor), and glucose metabolism pancreatic ß-cells are responsible for sensing and secreting the appropriate amount of insulin in response to a glucose stimulus **[Bedoya *et al,* 1986, Meglasson and Matschinsky, 1986, Arbuckle *et al,* 1996]**. Although this process involves a complex series of events, mitochondrial metabolism is critical in linking stimulus to secretion **[Morino *et al,* 2006]**. The mitochondria are both free radical generators and also their potential targets and therefore the ability of ROS to damage mitochondria and significantly attenuate insulin secretion is not surprising **[Morino *et al,* 2006]**.

Many studies have reported that ß-cell dysfunction is the result of chronic exposure to hyperglycaemia, chronic exposure to FFA or a combination of both hyperglycaemia and elevated FFA (and/or metabolites) and their effects appear to be dependent upon *1)* direct damage by ROS and *2)* the oxidative stress induction of several stress-sensitive pathways **[Evans *et al,* 2003 and Morino *et al,* 2006]**.

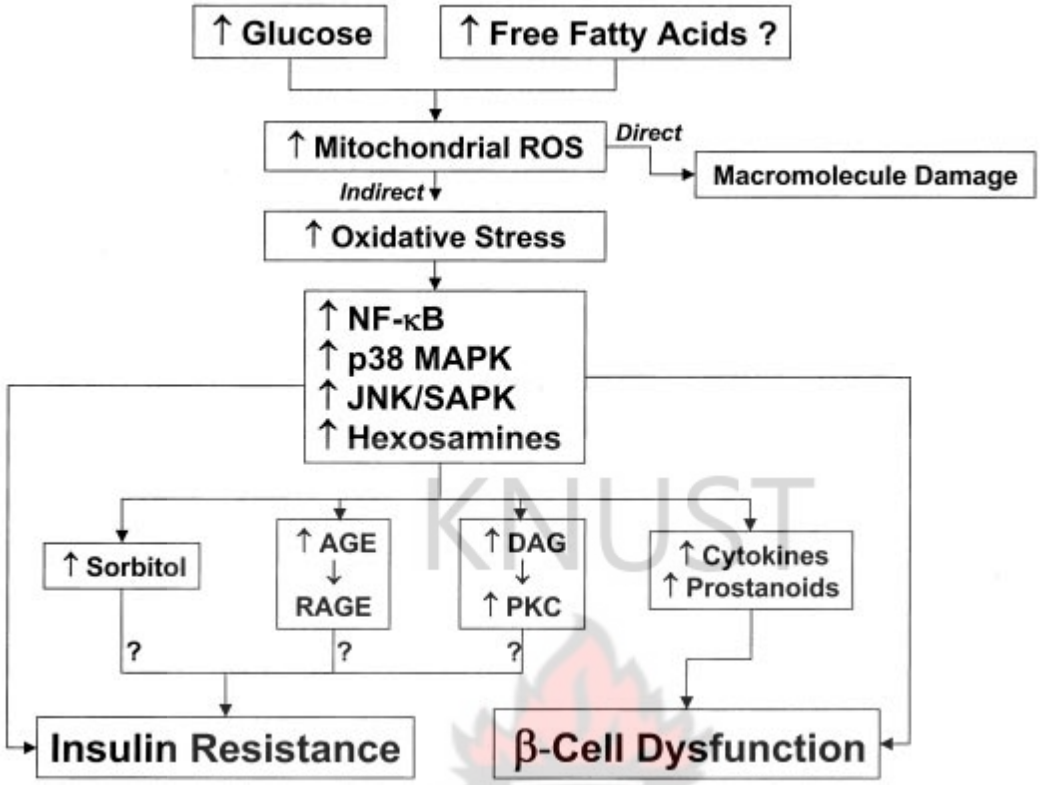


Figure 2.2. Proposed general theory of how elevated glucose and possibly FFA levels contribute to the pathophysiology of diabetes via the generation of ROS and consequent activation of numerous stresssensitive pathways.

The causative link among hyperglycemia, mitochondrial ROS generation, oxidative stress, and the development of diabetic complications has been previously suggested. ROS (and RNS), by inflicting macromolecular damage, may play a key direct role in the pathogenesis of diabetes. ROS also function as signaling molecules (analogous to second messengers) to activate several stresssensitive pathways (indirect role). In addition, in type 2 diabetes, there is growing evidence that activation of stress-sensitive pathways, such as NF-kB, p38 MAPK, JNK/SAPK, and hexosamine, by elevations in glucose and possibly FFA levels leads to both insulin resistance and impaired insulin secretion. Thus ROS and oxidative stress, induced by elevations in glucose and possibly FFA levels, may play a key role in causing insulin resistance and β-cell dysfunction by their ability to activate stress-sensitive signaling pathways. The proposed sequence of events may also include other stress pathways, such as the increased production of AGE, sorbitol, cytokines, and prostanoids along with PKC activation. DAG, diacylglycerol. **(Diagram courtesy Evans *et al,* 2003).**

2.6.0 STATEMENT OF PROBLEM

2.6.1. Diabetes is accompanied by severe oxidative stress

The development and progression of type 2 diabetes mellitus is caused by numerous metabolic events that occur well over a period of years (10-20 years) with hyperglycaemia as the central feature. However, the consequential effects are not limited to only carbohydrate metabolism; lipid and protein metabolism also play an important role in the progression of the disease **[Bao *et al,* 1996, Moller and Loft, 2003, Evans *et al,* 2003].**

A currently favoured hypothesis is that oxidative stress is the common pathogenic factor leading to insulin resistance, β-cell dysfunction, impaired glucose tolerance (IGT) and ultimately to type 2 DM (T2DM). Furthermore, this mechanism has been implicated as the underlying cause of both the microvascular and macrovascular complications associated with T2DM **[Moller and Loft, 2003, Evans *et al,* 2003, Morino *et al,* 2006].**

The most important tissues involved in the pathogenesis of insulin resistance are the muscle and adipose tissues. When caloric intake exceeds the energy expenditure, the substrate-induced increase in citric acid cycle activity generates an excess of both mitochondrial NADH (mNADH) and reactive oxygen species (ROS) **[Paolisso *et al,* 1996].** As described above, to protect themselves against harmful effects of ROS, cells may reduce the formation of ROS and/or enhance ROS removal. The prevention of ROS formation is accomplished by preventing the build-up of mNADH by inhibiting insulin-stimulated nutrient uptake and preventing the entrance of energetic substrates (pyruvate, fatty acids) into the mitochondria.

However, controversy exists as to whether free fatty acid (FFA) or glucose is the primary fuel source in the overnourished muscle and adipose tissue. In either case, an influx of substrates into the citric acid cycle generates mitochondrial acetyl-CoA and NADH **[Paolisso *et al,* 1996].** The acetyl-CoA, derived either from glucose through pyruvate or from beta-oxidation of FFA, combines with oxaloacetate to form citrate, which enters the citric acid cycle and is converted to isocitrate. NAD^+-dependent isocitrate dehydrogenase then generates NADH. When this excessive NADH cannot

be dissipated by oxidative phosphorylation (or other mechanisms), the mitochondrial proton gradient increases and single electrons are transferred to oxygen, leading to the formation of ROS, particularly superoxide anion **[Paolisso *et al,* 1996].** The generation of excessive NADH may be prevented in several ways, one of which is the inhibition of FFA oxidation **[Paolisso *et al,* 1996]**. The increase in intracellular FFA, in turn, leads to reduced GLUT4 translocation to the plasma membrane, resulting in resistance to insulin-stimulated glucose uptake in muscle and adipose tissue **[Paolisso *et al,* 1996]**. The insulin resistance, in this setting, may be considered a compensatory mechanism that protects the cells against further insulin-stimulated glucose and fatty acid uptake and therefore oxidative damage. Many studies, both *in vitro* and in animal models support this hypothesis and antioxidants have been shown to improve insulin sensitivity **[Paolisso *et al,* 1996].** Several clinical trials have demonstrated that treatment with vitamin E, vitamin C, or glutathione improves insulin sensitivity in insulin-resistant individuals **[Paolisso *et al,* 1996]**. The recent finding that insulin resistance is associated in humans with reduced intracellular antioxidant defense also supports this hypothesis **[Paolisso *et al,* 1996].**

It is a reasonable hypothesis that what happens in the muscle and fat cells may also occur in other cells, particularly in ß cells and endothelial cells. Furthermore, these cell types may be particularly affected by overfeeding since these cells are notably not dependent on insulin for glucose uptake, which in this case is via facilitative diffusion instead of insulin-regulated glucose transporters. Thus, if overfed, they cannot down regulate the influx of nutrients by means of insulin resistance, and must allow intracellular concentrations to increase further **[Evans *et al,* 2003]**.

Chronic hyperglycaemia has also been implicated as a secondary force that further damages the pancreatic ß-cells through glucose toxicity. The clinical state of diabetes mellitus is often accompanied by elevated blood levels of cholesterol, triglyceride, and free fatty acids and prolonged exposure of pancreatic beta cells to fatty acids has also been reported to inhibit insulin gene expression **[Robertson *et al,* 2004].** Therefore, the reasonable hypothesis is that the concomitant presence of hyperglycaemia and elevated fatty acid levels causes accumulation of cytosolic citrate, the precursor of malonyl-CoA, which inhibits carnitine palmitoyltransferase-1, the enzyme responsible

for fatty acid transport into the mitochondrion **[Robertson *et al,* 2004].** This model suggests that, in the presence of high glucose concentrations, elevated free fatty acids are not readily oxidized in mitochondria but are shunted toward esterification pathways. Glucose toxicity therefore can occur in the absence of hyperlipidaemia, whereas lipotoxicity attributable to hyperlipidaemia occurs only in the context of preexisting hyperglycaemia **[Robertson *et al,* 2004].** The pancreatic beta-cells are particularly sensitive to ROS because they are low in free-radical quenching (antioxidant) enzymes such as catalase, glutathione peroxidase, and superoxide dismutase **[Robertson *et al,* 2004]**. For example, it has been demonstrated that oxidative stress generated by short exposure of ß-cells to H_2O_2 increases production of p21 and decreases insulin mRNA, cytosolic ATP, and calcium flux in the cytosol and mitochondria **[Paolisso, 1996].** Hence, the ability of oxidative stress to damage mitochondria and markedly blunt insulin secretion.

The hyperlipidaemia associated with diabetes may also lead to increased lipid peroxidation possibly because an increased lipid load allows lipoproteins to reside for longer periods in the circulation and in vessel walls, giving them a prolonged exposure to any free radicals generated **[Walzem *et al,* 1995].** Furthermore, hyperketonaemia may also promote lipid peroxidation and glycation of lipoproteins render them more susceptible to peroxidation **[Sakata *et al,* 2001].**

The reactive species may not only directly damage cells by oxidizing DNA, protein, and lipids, but indirectly damage cells by activating a variety of stress-sensitive intracellular signaling pathways. The activation of these pathways results in the increased expression of numerous gene products that also cause cellular damage and play a major role in the aetiology of the cardiovascular disease **[Evans *et al,* 2003].**

2.6.2. The oxidative stress may precede the development of type 2 diabetes

Dysfunction of pancreatic ß-cells (loss of the normal insulin secretion pattern) and insulin resistance contribute to glucose intolerance and both can be found very early in the disease process, finally leading to type 2 diabetes mellitus **[Bao *et al,* 1996, DeFronzo, 1997].** Initially, the insulin resistance is compensated by hyperinsulinemia through which a normal glucose tolerance is preserved. Subsequent deterioration to

IGT occurs when insulin resistance increases further and/or the compensatory insulin secretory response decreases. An increase in insulin, FFA, and/or glucose levels can increase ROS production and oxidative stress, as well as the activation of stress-sensitive pathways **[Evans *et al,* 2003].** This, in turn, can worsen both insulin action and secretion and thereby accelerate the progression to overt type 2 diabetes mellitus. The loss of early-phase insulin response is a common event in subjects with impaired glucose metabolism. This alteration may not simply be a marker of the risk of diabetes, but more importantly a significant pathogenic mechanism causing excessive postprandial hyperglycaemia **[Ceriello *et al,* 2002].** Additionally, increased lipid peroxidation can be detected in the early stages of type 2 diabetes, well before the development of any diabetic complications **[Guzel *et al* 2000].** Lipid peroxides are well-known to cause tissue damage, hence the possibility that nephropathy, retinopathy, endothelial dysfunction and peripheral neuropathy associated with poor diabetic control involve free radical damage **[Oranje and Wolffenbuttel, 1999, Rosen *et al,* 2001]**. Elevated lipid peroxidation may precede the development of type 2 diabetes mellitus. There is a considerable variation in levels of lipid peroxides even in healthy subjects, hence the proposal that persons with elevated lipid peroxidation may be more prone to develop type 2 diabetes mellitus and cardiovascular disease **[Knept *et al,* 1999].** Facchini *et al* (2000) commented that lipid peroxidation may be increased in insulin-resistant individuals well before the onset of type 2 diabetes. Gopaul *et al* (2001) have also found increased lipid peroxidation in Indian Mauritian subjects with impaired glucose tolerance compared with control subjects. This means that not only may the sequelae of type 2 diabetes (hyperglycaemia and hyperlipidaemia) cause elevated peroxidation, but pre-existing high rates of lipid peroxidation may itself predispose to type 2 diabetes mellitus. Such elevated rates may reflect poor antioxidant intake **[Salonen *et al,* 1995]**, but could also involve inherited differences in rates of lipid metabolism and peroxidation **[Halliwell, 2000].** As discussed above, many studies on the relationship between oxidative stress and type 2 diabetes mellitus have shown that oxidative stress is associated with the development of diabetes complications. Chronic systemic oxidative stress has been shown to cause insulin resistance in rodents **[Houstis *et al,* 2006].** Subjects with pre-diabetes may therefore be predisposed to higher rates of ROS and other free radical attack well before the development of overt diabetes. However, there is limited data on the

relationship between pre-existing rates of oxidative stress and type 2 diabetes and other dysmetabolic conditions. The study focused on the relationship between pre-existing levels of oxidative stress and diabetes mellitus and other dysmetabolic conditions in the free-living and apparently healthy populations in urban and rural communities.

2.6.3 Study hypothesis

The fast progressing westernization of the Ghanaian culture is predisposing increasing numbers of the population to higher rates of systemic oxidative stress and this is associated with consequential increases in the incidence of type 2 diabetes mellitus and other dysmetabolic conditions.

2.7.0. RATIONALE OF STUDY

Type 2 diabetes mellitus is increasingly common throughout the world. The World Health Organization has predicted that between 1997 and 2025, the number of diabetics will double from 143 million to about 300 million **[WHO, 1998].** The incidence of type 2 diabetes mellitus is highest in economically developed nations, particularly the U.S., where approximately 6.5% of the population (17 million people) have either been diagnosed or undiagnosed diabetes. Additionally, there are an estimated 25 to 30 million people in the U.S. with impaired glucose tolerance. The incidence of type 2 diabetes mellitus in the U.S. has increased by about 33% over the past decade and is expected to increase further. However, the greatest rise in the prevalence of type 2 diabetes mellitus is projected to occur in developing countries **[King *et al,* 1998].**

Both type 2 diabetes mellitus and glucose intolerance increases with age. It is estimated that between 25% and 30% of the U.S. population aged 65 and older has type 2 diabetes mellitus or impaired glucose tolerance **[WHO, 1998].** Older adults exhibiting no clinical signs of diabetes may often demonstrate decreased glucose tolerance (i.e., decreased glucose disposal rate as determined by an oral glucose tolerance test). Although it is often considered a disease primarily of the elderly and middle-aged, type 2 diabetes mellitus has increased in all age groups over the past 10 years **[WHO, 1998].**

The greatest increase in type 2 diabetes mellitus has occurred among persons 30 to 50 years of age. For example, the incidence of type 2 diabetes mellitus among people aged 30 to 39 years increased approximately 70% during the past decade **[WHO, 1998].** In addition to age, the only other non-modifiable factor associated with an increased risk for the development of type 2 diabetes mellitus is ethnicity. During the past 10 years the prevalence of type 2 diabetes mellitus in the U.S. has increased across all ethnic groups **[WHO, 1998].** However, the greatest increase in the incidence of type 2 diabetes mellitus has occurred among African-Americans, Hispanics, and American Indians. Persons from these ethnic backgrounds are known to be at significantly greater risk for developing type 2 diabetes mellitus than are Caucasians. The apparent differences in risk among the various ethnic groups may ordinarily

reflect different dietary and lifestyle choices, or they may be due to a combination of genetic and environmental factors. In genetically susceptible persons, the development of type 2 diabetes mellitus appears to be facilitated by the adoption of the so-called western lifestyle which is a cluster of low physical activity pattern, cigarette smoking, and higher intakes of diets rich in calories (especially from saturated fat) and poor in fruits and vegetables. **[WHO, 1998, Cummings, 1988].** Though epidemiological data on the prevalence of diabetes in Ghana is scanty, diabetes mellitus seems to be a silent major non–communicable disease in Ghana. Type 2 diabetes is a major and growing problem in Ghana. It has been described as a silent-killer that is rubbing shoulders with the HIV/AIDS pandemic, and, strategies to delay its onset and ameliorate the side-effects of poor diabetes control are urgently needed. A recent study **[Amoah *et al,* 2002]**, reported a high prevalence rate of 6.3%. Also, a recent study on the prevalence and sociodemographic aspects of overweight and obesity among residents from rural and urban Accra reported an overall crude prevalence of 23.4% and 14.1%, respectively **[Amoah, 2003].**

Previous studies among the general population appear to show that dyslipidaemia was not a problem in Ghana **[Asibey-Berko and Avorkliyah, 1999].** However, a recent study showed that hyperlipidaemia is becoming a problem in Kumasi and Ashanti **[Eghan and Acheampong, 2003].** In recent years, the Ghanaian society has grown significantly, and the population mostly uses public and other forms of transportation. Most farmers, for example, do not walk to their farms anymore (walking represented one of the main forms of exercise for them). This is coupled with the changing lifestyle, characterized by increasing consumption of calorie-rich foods in the western diet. Overweight or obesity is considered a sign of well-being, respect, or beauty in this part of the country, and represents another part of the problem, especially when there is a regular inflow of foreign money to family members from relatives abroad **[Eghan and Acheampong, 2003]**. Obesity is known to be associated with insulin resistance. However, this relationship has become confusing since not all obese individuals are insulin resistant and that insulin resistance also occurs in individuals with normal BMIs **[Hansen *et al,* 2002].**

Modern life also confronts us with pollution, consumption of alcohol or medications, prolonged exposure to sunlight, smoking and sedentary lifestyle. All of these situations cause overproduction of ROS in our organism. This leads to a weakening of our antioxidant defenses (vitamins, oligoelements) and leading to oxidative stress and cell damage. To complicate the situation, our current diet is not healthy or balanced enough and thus provides inadequate amounts of the natural antioxidants needed to control the harmful effects of ROS.

