THYROID HORMONE LEVELS AND CARDIOMETABOLIC RISK FACTORS IN HYPERTENSIVE ADULT GHANAIANS

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DECLARATION

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

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ABSTRACT

Metabolic syndrome can be found in approximately one-third of patients who do not have diabetes but have hypertension. There are numerous correlations between the metabolic syndrome and hypertension, although this is not always the case. As metabolic syndrome and thyroid dysfunction are independent risk factors for the same disease process, namely cardiovascular disease, it is possible that patients suffering from both these disease entities may have a compounded risk. Our study will therefore attempt to determine the prevalence of metabolic syndrome and investigate the proposed association between these two disease entities and identify the factors that increase the risk of this association. This cross-sectional study was conducted at the Hypertension Clinic of the Department of Medicine, Komfo Anokye Teaching Hospital (KATH), Kumasi between April 2010 and November 2010. A total of 300 participants comprising of 200 hypertensives and 100 normotensives were enrolled. The prevalence of MetS among the hypertensive patients were significantly higher than the normotensive control (56.5% vrs 9.0%, 54.5% vrs 5.0% and 65.5%vrs15.0%, p<0.001) using NCEP ATP III, WHO and IDF criteria respectively. Irrespective of the criteria applied, all the components of MetS were significantly higher among the hypertensive patients as compared to the normotensive control. Among the hypertensive patients, the highest prevalence of cardiovascular risk factor was abdominal obesity as measured by WHR (77.0%), followed by reduced HDL-cholesterol (74.0%). From the univariate analysis, females were at about 3 times at risk of developing hypertension as compared to the male counterpart (OR = 2.7; 95% CI = 1.6-4.4; p = 0.0000). Reduced apolipoprotein A1 served as a risk factor (aOR = 13.4; 95% CI = 1.5-121.4; p = 0.0210) whilst high apolipoprotein A1 protects the individual from developing hypertension (aOR = 0.1; 95% CI = 0.0-0.2; p = 0.0000). High apolipoprotein B poses about 9 times risk of developing hypertension as compared to the normal level (aOR = 9.3; 95% CI = 4.2-20.9; p = 0.0000). Both Impaired fasting glucose and diabetes each pose more than 10 times risk of developing hypertension as compared to normoglycaemia. fT4 levels were positively associated to BMI and Apo A1 after adjustment for age. fT4 levels were however negatively associated to TC (β= -0.275; p<0.05), LDL-C (β= -0.337; p<0.05) and FBG (-0.121; p<0.05). We also demonstrated that, low normal FT4 levels were significantly associated with three of the cardiovascular risk factors. These findings are consistent with an increased cardiovascular risk in subjects with low normal thyroid function. In conclusion, the study demonstrated that, hypertension is more than just elevated blood pressure; it is intimately associated with the metabolic syndrome. There is therefore the need for metabolic screening of all hypertensives and increase awareness creation on the critical importance of public health strategies aimed at reducing risk factors in the entire population. Early detection and treatment (Multi-target approach) of the global risk profile should thus become a priority.

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Chapter 1

INTRODUCTION

1.1 GENERAL INTRODUCTION

Cardiovascular disease or cardiovascular diseases refers to the class of diseases that involve the heart or blood vessels (Hopkins *et al.,* 1993). While the term technically refers to any disease that affects the cardiovascular system, it is usually used to refer to those related to atherosclerosis (arterial disease). These conditions have similar causes, mechanisms, and treatments.

Hypertension is a major risk factor for cardiovascular disease, and the latter is the leading cause of morbidity and mortality worldwide. In developed countries, hypertension ranks as the top contributing factor for mortality and third in causing disability-adjusted life years (Ezzati *et al.*, 2002). Hypertension is a polygenic and complex disease with rising prevalence. More than 25% of the adult population is affected by hypertension, and two thirds of those individuals reside in developing countries (Kearney *et al.,* 2005). Europe shows an even higher prevalence of hypertension than North America (Wolf-Maier *et al.*, 2003). With the present trends, the prevalence of hypertension is predicted to increase to 30%, or ≈1.5 billion people, on the globe in the next 20 years (Kearney *et al.,* 2005). Mechanistically, endothelial dysfunction, increased renin-angiotensin system (RAS) activity, and sympathetic nervous system (SNS) hyperactivation have been considered as important risk factors of hypertension and hint at important events taking place at the interface of the endothelium, kidney, and SNS.

Obesity is a global epidemic in children and adults. In the United States, a steady increase of the prevalence of obesity has been found in all States (Ogden *et al.,* 2002). It is estimated that 65% of the population is overweight, which is judged by body mass index of 25.0 to 29.9, and 30% are obese ,body mass index of ≥30.0 (Mokdad *et al.,* 1999). These numbers have been continuously rising in the past 15 years (Ogden *et al,* 2006). The National Health and Nutrition Examination Survey

III for ≈18 000 adults found that body mass index is an associated risk factor for hypertension independent of age, sex, race, and smoking (Brown et al., 2000). A long-term weight/hypertension relationship study showed that weight loss of ≈10 kg is associated with a significant decrease of both diastolic and systolic blood pressure (Aucott et al., 2005). Obesity and hypertension are two complex disorders that are closely interrelated, but the precise underlying association remains elusive.

Recently, it has been questioned whether the metabolic syndrome as originally described prevails (Reaven, 1988) in most or all populations (Okosun et al., 1998; Toft et al., 1998; Osei & Schuster ,1996; Saad et al., 1991). The prevalent rates of different aspects of the syndrome vary in different racial and ethnic populations. For example, African Americans, especially women, are in general more obese and hypertensive, and have greater insulin resistance with hyperinsulinaemia but lower serum fasting triglycerides and higher high-density lipoprotein cholesterol concentrations than white Americans (Cowie et al., 1991). Titty et al. (2008) have previously reported the prevalence of metabolic syndrome among Ghanaian diabetics to be as high as 55.9 %.

Sub-clinical hypothyroidism (SCH) and overt hypothyroidism are recognized risk factors for atherosclerotic cardiovascular disease, hyperlipidemia, low grade inflammation and hypercoagulability (Serter *et al.*, 2004).

As metabolic syndrome (MetS) and hypothyroidism are independent risk factors for the same disease process, namely cardiovascular disease, it is possible that patients suffering from both these disease entities may have a compounded risk.

1.2 JUSTIFICATION

Cardiovascular diseases (CVD) have become a leading cause of mortality and morbidity in developing countries and rates are expected to rise further over the next few decades (Ezzati et al., 2002; Yusuf et al., 2001; Kearney et al., 2005; Wild et al., 2004). In particular, it has been estimated that high blood pressure (HBP) accounts for as much as 5.0% of the total mortality in middle-income countries, tobacco for 4.0%, high cholesterol for 2.1% and obesity for 2.7% (WHO, 2002). Ghana is no exception to the global trend, according to the 2007 annual report of the Ghana Health Service, hypertension features among the top ten (10) causes of morbidity at the Out Patient Department (OPD) level in all regions and accounted for 4.7 % of deaths. Urbanization in developing countries with adoption of sedentary lifestyle and its increased obesity has contributed significantly to the development of the condition.

The increasing burden of CVD has important economic implications. CVD occurs typically at a younger age in developing than developed countries with important consequences such as loss of revenue at household level and loss of productivity at a macroeconomic level. From a health system perspective, huge resources are needed for providing health care to large numbers of chronic patients for decades and for sustaining increasingly sophisticated equipment and more skilled and harder-to-replace workforce (Beaglehole & Yach, 2003).

In order to design effective messages for public education and good interventions there is the need to clearly define the risk factors involved. The risk factors are major contributors to the burden of ill-health in sub-Saharan Africa particularly in urban populations.

Sub-clinical hypothyroidism (SCH) and overt hypothyroidism are recognized risk factors for atherosclerotic cardiovascular disease, hyperlipidemia, low grade inflammation and hypercoagulability (Serter *et al.*, 2004).

There is however scanty data on the prevalence and the various associations of SCH, euthyroidism and overt hypothyroidism in Ghana.

As metabolic syndrome and hypothyroidism are independent risk factors for the same disease process, namely cardiovascular disease, it is possible that patients suffering from both these disease entities may have a compounded risk. Our study will therefore attempt to investigate the proposed association between these two disease entities and identify the factors that increase the risk of this association.

1.3 AIM OF STUDY

The study was primarily aimed at assessing the prevalence of metabolic syndrome and its components in patients with hypertension in comparison with controls.

1.4 SPECIFIC OBJECTIVES

- To determine the prevalence of metabolic syndrome and its components in hypertensives
- To determine the relationship between thyroid function and the traditional risk factors
- To determine the impact of cardiometabolic risk profile on blood pressure control
- Assess whether measurement of Apo B substantially improve risk identification
- Determine co- morbidities in hypertension

Chapter 2

LITERATURE REVIEW

2.1 CARDIOVASCULAR RISK FACTORS

Epidemiological data show that hypertensive individuals are remarkably different from normotensive persons in more than just high blood pressure (BP). A tendency exists for hypertension to occur in combination with other lifestyle, metabolic, and anthropometric factors, all independently associated with increased risk of cardiovascular diseases (CVD). According to data from the Framingham Heart Study, less than 20% of hypertension occurs in the absence of one or more risk factors, including high triglycerides and LDL cholesterol levels, reduced HDL cholesterol levels, glucose intolerance, hyperinsulinaemia, obesity, and left ventricular hypertrophy (Kannel, 2000).

2.1.1 HYPERTENSION

Hypertension is an important risk factor for cardiovascular disease. Although several risk factors for the development of hypertension have been identified, its etiology is still not fully understood (Chobanian *et al.,* 2003). Hypertension is commonly associated with other cardiovascular risk factors, such as obesity, diabetes, and dyslipidaemia (Reaven *et al.,* 1996). The presence of these cardiovascular risk factors and the resulting endothelial dysfunction may play a role in the pathophysiology of hypertension (Oparil *et al.,* 2003).

2.1.1.1PATHOPHYSIOLOGY OF HYPERTENSION

2.1.1.1.1

GENETICS

Evidence for genetic influence on blood pressure comes from various sources (Corvol et al., 1999). There is greater similarity in blood pressure within families than between families, which indicates a form of inheritance (Feinleib et al., 1977). And it was proved that this finding wasn't due to shared environmental factors

(Biron *et al.,* 1976). Single gene mutation is proved to cause Mendelian forms of high and low blood pressure (Lifton *et al.,* 2001). Almost 10 genes have been identified to cause this form of hypertension (Lifton *et al.,* 2001). These mutations affect blood pressure by altering renal salt handling. Recently and with the aid of newly developed genetic analysis techniques researchers found statistically significant linkage of blood pressure to several chromosomal regions, including regions linked to familial combined hyperlipidaemia (Hsueh *et al.,* 2000). These findings suggest that there are many genetic loci, each with small effects on blood pressure in the general population. Overall, however, identifiable single-gene causes of hypertension are uncommon, consistent with a multifactorial cause of essential hypertension (Corvol *et al.,* 1999).

2.1.1.1.2

AUTONOMIC NERVOUS SYSTEM

Also the autonomic nervous system, plays a central role in maintaining the cardiovascular homeostasis via pressure, volume, and chemoreceptor signals by altering peripheral vasculature, and kidneys, causing increased cardiac output, increased vascular resistance, and fluid retention. Disorder of the system, as in case of sympathetic nervous system overactivity, increases blood pressure and contributes to the development and maintenance of hypertension (Somers *et al.,* 1993). In addition, autonomic imbalance (i.e. increased sympathetic tone accompanied by reduced parasympathetic tone) has been associated with many metabolic and hemodynamic abnormalities that result in increased cardiovascular morbidity and mortality (Esler, 2000).

The mechanisms of increased sympathetic nervous system activity in hypertension are complex and involve alterations in baroreflex and chemoreflex pathways at both peripheral and central levels. Arterial baroreceptors are reset to a higher pressure in hypertensive patients, and this peripheral resetting reverts to normal when arterial pressure is normalized (Feinleib *et al.,* 1977). Furthermore, there is central resetting of the aortic baroreflex in hypertensive patients, resulting in

suppression of sympathetic inhibition after activation of aortic baroreceptor nerves. This baroreflex resetting seems to be mediated, at least partly, by a central action of angiotensin II. Additional small-molecule mediators that suppress baroreceptor activity and contribute to exaggerated sympathetic drive in hypertension include reactive oxygen species and endothelin. Some studies have shown that hypertensive patients manifest greater vasoconstrictor responses to infused norepinephrine than normotensive controls (Ziegler *et al.,* 1991). And that hypertensive patients do not show the normal response to increased circulating norepinephrine levels which generally induces downregulation of noradrenergic receptor, and its believed that this abnormal response is genetically inherited (Bianchetti *et al.,* 1986).

Exposure to stress increases sympathetic outflow, and repeated stress-induced vasoconstriction may result in vascular hypertrophy, leading to progressive increases in peripheral resistance and blood pressure (Oparil *et al.,* 2003). This could partly explain the greater incidence of hypertension in lower socioeconomic groups, since they must endure greater levels of stress associated with daily living. Persons with a family history of hypertension manifest augmented vasoconstrictor and sympathetic responses to laboratory stressors, such as cold pressor testing and mental stress that may predispose them to hypertension. This is particularly true of young African Americans. Exaggerated stress responses may contribute to the increased incidence of hypertension in this group (Calhoun *et al.,* 1993).

2.1.1.1.3

RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM

Another system maintaining the extracellular fluid volume, peripheral resistance and that if disturbed may lead to hypertension, is the renin-angiotensin-aldosterone system. Renin is a circulating enzyme that participates in maintaining extracellular volume, and arterial vasoconstriction, thus it contributes to regulation

of the blood pressure. It performs this function through breaking down (hydrolyzes) angiotensinogen secreted from the liver into the peptide angiotensin I, Angiotensin I is further cleaved by an enzyme that is located primarily but not exclusively in the pulmonary circulation bound to endothelium, that enzyme is angiotensin converting enzyme (ACE) producing angiotensin II, the most vasoactive peptide (Fujino et al., 2004). Angiotensin II is a potent constrictor of all blood vessels. It acts on the musculature of arteries and thereby raises the peripheral resistance, and so elevates blood pressure. Angiotensin II also acts on the adrenal glands too and releases aldosterone, which stimulates the epithelial cells of the kidneys to increase re-absorption of salt and water leading to raised blood volume and raised blood pressure. So elevation of renin level in the blood, which is normally in adult human is 1.98-24.6 ng/L in the upright position will lead to hypertension (Oparil et al., 2003).