Both in health and disease, there is an increasing interest into the significance of optimal vitamin and antioxidant status. Evidence has accumulated from epidemiological studies that serum vitamin E concentrations and mortality from ischemic heart disease are inversely related **[Salonen *et al,* 1995],** as are serum carotenoid concentrations and the incidence of several types of cancer **[Ford, 2001]**. Antioxidant status evidently varies considerably among different populations **[Gopaul *et al,* 2001]** and changes over time in dietary habits affect not only the intake of fruits and vegetables but also the consumption of polyunsaturated fatty acids, all of which influence the oxidant–antioxidant homeostasis. As already discussed above, impaired antioxidant status has been implicated in several disorders and intervention studies for correction of these deficiencies have been conducted or are still in progress. In all these situations, the definition of threshold values for initiation of intervention and successful treatment is critical. Preferably, baseline values obtained from the general healthy population living in the same area should be used to define this threshold.

Optimal nutrition in childhood and adolescence is increasingly becoming a general concern because development of certain diseases may be determined very early in life. For instance, autopsy studies confirm the presence of atherosclerotic lesions in the aortas and coronary arteries of teenagers and young adults **[Stary, 1989].**

Glucose tolerance is traditionally classified into three categories: normal (NGT), impaired (IGT), and diabetic **[WHO, 1998]**. The category of I G T, defined by an impaired glucose response 2 h after an oral glucose load (7.8–11.1 mmol/l) but a “normal” fasting plasma glucose concentration (<7.8 mmol/l), represents a metabolic state intermediate between normal and diabetic glucose homeostasis. Many cross-sectional studies have examined the metabolic characteristics of individuals with IGT

and have shown that they are, on average, more obese and more insulin resistant than individuals with NGT, and are also typically hyperinsulinemic **[Bogardus, 1996].** In 1997, the American Diabetes Association (ADA) released new diagnostic recommendations that promote the use of fasting rather than 2-h glucose concentrations for screening and diagnosis of diabetes. In addition to lowering the fasting plasma glucose level diagnostic of diabetes from 7.8 to 7.0 mmol/l, a new category was introduced, termed "impaired fasting glucose" (IFG) (fasting plasma glucose concentration 6.1–7.0 mmol/l) **[WHO, 1998].** Analogous to the category of IGT, this new diagnostic entity is meant to be an intermediate metabolic state between normal and diabetic glucose homeostasis. The choice of 6.1 mmol/l as the lower cutoff level for IFG was based largely in part on epidemiological data on the risk of micro- and macrovascular complications **[WHO, 1998]**, but it is also supported by pathophysiological data because it is near the level above which early phase insulin secretion is lost **[WHO, 1998]**. With the use of both WHO and the ADA diagnostic criteria, it is possible to classify impaired glucose homeostasis into three different subcategories: *1*) isolated IFG (impaired fasting but normal 2-h glucose), *2*) isolated IGT (normal fasting but impaired 2-h glucose), and *3*) combined IFG and IGT (impaired fasting and 2-h glucose). However, the metabolic abnormalities underlying these different groups of impaired glucose homeostasis remain to be elucidated. Results from epidemiological studies suggest that many subjects with pre-diabetes will eventually develop overt diabetes within 5-10 years **[Zimmet *et al*, 2002]** and strategies to help delay or prevent the conversion to overt diabetes are urgently needed. The increasing westernization of our society may predispose us to higher rates of ROS and other free radicals and defective antioxidant defenses leading to higher rates of oxidative stress. Increasing rates of oxidative stress in genetically predisposed societies may be the reason for the alarming increase in prevalence of pre-diabetes and the conversion to full diabetes.

However, there is limited data on the role of levels of naturally occurring antioxidant vitamins and oxidative stress in the metabolic characteristics underlying pre-diabetes. Additionally, there is inconsistent and inconclusive evidence of the beneficial role of high-dose antioxidant supplementation in preventing or delaying the onset and

progression of diabetes due to several methodological factors [**Moller and Loft, 2002].**

As already discussed, type 2 diabetic patients have an excess risk of developing atherosclerosis, resulting in high cardiovascular disease morbidity and mortality [**Haffner *et al,* 1990, Evans *et al,* 2003].** Hence, with the rise of the prevalence of diabetes, it may be expected that the global burden of cardiovascular disease will also increase. To guard against these potentially devastating degenerative complications, it's important that both oxidative stress and metabolic control of glucose and lipids be carefully kept in check and monitored regularly in patients with, or at risk of, type-2 diabetes mellitus.

With the above discussed background, this study will therefore investigate the comparative risk of development of type 2 diabetes mellitus among subjects in rural and urban communities in Ashanti region and the relationship between metabolic status, levels of antioxidants and oxidative stress in type 2 diabetes and other dysmetabolic conditions.

2.8.0. OBJECTIVES OF RESEARCH

The objectives of the research included the following:

- Conduct trial to investigate the differences in prevalence of type 2 diabetes in urban and rural communities in the Ashanti region;
- Conduct trial to investigate the differences in the plasma level of malondialdehyde and antioxidants;
- Conduct trial to investigate the differences in the prevalence of the metabolic syndrome and the contribution of the various components in the classification of the metabolic syndrome;
- Develop a simple tool that can be used for the identification of Ghanaians who are at high risk of development of type 2 diabetes and other dysmetabolic states.

Chapter 3

MATERIALS AND METHODOLOGY

3.1.0. Subjects

The study aimed at comparing the relationship between oxidative stress and dysmetabolism in urban and rural adult populations. The community-based study included 210 adults (90 females and 120 males) aged between 32-76 years (mean 51.08 ± 0.59) living in urban communities in Kumasi and 180 (129 females and 51 males) aged between 36 – 97 years (mean 59.43 ± 1.06) living in rural communities in the Ashanti region the second largest city in Ghana. The urban subjects were selected from various government and private institutions and churches in Kumasi whiles rural subjects were selected from three rural communities (Abore, Manso Nkwanta and Atwedie) in the Ashanti region after a diabetes awareness talk had been given. Sociodemographic data, dietary habits and physical activity were ascertained with a structured questionnaire (Appendix 2).

3.2.1. Inclusion criteria:

1. Non-diabetic adults aged 30 years and above,
2. Relatives of known diabetics,
3. Subjects who have ever been diagnosed of impaired glucose regulation.

3.2.2. Exclusion criteria:

1. Pregnancy,
2. History of any other chronic conditions such as previously diagnosed diabetes, hypertension, chronic hepatitis, cholestatic jaundice, neurodegenerative disease, cancer, HIV/AIDS, nephropathy, gastrointestinal insufficiency, Cushing's syndrome, acromegaly, thyroid dysfunction or current illness.
3. Medication affecting glucose or lipid metabolism,
4. Regular antioxidant supplementation for at least one month before the start of the study.

The study was approved by the Committee on Human Research, Publications and Ethics (CHRPE), School of Medical Sciences, Kwame Nkrumah University of Science & Technology (KNUST), Kumasi. Participation in the study was voluntary and all patients enrolling in the study completed a written informed consent form.

3.3.0. Anthropometric variables

The subjects were weighed on a bathroom scale while barefooted and in light clothing and their height was measured with a wall-mounted ruler. BMI was calculated by dividing weight (kg) by height squared (m^2). Waist circumference was measured with a Gulick II springloaded measuring tape (Gay Mills, WI) midway between the inferior angle of the ribs and the suprailiac crest and recorded in centimeters. Blood pressure was measured by qualified nurses using a mercury sphygmomanometer and stethoscope. Measurements were taken from the left upper arm after at least 5 minutes rest in a sitting position. Duplicate measurements were taken with a 5 min rest interval between measurements and the mean value was recorded in mmHg.

3.4.0. Sample preparation

Blood samples were collected from the ante cubital vein after at 12 hours of overnight fast. Rubber tourniquet was applied for less than one minute and the site to be punctured cleaned with 70% methylated spirit. Blood (10ml) was taken into separate vacutainers tubes. Two (2) milliliters of blood was dispensed into tubes containing fluoride oxalate, 5ml of blood was dispensed into EDTA tubes whiles the rest was dispensed into plain tubes and allowed to clot. The tubes were then placed in a centrifuge and spun at 3000 x g for 10 minutes to obtain the plasma and sera. Plasma glucose was measured immediately and the serum and plasma for the measurement of other biochemical variables and oxidative stress indices were stored at -80°C until analysis.

3.5.0. Biochemical variables

Various biochemical assays including glucose, total cholesterol, HDL-cholesterol, triglycerides, total bilirubin, total protein, albumin and uric acid were analysed using the ATAC® 8000 Random Access Chemistry System (Elan Diagnostic Systems,

Smithfield, RI, USA). For the measurement of biochemical variables, aliquots of serum were dispensed into cryotubes after thawing. The tubes with the sera were then placed at pre-programmed positions in the autoanalyser and the analysis was done in batches. All the reagents used in the analysis were made by the same manufacturer. LDL-cholesterol was calculated according to Friedewald equation **[Friedewald *et al,* 1972]**: LDL-cholesterol = total cholesterol – HDL-cholesterol – (triglycerides/2.19). Dyslipidaemia was classified based on the National Cholesterol Education Panel (appendix 3).

3.5.1. Glucose

The method used for the analysis of glucose was the glucose hexokinase method based on a modification of **Slein (1963)** using hexokinase and glucose-6-phosphate-dehydrogenase to catalyze the reaction. Glucose is phosphorylated with adenosine triphosphate (ATP) in the reaction catalyzed by hexokinase (HK). The product, glucose-6-phosphate is then oxidized with the concomitant reduction of nicotinamide adenine dinucleotide (NAD) to NADH in the reaction catalyzed by glucose-6-phosphate-dehydogenase. The formation of NADH causes an increase in absorbance at 340nm. The increase is directly proportional to the amount of glucose in the sample.

3.5.2. Total cholesterol

The method of analysis was first described by **Trinder (1969)**. Cholesterol esters are broken down to cholesterol and fatty acids. The cholesterol is then oxidized to chole-4-en-3-one and hydrogen peroxide. The hydrogen peroxide is the hydrolyzed by a peroxidase to a red dye (quinoneimine).

Cholesterol Esters $\xrightarrow{\text{C. Esterase}}$ Cholesterol + Faty acids

Cholesterol + 0_2 $\xrightarrow{\text{C. Oxidase}}$ Cholest-4-en-3-one + H_20_2

$2H_2\,0_2$ + Hydroxybenzoic acid + 4-AAP $\xrightarrow{\text{Peroxidase}}$ Quinoneimine + $4H_20$

(red dye)

The intensity of the red colour produced is directly proportional to the total cholesterol in the sample when read at 540-550 nm.

3.5.3. HDL-cholesterol

The method is based on a modified polyvinyl sulfonic acid (PVS) and polyethylene-glycol methyl ether (PEGME) coupled classic precipitation method with the improvements in using optimized quantities of PVS/PEGME and selected detergents **[Hongbing, 2002]**. LDL, VLDL and chylomicrons react with PVS and PEGME and the reaction results in inaccessibility of LDL, VLDL and chylomicrons by cholesterol oxidase and cholesterol esterase. The enzymes selectively react with HDL to produce hydrogen peroxide through a Trinder reaction in the presence of N,N-Bis(4-sulfobutyl)-3-methylaniline (TODB). The intensity of the red colour produced is directly proportional to the HDL-cholesterol in the sample when read at 560 nm.

$$\text{HDL + LDL + VLDL + CM} \xrightarrow[\text{PEGME}]{\text{PVS}} \text{HDL + (LDL + VLDL + CM) PVS/PEGME}$$

$$\text{HDL + CHOD + CHER} \longrightarrow \text{Fatty acid + } H_20_2$$

$$2H_20_2\text{+4-AA+ TODB} \xrightarrow[\text{(560nm)}]{\text{Peroxidase}} \text{Quenone + 5 } H_20$$

3.5.4. Triglycerides

The method for the analysis is a modification of that of **Trinder (1969)**. Triglycerides in the sample are hydrolyzed by lipase to glycerol and fatty acids. The glycerol is then phosphorylated by adenosine-5-triphosphate to glycerol-3-phosphate and adenosine-5-diphosphate in a reaction catalyzed by glycerol kinase (GK). Glycerol-3-phosphate is then converted to dihydroxyacetone phosphate (DAP) and hydrogen peroxide by glycerophosphate oxidase. The hydrogen peroxide then reacts with 4-aminoantipyrine (4-AAP) and 3-5 dichloro-2-hydroxybenzene (3,5-DHBS)in a reaction catalyzed by peroxidase (POD) to yield a red coloured quinoneimine dye in the sample. The intensity of the color produced is directly proportional to the concentration of Triglycerides in the sample.

$$\text{Triglycerides} + H20 \xrightarrow{\text{Lipase}} \text{Glycerol} + \text{Fatty acids}$$

$$\text{Glycerol} + ATP \xrightarrow{GK} G_3P + ADP$$

$$G_3P + 0_2 \xrightarrow{GP0} DAP + H_20_2$$

$$H_20_2 + \text{4-AAP} + \text{3,5-DHBS} \xrightarrow{POD} \text{Quinoneimine Dye} + 2H_20$$

3.5.5. Uric acid

The method for the analysis is based on the procedure of **Klackar (1947)**. Uric acid is oxidized by Uricase to allantoin and hydrogen peroxide. 4-aminoantipyrine then reacts with the hydrogen peroxide in a reaction catalyzed by a peroxidase in the presence of a colour reagent. The colour intensity at 520 nm is proportional to the concentration of uric acid in the sample.

$$\text{Uric acid} + 0_2 + H_20 \xrightarrow{\text{Uricase}} \text{Allantoin} + CO_2 + H_20_2$$

$$H_20_2 + \text{4 – Aminoantipyrine} + \text{TBHB} \xrightarrow[(520nm)]{\text{Peroxidase}} \text{Quinoneimine} + H_20$$

3.5.6. Total Bilirubin

The method used for this assay was first described by **Ehrlich (1884)** where the clinical determination of serum bilirubin was done by coupling the serum with diazotised sulphanilic acid which produced an azobilirubin dye. This method was modified by **Malloy and Evelyn, (1937)** where they introduced methanol. Sodium nitrate was added to the sulphanilic acid which formed diazotised sulphanilic acid. Bilirubin in the sample reacted with diazotised sulphanilic acid which produced azobilirubin which absorbed strongly at 550nm. The absorbance measured was directly proportional to the total bilirubin concentration in the sample.

3.5.7. Total Protein

The method used for this assay was based on a colour reaction of protein molecules with cupric ions and this was known as the Biuret colour reaction. This procedure was first described by **Riegler (1914)**. This procedure was later modified by **Weichselbaum (1946) and Gornall (1949)** where sodium potassium tatrate was introduced to stabilise the cupric ions in the alkaline reagent.

$$\text{Protein} + Cu^{2+} \xrightarrow{\text{Alkali}} \text{Coloured complex}$$

Protein in the serum formed a blue coloured complex when it reacted with cupric ions in the alkaline solution. The intensity of the violet colour was directly proportional to the amount of protein present in the serum and this was measured at 540 nm.

3.5.8. Albumin

The method used for this assay was based on that of **Doumas *et al*, 1971** where at a controlled pH, bromocresol green formed a coloured complex with albumin. The intensity of the colour at 630nm was directly proportional to the albumin in the serum.

3.6.0. Oxidative stress indices

3.6.1. Malondialdehyde

MDA levels were determined by the MDA-Thiobarbituric acid (TBA) test which is the colorimetric reaction of MDA and TBA in acid solution. TBA reacted with MDA, a secondary product from lipid peroxidation, which generated an adduct of red colour, which was detected spectrophotometrically. This method is a fast, sensitive, and low-cost method that can be used to indicate the extent of lipid peroxidation in a variety of systems **[Shlafer and Shepard, 1984].**

The method used for this assay was based on that of **Kamal *et al.*, 1989** All reagents for the measurement were from Sigma chemicals, St. Louis, USA. Serum (0.5ml) was treated with 2.5 ml of 20% trichloroacetic acid (TCA) and then 1 ml of 0.67% TBA. The mixture was incubated at 100^{o}C for 30 minutes. After cooling, the sample was extracted with 4 ml n-butanol and centrifuged at 3000 rpm for 10 min. The absorbances of supernatant were measured at 535 nm and the results were expressed as µmol/l, using the extinction coefficient of 1.56 x 10^{5}l/mmol cm.