Recent studies claim that obesity is a risk factor for hypertension because of activation of the renin-angiotensin system (RAS) in adipose tissue, and also linked renin-angiotensin system with insulin resistance, and claims that anyone of these can cause the other (Saito, 2009). Local production of angiotensin II in various tissues, including the blood vessels, heart, adrenals, and brain, is controlled by ACE and other enzymes, including the serine proteinase chymase. The activity of local renin–angiotensin systems and alternative pathways of angiotensin II formation may make an important contribution to remodeling of resistance vessels and the development of target organ damage (i.e. left ventricular hypertrophy,

congestive heart failure, atherosclerosis, stroke, end-stage renal disease, myocardial infarction, and arterial aneurysm) in hypertensive persons (McConnaughey et al., 1999).

2.1.1.1.4

ENDOTHELIAL DYSFUNCTION

The endothelium of blood vessels can produce an extensive range of substances that influence blood flow and, in turn, is affected by changes in the blood and the pressure of blood flow. For example, local nitric oxide and endothelin, which are secreted by the endothelium, are the major regulators of vascular tone and blood pressure. In patients with essential hypertension, the balance between the vasodilators and the vasoconstrictors is upset, which leads to changes in the endothelium and sets up a "vicious cycle" that contributes to the maintenance of high blood pressure. In patients with hypertension, endothelial activation and damage also lead to changes in vascular tone, vascular reactivity, and coagulation and fibrinolytic pathways. Alterations in endothelial function are a reliable indicator of target organ damage and atherosclerotic disease, as well as prognosis (O'Brien *et al.*, 2007).

Multiple evidences suggest that oxidant stress alters many functions of the endothelium, including modulation of vasomotor tone. Inactivation of nitric oxide (NO) by superoxide and other reactive oxygen species (ROS) seems to occur in conditions such as hypertension. Normally nitric oxide is an important regulator and mediator of numerous processes in the nervous, immune and cardiovascular systems, including smooth muscle relaxation thus resulting in vasodilation of the artery and increasing blood flow, suppressor of migration and proliferation of vascular smooth-muscle cells (Oparil *et al.*, 2003). It has been suggested that angiotensin II enhances formation of the oxidant superoxide at concentrations that affect blood pressure minimally (Fukui *et al.*, 1997).

Endothelin is a potent vasoactive peptide produced by endothelial cells that has both vasoconstrictor and vasodilator properties. Circulating endothelin levels are increased in some hypertensive patients (Touyz & Schiffrin, 2003), particularly African Americans and persons with hypertension.

2.1.2 AGE

The risk of cardiovascular events increases as people get older. In many epidemiologic surveys, age remains one of the strongest predictors of disease. More than half of those who have heart attacks are 65 or older, and about four out of five who die of such attacks are over age 65 (Black, 1980). Of course, nothing can be done to reduce age. However, careful attention to diet and maintaining fitness may delay the degenerative changes associated with aging.

2.1.3 GENDER

Men are more likely than women to develop coronary heart disease, stroke, and other cardiovascular diseases that are manifestations of atherosclerosis. Whether this is because male hormones—androgens —increase risk or because female hormones—estrogens—protect against atherosclerosis is not completely understood. It is likely that both play a role, but that the protective role of estrogens is the predominant factor. This seems to be supported by the fact that heart disease risk for women rises dramatically after menopause, when their bodies stop producing estrogen (Black, 1980).

2.1.4 HEREDITY

There is no question that some people have a significantly greater likelihood of having a heart attack or stroke because they have inherited a tendency from their parents. In some instances, such as familial hypercholesterolemia, the pattern of inheritance is well understood and the specific biochemical defects are well characterized (Black, 1980). For most cardiovascular risk factors, however, the specific way in which inheritance plays a role is not at all clear. As in almost all situations in medicine, both heredity and environment play a role and it is often difficult to know where one stops and the other begins. Prior generations did not

have the level of medical care as enjoyed currently, nor the general awareness about health; the details of the illness that one's grandparents or even parents had may not be precise.

In practical terms, anyone who has a family history of heart disease that occurred at an early age (below 55) should be especially careful to reduce the impact of any risk that can be controlled. Even if one can successfully control known risk factors, there are, unfortunately, a number of inherited characteristics that we have not yet identified and so cannot favourably control. Individuals with a history of atherosclerotic cardiovascular disease in the family simply have to be more vigilant if they wish to avoid heart attacks and strokes. However, almost every family has some member who died of a heart or blood vessel disease, since about half of all deaths are attributable to these diseases. If these episodes occurred in relatives who were 75 - 80, it may not be a major cause for concern.

Heredity also includes race. For reasons that are not completely understood, African-Americans have considerably higher rates of diabetes and both moderate and severe high blood pressure, adding to their overall risk of heart disease.

2.1.5 CIGARETTE SMOKING

Cigarette smoking is a major contributor to coronary heart disease, stroke, and peripheral vascular disease—even though smokers tend to be thinner and to have lower blood pressure than nonsmokers (Black, 1980). Overall, it has been estimated that 30 to 40 percent of the approximately 500,000 deaths from coronary heart disease each year can be attributed to smoking. Individuals, who smoke, regardless of their level of other risk factors or family history, are at significant risk of premature coronary disease and death. Smokers, for example, have less of a chance of surviving a heart attack than nonsmokers. Evidence from the Framingham Heart Study shows that the risk of sudden death increases more than ten fold in men and almost five fold in women who smoke. Smoking is the number one risk factor for sudden cardiac death and for peripheral vascular disease. Smoking

cigarettes that are low in nicotine and tar does not decrease the risk of heart disease, which is increased by the effect of smoke on blood vessel walls. In fact, some people tend to smoke more and inhale deeply when they switch to this type of cigarette, increasing their exposure to the carbon monoxide in the smoke itself.

Fortunately, the risk of heart disease begins to decline rapidly as soon as smokers—even heavy, longtime smokers—stop. Ultimately, their level of risk is almost the same as that of people who have never smoked.

2.1.6 OBESITY

The metabolic view of adiposity is that of another state of insulin resistance. Obesity, or an excess of body fat, favours the expression of the phenotypes synonymous to those described in other forms of insulin resistance, namely hypertension, fasting and postprandial hyperglycemia, and a dyslipidemia characterized by elevations in triglycerides, production of small, dense LDL particles and reduced HDL cholesterol. Excess fat is, on the average, harmful but it is most evident when it is carried intra-abdominally (Nieves *et al.*, 2003).