3.6.2. Plasma retinol and beta-carotene

The High Performance Liquid Chromatography (HPLC) method used for the analysis was that of **Sowell *et al*, 1994.** The measurement of plasma retinol and beta-carotene was done with Shimadzu (Tokyo) LC10A HPLC system fitted with SPD10AV UV-visible detector and a C18 column. All the reagents used were HPLC grade from Sigma chemicals, St Louis, USA. Prior to the analysis, the frozen samples were left to stand at room temperature to thaw, then inverted several times to mix properly. The extraction procedure for the assay was as follows: 200 ul of plasma was added to 250 ul of distilled water and 50 ul of internal standard (retinyl acetate). 400 ul of ethanol solution was added and the contents vortexed for 5 minutes. 1 ml hexane was then added, the mixture centrifuged (4500 r.p.m for 10 minutes), and the top layer transferred to another tube containing 200 ul of methanol. A further 1 ml hexane was added to the original tube for a second extraction step. The mixture was vortexed-mixed and centrifuged. The top layer was removed and combined with the previous hexanae layer from the first extraction step. This was the vacuum-dried and the residue

re-dissolved in 150 ul of mobile phase 70% acetonitrile, 20% dichloromethane, 10% methanol) for injection into the HPLC system. Chromatograms were extracted for 325 nm for retinol and 446 nm for beta-carotene.

3.6.3. Plasma ascorbic acid

The method used for the analysis of plasma ascorbic acid was that of **Omaye *et al,* 1971**. All reagents were from Sigma chemicals, St. Louis, USA. Prior to the analysis, the frozen samples were left to stand at room temperature to thaw, then inverted several times to mix properly.0.4 ml of plasma was added to 1.6 ml of 10% trichloroacetic acid and the mixture vortexed and allowed to stand for 5 minutes at room temperature. The mixture was then centrifuged at 2000 r.p.m and the supernatant transferred into another tube. 1 ml of the supernatant was then added to 0.4 ml of DTC reagent and incubated at 37ºC for 3 hours. The mixture was then chilled in an icebath and 1.6 ml of 65% sulphuric acid was added and vortexed. The mixture was then allowed to stand for another 30 minutes after which the wavelength was read on a Stat Fax (Awareness Technology Inc, USA) spectrophotometer at 320 nm against a reagent blank (1ml 10% trichloroacetic acid and 0.4 ml DTC reagent). 10 mg/dl ascorbic acid standard was treated the same way as the sample. Concentration of ascorbic acid in the plasma samples is directly proportional to the intensity of the colour developed.

3.6.4. Plasma catalase

The method used for the measurement of plasma catalase was that of **Takahara *et al,* 1960** with slight modification. All reagents were from Sigma, St Louis, USA. 0.2 ml of plasma was added to 1.2 ml 50 mM phosphate buffer (pH 7.0). 1 ml of 30mM hydrogen peroxide was then added to the mixture and the change in absorbance was measured with a Stat Fax (Awareness Technology Inc, USA) spectrophotometer at 240nm at 30 seconds interval for 2 minutes against an enzyme blank (1.0 ml distilled water). The catalase activity was expressed as umol of hydrogen peroxide decomposed per minute.

3.6.5 Data analysis

On the basis of BMI, subjects were classified into four groups: under weight (BMI<19 kg m^{-2}), normal (BMI between 19 and 24.9 kg m^{-2}), overweight (BMI between 25 and 29.9 kg m^{-2}) and obese (BMI≥30 kg m^{-2}).

Diabetes and impaired fasting glucose were defined in accordance with the new ADA and WHO criteria **[ADA, 1997 and WHO, 1998].** Diabetes was defined as fasting plasma glucose ≥7.0 mmol/L and IFG was defined as fasting plasma glucose 6.1- 6.9 mmol/L and hypertension was defined as ≥130/85 mmHg according to the International Diabetes Federation (IDF) criteria. Based on the results, all subjects were classified into six metabolic groups as follows: IFG only, both IFG and hypertension, diabetes only, both diabetes and hypertension, hypertension only and controls (subjects without any of these conditions)

Subjects were classified as having the metabolic syndrome based on the International Diabetes Federation criteria **[Zimmet *et al,* 2005]** as shown in figure 3.1 below;

Central Obesity (waist circumference)	**Men: ≥94cm** **Women: ≥80cm**
Plus any two of the following conditions	
Raised Triglycerides	**≥1.7 mmol/L**
Reduced HDL-cholesterol	**Men: <1.03 mmol/L** **Women: <1.29 mmol/L**
Raised blood pressure	**Systolic: ≥130 mmHg or** **Diastolic: ≥85 mmHg**
Hyperglycaemia	**Fasting plasma glucose ≥5.6 mmo/L**

Figure 3.1. International Diabetes Federation criteria for classification of the metabolic syndrome [Zimmet *et al,* 2005].

The data analysis was done using Graph Pad Prism version 5.00 for windows (GraphPad Software, San Diego California, USA). Demographic differences between the subject populations were expressed as percentages and baseline characteristics were expressed as mean ± standard error of mean (SEM). Student unpaired, two-sided *t*-test was used to test for differences in means between two subject groups and one-way analysis of variance (ANOVA) with Bonferroni's post test was used for comparison between metabolic groups. *P* value <0.05 was considered significant.

Chapter 4

RESULTS

4.1.0. Introduction

The results show the differences in demographic, anthropometric, biochemical and oxidative stress variables in both the urban and rural populations.

4.1.1. Sociodemographic Variables

A summary of the demographic characteristics of the subject population is shown in table 4.1. All the subjects in the urban population have had over nine years of formal education and are either professionals or paraprofessionals whilst majority of the subjects in the rural population have had less than nine years of formal education and are either traders or farmers. Most of the urban dwellers are also likely to be living a more sedentary lifestyle as their job-related physical activity is very low and they are less likely to engage in any physical activity in their leisure time. Although the rural subjects are also not likely to engage in any physical activity in their leisure time, their occupation and means of transportation involves a lot of physical activity. The urban subjects are more likely to be smokers with a positive family history of diabetes mellitus. Additionally, the urban subjects are likely to consume more meals and desert but less fresh fruits and vegetables than the rural subjects.

Table 4.1. Distribution of demographic variables in the study population.

Demographic variable	**Urban (210)**	**Rural (180)**
≤ 9 years education	0	93.9
> 9 years education	100	6.1
Occupation		
Professional/paraprofessional	100	1.1
Trader	0	2.2
Farmer	0	96.7
Sedentary/light job-related physical activity	100	3.3
Heavy job-related physical activity	0	96.7
Leisure-time physical activity		
None/once a while	92.4	1.7
Regular	7.6	0
Never	0	98.3
Not smoked for at least 6 months	0	0
≥ 5 cigarettes per day	4.8	1.7
Positive family history of diabetes	18.6	11.7
Negative family history of diabetes	81.4	88.3
Food frequency		
2/day	14.3	18.5
3/day	82.6	81.5
>3/day	3.1	0.0
Daily intake of desert after meals	31.4	7.4
Daily intake of fruits and vegetables	8.1	27.2

Results are expressed as percentages.

4.1.2.0. Distribution of dysmetabolism in the subject population

Graph 1. Distribution of dysmetabolism in the subject population

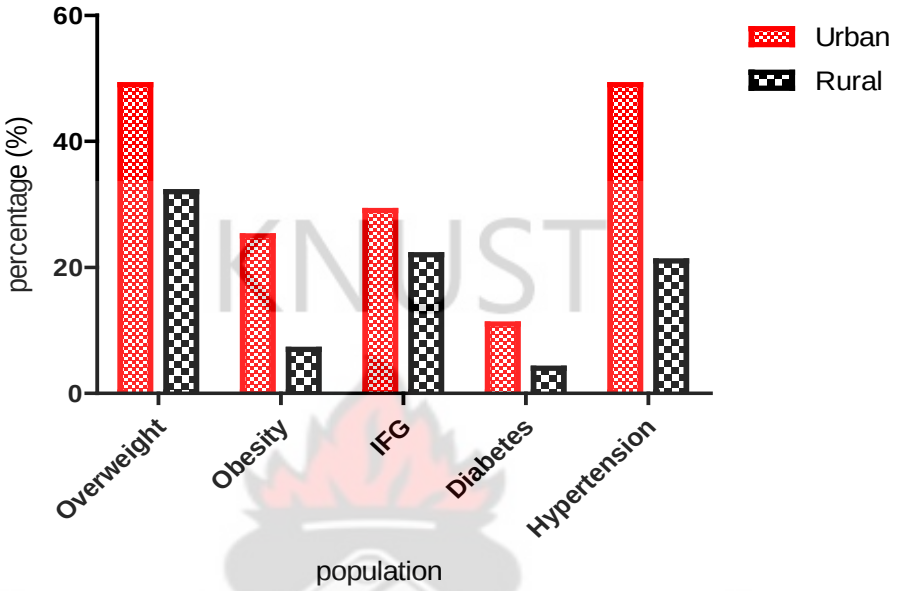


Figure 4.1. Distribution of dysmetabolism in the subject population IFG: Impaired fasting glycaemia.

Graph 2. Distribution of dysmetabolism in the urban population

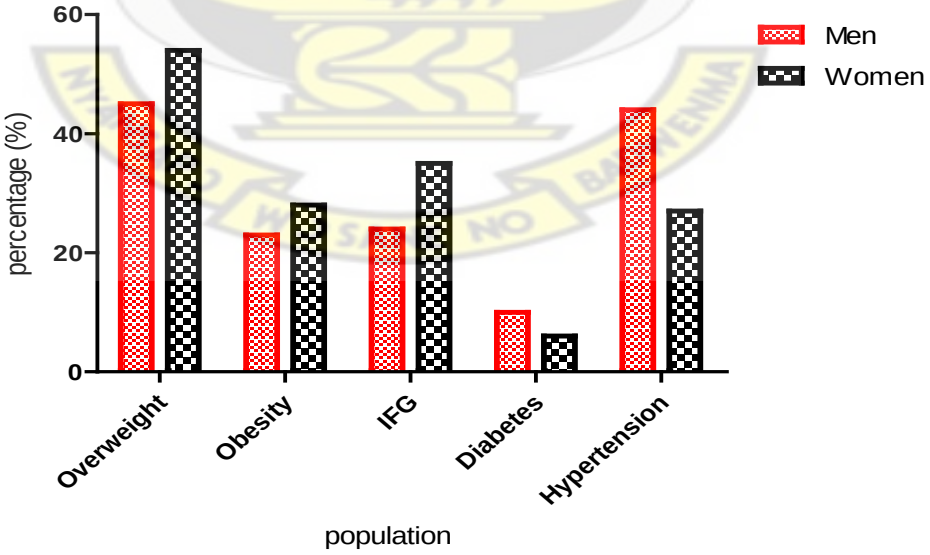


Figure 4.2. Distribution of dysmetabolism in the urban population. IFG: Impaired fasting glycaemia

Graph 3. Distribution of dysmetabolism in the rural population

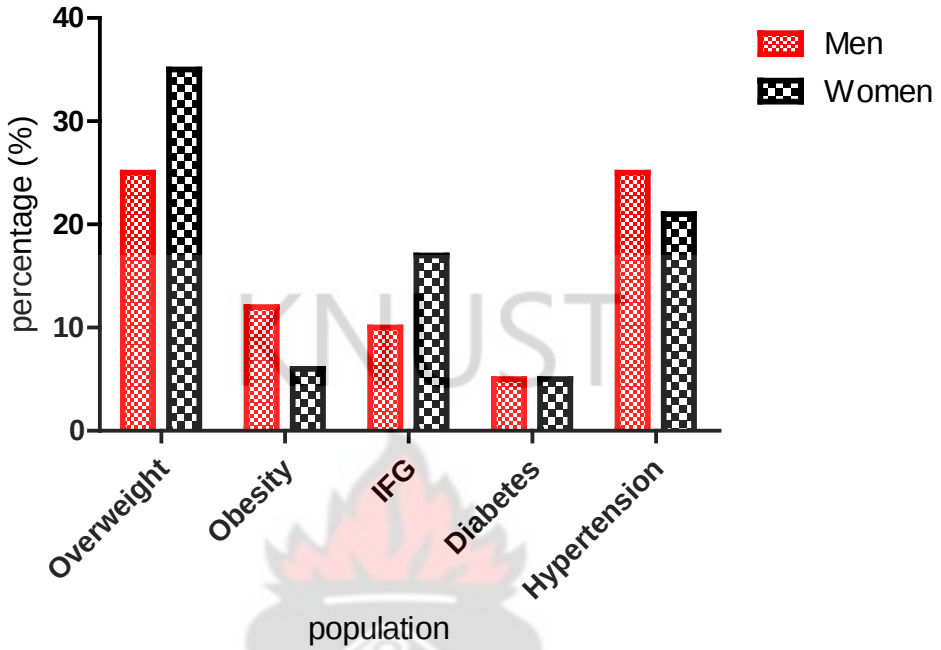


Figure 4.3. Distribution of dysmetabolism in the rural population. IFG: Impaired fasting glycaemia.

Graph 4. Distribution of dyslipidaemias in the subject population

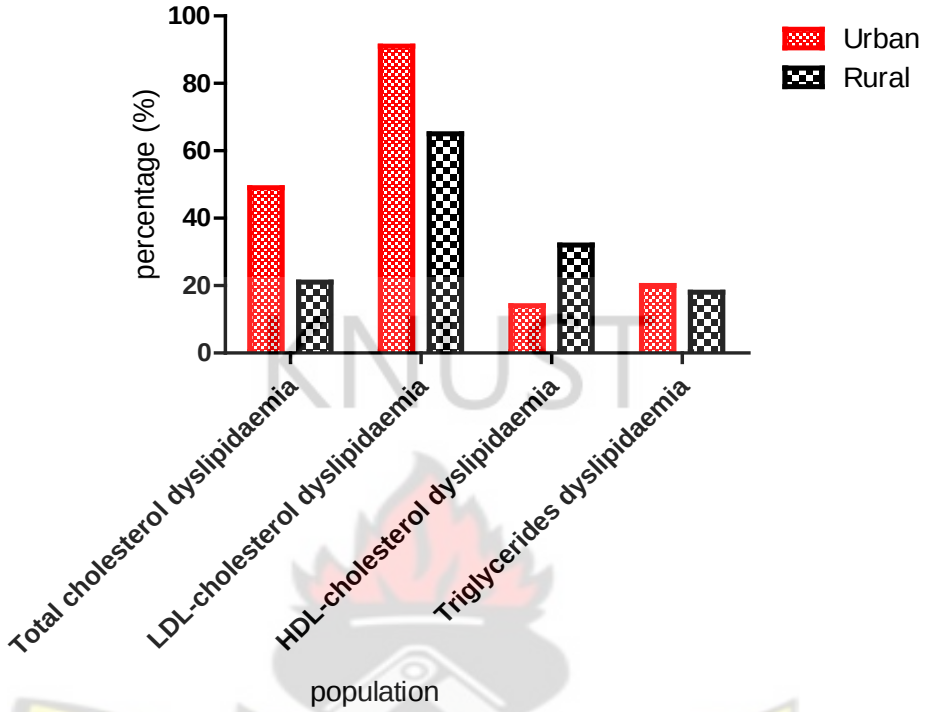


Figure 4.4. Distribution of dyslipidaemia in the subject population.

Graph 5. Distribution of dyslipidaemias in the urban population

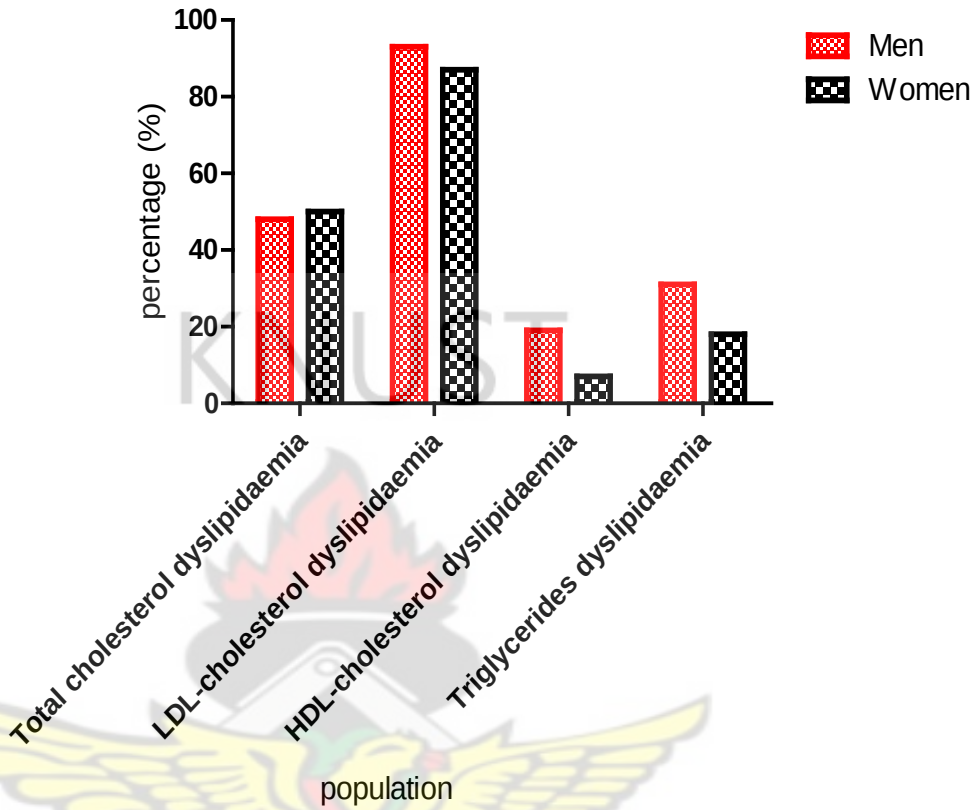


Figure 4.5. Distribution of dyslipidaemia in the urban population.

Graph 6. Distribution of dyslipidaemias in the rural population

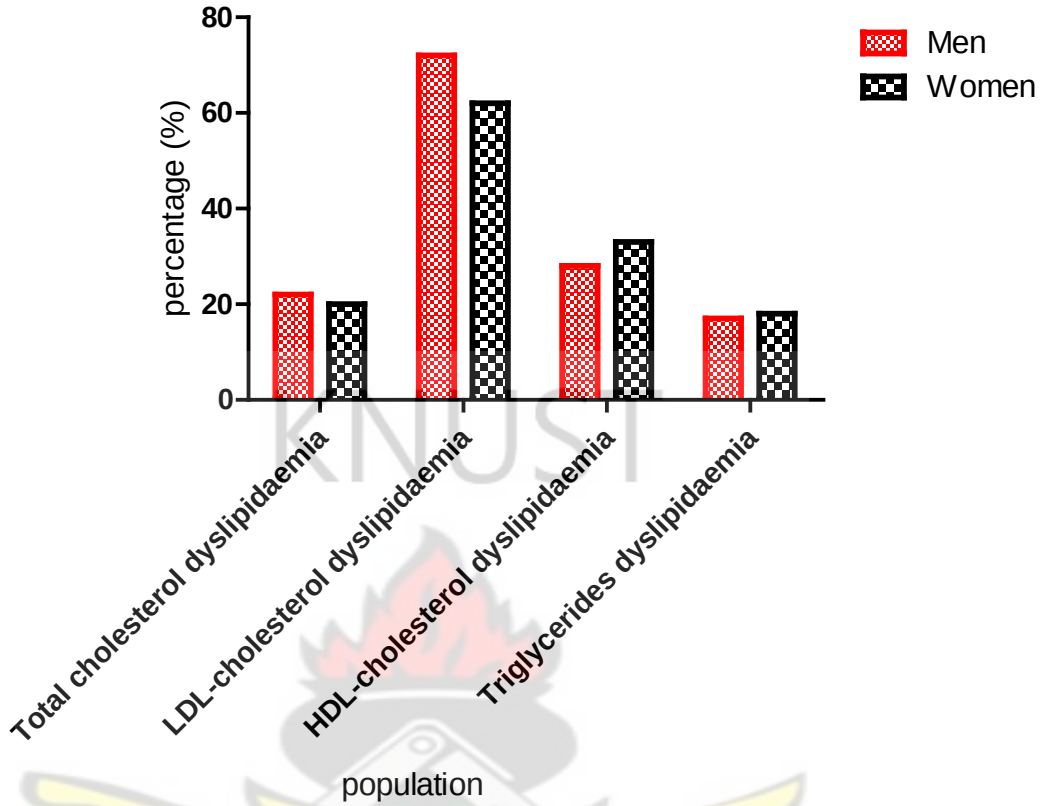


Figure 4.6. Distribution of dyslipidaemia in the rural population.

4.1.2.1. Anthropometric parameters

The summary of the anthropometric characteristics of the subject populations are shown in table 4.2.

4.1.2.2. Age

The subjects in the rural population were significantly older than the urban subjects (59.43 ± 1.06 and 51.08 ± 0.59 respectively, $p < 0.0001$). However, tables 4.3 and 4.4 show that there was no significant difference in age between men and women in both the urban and rural populations. Additionally, there was no significant difference in age between subjects in the various metabolic groups as shown in table 4.5.

Table 4.2 Anthropometric characteristics of urban and rural subjects.

Parameter	Urban		Rural		*P*-value	Total	
	N = 210		N = 180			N = 390	
	Mean	SEM	Mean	SEM		Mean	SEM
Age (years)	51.08	0.59	59.43	1.06	***	53.83	0.0.89
S. BP (mmHg)	129.3	0.86	132.3	1.57	Ns	132.3	1.41
D. BP (mmHg)	85.46	0.86	77.44	0.93	***	84.97	0.98
BMI (kg/m²)	27.10	0.28	23.78	0.29	***	25.64	0.33
WC (cm)	88.92	0.77	79.19	0.69	***	85.78	0.89

The results are expressed as mean ± SEM. All *p*-values: *<0.05, **<0.001, ***<0.0001 and Ns: Not Significant. S.BP: systolic blood pressure, D.BP: diastolic blood pressure, BMI: body mass index, WC: waist circumference.

Table 4.3 Differences in anthropometric characteristics between men and women in urban subjects.

Parameter	Men (n = 120)	Women (N = 90)	*P* value
Age (years)	50.43 ± 0.72	51.93 ± 0.96	Ns
S. BP (mmHg)	130.6 ± 1.60	127.6 ± 1.92	Ns
D. BP (mmHg)	87.24 ± 1.19	83.08 ± 1.19	*
BMI (kg/m²)	26.49 ± 0.37	27.91 ± 0.42	*
WC (cm)	88.47 ± 1.06	89.52 ± 1.11	Ns

The results are expressed as mean ± SEM. All *p*-values: *<0.05, and Ns: Not Significant. S.BP: systolic blood pressure, D.BP: diastolic blood pressure, BMI: body mass index, WC: waist circumference.

Table 4.4 Differences in anthropometric characteristics between men and women in rural subjects.

Parameter	Men (n = 51)	Women (N = 129)	P value
Age (years)	61.82 ± 2.05	58.48 ± 1.23	Ns
S. bp (mmHg)	136.4 ± 2.7	130.7 ± 1.88	Ns
D. bp (mmHg)	79.63 ± 1.45	76.57 ± 1.16	Ns
BMI (kg/m²)	23.73 ± 0.56	23.87 ± 0.32	Ns
WC (cm)	88.47 ± 1.06	79.09 ± 0.82	Ns

The results are expressed as mean ± SEM. All *p*-values Ns: Not Significant. S.BP: systolic blood pressure, D.BP: diastolic blood pressure, BMI: body mass index, WC: waist circumference.

4.1.2.3. Blood pressure

Table 4.2 shows the distribution of blood pressure in both the urban and rural populations. Though there was no significant difference in systolic blood pressure between the urban and rural subjects, the mean diastolic blood pressure of the urban subjects was significantly higher than the rural subjects and the mean diastolic blood pressure was also significantly higher in men than in women in both the urban and rural populations as shown in tables 4.3 and 4.4 respectively. As shown in graph 1, hypertension in general was more prevalent among the urban subjects than the rural subjects (36.7% and 20.6% respectively). Additionally, figures 4.2 and 4.3 show that hypertension was also more common in men than in women in both the urban and rural populations.

4.1.2.4. Body mass index (BMI)

Table 4.2 shows the differences in BMI in both the urban and rural populations. The urban subjects have significantly higher BMI than the rural subjects (p <0.0001) and women tend to have higher BMI than men in both the urban and rural populations as shown in tables 4.3 and 4.4. BMI tend to be higher in the presence of both type 2 diabetes and hypertension as shown in table 4.5. Overweight and obesity were also more prevalent among urban subjects than in the rural subjects as shown in figure 4.1. Almost half (49.0%) of the urban population were overweight and obesity had a prevalence of 24.8%. However, the prevalence of overweight and obesity in the rural population were 32.2% and 7.2% respectively. Overweight and obesity were also found to be more common in women than men in both the urban and rural populations as shown in figures 4.2 and 4.3 respectively.

4.1.2.5. Waist circumference

Table 4.2 shows the differences in waist size between the urban and rural populations. The urban subjects had significantly bigger waist circumference than the rural subjects (p <0.0001) although there was no significant gender difference in waist size in both the urban and rural populations as shown in table 4.3 and 4.4. Additionally, just like BMI, the metabolic group with both diabetes and hypertension had the biggest waist circumfernce as shown in table 4.5.

Table 4.5 Distribution of anthropometric variables in the various metabolic groups in the total population.

	IFG only	IFG + Hypertension	Diabetes only	Diabetes + Hypertension	Hypertension only	Controls
	N = 49	N = 37	N = 16	N = 13	N = 181	N = 94
Age (years)	49.00 ±1.19	57.95 ±2.00	51.88 ± 2.54	54.83 ± 2.98	57.47 ± 0.94	52.64 ± 1.27
S.BP(mmHg)	111.1 ± 1.22	142.4 ± 2.09	112.2 ± 1.79	139.3 ± 3.05	142.9 ± 1.21	115.1 ±1.27
D.BP(mmHg)	75.61 ± 1.01	88.97 ± 2.20	72.81 ± 1.91	87.92 ± 3.45	87.16 ± 0.99	72.36 ± 0.92
BMI(kg/m²)	26.84 ± 0.56	25.57 ± 0.82	27.00 ± 1.17	27.17 ± 1.12	25.61 ± 0.32	24.45 ± 3.99
WC (cm)	86.76 ± 1.37	86.03 ± 2.19	86.94 ± 2.83	90.00 ± 3.42	84.71 ± 0.87	80.94 ± 1.07

Results are expressed as mean ± SEM. One-way ANOVA with Bonferroni's post-test. S.BP: systolic blood pressure, D.BP: diastolic blood pressure, BMI: body mass index, WC: waist circumference.

4.1.3.0. Biochemical Parameters

4.1.3.1. Type 2 diabetes mellitus and impaired fasting glycaemia

A summary of the prevalence of type 2 diabetes mellitus and impaired fasting glycaemia is shown in figures 4.1, 4.2 and 4.3. As shown in figure 4.1, the prevalence of type 2 diabetes mellitus and IFG were considerably higher among the urban subjects as compared to the rural population (10.5% and 28.6% respectively for urban subjects and 3.8% and 22.1% respectively for rural subjects). Figures 4.2 and 4.3 show the gender distribution of type 2 diabetes mellitus and IFG in the urban and rural populations. Although there was no gender difference in the prevalence of type 2 diabetes in the rural population, it was found to be more prevalent in men than in women in the urban population. However, IFG was more common among women than in men in both the urban and rural populations.

4.1.3.2. Dyslipidaemia in the subject population.

Figure 4.4, 4.5 and 4.6 above show the distribution of the types of dyslipidaemia in the various subject populations. In general, LDL dyslipidaemia was the most prevalent type of dyslipidaemia in both the urban and rural populations. Total cholesterol, LDL and triglycerides dyslipidaemias were more common in the urban population than in the rural subjects. However HDL dyslipidaemia was more common among the rural population than in the urban subjects. Additionally, LDL, HDL and triglycerides dyslipidaemias were more common in men than in women among the urban subjects. However, the percentage of female subjects with HDL cholesterol dyslipidaemia was higher in the rural population.

4.1.3.3. Fasting Plasma Glucose

Table 4.6 shows the general distribution of biochemical parameters in the urban and rural populations. There was a significant ($p < 0.001$) difference between the urban and rural populations in terms of fasting plasma glucose with urban subjects having a higher mean plasma glucose than the rural subjects. However, as shown in tables 4.7 and 4.8, there were no significant gender differences in fasting plasma glucose in both the urban and rural populations. One-way ANOVA and Bonferroni's post test was

used to analyze differences in fasting plasma in the metabolic groups. Fasting plasma glucose was shown to be higher in the metabolic group with both type 2 diabetes and hypertension as compared to the other groups as indicated in table 4.9.

4.1.3.4. Total cholesterol

As shown in table 4.6, the mean serum total cholesterol level was significantly higher among the urban subjects than in the rural population ($p< 0.0001$). However, there were no significant differences in gender in both the urban and rural populations as shown in tables 4.7 and 4.8. Upon analysis with one-way ANOVA and Bonferroni's post test, serum total cholesterol was found to be significantly higher in the group with both type 2 diabetes and hypertension as compared to the other metabolic groups as indicated in table 4.9 ($F = 22.11$, $p<0.0001$).

4.1.3.5. LDL-cholesterol

As shown in tables 4.6 and 4.7, the mean LDL cholesterol was significantly higher among urban subjects than in the rural population and was also higher among men than women in the urban population. Like total cholesterol, table 4.9 shows that LDL-cholesterol was found to be higher in the metabolic group with both type 2 diabetes mellitus and hypertension as compared to the other groups ($F = 3.16$, $p<0.0001$ with one-way ANOVA and Bonferroni's post test).

Table 4.6 Distribution of biochemical variables in urban and rural populations.

	Urban (210)		Rural (180)		P-value
Parameter	Mean	SEM	Mean	SEM	
FPG	5.76	0.10	5.32	0.10	**
Cholesterol	5.32	0.06	4..57	0.06	***
HDL	1.46	0.02	1.36	0.02	**
LDL	3.61	0.06	2.97	0.06	***
Triglycerides	1.21	0.03	1.24	0.03	Ns
Protein	78.88	0.31	78.22	0.41	Ns
Albumin	39.28	0.25	38.74	0.43	Ns
Bilirubin	12.06	0.22	13.57	0.24	***
Uric acid	331.5	5.52	332.5	5.56	Ns

The results are expressed as mean ± SEM. All *p*-values: *<0.05, **<0.001, ***<0.0001 and Ns: Not Significant. FPG: fasting plasma glucose, HDL: HDL-cholesterol, LDL: LDL-cholesterol

4.1.3.6. HDL-cholesterol

Table 4.6 shows that the mean serum HDL-cholesterol was significantly higher among the urban population than in the rural population (p <0.001). There was however no significant differences in the serum HDL-cholesterol concentrations in the metabolic groups as shown in tables 4.9. The other significant difference in HDL-cholesterol was found in the gender distribution with women having significantly higher mean HDL-cholesterol than men in both the urban and rural populations as shown in tables 4.7 and 4.8.

4.1.3.7. Triglycerides

As shown in table 4.6, there was no significant difference in the mean serum triglycerides concentrations between the urban and rural populations. Additionally, there were no significant differences in the serum triglycerides concentration between men and women in both the urban and rural populations as shown in tables 4.7 and 4.8 respectively. Like total cholesterol and LDL-cholesterol, the mean triglycerides concentration was found to be higher in the presence of both type 2 diabetes mellitus and hypertension as compared to the other metabolic groups as shown in table 4.9.

4.1.3.8. Total Protein

As shown in tables 4.6, 4.7, 4.8 and 4.9, there were no significant differences in the mean serum total protein levels between the urban and rural populations and a similar trend of non significant difference was also seen in the gender and metabolic groups.

4.1.3.9. Albumin

The mean serum albumin like total protein did not show any significant differences when compared between the urban and rural populations and also showed a similar trend of non significant difference in the gender and metabolic groups as shown in tables 4.6, 4.7, 4.8 and 4.9.

4.1.3.10. Total Bilirubin

Table 4.6 shows that, the mean serum total bilirubin was significantly higher in the rural subjects than in the urban subjects (p <0.0001). However, there were no significant differences in the mean serum total bilirubin levels in the gender, and metabolic groups as shown in tables 4.7, 4.8 and 4.9.

4.1.3.11. Uric Acid

Table 4.6 shows that there was no significant difference in the mean serum uric acid levels between the urban and rural populations. A significantly higher mean serum uric acid was seen in men than in women in both populations as shown in tables 4.7 and 4.8 respectively. One-way ANOVA and Bonferroni's post test showed that the

serum uric acid was higher in the metabolic group with hypertension alone as compared to the other groups as shown in table 4.9.

Table 4.7 Distribution of biochemical variables in the urban population.

	Urban men		**Urban women**		**P-value**
	n = 120		n = 90		
Parameter	Mean	SEM	Mean	SEM	
FPG	5.70	0.14	5.84	0.14	Ns
Cholesterol	5.28	0.09	5.38	0.11	Ns
HDL	1.35	0.02	1.60	0.02	***
LDL	3.68	0.08	3.53	0.10	**
Triglycerides	1.19	0.05	1.23	0.06	Ns
Protein	78.44	0.40	79.46	0.49	Ns
Albumin	39.34	0.34	39.21	0.39	Ns
Bilirubin	12.35	0.31	11.66	0.29	Ns
Uric acid	346.1	7.33	312.0	4.98	*

The results are expressed as mean ± SEM. All p-values: *<0.05, **<0.001, ***<0.0001 and Ns: Not Significant. FPG: fasting plasma glucose, HDL: high density lipoprotein, LDL: low density lipoprotein.

Table 4.8 Distribution of biochemical variables in the rural population.

	Rural men		Rural women		P-value
	n = 51		n = 129		
Parameter	Mean	SEM	Mean	SEM	
FPG	5.70	0.21	5.43	0.13	Ns
Cholesterol	4.55	0.12	4.58	0.07	Ns
HDL	1.22	0.04	1.41	0.02	***
LDL	3.09	0.12	2.92	0.08	Ns
Triglycerides	1.22	0.05	1.26	1.26	Ns
Protein	78.79	0.72	78.00	0.49	Ns
Albumin	39.36	0.76	38.49	0.52	Ns
Bilirubin	13.00	0.43	13.80	0.29	Ns
Uric acid	355.8	10.08	323.2	6.49	*

The results are expressed as mean ± SEM. All p-values: *<0.05, ***<0.0001 and Ns: Not Significant. FPG: fasting plasma glucose, HDL: high density lipoprotein, LDL: low density lipoprotein.