2.1.6.1 THE DYSLIPIDEMIA OF OBESITY

The largest survey of the relationship of obesity on blood lipids is the National Health and Nutrition Examination Survey. Separate reports on the dyslipidaemia of men (Denke et al., 1994), women (Sundquist et al., 2001) and children (Hickman et al., 1998) and among other ethnic groups reflect a common dyslipidaemia pattern as one of increased triglycerides, elevated non-HDL cholesterol, and lower HDL cholesterol. In young obese men and women, the NHANES data demonstrated that total and LDL cholesterol levels were higher in the obese than the non-obese. It is important to point out that fatness per se, without separation by degree of obesity (overweight vs. obese) or distribution (central vs. peripheral)

exerts a dose-response effect on blood lipids, specifically as increased VLDL triglycerides and cholesterol, reduced HDL cholesterol and a relative increase in small, dense LDL particles. On the average, the more fat, the more likely an individual will be dyslipidemic and to express elements of the metabolic syndrome. However, gram-for-gram, fat cells exert the most evident deleterious impact when they are located centrally (Rexrode et al., 1998). In comparison to peripheral fat, central fat is insulin resistant and more rapidly recycles fatty acids through lipolysis (Mittelman et al., 2002; Bergman et al., 2001; Bjorntorp, 1991). Age and gender also are important modifiers of the impact of obesity on blood lipids. The younger obese has relatively larger changes in blood lipids at any given level of obesity. Overweight women may have somewhat different patterns than obese men. For young women, excess body weight seems to be associated with higher total, non-HDL and LDL cholesterol levels, higher triglyceride levels, and lower HDL cholesterol levels. Total cholesterol: HDL cholesterol ratios seem to be highest in obese postmenopausal women, due to the much lower HDL cholesterol concentrations.

Overweight boys and girls also demonstrate this dyslipidemic atherogenic pattern reflected by positive correlations of BMI with triglycerides, LDL cholesterol, and triglycerides and a negative association with HDL cholesterol (Zwiauer et al., 1992). The Bogalusa Study found adverse serum lipoprotein elevations primarily in obese girls but not in boys (Tershakovec & Kuppler, 2003). These ominous changes in lipoproteins are probably reflected in arterial fatty streaks appearing in the early decades of life (McGill et al., 2002; Zieske et al., 2002).

The dyslipidemic pattern described among American men, women and children has also been found in a variety of ethnic populations including Asians living in Singapore (Lim et al., 2002), Hispanic Americans (Sundquist et al., 2001) and American Indians (Hu et al., 2000).

2.1.6.2 PATHOPHYSIOLOGY OF THE DYSLIPIDEMIA OF OBESITY

Central obesity is the main cause of the resistance to insulin-mediated glucose disposal and compensatory hyperinsulinemia, which are in turn responsible for most, if not all, of the associated lipoprotein abnormalities. There are three major components of the dyslipidemia that occur in obesity: increased fasting and postprandial triglyceride-rich lipoproteins (TRLs), decreased HDL, and increased small, dense LDL particles. Since the metabolism of all lipoproteins is highly interrelated (Figures 1 and 2), it is likely that a common fundamental metabolic defect explains all of the lipoprotein changes in the dyslipidemia of insulin resistant states. It is indeed rare that they are found separately in insulin resistant or obese individuals.

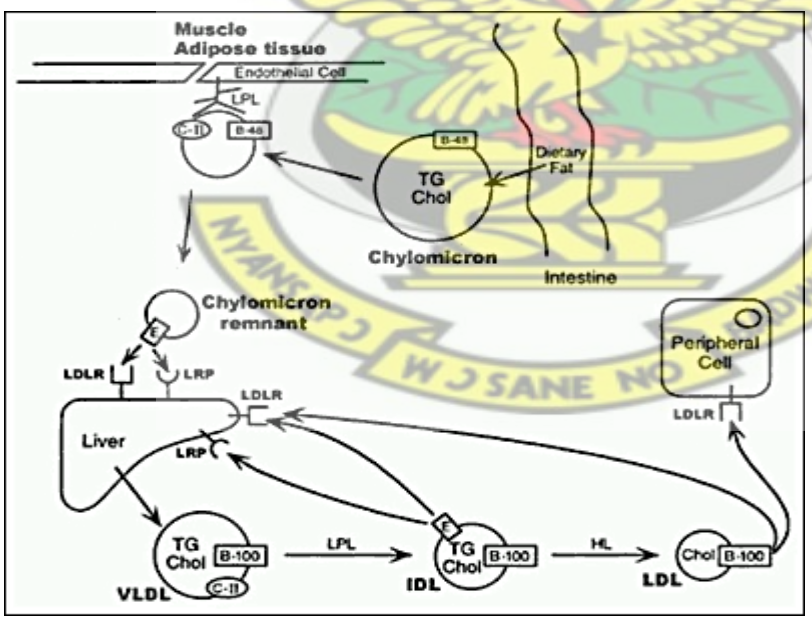

Figure 1.Metabolism of fasting and postprandial TRLs. TG indicates triglycerides; chol, cholesterol; B-48, B-100, C-II and E, specific apolipoproteins; LPL, lipoprotein lipase; HL, hepatic lipase; LDLR, LDL receptor; LRP, LDLR-related protein. Apo C-II's role is LPL activation, whereas apo E is fundamental for TRLs clearance. Source: Howard et al., (2000)

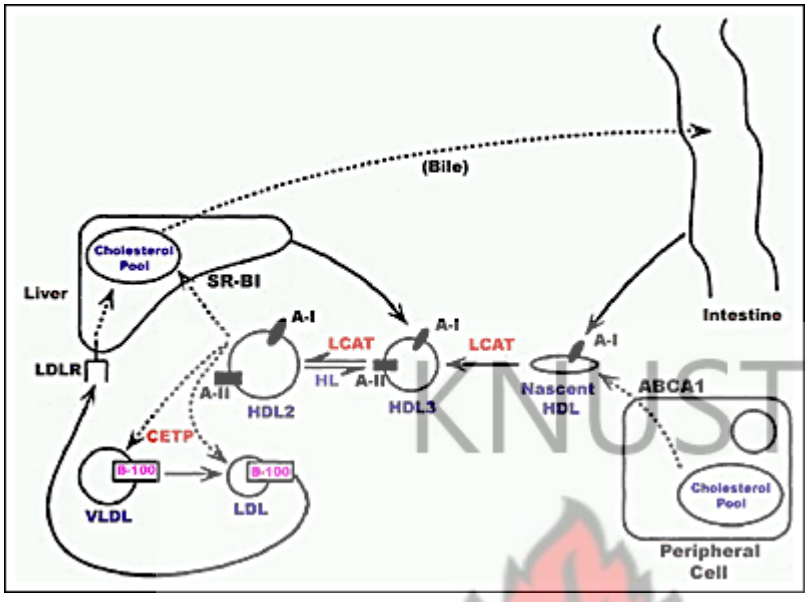

Figure 2. HDL metabolism. B-100, A-I and A-II indicate specific apolipoproteins; ABCA1, ATP-binding cassette A1; CETP, cholesteryl ester transfer protein; HL, hepatic lipase; LDLR, LDL receptor. LCAT (lecithin:cholesterol acyltransferase) is a key enzyme of reverse cholesterol transport, and esterifies all circulating unesterified cholesterol molecules. SR-BI (scavenger receptor class B, type I) is a HDL receptor that mediates selective cholesteryl esters uptake by cells. Source: Howard et al., (2000)

Population-based studies have universally and consistently found positive associations of measures of insulin resistance with plasma total or VLDL triglyceride, and negative associations with HDL cholesterol concentration. The associations remained significant when adjusted for main covariates like age, smoking and physical activity for example, and appear to be consistent in both genders and among various populations, such as Whites ,Framingham Heart Study (Castelli, 1992), Paris Prospective Study (Balkau & Eschwege, 1999), Quebec Cardiovascular Study (Lamarche *et al.*, 1998), Blacks, CARDIA (Manolio *et al.*, 1990), Hispanics ,San Antonio Heart Study (Haffner *et al.*, 1993), Asians (Bhopal *et al.*, 1999; Laws *et al.*, 1994) and American Indians ,Pima Indians (Howard *et al.*,

1984), Strong Heart Study (Gray *et al.,*1998). These studies clearly show a strong correlation of dyslipidemia with obesity, especially central deposition of fat.