Table 4.9 Differences in distribution of biochemical variables in the metabolic groups

Parameter	IFG only	IFG and Hypertension	Diabetes only	Diabetes and Hypertension	Hypertension only	Controls
	N = 49	N = 37	N = 16	N = 13	N = 181	N = 94
FPG	6.41 ± 0.04	6.47 ± 0.04	8.40 ± 0.48	9.43 ± 0.73	4.91 ± 0.05	5.02 ± 0.07
Cholesterol	4.98 ± 0.09	5.34 ± 0.21	5.66 ± 0.23	6.77± 0.30***	4.98 ± 0.06	4.47 ± 0.08
HDL	1.52 ± 0.03	1.46 ± 0.05	1.45 ± 0.07	1.49 ± 0.08	1.38 ± 0.02	1.38 ± 0.03
LDL	3.21 ± 0.09	3.58 ± 0.19	3.93 ± 0.25	4.81 ± 0.31**	3.37 ± 0.07	2.87 ± 0.08
Triglycerides	1.20 ± 0.06	1.28 ± 0.09	1.40 ± 0.10	1.75 ± 0.15**	1.17 ± 0.03	1.13 ± 0.04
Protein	78.48 ± 0.75	79.38 ± 0.80	77.18 ± 1.08	77.01 ± 1.37	78.70 ± 0.38	78.54 ± 0.48
Albumin	39.08 ± 0.61	39.63 ± 0.91	37.34 ± 1.03	36.84 ± 1.12	39.05 ± 0.33	39.32 ± 0.54
Bilirubin	11.94 ± 0.42	12.82 ± 0.47	12.24 ± 0.72	13.73 ± 0.92	12.75 ± 0.26	13.18 ± 0.35
Uric acid	324.0 ± 7.93	319.0 ± 11.4	334.4 ±19.24	325.3 ± 27.56	349.0 ±5.73**	309.3 ± 7.93

The results are expressed as mean ± SEM. *p* values: *** $p < 0.0001$ for significance in comparison to other groups, ** $p < 0.001$ in comparison to other groups (One-way ANOVA and Bonferroni's post test). FPG: fasting plasma glucose, HDL: high density lipoprotein, LDL: low density lipoprotein.

4.1.4.0. Oxidative stress indices

Table 4.10 shows the distribution of oxidative stress indices in the urban and rural populations

4.1.4.1. Ascorbic acid

The mean plasma ascorbic acid level was significantly higher in the rural population than in the urban subjects. Although there was no significant difference in the mean plasma level of ascorbic acid in the rural population as shown in table 4.12, there was a significant difference in the urban population with women having lower mean plasma level than men as shown in table 4.11. Plasma ascorbic acid also showed significantly lower levels in the metabolic groups with either type 2 diabetes mellitus alone or both type 2 diabetes mellitus and hypertension ($F = 9.75$, $p<0.0001$) when analyzed with one-way ANOVA and Bonferroni's post test as shown in table 4.13. The highest level of ascorbic acid was however found in the control subjects without any morbidity.

4.1.4.2. Retinol

Table 4.10 again shows that the mean plasma retinol was significantly lower in the urban subjects than in the rural subjects ($p <0.0001$). There was however no significant difference in plasma retinol between men and women in both the urban and rural populations as shown in table 4.11 and 4.12 respectively. One-way ANOVA and Bonferroni's post test showed that plasma retinol was significantly lower in the presence of either type 2 diabetes alone or both type 2 diabetes and hypertension ($F = 27.76$, $p<0.0001$) as shown in table 4.13. The highest level of plasma retinol was observed in the control group as it was in the case of ascorbic acid.

4.1.4.3. Beta-carotene

Table 4.10 shows that the urban subjects had a significantly lower mean plasma level than the rural subjects. Although there was no significant difference in the mean plasma level of beta-carotene in the urban population as shown in table 4.11, there was a significant difference in the rural population with women having lower mean plasma level than men as shown in table 4.12. There was however no significant differences in the mean plasma beta-carotene in the various metabolic groups as shown in table 4.13.

4.1.4.4. Catalase

Tables 4.10, 4.11 and 4.12 show that there was no significant difference in the plasma catalase level between the urban and rural populations and also there were no differences in plasma catalase levels between the gender groups in both the urban and rural populations. However, subjects with either type 2 diabetes mellitus alone or both type 2 diabetes mellitus and hypertension had significantly lower mean levels as compared to the other metabolic groups (Catalase $F = 55.96$ $p<0.0001$) when analyzed with one-way ANOVA and Bonferroni's post test as shown in table 4.13.

4.1.4.5. Malondialdehyde

Table 4.10 shows that there was a significant difference in the plasma malondialdehyde as a measure of the lipid peroxidation status of the subject population. The urban population had a higher mean level than the rural population. Tables 4.11 and 4.12 also show that there was no significant difference in lipid peroxidation between men and women in both urban and rural populations. In sharp contrast to their antioxidant levels, table 4.13 shows that subjects with either type 2 diabetes alone or both type 2 diabetes and hypertension had higher levels of malondialdehyde as compared to subjects in the other metabolic groups.

Table 4.10 Differences in the distribution of oxidative stress indices in the urban and rural populations.

	Urban		Rural		P-value
	N = 210		N = 180		
Parameter	Mean	SEM	Mean	SEM	
Ascorbic acid	1.12	0.03	1.15	0.01	*
Retinol	26.56	0.24	28.62	0.43	***
Beta-carotene	57.42	0.32	60.85	0.21	***
Catalase	591.2	3.61	598.5	3.50	Ns
MDA	2.03	0.03	1.95	0.02	*
10-yr CHD risk	6.67	0.43	7.23	0.49	Ns
DRS	6.02	0.15	4.04	0.11	***

The results are expressed as mean ± SEM. All p-values: *<0.05, ***<0.0001 and Ns: Not Significant. MDA: malondialdehyde, 10-yr CHD risk: 10-year coronary heart disease risk, DRS: Ghana diabetes risk score.

Table 4.11 Distribution of oxidative stress indices in the urban population.

	Men		Women		P-value
	n = 120		n = 90		
Parameter	Mean	SEM	Mean	SEM	
Ascorbic acid	1.13	0.01	1.11	0.01	*
Retinol	26.59	0.33	26.53	0.35	Ns
Beta-carotene	57.51	0.42	57.31	0.50	Ns
Catalase	592.3	4.94	589.7	5.27	Ns
MDA	2.01	0.04	2.06	0.03	Ns
10-yr CHD risk	8.69	0.67	4.00	0.28	***
DRS	5.71	0.22	6.43	0.17	*

The results are expressed as mean ± SEM. All p-values: *<0.05, ***<0.0001 and Ns: Not Significant. MDA: malondialdehyde, 10-yr CHD risk: 10-year coronary heart disease risk, DRS: Ghana diabetes risk score.

Table 4.12. Distribution of oxidative stress indices in the rural population.

	Men		Women		P-value
	N = 51		n = 129		
Parameter	Mean	SEM	Mean	SEM	
Ascorbic acid	1.17	0.02	1.14	0.01	Ns
Retinol	29.39	0.76	28.32	0.52	Ns
B-carotene	61.62	0.39	60.55	0.25	*
Catalase	599.2	5.62	598.2	4.37	Ns
MDA	1.92	0.03	1.96	0.02	Ns
10-yr CHD risk	11.90	1.26	5.38	0.04	***
DRS	3.86	0.19	4.12	0.14	Ns

The results are expressed as mean ± SEM. p-values: *<0.05, ***<0.0001 and Ns: Not Significant. MDA: malondialdehyde, 10-yr CHD risk: 10-year coronary heart disease risk, DRS: Ghana diabetes risk score.

Table 4.13 Differences in the distribution of oxidative stress variables in the various metabolic groups.

Parameter	IFG only	IFG + Hypertension	Diabetes only	Diabetes + Hypertension	Hypertension only	Controls
	N = 49	N = 37	N = 16	N = 13	N = 181	N = 94
Ascorbic acid	1.10 ± 0.01	1.11 ± 0.01	1.02 ± 0.03***	1.00 ± 0.04***	1.16 ± 0.01	1.15 ± 0.01
Retinol	24.83 ± 0.70	25.05 ± 0.69	21.10 ± 0.58***	21.56 ± 1.29**	29.15 ± 0.31	28.68± 0.39
Beta-carotene	55.69 ± 0.46	56.06 ± 0.53	52.17 ± 0.83	50.72 ± 1.11	60.89 ± 0.22	60.52± 0.37
Catalase	559.5 ± 5.05	561.9 ± 4.87	494.0 ± 22.22**	519.4 ± 9.37**	615.8 ± 2.42	612.6± 3.47
MDA	2.13 ± 0.02	2.12 ± 0.03	2.71 ± 0.10	2.68 ± 0.12	1.87 ± 0.02	1.89 ± 0.02
10-yr CHD risk	3.08 ± 0.28	8.35 ± 0.79	7.94 ± 2.05	19.08 ± 4.41**	8.31 ± 0.44	3.97 ± 0.33
DRS	4.78 ± 0.25	6.35 ± 0.34	4.88 ± 0.52	6.83 ± 0.75***	5.71 ± 0.14	3.46 ± 0.16

The results are expressed as mean ± SEM. *** $p< 0.0001$, ** $p<0.001$ in comparison to other groups (One way ANOVA and Bonferroni's post test). MDA: malondialdehyde, 10-yr CHD risk: 10-year coronary heart disease risk, DRS: Ghana diabetes risk score.

4.1.5.1. Associations between anthropometric variable

Tables 4.14 and 4.15 show the Pearson's correlation table for the anthropometric variables the urban and rural populations respectively. There was a strong positive correlation between age and systolic blood pressure (r = 0.265, p < 0.0001) and a significantly negative correlation between age and BMI and waist circumference (r = - 0.222, p< 0.0001 and r = - 0.170, p= 0.001 respectively). However, age showed a significant positive correlation with only systolic blood pressure in both the urban and rural populations (r = 0.207, p< 0.05 and r = 0.290, p< 0.0001 respectively) but did not show any significant correlation with the other anthropometric variables in both the urban and rural populations as shown in tables 4.14. and 4.15 respectively. Age however did not show any significant correlation with the other biochemical variables except for uric acid in which case there was a significant positive correlation in both the urban (r = 0.145, p< 0.05) and rural (r = 0.315, p< 0.0001) populations as shown in tables 4.16 and 4.17 respectively. Additionally, there was no significant correlation between age and oxidative stress indices in both the urban and rural populations as shown in tables 4.18 and 4.19 respectively.

Tables 4.14 and 4.15 show the Pearson's correlation tables of anthropometric variables in urban and rural populations respectively. As shown in the tables below, there was a significant positive correlation between age and systolic blood pressure in both the urban and rural populations but age did not show any significant correlation with the other anthropometric variables in the urban population. A strong positive correlation was also observed between diastolic blood pressure and BMI and waist circumference in the rural population. Additionally, a significant correlation was observed between age and BMI and waist circumference in the rural population. Waist circumference correlated strongly with BMI suggesting an interchangeable use of these anthropometric indices.

Table 4.14 Pearson's correlation table of anthropometric variables in the urban population

	AGE	S. BP	D. BP	BMI
AGE				
S. BP	0.290(***)			
D. BP	0.006(Ns)	0.604(***)		
BMI	-0.145(Ns)	-0.057(Ns)	0.035(Ns)	
WC	-0.119(Ns)	-0.0004(Ns)	0.020(Ns)	0.737(***)

P values are in parenthesis: *** $p < 0.0001$, Ns: not significant. S.BP: systolic blood pressure, D.BP: diastolic blood pressure, BMI: body mass index, WC: waist circumference.

Table 4.15 Pearson's correlation table of anthropometric variables in the rural population.

	AGE	S. BP	D. BP	BMI
AGE				
S. BP	0.265(***)			
D. BP	-0.063(Ns)	0.582(***)		
BMI	-0.222(***)	0.002(Ns)	0.224(***)	
WC	-0.170(**)	0.081(Ns)	0.237(***)	0.779(***)

P values are in parenthesis. ***p <0.0001, **p <0.001, Ns: not significant. S.BP: systolic blood pressure, D.BP: diastolic blood pressure, BMI: body mass index, WC: waist circumference.

4.1.5.2. Associations between anthropometric and biochemical variables

Tables 4.16 and 4.17 show the Pearson's correlation tables of anthropometric and biochemical variables in urban and rural populations respectively. There was a significant correlation between age and uric acid in both the urban and rural populations. However, there was no significant correlation between age and the other variables. Systolic blood pressure also showed a strong association with total cholesterol in both populations and a strong positive association with uric acid in the rural population. This suggests that uric acid may be implicated in raised blood pressure associated with advancing age. Waist circumference and BMI also correlated positively with total cholesterol but this was observed in only the urban population.

Table 4.16 Pearson's correlation table of anthropometric and biochemical variables in the urban population.

	FPG	**Cholesterol**	**HDL**	**LDL**	**Triglycerides**	**Uric acid**
AGE	0.039(Ns)	0.031(Ns)	0.052(Ns)	0.013(Ns)	0.066(Ns)	0.145(*)
S.BP	-0.178(*)	0.146(*)	-1.08(Ns)	0.189(*)	0.017(Ns)	0.086(Ns)
D.BP	-0.131(Ns)	0.134(Ns)	-0.091(Ns)	0.16(*)	0.066(Ns)	-0.067(Ns)
BMI	0.067(Ns)	0.249(***)	0.063(Ns)	0.225(**)	0.181(*)	-0.094(Ns)
WC	0.065(Ns)	0.279(***)	-0.088(Ns)	0.302(***)	0.145(*)	0.021(Ns)

P values are in parenthesis. * p <0.05, ** p <0.001, ***p <0.0001, Ns: not significant. FPG: fasting plasma glucose, S.BP: systolic blood pressure, D.BP: diastolic blood pressure, BMI: body mass index, WC: waist circumference, HDL: high density lipoprotein, LDL: low density lipoprotein.

Table 4.17 Pearson's correlation table of anthropometric and biochemical variables in the rural population.

	FPG	Cholesterol	HDL	LDL	Triglycerides	Uric acid
AGE	-0.022(Ns)	0.044(Ns)	-0.098(Ns)	0.091(Ns)	-0.012(Ns)	0.315(***)
S.BP	-0.144(Ns)	0.236(**)	-0.015(Ns)	0.226(*)	0.078(Ns)	0.506(***)
D.BP	-0.077(Ns)	0.183(*)	0.035(Ns)	0.145(Ns)	0.120(Ns)	0.262(***)
BMI	0.086(Ns)	0.051(Ns)	-0.073(Ns)	0.080(Ns)	0.065(Ns)	0.078(Ns)
WC	0.055(Ns)	0.055(Ns)	-0.011(Ns)	0.098(Ns)	0.050(Ns)	0.098(Ns)

P values are in parenthesis. *p <0.05, **p <0.001, ***p <0.0001, Ns: not significant. FPG: fasting plasma glucose, S.BP: systolic blood pressure, D.BP: diastolic blood pressure, BMI: body mass index, WC: waist circumference, HDL: high density lipoprotein, LDL: low density lipoprotein.

4.1.5.3. Associations between anthropometric variables and oxidative stress indices

Tables 4.18 and 4.19 show the Pearson's correlation tables of anthropometric variables and oxidative stress indices. The anthropometric variables showed negative correlation with plasma antioxidants but this was not statistically significant.

Table 4.18 Pearson's correlation table of anthropometric variables and oxidative stress indices in the urban population.

	Ascorbic acid	Retinol	Beta-carotene	Catalase	MDA
AGE	0.071(Ns)	0.053(Ns)	-0.058(Ns)	0.017(Ns)	0.050(Ns)
S.BP	0.178(Ns)	0.204(Ns)	0.231(Ns)	0.136(Ns)	0.179(Ns)
D.BP	0.189(Ns)	0.156(Ns)	0.161(Ns)	0.119(Ns)	0.118(Ns)
BMI	0.003(Ns)	-0.04(Ns)	-0.116(Ns)	-0.611(Ns)	0.002(Ns)
WC	-0.035(Ns)	-0.029(Ns)	-0.082(Ns)	-0.601(Ns)	0.009(Ns)

P values are in parenthesis, Ns: not significant. MDA: malondialdehyde, S.BP: systolic blood pressure, D.BP: diastolic blood pressure, BMI: body mass index, WC: waist circumference.

Table 4.19 Pearson's correlation table of anthropometric variables and oxidative stress indices in the rural population.

	Ascorbic acid	Retinol	B-carotene	Catalase	MDA
AGE	-0.007(Ns)	0.068(Ns)	0.004(Ns)	0.037(Ns)	0.073(Ns)
S.BP	0.008(Ns)	0.155(0.040)	0.149(Ns)	0.138(Ns)	0.099(Ns)
D.BP	0.052(Ns)	0.128(Ns)	0.099(Ns)	0.104(Ns)	0.071(Ns)
BMI	-0.098(Ns)	0.074(Ns)	-0.077(Ns)	0.107(Ns)	0.107(Ns)
WC	-0.093(Ns)	0.078(Ns)	-0.132(Ns)	0.084(Ns)	0.084(Ns)

P values are in parenthesis, Ns: not significant. MDA: malondialdehyde, S.BP: systolic blood pressure, D.BP: diastolic blood pressure, BMI: body mass index, WC: waist circumference.

4.1.5.4. Associations between biochemical variables

Tables 4.20 and 4.21 show the Pearson's correlation tables of biochemical variables in urban and rural populations respectively. Most of the biochemical variables correlated well with each other in both populations. There was a positive correlation between plasma glucose and cholesterol in both the urban population and rural populations. Uric acid however did not show any significant correlation with any of the biochemical variables in both the urban and rural population.

Table 4.20 Pearson's correlation table of biochemical variables in the urban population.

	FPG	Cholesterol	HDL	Triglycerides	LDL
FPG					
Cholesterol	0.438(***)				
HDL	0.234(**)	0.144(*)			
Triglycerides	0.342(***)	0.609(***)	0.035(Ns)		
LDL	0.347(***)	0.955(***)	-0.117(Ns)	0.522(***)	
Uric Acid	-0.040(Ns)	-0.085(Ns)	-0.050(Ns)	-0.033(Ns)	-0.058(Ns)

P values are in parenthesis. *p<0.05, ** p< 0.001, *** p< 0.0001, Ns: not significant. FPG: fasting plasma glucose, HDL: high density lipoprotein, LDL: low density lipoprotein.

Table 4.21. Pearson's correlation table of biochemical variables in the rural population.

	FPG	Cholesterol	HDL	Triglycerides	LDL
FPG					
Cholesterol	0.050(Ns)				
HDL	0.051(Ns)	0.037(Ns)			
Triglycerides	0.112(Ns)	0.385(***)	-0.076(Ns)		
LDL	0.025(Ns)	0.934(***)	-0.284(***)	0.318(***)	
Uric acid	-0.096(Ns)	0.134(Ns)	-0.029(Ns)	0.114(Ns)	0.131(Ns)

P values are in parenthesis. *** p< 0.0001, Ns: not significant. FPG: fasting plasma glucose, HDL: high density lipoprotein, LDL: low density lipoprotein.

4.1.5.5. Associations between biochemical variables and oxidative stress indices

Tables 4.22 and 4.23 show the Pearson's correlation tables of biochemical variables and oxidative stress indices. There was a significant negative association between plasma glucose and the plasma antioxidants and a significant positive correlation between plasma glucose and malondialdehyde in both the urban and rural populations. However, a similar relationship was observed with total cholesterol, LDL-cholesterol and triglycerides in only the urban population. This suggests that higher levels of plasma glucose and lipid lead to a depletion in the antioxidant defenses and a consequential increase in lipid peroxidation. Uric acid however did not show any significant correlation with the plasma antioxidants and lipid peroxidation.

Table 4.22 Pearson's correlation table of biochemical and oxidative stress variables in the urban population.

	Ascorbic acid	Retinol	Beta-carotene	Catalase	MDA
FPG	-0.488(***)	-0.683(***)	-0.745(***)	-0.711(***)	0.834(***)
Cholesterol	-0.149(*)	-0.399(***)	-0.284(***)	-0.349(***)	0.400(***)
HDL	-0.138(*)	-0.107(Ns)	-0.124(Ns)	-0.170(*)	0.202(**)
Triglycerides	-0.126(Ns)	-0.372(***)	-0.297(***)	-0.327(***)	0.313(***)
LDL	-0.092(Ns)	-0.346(***)	-0.217(**)	-0.282(***)	0.319(***)
Uric acid	0.024(Ns)	0.123(Ns)	0.108(Ns)	0.054(Ns)	-0.009(Ns)

P values are in parenthesis. * $p< 0.05$, ** $p< 0.001$, *** $p< 0.0001$, Ns: not significant. FPG: fasting plasma glucose, MDA: malondialdehyde, HDL: high density lipoprotein, LDL: low density lipoprotein.

Table 4.23 Pearson's correlation table of biochemical and oxidative stress variables in the rural population.

	Ascorbic acid	Retinol	Beta-carotene	Catalase	MDA
FPG	-0.31(***)	-0.354(***)	-0.582(***)	-0.582(***)	0.696(***)
Cholesterol	-0.035(Ns)	0.058(Ns)	-0.034(Ns)	-0.088(Ns)	0.041(Ns)
HDL	-0.139(Ns)	-0.057(Ns)	-0.082(Ns)	-0.036(Ns)	-0.027(Ns)
Triglycerides	-0.073(Ns)	0.040(Ns)	-0.008(Ns)	-0.156(*)	0.088(Ns)
LDL	-0.044(Ns)	0.078(Ns)	-0.0130(Ns)	-0.055(Ns)	0.041(Ns)
Uric acid	-0.021(Ns)	0.198(Ns)	0.076(Ns)	0.140(Ns)	-0.064(Ns)

P values are in parenthesis. * $p< 0.05$, *** $p< 0.0001$, Ns: not significant. FPG: fasting plasma glucose, MDA: malondialdehyde, HDL: high density lipoprotein, LDL: low density lipoprotein.

4.1.5.6. Associations between oxidative stress indices

Tables 4.24 and 4.25 show the Pearson's correlation tables of oxidative stress indices in urban and rural populations respectively**.** All the plasma antioxidants correlated positively with each other in both urban and rural populations suggesting a synergistic relationship in their antioxidant mechanism. The plasma antioxidants however showed a significantly negative correlation with malondialdehyde in both the urban and rural populations and this supports the earlier suggestion above that reduction of antioxidant defenses leads to a rise in oxidative stress.

Table 4.24 Pearson's correlation of oxidative stress indices in the urban population.

	MDA	Vitamin C	Vitamin A	Beta-carotene
Vitamin C	-0.484(*)			
Vitamin A	-0.638(***)	0.412(***)		
Beta-carotene	-0.603(***)	0.423(***)	0.639(***)	
Catalase	-0.615(***)	0.437(***)	0.569(***)	0.589(***)

P values are in parenthesis. * $p< 0.05$, ** $p< 0.001$, *** $p< 0.0001$, Ns: not significant. MDA: malondialdehyde

Table 4.25 Pearson's correlation of oxidative stress indices in the rural population.

	MDA	Vitamin C	Vitamin A	Beta-carotene
Vitamin C	-0.213(**)			
Vitamin A	-0.278(***)	0.046(Ns)		
Beta-carotene	-0.403(***)	0.213(**)	0.288(***)	
Catalase	-0.458(***)	0.171(*)	0.267(**)	0.408(***)

P values are in parenthesis. * $p< 0.05$, ** $p< 0.001$, *** $p< 0.0001$, Ns: not significant. MDA: malondialdehyde.

4.1.6.0. Metabolic syndrome in the general population.

The summary of the prevalence and distribution of the metabolic syndrome in the urban and rural subjects is shown in table 4.26 and 4.27 respectively. Classification of the metabolic syndrome was based on the IDF criteria **[Zimmet *et al,* 2005].** Out of the 11 possible types of clustering of metabolic components that are used in the classification of the metabolic syndrome, the clustering of central obesity, raised fasting glucose and the presence of hypertension is the most prevalent form of the metabolic syndrome in both the urban and rural subjects and a similar picture was observed in the gender**.** The metabolic syndrome was observed to be more prevalent in the urban population (17.6%) than in the rural population (7.2%). The metabolic syndrome was also found to be more prevalent in women than in men (24.4% and 12.5% respectively in the urban population and 9.3% and 2.0% respectively in the rural population as shown in tables.

Table 4.26. Contributions of various components of the metabolic syndrome in the urban population.

Morbidity	Men (N = 120)	Women (N = 90)	Total (210)
WC + H + FPG	15 (12.5%)	13 (24.4%)	37 (17.6%)
WC + H + HDL	6 (5.0%)	5 (6.0%)	11 (5.0%)
WC + H + TG	6(5.0%)	5(6.0%)	11 (5.0%)
WC + H + FPG + HDL	5(4.0%)	2(2.0%)	7 (3.0%)
WC + H + FPG + TG	6 (5.0%)	4(4.0%)	10 (5.0)
WC + H + HDL +TG	2 (2.0%)	2 (2.0%)	4 (2.0%)
WC + H + FPG + TG + HDL	2 (2.0%)	1(1.0%)	3 (1.0%)
WC + FPG + HDL	6 (5.0%)	5 (6.0)	11 (5.0%)
WC + FPG + TG	6 (5.0%)	9 (1.0%)	15 (7.0%)
WC + FPG + TG + HDL	2 (.02%)	2 (2.0%)	4 (2.0%)
WC + HDL + TG	4 (4.0%)	3 (.03%)	7 (3.0%)

The results are expressed as N (percentage). WC: waist circumference, FPG: high fasting plasma glucose, H: hypertension, HDL: low plasma high density lipoprotein cholesterol, TG: hypertriglyceridaemia

Table 4.27. Contributions of various components of the metabolic syndrome in the rural population. .

Morbidity	Men (N = 51)	Women (N = 129)	Total (180)
WC + H + FPG	1 (2.0%)	12 (9.3%)	13 (7.2%)
WC + H + HDL	0 (0.0%)	11(8.5%)	11 (6.1%)
WC + H + TG	0 (0.0%)	0 (0.0%)	0 (0.0%)
WC + H + FPG + HDL	0 (0.0%)	3(2.3%)	3(1.7%)
WC + H + FPG + TG	0 (0.0%)	2(1.7%)	2 (1.1%)
WC + H + HDL +TG	0 (0.0%)	2 (1.7%)	2 (1.1%)
WC + H + FPG + TG + HDL	0 (0.0%)	1(0.7%)	1 (0.5%)
WC + FPG + HDL	0 (0.0%)	4 (3.1%)	4 (2.2%)
WC + FPG + TG	0 (0.0%)	0 (0.0%)	0 (0.0%)
WC + FPG + TG + HDL	0 (0.0%)	1(0.7%)	1 (0.5%)
WC + HDL + TG	1 (2.0%)	2(1.7%)	3 (1.7%)

The results are expressed as N (percentage). WC: waist circumference, FPG: high fasting plasma glucose, H: hypertension, HDL: low plasma high density lipoprotein cholesterol, TG: hypertriglyceridaemia

A summary of the general characteristics of the subjects with the metabolic syndrome is shown in table 4.28 below. The subjects with the metabolic syndrome had significantly higher systolic and diastolic blood pressures, BMI and waist circumference than the control group. The subjects with the metabolic syndrome also had higher fasting plasma glucose and cholesterol than the controls. However, the higher uric acid level difference in the subjects with the metabolic syndrome did not show any statistical significance. Additionally, the metabolic syndrome subjects had significantly lower levels of plasma antioxidants and higher mean plasma malondialdehyde concentrations than the control group.

Table 4.28. General characteristics of subjects with the metabolic syndrome.

Parameter	Metabolic Syndrome		Controls		P-value
	N = (50)		N = (94)		
	Mean	SEM	Mean	SEM	
Age (years)	57.26	1.62	52.64	1.27	*
S.BP(mmHg)	143.1	1.86	115.1	0.93	***
D. BP (mmHg)	89.30	2.08	72.36	0.74	***
BMI (kg/m²)	28.20	0.62	24.45	0.41	***
WC (cm)	94.26	1.38	80.94	1.07	***
FPG (mmol/l)	6.77	0.26	5.02	0.07	***
Cholesterol(mmol/l)	5.74	0.18	4.47	0.08	***
HDL (mmo/l)	1.49	0.04	1.38	0.03	*
LDL (mmol/l)	3.94	0.16	2.87	0.07	***
Triglycerides (mmol/l)	1.37	0.08	1.13	0.04	*
Uric acid (mmol/l)	322.6	10.32	309.3	7.93	Ns
MDA (umo/l)	2.18	0.05	1.89	0.02	***
Ascorbic acid (mg/dl)	1.11	0.01	1.15	0.01	**
Retinol (ug/dl)	25.84	0.57	28.68	0.38	***
Beta-carotene (ug/dl)	55.89	0.56	60.52	0.37	***
Catalase (umol/l)	571.1	5.45	612.3	3.47	***
10-yr CHD risk	9.38	1.17	3.97	0.33	***
DRS	7.44	0.27	3.44	0.16	***

The results are expressed as mean ± SEM. * $p< 0.05$, ** $p< 0.001$, *** $p< 0.0001$, Ns: not significant. S.BP: systolic blood pressure, D.BP: diastolic blood pressure, BMI; body mass index, WC: waist circumference, FPG: fasting plasma glucose, HDL: high density lipoprotein, LDL: low density lipoprotein, MDA: malondialdehyde, 10-yr CHD risk: 10-year coronary heart disease risk, DRS: Ghanaian diabetes risk score.

4.1.7.0. Differences between subjects based on fruits and vegetables consumption

The results of table 4.29 show the general characteristics of subjects who consume fresh fruits and vegetables everyday and those who do not. Subjects who consume fresh fruits and vegetables daily had significantly lower levels of systolic blood pressure, fasting plasma glucose and total cholesterol. Additionally, this translated in the higher plasma levels of vitamins A, C and beta–carotene. Their mean plasma lipid peroxidation level was also lower than those who do not consume fresh fruits and vegetables everyday ($p< 0.0001$). There was however no significant differences in the BMI, waist circumference and plasma catalase activity between the two groups.

4.1.8.0.10-year Coronary Heart Disease Risk

Tables 4.10, 4.11 and 4.12 show the differences in the 10-year CHD risk between the urban and rural populations. There was no significant difference in the 10-year CHD risk between the urban and rural populations. However, men had significantly higher 10-year CHD risk than women in both the urban and rural populations. Additionally, as shown in table 4.13, one-way ANOVA and Bonferroni's post test showed that the 10-year CHD risk was higher in the metabolic group with both type 2 diabetes mellitus and hypertension. Subjects with the metabolic syndrome also had higher 10-year CHD risk than the controls as shown in table 4.28.

Table 4.29. Distribution of anthropometric and biochemical parameters among subjects who consume fresh fruits and vegetables daily and those who do not.

Parameter	Daily (N = 66)		Not daily (N = 324)		P-value
	Mean	SEM	Mean	SEM	
Age (years)	57.53	1.73	54.40	0.66	Ns
S. BP (mmHg)	136.4	2.65	129.5	1.05	**
D. BP (mmHg)	82.14	1.69	81.68	0.72	Ns
BMI (kg/m²)	25.20	0.51	25.67	0.24	Ns
WC (cm)	81.73	1.22	84.98	0.65	*
FPG (mmol/l)	4.99	0.09	5.68	0.08	***
Cholesterol(mmol/l)	4.65	0.10	5.04	0.06	**
HDL (mmo/l)	1.39	0.03	1.42	0.02	Ns
LDL (mmol/l)	3.01	0.11	3.38	0.05	**
Triglycerides (mmol/l)	1.25	0.06	1.22	0.03	Ns
Uric acid (mmol/l)	339.1	9.20	330.5	4.33	Ns
MDA (umo/l)	1.79	0.03	2.03	0.02	***
Ascorbic acid (mg/dl)	1.16	0.01	1.13	0.01	*
Retinol (ug/dl)	29.98	0.64	27.01	0.25	***
Beta-carotene (ug/dl)	60.95	0.37	58.61	0.25	***
Catalase (umol/l)	592.4	2.87	605.2	4.81	Ns
10-yr CHD	7.83	0.85	6.74	0.35	Ns
DRS	4.35	0.22	5.26	0.12	**

The results are expressed as mean ± SEM. * $p< 0.05$, ** $p< 0.001$, *** $p< 0.0001$, Ns: not significant. S.BP: systolic blood pressure, D.BP: diastolic blood pressure, BMI; body mass index, WC: waist circumference, FPG: fasting plasma glucose, HDL: high density lipoprotein, LDL: low density lipoprotein, MDA: malondialdehyde, 10-yr CHD risk: 10-year coronary heart disease risk, DRS: Ghanaian diabetes risk score.

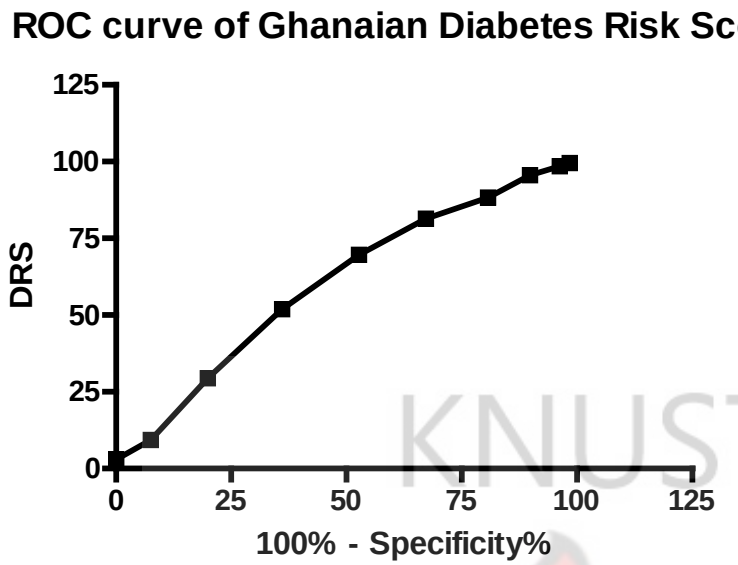
4.1.9.0. The Ghanaian Diabetes Risk Score

Some risk scores have been developed to predict the probability of development of type 2 diabetes mellitus in various populations. Most risk scores tend to be specific to the population used to develop the risk score. Another objective of the study was to develop a diabetes risk score that can be used to predict the likelihood of the presence of undiagnosed hyperglycaemia and for that matter the identification of individuals at high risk of the development of type 2 diabetes and other dysmetabolic conditions in a Ghanaian population. Because the risk score was just to identify the presence of undiagnosed hyperglycaemia in the subjects, beta-coefficients to actually calculate weight of score of the various parameters were not done. Parameters were given individual scores ranging from 0 to 3 (appendix 1).

The mean Diabetes Risk Score in the urban population was higher than the rural population as shown in table 4.10. Additionally, tables 4.11 and 4.12 above show that women had a significantly higher mean Diabetes Risk Score than men in both the urban and rural populations.

The Ghanaian Diabetes Risk Score was able to identify those at high risk of development of dysmetabolic states. As shown in table 4.28, subjects with the metabolic syndrome were observed to have higher risk scores than those at lower risk. Additionally, a linear regression model (figure 4.8) showed that the Ghanaian Diabetes Risk Score can be used to predict the presence of undiagnosed hyperglycaemia in apparently healthy adults ($r^2 = 0.023$, $p = 0.003$) in the subject population. Body mass index and waist circumference were the major contributors to the risk score ($r = 0.444$ and $r = 0.490$ respectively, $p<0.0001$). With Receiver Operator Characteristics, the Ghanaian Diabetes Risk Score had an area of 0.6031, $p = 0.0004$.

Figure 4.7. Reciever Operative Analysis of Ghanaian Diabetes Risk Score.