2.1.6.3 PATHOGENESIS

2.1.6.3.1

Elevated Fasting Triglycerides

The hepatic overproduction of VLDL appears to be the primary and crucial defect of the insulin resistant state accompanying obesity and compensatory hyperinsulinemia (Figure 3). Inability to suppress hepatic glucose production, impaired muscle glucose uptake and oxidation, and inability to suppress release of nonesterified fatty acids (NEFA) from adipose tissue are the most important consequences of insulin resistance in liver, muscle and adipose tissue, respectively. These events give rise to increased NEFA and glucose flux to the liver, an important regulator of hepatic VLDL production (Sparks & Sparks, 1994).

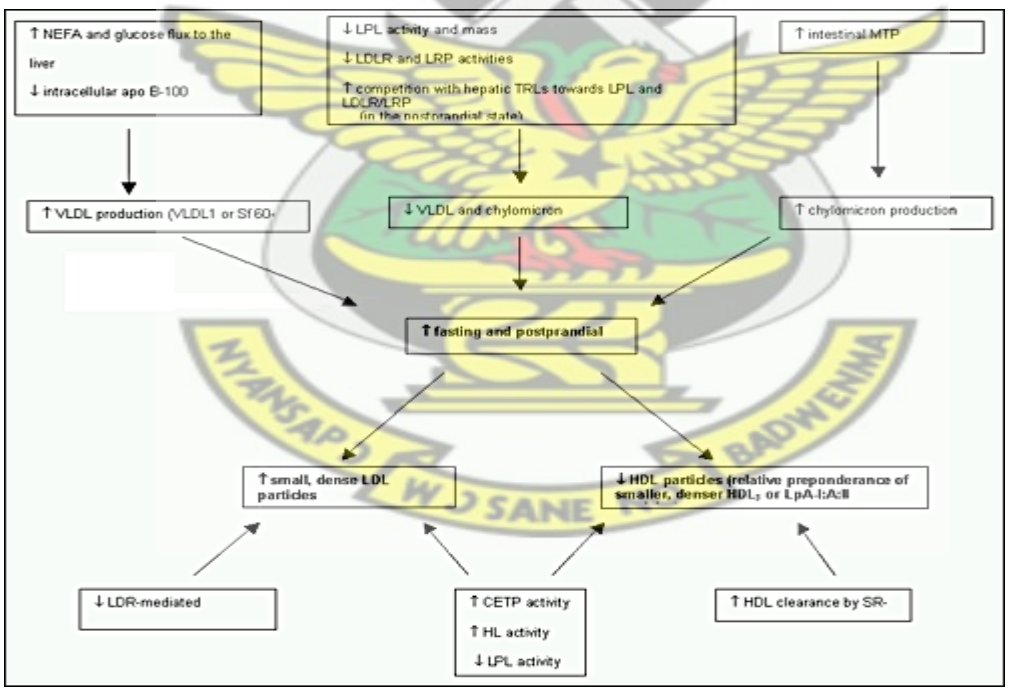

Figure 3. Pathogenesis of dyslipidaemia in obesity. Central role of fasting and postprandial TRLs. Source: Howard et al., (2000)

Another key site in the regulation of VLDL secretion is the rate of apo B-100 degradation. Newly synthesized apo B-100 remains associated with the rough endoplasmic reticulum (RER) and is degraded by the ubiquitin/proteasome system, or is translocated into the lumen and incorporated into lipid-poor VLDL precursors. Next, the lumenal apo B-100 is either degraded or advances, acquiring the remaining VLDL lipids in the smooth endoplasmic reticulum (SER)/cis-Golgi. Apo B-100 is stabilized and protected from degradation by the heat shock protein 70 (HSP-70). Lipids and microsomal triglyceride protein (MTP), a heterodimeric lipid transfer protein that is required for the assembly of apo B-containing lipoproteins, play a major role in the translocation of apo B-100. If it does not occur, then the apo B-100 is degraded. Insulin seems to be an important factor for the intracellular degradation of freshly translated apo B-100. Therefore, in the insulin resistant state there is inability to suppress apo B-100 degradation, and consequent imbalance between secretion and degradation in favor of the former (Kendrick *et al.*, 1998).

However, hepatic VLDL apo B overproduction in the fructose-fed hamster, a novel animal model of insulin resistance, appears to result from both increased intracellular stability of nascent apo B and enhanced expression of MTP (Taghibiglou *et al.*, 2000). In fact, insulin also negatively regulates MTP gene expression, resulting in a decrease of MTP transcription, even though sustained changes in MTP mRNA levels would be required to affect MTP protein levels in humans (Lin *et al.*, 1995; Sato *et al.*, 1999). In addition, neither MTP nor newly synthesized triglycerides seems necessary for the later stages of apo B100-lipoprotein assembly and secretion in either HepG2 or McA-RH7777 cells (Pan *et al.*, 2002).

Therefore, the end result in insulin resistant states is an increased assembly and secretion of VLDL.

In addition to increased synthesis, the insulin resistance of obesity is characterized by decreased clearance of TRLs. Insulin is a stimulator of lipoprotein lipase (LPL) activity, by increasing LPL mRNA, and therefore enhancing its rate of synthesis. LPL activity in skeletal muscle of insulin resistant subjects has been shown to be lower, suggesting a defective insulin regulation of LPL. Therefore, the decreased LPL activity and mass in insulin resistance slow down the normal lipoprotein metabolic cascade, resulting in decreased clearance of VLDL (Miyashita *et al.*, 2002).

VLDL particles are mainly cleared from circulation by the LDL receptor (LDLR), also referred to as apo B/E receptor. The transcription of the LDLR gene is regulated by intracellular cholesterol concentration, hormones, and growth factors. Sterol regulatory element binding protein-1 (SREBP-1) is selectively involved in the signal transduction pathway of insulin and insulin-like growth factor-I (IGF-I) leading to LDLR gene activation (Streicher, 1996). The insulin resistance associated with obesity may also impair LDLR activity, thus contributing to the delayed VLDL particle clearance accompanying this condition.

Insulin acutely suppresses the total production rate of VLDL particles by decreasing mainly the production of large, VLDL1 (Sf 60-400), without affecting that of small TRLs, VLDL2 (Sf20-60) (Malmstrom et al., 1997). This effect seems to be independent of the availability of NEFA (Malmstrom et al., 1998). In type 2 diabetes insulin appears unable to inhibit acutely the release of VLDL1 from the liver, despite efficient suppression of serum NEFA. However, the decrease in circulating VLDL particles following acute insulin action in insulin sensitive

individuals appears to be the result not only of a decreased hepatic production (Ruotolo et al., 1990), but also an increased clearance.