Area under the ROC curve (Area = 0.6031, Std. Error = 0.02866, 95% confidence interval = 0.5469 to 0.6592, P value = 0.0004)

Cutoff	Sensitivity%	95% CI	Specificity%	95% C	Likelihood ratio
< 1.500	2.941	1.087% to 6.292%	100.0	98.04% to 100.0%	
< 2.500	9.314	5.701% to 14.16%	92.47	87.69% to 95.82%	1.24
< 3.500	29.41	23.26% to 36.18%	80.11	73.64% to 85.59%	1.48
< 4.500	51.96	44.87% to 58.99%	63.98	56.63% to 70.87%	1.44
< 5.500	69.61	62.80% to 75.84%	47.31	39.96% to 54.75%	1.32
< 6.500	81.37	75.34% to 86.47%	32.80	26.10% to 40.05%	1.21
< 7.500	88.24	83.00% to 92.31%	19.35	13.94% to 25.77%	1.09
< 8.500	95.59	91.79% to 97.96%	10.22	6.263% to 15.49%	1.06
< 9.500	98.53	95.76% to 99.70%	3.763	1.526% to 7.600%	1.02
< 10.50	99.51	97.30% to 99.99%	1.613	0.3339% to 4.641%	1.01

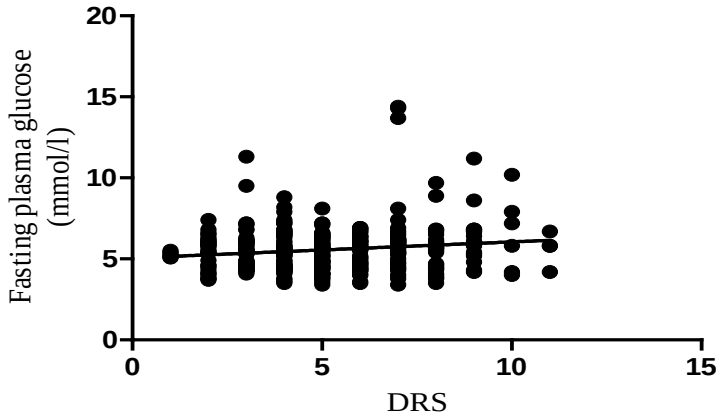


Figure 4.8. Linear regression of Diabetes risk score and plasma glucose in the subject population (r^2 = 0.023, p = 0.003).

Chapter 5

DISCUSSION

5.1.0. Obesity

Modernization has its own benefits and disadvantages. As shown in the results above, many of the relatively younger urban dwellers have had more than 9 years of formal education and are either professionals or paraprofessionals who live a considerably more sedentary lifestyle than their rural counterparts. A similar sociodemographic picture has been observed in another study in Ghana **[Amoah *et al,* 2002].**

Evidence **[Astrup and Finer, 2000]** has shown that sedentary lifestyle is associated with obesity and other cardiovascular risk factors like hypertension, dyslipidaemia and type 2 diabetes mellitus.

The degree of overweight can be expressed in several ways, but the most useful is the body mass index (BMI). This index is the body weight in kilograms divided by the square of the height in metres (weight/ (height) ^{2}). Healthy weight is defined as a BMI between 19 and 25 kg/m^2. Overweight is a BMI of 25-30 kg/m^2 and is associated with lower risk of development of type 2 diabetes and other cardiovascular diseases **[Astrup and Finer, 2000]**. A BMI greater than 30 kg/m^2 is almost always associated with an increase in body fat and is classified as obesity, which is prevalent worldwide and is associated with increased mortality, increased cardiovascular diseases and type 2 diabetes mellitus **[Astrup and Finer, 2000, Liu and Manson, 2001].** These relative risks range from 2 to 8 when the BMI is in the range 35-50 kg/m^2 and contrasts with the low risk to health of people with healthy body weight **[Astrup and Finer, 2000, Liu and Manson, 2001].** Although the exact biochemical mechanisms responsible for the association between obesity and the above diseases have not been completely elucidated, it is known fact that increases in triglyceride stores is always associated with a linear increase in the production of cholesterol **[Astrup and Finer, 2000, Liu and Manson, 2001].**

In most parts of the world including Ghana, there are increasing trends of sedentary lifestyle and obesity **[Amoah, 2003, Owiredu *et al,* 2008].** In this study, the mean BMI of the urban subjects was significantly higher than that of the rural subjects

(27.10 ± 0.28 and 23.78 ± 0.29, $p<0.0001$ respectively). Additionally, the mean waist circumference of the urban subjects was significantly higher than the rural subjects. It was also evident that most of the urban subjects presented with overweight or obesity as compared to the rural subjects. Almost half (49.0%) of the urban population were overweight and obesity had a prevalence of 24.8%. However, the prevalence of overweight and obesity in the rural population were 32.2% and 7.2% respectively. This finding is similar to those found in other West African countries **[Balde *et al,* 2002].** Overweight and obesity were also found to be more common in women than men in both the urban and rural populations. Similar findings have been reported in other studies in Ghana **[Amoah 2003, Owiredu *et al,* 2008].** In about 10 years the rate of obesity in Ghanaians has increased several times, from less than 1 to 14% in some populations **[Amoah, 2003]**. It has been observed that an increase in the prevalence of obesity within a population is often noted before a rise in the occurrence of chronic non-communicable diseases such as diabetes, hypertension, stroke, coronary artery disease and some forms of cancer. With the increasing rate of obesity, the stage is thus set for non-communicable diseases to emerge and threaten the health of Ghanaians. The finding that the metabolic group with both type 2 diabetes and hypertension had the highest mean BMI gives credence to the fact that obesity is directly linked to the development of type 2 diabetes and other cardiovascular diseases.

5.1.2 Hypertension

Previous studies in Ghana have reported crude prevalence of hypertension between 25% and 48% **[Pobee *et al,* 1979, Agyemang *et al,* 2006, Addo *et al,* 2006, Owiredu *et al,* 2008].** In this study, hypertension was more prevalent among the urban subjects than the rural subjects (36.7% and 20.6% respectively). The finding of hypertension being more prevalent in the urban subjects than the rural subjects has been observed in other studies **[Amoah, 2003, Addo *et al,* 2006, Agyemang, 2006].** Additionally, the results of this study show that hypertension is more common in men as compared to women in both the urban and rural populations. Previous studies have also reported hypertension to be more prevalent in men than women **[Pobee *et al,* 1979, Agyemang *et al,* 2006, Addo *et al,* 2006, Owiredu *et al,* 2008].**

5.1.3. Fasting plasma glucose

There was a significant ($p< 0.001$) difference between the urban and rural populations in terms of fasting plasma glucose with urban subjects having a higher mean plasma glucose than the rural subjects. However, as shown in tables 4.7 and 4.8, there were no significant gender differences in fasting plasma glucose in both the urban and rural populations.

In the late 1950s, the prevalence of diabetes was estimated at less than 0.5% in urban areas in Ghana **[Dodu, 1958]**; in a recent study the prevalence of type 2 diabetes in adult Ghanaians from the city of Accra and its environs was estimated at 6.3% **[Amoah *et al*., 2002**]. In this study, the prevalence of type 2 diabetes mellitus and IFG were considerably higher among the urban subjects as compared to the rural population (10.5% and 28.6% respectively for urban subjects and 3.8% and 22.1% respectively for rural subjects). A recent study by Owiredu *et al* (2008) in Kumasi also showed a similar picture. Although these prevalence rates were not directly comparable as a result of different sampling methods, they are in accordance with the rising trend of obesity **[Amoah *et al,* 2002]** in the present population.

Figures 4.2 and 4.3 show the gender distribution of type 2 diabetes mellitus and IFG in the urban and rural populations. Type 2 diabetes was more prevalent in men than in women in the urban population but IFG was more common among women than in men in both the urban and rural populations. There was however no gender difference in type 2 diabetes mellitus prevalence in the rural population. These results are similar to those reported elsewhere in Sub-Sahara Africa where subjects in the urban area had twice as much diabetes mellitus as compared to the rural subjects **[Balde *et al,* 2002].**

Fasting plasma glucose was shown to be higher in the group with both type 2 diabetes and hypertension as compared to the other groups as indicated in table 4.9. Eghan and Acheampong (2003) also reported similar results of mean plasma glucose being higher in the presence of type 2 diabetes and hypertension.

In the study, there was a negative correlation between fasting plasma glucose and antioxidants but, fasting plasma glucose showed a positive correlation with malondialdehyde. Several studies have showed that hyperglycaemia leads to

overproduction of reactive oxidants through several pathways that may lead to destruction of various macromolecules in the body including lipids through the mechanism of oxidative stress **[Evans *et al,* 2003].** The positive association between plasma glucose and lipid peroxidation observed from this study gives evidence to this biological mechanism.

5.1.4. Dyslipidaemia

Previous studies among the general population showed that dyslipidaemia was not a problem in Ghana **[Asibey-Berko and Avorkliyah, 1999]**. However, recent studies have shown an increasing trend of dyslipidaemia in the Ghanaian society **[Eghan and Acheapong, 2003, Owiredu *et al,* 2008]**.

In general, LDL dyslipidaemia was the most prevalent type of dyslipidaemia in both the urban and rural populations. Total cholesterol, LDL and triglycerides dyslipidaemia were more common in the urban population than in the rural subjects. Almost half (>40%) of the urban adults had total cholesterol dyslipidaemia as compared to about 20% of the rural subjects. Additionally, over 80% of the urban adults had LDL dyslipidaemia as compared to about 60% in the rural population. Other studies have also reported higher prevalence of dyslipidaemia in urban subjects **[Balde *et al,* 2002, Kamadjeu *et al,* 2006].** Dyslipidaemia in general was more common in men than in women. This is in accordance with the observation that hypertension was also more common in men than in women as has been shown in other studies **[Pobee *et al,* 1979, Agyemang *et al,* 2006, Addo *et al,* 2006, Owiredu *et al,* 2008].** The mean plasma levels of total cholesterol, LDL-cholesterol and triglycerides were not much different between men and women in both populations except for HDL-cholesterol which was found to be higher in women than in men. Eghan and Acheampong (2003) and Ko *et al,* (2001) have also reported higher mean plasma HDL-cholesterol in women in both the Ghanaian and Chinese populations respectively. In general, dyslipidaemia was more common in the metabolic group with both hypertension and type 2 diabete mellitus.This is contrary to the results of Eghan and Acheampong (2003) who reported serum total cholesterol dyslipidaemia to be more common in the metabolic group with only hypertension. The difference may be due to sampling differences.

In this study, there was a negative correlation between plasma antioxidants and total cholesterol, LDL-cholesterol and triglycerides. However, hyperlipidaemia showed a positive correlation with malondialdehyde. Some studies have showed that hyperlipidaemia leads to overproduction of reactive oxidants that may lead to destruction of various macromolecules in the body including lipids through the mechanism of oxidative stress **[Evans *et al,* 2003].** This shows that both hyperlipidaemia and hyperglycaemia may lead to overproduction of reactive oxidants with a consequential damage to macromolecules.

5.1.5. Uric acid

The prevalence of hyperuricaemia has been increasing in recent years, not only in advanced countries but also in developing countries, along with the development of their economies **[Cirrilo *et al,* 2006, Heinig and Johnson, 2006]**. In the study however, there was no significant difference in the mean serum uric acid between the urban and rural populations.

A significantly higher mean serum uric acid was seen in men than in women in both populations. Additionally, it was observed that the serum uric acid was higher in the metabolic group with hypertension alone as compared to the other groups. There was however no significant relationship between uric acid and the other indices of oxidative stress. Some studies have suggested a positive association between hyperuricaemia and hypertension, obesity and the metabolic syndrome **[Li-ying *et al,* 2007, Nagakawa *et al,* 2008, Cirrilo *et al,* 2006, Heinig and Johnson, 2006].** However, this study did not find any significant association between uric acid and the metabolic syndrome as being suggested by some researchers and needs further investigation. This study showed a positive association between age and systolic blood pressure and uric acid. This suggests that ageing may lead to hypertension through a hyperuricaemia-mediated factor.

5.1.6. Oxidative Stress indices

Oxidative stress results from an imbalance between the pro-oxidant and antioxidant factors in the human body that favours the pro-oxidant species **[Sies, 1993]**. As shown from the results of the study shows that there was a significant negative association between the plasma antioxidant and malondialdehyde. Depletion of the body's antioxidant defenses leads to higher rates of oxidative stress with a consequential destruction of body lipids, resulting in higher levels of plasma malondialdehyde **[Evans *et al,* 2003]**. The results of the study also showed that the antioxidants correlated positively with each other, confirming the biological mechanism of synergism that has been observed in some studies **[Halliwell and Gutteridge, 1990]**. It was also evident from the results of the study that the mean plasma retinol, beta-carotene, and ascorbic acid of the rural population was significantly higher in the rural population than in the urban population whiles the mean level of malondialdehyde was significantly higher in the urban population than the rural population. The study showed that BMI, waist circumference, plasma glucose, and cholesterol all showed a negative correlation with plasma antioxidants but a positive association with malondialdehyde. The results also showed that oxidative stress was higher in the presence of either diabetes mellitus alone or both diabetes mellitus and hypertension. Research data to date indicate that increased levels of lipid peroxides are associated with premature aging, atherosclerotic process, hypertension, and cancer **[Evans *et al,* 2003]**. Lipid peroxides, produced inevitably from polyunsaturated fatty acids, may damage cell membranes and might accelerate cellular aging process. Various environmental pollutants and industrial toxicants have elevated the levels of lipid peroxides. Indeed higher levels of lipid peroxides have been reported in industrial workers exposed to smoke, tar, and high furnace temperatures, compared with rural workers **[Reddy *et al,* 1994].** It has been shown that such elevated levels may increase the risk for cardiovascular and other degenerative diseases.

Despite the fact that it is the prevalent view that insulin resistance is the main genetic factor predisposing to development of type 2 diabetes, review of several lines of evidence in current literature indicates a lack of overwhelming support for this concept **[Gerich, 1998]**. In fact, current literature better supports the case of impaired insulin secretion being the initial and main genetic factor predisposing to type 2 diabetes,

especially 1) the studies in people at high risk to subsequently develop type 2 diabetes (discordant monozygotic twins and women with previous gestational diabetes), 2) the studies demonstrating compete alleviation of insulin resistance with weight loss, and 3) the studies finding that people with type 2 diabetes or IGT can have impaired insulin secretion and no insulin resistance compared with well matched NGT subjects. This shows that a superimposition of acquired insulin resistance on a genetic predisposition drives individuals to the development of type 2 diabetes **[Gerich, 1998]**. This shows that subjects who are exposed to the effects of westernization of the Ghanaian society are predisposed to higher rates of systemic oxidative stress which may act as the mediating factor in the superimposition of the insulin resistance on an intial genetically defective insulin secretion and a consequential development of type 2 diabetes mellitus.

5.1.7. Metabolic syndrome

The metabolic syndrome is a cluster of various dysmetabolic states with the central figure being central obesity and is a growing medical problem in the industrialized countries **[Ford, 2002].** Of all the 11 possible combinations, the clustering of central obesity raised fasting glucose and the presence of hypertension was the most prevalent form of the metabolic syndrome in both the urban and rural subjects and a similar picture was observed in the gender groups. The percentage of subjects with this type of clustering in the classification of the metabolic syndrome was higher in the urban population (17.6%) than the rural population (7.2%). Additionally, this type of clustering was observed to be more common in women than men (24.4% and 12.5% respectively in the urban population and 9.3% and 2.0% respectively in the rural population. The study also showed that subjects with the metabolic syndrome had significantly higher systolic and diastolic blood pressures, BMI and waist circumference than the control group. The subjects with the metabolic syndrome also have higher fasting plasma glucose and cholesterol than the controls. However, the higher uric acid level difference in the subjects with the metabolic syndrome did not show any statistical significance. As already stated above, hyperuricaemia has been suggested by some researchers **[Li-ying *et al,* 2007 and Nagakawa *et al,* 2008]** as another facet of the metabolic syndrome however this study did not find any significant association between uric acid and the metabolic syndrome. Additionally,

the metabolic syndrome subjects had significantly lower levels of plasma antioxidants and higher mean plasma malondialdehyde concentrations than the control group. This observation is in accordance with other studies **[Shigetada *et al,* 2004]** that have shown that subjects with the metabolic syndrome have increased levels of systemic oxidative stress.

5.1.8. The Ghanaian Diabetes Risk Score

Variability is an inherent feature of the biological world. The world today faces an epidemic of non-communicable diseases (NCD), which will soon surpass communicable diseases both in the developing and developed world and Ghana is no exception. However, in NCD due to lack of a clear aetiological agent it is heavily dependent on identifying and tackling potential risk factors. The risk factors like age, gender, family history are non-modifiable while others like smoking, diet, physical activity, hypertension, etc are largely modifiable. Thus for NCD a classical screening alone may not work and principles of primordial prevention have to be applied. Thus the need for identification of "Risk Scores or Tests" to evaluate an individual's risk of development of such chronic conditions. Risk scores for diabetes mellitus may fall into two primary categories that are conceptually distinct. Although risk scores are usually thought to quantify an individual's risk of developing a particular disease, as with the Framingham Risk Score for coronary heart disease, most self-identified diabetes risk scores do not necessarily assess the risk of developing the disease; rather, they assess the likelihood of having undiagnosed diabetes mellitus **[Herman *et al,* 1995, Griffin *et al,* 2000, Glumer *et al,* 2004].** Some risk scores however assess the risk of developing diabetes **[Lindstrom and Toumilehto, 2003]**. The Ghanaian Diabetes Risk Score developed in this study falls in the former category of identification of undiagnosed hyperglycaemia in apparently healthy subjects.

The results of this study show that the mean Diabetes Risk Score in the urban population was higher than the rural population. Additionally, the results show that women had a significantly higher mean Diabetes Risk Score than men in both the urban and rural populations.

The Ghanaian Diabetes Risk Score was able to identify undiagnosed hyperglycaemia and for that matter individuals at high risk of development of dysmetabolic states. Subjects at high risk of development of dysmetabolic states were observed to have

higher risk scores than those at low risk. Additionally, a linear regression model showed that the Ghanaian Diabetes Risk Score can be used to predict the presence of hyperglycaemia in apparently healthy adults (r^2 = 0.023, p = 0.003) in the subject population. Body mass index and waist circumference were the major contributors to the risk score (r = 0.444 and r = 0.490 respectively, p<0.0001). With Receiver Operator Characteristics, the Ghanaian Diabetes Risk Score had an area of 0.6031, p = 0.0004 under the curve. The sensitivity and specificity observed in this study were lower than those reported elsewhere **[Herman *et al,* 1995, Griffin *et al,* 2000, Mathias *et al,* 2007]** and this may be due to the relatively smaller sample population used in this study.

As the prevalence of diabetes rises, and more young adults and adolescents develop diabetes mellitus, it is critical from a clinical and public health perspective to be able to identify high-risk populations. Several diabetic risk scores or risk tools have been devised for the last decade. Recent evidence has suggested that risk assessment strategies may need to differ depending on which racial or ethnic population is being evaluated since some risk scores have been found not to work well in some populations **[Glumer *et al,* 2006].**

The Ghanaian Diabetes Risk Score should be tested in other larger population-based studies in rural and urban. Prospective follow up studies on non-diabetic subjects with high-risk score are needed to assess the predictive nature of this risk score. This risk score may be predictive of metabolic syndrome and cardiovascular disease as four of the factors (age, physical activity, waist circumference and blood pressure) are risk factors for both metabolic syndrome and cardiovascular disease. This risk score uses two modifiable risk factors (waist circumference and physical inactivity) and two non-modifiable risk factors (age and family history of diabetes), providing a clear message that if modifiable risk factors are altered, the risk score can be considerably reduced. Subjects with high risk scores regardless of their blood sugar status, are ideal candidates for life style modification as these are risk factors for not only diabetes but also for cardiovascular disease. The new Ghanaian Diabetes Risk Score is simple and user friendly and it will need validation in other population based studies from larger prospective trials in Ghana. It will also get modified in the process to increase the specificity, sensitivity and predictive values.

5.1.9. Summary

From the results of the study it has been evident that the modern society has brought with it profound changes in lifestyle and an increased incidence of type 2 diabetes and other cardiovascular risks. Previous studies in the Ghanaian population has shown that type 2 diabetes, obesity, and dyslipidaemia were not major problems. However, recent studies have shown evidence of an upsurge in the incidence of these dysmetabolic states. Body weights are on the rise, diets are becoming less healthy, and people are becoming increasingly sedentary, resulting in metabolic alterations that increase systemic oxidative stress levels. It has been shown that obesity, the metabolic syndrome, and type 2 diabetes mellitus are becoming a public health problem of epidemic proportions. Majority of type 2 diabetes mellitus risk in the developed and developing countries is attributable to overweight and obesity **[WHO, 1998, Ford, 2002].**

Ghanaians generally associate fatness with beauty and in women and well-being and success in both sexes. It is, therefore, not surprising that some women and indeed some men are now going out of their way to put on weight in order to appear beautiful or prosperous. Ghanaian men are also perceived to prefer plumb and bigger women to thin ones and this may conceivably contribute to the higher rates of overnutrition among females. It also appears that Ghanaians are now eating more fried and fatty foods, sauces and soups than they did several decades before because of the proliferation of fast food outlets in the urban and peri-urban centres as part of the consequences of urbanization and westernization**.** In the past two decades, understanding of the role of nutrients and foods likely to promote cardiac health has grown substantially owing to studies of the molecular mechanisms of atherosclerosis and the metabolic effects of various nutrients and foods. Dietary patterns that emphasize whole grain foods and legumes, and vegetables and fruits, and that limit red meat, full-fat dairy products, and food and beverages high in added sugars are known to be associated with decreased risk of a variety of chronic disease **[Hu and Willet, 2002].** In a more general sense, adherence to healthy lifestyle practices, which of course also include healthy diets, has been found to be associated with significant reduction in the rate of type 2 diabetes mellitus and other cardiovascular diseases.

[Rimm and Stampfer, 2004]. Despite this significant evidence, the 'Western' diets have shifted unfavourably. Vegetable and fruit consumption of both adults and youth continues to be below recommended levels. It has been reported that only 24.5% of adults and 21.4% of youth consume at least five servings each day, whereas consumption of refined grains and food high in added sugars are on the rise. It therefore not surprising that low consumption of fruit and vegetables, together with physical inactivity, are now among the top 10 causes of mortality in developed countries **[The World Health Report, 2004].**

Nutrition represents a characteristic lifestyle element that can be controlled, and that can directly influence health; therefore preventative nutrition and weight control should become a main focus of both consumers and prepared-food providers **[Feskens *et al,* 1995]**. The Westernization of diets, with an increase in availability of high calorie foods certainly contributes to the epidemic of metabolic syndrome. In the past, physicians and researchers have made an association between dietary energy from fat and body fat. Although a large market has developed for the popularity and promotion of low fat diets, the decline in dietary fat consumption has not corresponded to a decrease in obesity. In fact, the opposite trend has rather emerged **[Hu and Willet, 2002]**. Diets high in saturated fats have been shown to induce weight gain, insulin resistance, and hyperlipidaemia in humans and animals **[Feskens *et al,* 1995]** but the emphasis on dietary fat reductions has had no significant benefits relative to the obesity epidemic. Moreover, the focus on dietary fat seem more likely a distraction to more significant causes of metabolic syndrome **[Hu and Willet, 2002]**. If fat is not the key culprit in metabolic disorders, then another factor needs to be identified. In fact, increasing evidence now suggests that the rise in consumption of carbohydrates, particularly refined appears to be at least one very important contributing factor.

Obesity is known to be associated with insulin resistance. In fact, it is generally accepted that both obesity and insulin resistance are part of a common pathological mechanism **[Rosmund, 2005]**. However, this relationship has become confusing since not all obese individuals are insulin resistant and that insulin resistance occurs in individuals with normal BMIs **[Ferrannini *et al,* 1997].** Mounting evidence shows that the metabolic syndrome process begins early in life and persistence from childhood to adolescent/adult life produces type 2 diabetes mellitus and cardiovascular

disease **[Freedman *et al,* 2001]**. The symptoms of the metabolic syndrome may not necessarily be manifestations of age, but develop over a predisposed background established at a young age **[Kohen-Avramoglu *et al,* 2003]**.

As discussed above, many studies on the relationship between oxidative stress and type 2 diabetes have shown that oxidative stress is associated with the development of diabetes complications. Chronic systemic oxidative stress has been shown to cause insulin resistance in rodents **[Houstis *et al,* 2006].** Subjects with pre-diabetes may therefore be predisposed to higher rates of ROS and other free radical attack well before the development of overt diabetes. However, there is limited data on the relationship between pre-existing rates of oxidative stress and type 2 diabetes and other dysmetabolic conditions. This study focused on the relationship between pre-existing levels of systemic oxidative stress and diabetes mellitus and other dysmetabolic conditions in the free-living and apparently healthy populations in urban and rural communities.

Because of this cross-sectional nature of this study, it did not aim at establishing a direct causal relationship between oxidative stress and the development of type 2 diabetes. However, the study aimed at creating a hypothesis that the westernisation of the Ghanaian society is associated with increased rates of systemic oxidative stress and this is accompanied by the increasing incidence of accelerated ageing diseases like type 2 diabetes and other dysmetabolic conditions.

Chapter 6

CONCLUSION

From the results of the study it has been evident that the modern society has brought with it profound changes in lifestyle and an increased incidence of type 2 diabetes and other cardiovascular risks. Although previous studies in the Ghanaian population has shown that type 2 diabetes, obesity, and dyslipidaemia were not major problems, recent studies have shown evidence of an upsurge in the incidence of these dysmetabolic states. Body weights are on the rise, diets are becoming less healthy, and people are becoming increasingly sedentary, resulting in metabolic alterations that increase cardiovascular risk. It has been shown that obesity, the metabolic syndrome, and type 2 diabetes mellitus are becoming a public health problem of epidemic proportions. Majority of type 2 diabetes risk in the developed and developing countries is attributable to overweight and obesity. Although the pathogenic mechanism that leads to insulin resistance and type 2 diabetes mellitus and the metabolic syndrome is not fully elucidated, obesity is known to be associated with insulin resistance and consequently type 2 diabetes mellitus. However, not all insulin-resistant individuals are obese and additionally, not all obese individuals are insulin-resistant. These findings suggest that other factors may play a role in the pathogenesis of type 2 diabetes and other dysmetabolic conditions. Since the development of type 2 diabetes spans over years, it suggests that increased exposure to the pathogenic mechanism may predispose many individuals to the development of type 2 diabetes and other dysmetabolic conditions. Although oxidative stress has been shown to be the pathogenic mechanism involved diabetes complications, results of the beneficial role of antioxidants in intervention trials have been inconsistent. Additionally, there is limited data on the relationship between pre-existing rates of oxidative stress and type 2 diabetes mellitus and other dysmetabolic conditions in the free-living and apparently healthy populations.

In sub-Saharan Africa, the increasing non-communicable disease burden is compounded by lack of a coherent policy on chronic disease prevention, control, surveillance, and research. Furthermore, inadequate financing and inadequate and dwindling numbers of trained healthcare personnel constitute major barriers to the

control of diabetes and its complications. Other limitations include the failure to provide key decision makers with clear and up-to-date evidence on the burden of diabetes and other chronic diseases, a lack of understanding of the economic factors that influence disease risks, and the current orientation of health systems toward acute care. The economic cost of diabetes and its complications are unaffordable by most individuals and families in sub-Saharan Africa. Their incomes are insufficient to purchase insulin, oral hypoglycaemic agents, and other supplies for the management of diabetes. The limited resources available to sub-Saharan African countries are shared between fighting poverty, implementing education strategies, provision of housing and appropriate sanitation, and the socioeconomic and health burden of fighting the increasing incidence and prevalence of HIV/AIDS. Diabetes poses an additional burden on the limited healthcare delivery system and resources. Many key decision makers still believe that diabetes and non-communicable diseases afflict only the affluent and the elderly and arise only from freely acquired risks and that their control is ineffective and too expensive and should wait until control of infectious diseases is addressed. There is this misconception that risk exposure is solely the responsibility of individuals. However, it is well known that sub-Saharan African countries are facing marketing pressure, with risk exposure habits such as smoking, fast foods, and drinking, which is highly promoted among vulnerable groups like children. It also appears that Ghanaians are now eating more fried and fatty foods, sauces and soups than they did several decades before because of the proliferation of fast food outlets coupled with reduced physical activity and low consumption of fresh fruits and vegetables in the urban and peri-urban centres as part of the consequences of urbanization and westernization.

In conclusion, the study hypothesizes that the fast increasing westernization of the Ghanaian society predisposes individual to higher rates of systemic oxidative stress independent of BMI and this is associated with accelerated ageing process in the society as evident in the increasing incidence of type 2 diabetes mellitus and other dysmetabolic conditions. To guard against these potentially devastating degenerative complications, it's important that both oxidative stress and metabolic control of glucose and lipids be carefully kept in check and monitored regularly in patients with, or at risk of type-2 diabetes.

Among the novel findings of this study are

1. Pre-existing higher rates systemic oxidative stress is associated with increased risk to the development of type 2 diabetes and other dysmetabolic conditions. This may have implications for prevention and management of type 2 diabetes.
2. The results of this can be used for future research since it provides baseline reference levels of antioxidants and lipid peroxidation in the apparently healthy adults in both and urban and rural communities in the Ashanti region.
3. Additionally, a simple risk tool like the Ghanaian Diabetes Risk Score may be used to identify individuals with undiagnosed hyperglycaemia and at high risk of development of type 2 diabetes and other dysmetabolic conditions in the Ghanaian society.

6.1. Recommendations

Based on the results of this study and those of other past and recent studies the following ideas are highly recommended:

- There is a need for further research to investigate the beneficial role of measurement and control of systemic oxidative stress in the prevention of type 2 diabetes.
- There is a need for further research to investigate the beneficial role of measurement and control of systemic oxidative stress in the routine management of type 2 diabetes.
- Further research is needed to understand how specific ROS and antioxidants act in normal physiology and the development of small-molecule catalase, peroxidase and other endogenous antioxidant mimetics may be critical in an attempt to elucidate this paradox of the inconsistent results on the beneficial role of antioxidants in intervention trials.
- While not advocating for mass screening for diabetes, the policy makers in the health sector should utilize a simple tool like the Ghanaian Diabetes Risk Score to identify individuals at high risk of development of these chronic dysmetabolic conditions. It is therefore recommended that the Ghanaian

Diabetes Risk Score should be validated in a larger prospective study to assess the true specificity, sensitivity and predictive value of the risk score and modify it if necessary.

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APPENDIX 1. THE GHANAIAN DIABETES RISK SCORE

PARAMETER		**SCORE**
AGE (years)		
< 35		0
35 – 54		2
≥ 54		3
BMI (kg/m²)		
≤ 25		0
26 – 29		1
≥ 30		3
WAIST CIRCUMFERENCE (cm)		
< 80 (women)	< 90 (men)	0
≥ 80 – 89 (women)	≥ 90 -99 (men)	1
≥ 90 (women)	≥ 100 (men)	3
PHYSICAL ACTIVITY		
Regular exercise and/ or strenous work on most days		0
No exercicise and sedentary work		2
FAMILY HISTORY OF DIABETES		
No parent or sibling		0
Sister or brother		1
Either parent		2
Both parents		3
INTAKE OF FRUITS AND VEGETABLES		
Everyday		0
Not everyday		1
Maximum score		**15**

APPENDIX 2: **QUESTIONNAIRE**

PARTICIPANT ID: DATE: ……..

STUDY SITE: …….

A. PERSONAL DETAILS

SEX: MALE ☐ FEMALE ☐

DATE OF BIRTH: …… OR APPROXIMATE AGE: ……

MARITAL STATUS:
Are you now married, widowed, divorced or never been married?

Married ☐ Divorced ☐

Widowed ☐ Never been married ☐

Are you currently pregnant? Yes ☐ No ☐

How many children have you had?
Alive children ☐ Deceased children ☐

RELIGION:

What is your religion? Christian ☐ Moslem ☐

☐ Other, please specify:

B. EDUCATION
What is the highest grade that you completed?

Never attended ☐ Primary ☐

Secondary/Vocational ☐ University/Tertiary ☐

C. PHYSICAL ACTIVITY PATTERN:

Occupation:

Are you currently retired? YES ☐ NO ☐

(If so please indicate previous occupation)

How often do you exercise? Never exercise ☐

Once a while ☐

≥1 day/week ☐

Specify nature of exercise: ……

What is your usual means of transportation? Walking ☐ Driving/Motorbike ☐

Bicycle ☐ Public transport ☐

D. LIFE STYLE

Smoking:

Smoker: has smoked cigarettes regularly for at least 6 months ☐

Ex-smoker: stopped for at least 1 year after regular smoking ☐

Never ☐

Drinking:

DRINK	**FREQUENCY(at least for the past 6 months)**			
	Never	Once a while	<3/week	≥3/week
Beer				
Palm wine				
Whisky/Gin				
Local liquor				

E. FAMILY HISTORY

Has a doctor ever diagnosed any of your relatives of the following conditions?

Diabetes: Yes ☐ No ☐ Hypertension: Yes ☐ No ☐

F. MEDICAL HISTORY

Has a doctor ever told you that you have any of the following conditions?

(If yes please specify date of diagnosis)

Diabetes	Yes ☐	No ☐
Impaired glucose regulation:	Yes ☐	No ☐
Liver disease:	Yes ☐	No ☐
High blood pressure (doctor gave pills):	Yes ☐	No ☐
Kidney disease:	Yes ☐	No ☐
Cancer:	Yes ☐	No ☐
HIV/AIDS:	Yes ☐	No ☐
Cushing's syndrome:	Yes ☐	No ☐
Thyroid disease:	Yes ☐	No ☐
Recent infection or illness:	Yes ☐	No ☐

MEDICATIONS:

List any medications that you are currently taking

Have you ever taken any antioxidant supplements for at least the past month?

Yes ☐ No ☐

Yes No
(If yes please specify): ……
……
……

H. DIETARY PATTERN

How many times do you usually eat in a day?
Once a day ☐ 2 times a day ☐ 3 times a day ☐ >3 times a day ☐

Meals usually prepared at home

Breakfast ☐ Lunch ☐ Supper ☐

Consumption of staples, animal, legumes and their products

Variable	Frequency				
	Daily	Weekly	Monthly	Occasionally	Never
Yam					
Plantain					
Cassava					
Cocoyam					
Potato					
Sweet potato					
White rice					
Brown rice					
Fried rice					
Spaghetti					
Maize					
Millet					
Sorghum					
Beans					
Cowpea					
Bread					
Oats					
Meat					
Fish					
Milk					
Egg					

Consumption of desert

Variable	Frequency				
	Daily	Weekly	Monthly	Occasionally	Never
Cake/pie/pastries/biscuits					
Soft drink					
Ice cream					
Yoghurt/cheese					

Consumption of fruits and vegetables in their season

Variable	Frequency				
	Daily	Weekly	Monthly	Occasionally	Never
Orange					
Mango					
Pineapple					
Pawpaw					
Pear					
Watermelon					
Banana					
Tomato					
Pepper					
Onion					
Okro					
Garden eggs					
Carbbage					
Carrot					
Local leafy vegetables					

APPENDIX 3. National Cholesterol Education Program Adult Treatment Panel III Approach to Dyslipidemias

TC (mmol/L)	
<5.17	Desirable
5.17–6.18)	Borderline high
≥ 6.20	High
LDL-cholesterol	
<2.58	Optimal
2.58–3.33	Near optimal/above optimal
3.36–4.11	Borderline high
4.13–4.88	High
≥ 4.91	Very high
HDL-cholesterol	
< 1.03	Low
≥ 1.55	High
TG	
< 1.695	Desirable
1.695–2.249	Borderline high
2.26–5.639	High
≥ 5.65	Very high

Data from the Third Report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. National Institutes of Health, National Heart, Lung, and Blood Institute, 2001. *Circulation* 110:227–239, 2004.