2.1.6.3.2

Elevated Postprandial Lipaemia

Less is known about the mechanisms responsible of the association of insulin resistance with increased postprandial lipemia. During the postprandial state, dietary fatty acids are transported from the intestine to peripheral tissues as chylomicron triglycerides. In the capillary beds of peripheral tissues, chylomicron triglycerides are lipolyzed by LPL, allowing the delivery of NEFA to cells and resulting in production of smaller, cholesteryl ester-enriched chylomicron remnants. These particles are rapidly removed from the blood primarily by the liver through two receptors, LDLR and LDLR-related protein (LRP), acting in association with heparan sulfate proteoglycans (HSPGs) and/or hepatic lipase (HL) (Yu & Cooper, 2001).

Some investigators have examined the relation between postprandial lipaemia and insulin resistance, plasma glucose and insulin response to a meal in healthy nondiabetic subjects (Jeppesen *et al.,* 1995). Postprandial triglyceride levels, as an indirect measure of chylomicron remnant particles, were found to be significantly related to insulin action. A significant relation of triglyceride levels to postheparin plasma LPL activity was also demonstrated. Since LPL is an insulin-sensitive enzyme, which is suppressed in insulin resistant individuals, its deficiency might contribute to the abnormal levels of remnant particles in obesity and other insulin resistant states.

The relation of fasting insulin concentrations to postprandial lipoproteins has also been evaluated in a population-based study of healthy middle-aged men with apo E3/3 genotype (Boquist *et al.,* 2000). Besides postprandial triglycerides, postprandial TRL apo B-48 and apo B-100 concentrations were also determined, as a measure of chylomicron and VLDL remnant particle concentrations. Fasting

plasma insulin was associated with the triglyceride response to the test meal, independently of obesity measures, blood glucose and fasting triglyceride concentrations. Exaggerated and prolonged postprandial lipemia in subjects in the upper quartile of the plasma insulin distribution was largely accounted for by large TRLs (Sf>60). However, insulin relations to large postprandial TRLs exclusively reflected the association between plasma insulin and the fasting plasma concentrations of these lipoprotein species. On the other hand, plasma insulin and late postprandial plasma concentrations of small TRLs (Sf 20-60) were related independent of insulin influences on fasting concentrations. Indeed this slow removal of chylomicron remnants is a common observation in insulin resistant individuals. This study concluded that the degree of insulin sensitivity is a major determinant of postprandial lipemia, and supports the hypothesis that the preferential clearance of chylomicron triglycerides by LPL leads to accumulation of hepatogenous VLDL during the alimentary period (Schneeman *et al.,* 1993). Because postprandial particles may play an important role in the pathogenesis of CVD, the increased postprandial lipemia in insulin resistance may contribute to increased CVD risk (Boquist *et al.,* 1999).

Insulin does not seem to influence LRP mRNA and protein expression acutely, while stimulates recycling of LRP from an endosomal pool to the plasma membrane thus increasing the cell surface presentation of LRP (Descamps *et al.,* 1993; Ko *et al.,* 2001). The diminished insulin action on both receptors, LDLR and LRP, could theoretically contribute to the increased postprandial lipemia of the metabolic syndrome, even though this process is far from saturable in normal functioning receptors.

It is not clear yet if an overproduction of intestinal TRLs (chylomicrons) has a role in the postprandial lipemia of diabetes in humans. Animal studies (obese Zucker rats, and diabetic New Zealand white rabbits) have shown a higher secretion of lymph chylomicron particles in the insulin resistant animals compared with the controls (lean rats and nondiabetic rabbits) (Phillips *et al.,* 2002). These animal

studies suggest that intestinal MTP could play some role in the postprandial dyslipidaemia of diabetes in humans.

2.1.6.3.3

Increased small, dense LDL particles

Elevated LDL cholesterol is not a uniform characteristic of the dyslipidemia of obesity. In the insulin resistant state, the composition and distribution of LDL particles are altered, resulting in an increased concentration of small, dense LDL. The LDL particle is characterized by a core consisting primarily of cholesteryl ester surrounded by apo B-100. In insulin resistance, the lipid content of the core changes since cholesteryl ester decreases and triglyceride increases relatively, leading to a decreased number of cholesterol molecules per apo B-100 (or LDL) particle. Fasting triglyceride and small, dense LDL concentration are positively correlated, since the formation of small, dense LDL depends largely on the metabolism of VLDL particles. In insulin resistant states, the increased concentration and delayed clearance of VLDL particles induce an increased exchange between cholesteryl esters in LDL and triglycerides in VLDL, mediated by cholesteryl ester transfer protein (CETP). This exchange produces LDL particles enriched in triglycerides, which are rapidly lipolyzed by HL leaving smaller, denser LDL particles. The activities of both CETP and HL appear to be increased in the metabolic syndrome. This exchange process also leads to highly atherogenic cholesteryl ester-enriched VLDL particles. Small, dense LDL particles seem to be more prone to modifications, such as oxidation and glycation (increased in the presence of high glucose levels), which could lead to increased production of

antibodies against the modified apo B-100 and formation of immunocomplexes. In addition, the reduced diameter of these particles increases the probability of movement through endothelial fenestrations placing them in the subendothelial space where inflammation, leukocyte ingestion and transformation into plaque occur (Kwiterovich, 2002). All these modifications might result in a decreased LDLR-mediated clearance of small, dense LDL particles (Lund-Katz, 1998), which could contribute to their elevated plasma levels. The modified LDL is mostly taken up by macrophage scavenger receptors, rather than the normal LDLR pathway, thus inducing atherosclerosis. The association between LDL subclass patterns and plasma insulin, as a measure of insulin resistance, has been demonstrated in many population-based studies, even independently of plasma triglycerides and HDL cholesterol (Haffner et al., 1993; Howard et al., 2000; Reaven et al., 1993).

2.1.6.3.4

Decreased HDL Cholesterol

HDL particles are the smallest lipoprotein particles, with cholesterol ester in the central core and a variety of apolipoproteins that govern their metabolism. Although the mechanisms that regulate HDL are not completely understood, the atherogenic potential of low HDL levels is well known (Goldbourt et al., 1997). Several mechanisms can contribute to the decreased HDL in the insulin resistance of obesity, and as in the formation of small, dense LDL particles, TRL metabolism plays an important role. Most studies of lipoproteins have shown an inverse relationship between VLDL triglycerides and HDL cholesterol (Frenais et al., 2001).

Impaired TRL lipolysis leads to reduced HDL concentration, by decreasing the transfer of apolipoproteins and phospholipids from TRL to the HDL compartment. In addition, the delayed cleareance of TRLs facilitates the CETP-mediated exchange between cholesterol esters in HDL and triglycerides in VLDL. The increased activity of HL in insulin resistant states such as obesity produces smaller HDL particles and facilitates HDL clearance (Frenais et al., 2001). Finally, insulin could also have a direct effect on the production of apo A-I or hepatic secretion of nascent HDL. Therefore, in insulin resistance there is a substantial decrease of HDL particles, especially the larger HDL2 (compared to the smaller HDL3) and HDL containing mostly apo A-I (referred to as LpA-I particles). The LpA-I particles are more effective than LpA-I:A-II particles in the reverse cholesterol process, and therefore are considered more antiatherogenic. The function of the other major apolipoprotein of HDL, apo A-II, is not clear yet. Recent data have suggested a possible role of apo A-II in visceral fat accumulation, even though no direct relationship with insulin resistance has been demonstrated in humans (van't Hooft et al., 2001). However, studies on knockout and transgenic human apo A-II mice have shown a clear role of this apolipoprotein in insulin sensitivity.

Leptin, tumor necrosis factor-a (TNF-a), resistin, and adiponectin represent the major hormone-like peptides, or adipocytokines, secreted by the adipocyte. Plasma leptin, tumor necrosis factor-a (TNF-a), and resistin levels are increased, whereas adiponectin levels are decreased in obesity. These adipocytokines have many metabolic effects on both glucose and lipoprotein metabolism, largely accounted

for by the insulin resistant state accompanying obesity. However, a positive correlation between plasma adiponectin and HDL cholesterol levels seems to be independent of body fat mass and insulin sensitivity (Tschritter et al., 2003).

2.2 OBESITY AND HYPERTENSION

The majority of patients with high blood pressure are overweight (Stamler *et al.*, 1978). Hypertension is about six (6) times more frequent in obese subjects than in lean men and women (Stamler *et al.*, 1978). Not only is hypertension more frequent in obese subjects than in normal weight control subjects, but also weight gain in young people is a potent risk factor for subsequent development of hypertension.

A 10-kg higher body weight is associated with a 3.0-mm Hg higher systolic and a 2.3-mm Hg higher diastolic blood pressure. These increases translate into an estimated 12% increased risk for CHD and 24% increased risk for stroke. However, results from NHANES III reported more specific estimates for the prevalence of high blood pressure per age group and BMI group (Brown *et al.*, 2000). Among men, the prevalence of high blood pressure increased progressively with increasing BMI, from 15% at a BMI of 25 kg/m^2 to 42% at a BMI of 30 kg/m^2. Women showed a pattern similar to that of men; prevalence of hypertension being 15% at a BMI of 25 kg/m^2 to 38% at a BMI of 30 kg/m^2 (Brown *et al.*, 2000). The trend of higher prevalence of high blood pressure with increasing BMI was similar for white, black, and Mexican Americans of both genders, and the age-adjusted rates were highest among blacks at every level of BMI (Brown *et al.*, 2000). It is well recognized that technical difficulties exist in the indirect measurement of blood pressure in the obese patient that may result in an overestimation of the level of blood pressure (Kirkendall *et al.*, 1980). Nevertheless, obesity is strongly associated with higher-than optimal blood pressure (Johnson *et al.*, 1975; Voors *et al.*, 1977). This increase in blood pressure is greatest when the obesity is of abdominal

distribution (Bjorntorp, 1990a; Bjorntorp, 1990; Poirier *et al.*, 2005; Muller *et al.*, 1993).

Factors to be considered in linking obesity to an increase in blood pressure are related to changes in cardiac output and peripheral vascular resistance, because BP= CO x SVR, where BP is blood pressure, CO is cardiac output, and SVR is systemic vascular resistance. These factors include direct effects of obesity on hemodynamics and mechanisms linking obesity and an increase in peripheral vascular resistance: endothelial dysfunction, insulin resistance, sympathetic nervous system, substances released from adipocytes (IL-6, TNF-α), and sleep apnea.

Obesity per se is associated with alterations in hemodynamics (Reisin, 1986). An increase in oxygen demand produced by excess adipose tissue (1.5 ml/kg per minute) requires an increase in cardiac output. Also, a parallel increase occurs in blood volume. Thus, obese individuals have an increase in blood volume, stroke volume, and cardiac output. This high-output state is associated with a reduction in peripheral vascular resistance in individuals with a normal blood pressure, as would be predicted from the Poiseuille formula: $R= \Delta P/ F= (8/\pi)x(\eta)x(l/r^4)$, where R is resistance, $8/\pi$ is a numerical factor, η is blood viscosity, and l/r^4 is a geometric factor that includes vessel characteristics (Reisin, 1986). Because of the marked influence of the geometric factor (to the fourth power) in the equation, resistance is decreased. However, obese persons with a greater-than-optimal increase in blood pressure (ie, hypertension) have a peripheral vascular resistance that is either inappropriately "normal" or increased. Therefore, although an increase in cardiac output may add to the increase in blood pressure, in the obese individual, an abnormal increase in blood pressure is primarily dependent on an increase in peripheral vascular resistance.

2.3 HYPERTENSION AND THE PATHOGENESIS OF ATHEROSCLEROSIS

Hypertension is a risk factor for the development of atherosclerosis, although the mechanisms have not been well elucidated. As the cellular and molecular mechanisms of the pathogenesis of atherosclerosis and the effects of hypertension are being more clearly defined, it becomes apparent that the two processes have certain common mechanisms. The endothelium is a likely central focus for the effect of both diseases. There is increasing evidence that atherosclerosis should be viewed fundamentally as an inflammatory disease (Munro & Cotran, 1988). Atherogenic stimuli such as hyperlipidemia appear to activate the inflammatory response by causing expression of mononuclear leukocyte recruiting mechanisms. The gene for one of these, the vascular cell adhesion molecule-1, is controlled at least in part by transcriptional factors regulated by oxidative stress, which modifies the redox state of the endothelial cell (Alexander, 1995). Alterations in the redox state of the arterial wall also may contribute to vascular smooth muscle cell growth. In a somewhat parallel fashion, there is evidence that hypertension may also exert oxidative stress on the arterial wall.

2.3.1 The Effects of Hypertension on the Arterial Wall

The pathogenesis of hypertension is a multifactorial process that involves the interaction of genetic and environmental factors. In varying degrees, abnormalities of volume regulation, enhanced vasoconstriction, and remodeling of the arterial wall (decreasing lumen diameter and increasing resistance) contribute to the development of hypertension (Alexander, 1995). Various abnormalities in ion transport have been described in subsets of hypertensive individuals and in experimental models. These generally involve changes in sodium, calcium, and/or

proton fluxes or concentrations. These changes in electrolyte metabolism enhance contractile response and hypertrophy and proliferation of vascular smooth muscle cells. Increases in blood pressure cause ongoing adaptive responses in the microvasculature (Griendling & Alexander, 1994).

The effects of blood pressure are also exhibited in larger arteries. The increased growth response of vascular smooth muscle is one of the characteristics of atherosclerosis in large arteries. Thus, increased vascular smooth muscle cell growth is another common feature in the pathogenesis of both atherosclerosis and hypertension. The growth of vascular smooth muscle is controlled to an important extent by the endothelium (Griendling & Alexander, 1994). The normal endothelium appears to exhibit an inhibitory influence on vascular smooth muscle cell growth. Dysfunctional endothelium in either atherosclerosis or hypertension may contribute to or permit vascular smooth cell growth, which contributes to narrowing of the lumen.

Another effect of medial thickening (whether from hypertrophy and hypertension or from atherosclerosis) is to increase the distance required for diffusion of oxygen from the lumen. A decrease in PO2, in turn, would result in incomplete oxidation and probably lead to increased concentrations of free radicals and abnormalities of the redox state (Sharma et al., 1992). This oxygen radical formation would contribute to tissue damage and lipid oxidation, with many of the implications discussed above.

There is increasing evidence that hypertension, like hyperlipidemia, induces oxidative stress in the arterial wall. It has even been suggested that superoxide anions might trigger the development of hypertension in some models, presumably by inactivating endothelium-derived nitric oxide and thus mitigating this important vasodilator mechanism (Nakazono et al., 1991). A fusion protein was developed consisting of human copper/zinc superoxide dismutase (SOD) and a C-terminal basic peptide that would provide high affinity for heparans on

endothelial cells. SOD targeted to the endothelium would dismutate oxygen free radicals to H_2O_2. This SOD fusion protein bound vascular endothelial cells when injected intravenously and localized within the vessel wall, reducing blood pressure in spontaneously hypertensive rats but not normal controls (Nakazono et al., 1991). Blood pressure was also reduced by xanthine oxidase inhibitors (Nakazono et al., 1991). These observations suggest that oxygen free radicals may be important in the pathogenesis of hypertension in this model and that xanthine oxidase may be one potential source of the oxygen free radicals. An inferred mechanism of blood pressure elevation here would be the destruction of nitric oxide by excessive production of oxygen free radicals, although this has not been demonstrated in this model.

Additional data in other models of hypertension support the notion that oxygen free radicals contribute to either the causes or consequences of hypertension (Zhang & Ellis, 1991). Infusion of SOD in rats in which hypertension was induced by the administration of systemic norepinephrine shifted the norepinephrine–blood pressure response curves to the right. There was also improved survival in all of the SOD-treated rats, a result consistent with the possibility that oxygen free radicals produced by the arterial wall compromise vascular structural integrity in this model. These data are consistent with the possibility that this model is associated with increased production of oxygen free radicals that destroy endothelium-derived nitric oxide and contribute to hypertension. This same group has shown that acute hypertension caused by experimental acute brain injury or by pressor agents is associated with abnormalities of cerebral arterioles, including the development of endothelial lesions and increased permeability that is thought to result from enhanced production of free radicals (Zhang & Ellis, 1991). Similar

conclusions have been reached from studies on intestinal microvascular damage in a rat model of acute angiotensin II–induced hypertension (Wilson, 1990).

Additional evidence that hypertension induces an oxidative stress on the arterial wall comes from a rabbit suprarenal aortic coarctation model of hypertension. Here, antioxidant defense enzymes related to the generation of reduced glutathione and thiobarbituric acid–related substances (TBARS), formed when oxygen radicals interact with fatty acids, were increased in the suprarenal but not in the infrarenal aortic segment (Cuccurullo et al., 1991).

2.3.2 Hypertension and Atherosclerosis May Act Together to Enhance Arterial Oxidative Stress

Atherosclerosis and hypertension each may enhance the oxidative stress of the arterial wall. One might expect additive effects from the presence of both conditions, and indirect evidence supports this notion. In rabbits, hypertension and hyperlipidaemia each enhance arterial expression of antioxidant scavenger enzymes. The presence of both conditions is an even more potent stimulus, suggesting that through a common mechanism both conditions enhance the oxidative stress of the arterial wall (Sharma *et al.*, 1992). Additional provocative evidence supporting the concept that hypertension and atherosclerosis have certain common physiological mechanisms comes from observations in cholesterol-fed monkeys; here, hypertension sustained coronary artery plaque progression despite the return of cholesterol levels toward normal with dietary manipulation (Xu *et al.*, 1991).

Chobanian has called attention to and summarized similarities in the effects of hypertension and atherosclerosis on the arterial wall (Chobanian, 1990). The argument presented above suggests that oxidative stress is a manifestation common to both conditions. The mechanistic data showing that monocyte recruitment mechanisms involve redox-sensitive steps have been previously summarized and would lead one to predict that hypertension per se, even in the

absence of the metabolic stress of hyperlipidaemia, might be associated with increased recruitment of mononuclear cells into the arterial wall (Chobanian, 1990). In fact, this appears to be the case. Hypertension in animal models is associated with leukocyte adhesion, macrophage accumulation, smooth muscle cell migration and proliferation, and intimal thickening (Chobanian, 1990). Lipid accumulation in foam cells and formation of atherosclerotic plaque are generally not observed if plasma lipoproteins are low (Chobanian, 1983). Thus, one reason that hypertension facilitates the development and progression of atherosclerosis may be that it oxidatively stresses or injures the endothelium, resulting in activation of redox-sensitive mechanisms that recruit mononuclear leukocytes into the arterial wall.

2.4 HYPERTENSION AND METABOLIC SYNDROME

The mechanism and pathophysiology of hypertension are associated with the metabolic abnormalities seen in the metabolic syndrome. Because of the compensatory hyperinsulinaemia caused by insulin resistance (IR), the sympathetic nervous system is stimulated, causing vasoconstriction, increased cardiac output, and renal absorption of sodium, which, in turn, leads to elevated blood pressure sufficient to override the direct normal vasodilatation action of insulin in obese and hypertensive patients. Studies show that 50% of patients with hypertension have IR and hyperinsulinaemia caused by resistance to insulin-mediated glucose disposal. In patients with insulinomas, the hyperinsulinaemia is primary and not caused by IR; these patients do not have a greater prevalence of hypertension than those without insulinomas (Reaven *et al.*, 1996).

2.5 EFFECTS OF THYROID HORMONES ON CARDIOVASCULAR SYSTEM

Thyroid hormone has relevant effects on the cardiovascular system (Klein and Ojamaa, 2001). Many symptoms and signs recognized in patients with overt hyperthyroidism and hypothyroidism are due to the increased or reduced action of thyroid hormone on the heart and the vascular system, respectively, and the related hemodynamic derangements.

2.5.1 CELLULAR EFFECTS OF THYROID HORMONE ON THE CARDIOVASCULAR SYSTEM

Most of the molecular and cellular mechanisms responsible for the cardiovascular effects of thyroid hormone have been clarified. Thyroid hormone may exert both genomic and nongenomic effects on cardiac myocytes. The genomic effects of thyroid hormone are mediated by the transcriptional activation or repression of specific target genes that encode both structural and functional proteins (Dillmann, 1990). This process begins with the entry of triiodothyronine (T3), the biologically active thyroid hormone, into the cardiomyocyte through specific transport proteins located within the cell membrane (Everts et al., 1996). To date, there is no clear evidence of a biologically relevant conversion of thyroxine (T4) to T3 in cardiomyocytes (Everts et al., 1996). Once in the cardiomyocyte, T3 enters the nucleus and interacts with specific transcriptional activators (nuclear receptor α1) or repressors (nuclear receptor α2). Occupancy of these receptors by T3, in combination with recruited cofactors, allows the thyroid hormone-receptor complex to bind (nuclear receptor α-1) or release (nuclear receptor α-2) specific sequences of DNA (thyroid-responsive elements) that, in turn, by acting as cis- or trans-regulators, modify the rate of transcription of specific target genes (Brent, 1994).

In addition to these genomic effects, thyroid hormone produces changes in cardiac inotropism and chronotropism more rapidly than would be expected from regulation of gene expression, which usually take minutes to hours to be phenotypically and functionally appreciable. This calls into question the involvement of nongenomic mechanisms (Davis and Davis, 1993; Walker et *al.*, 1994). Some evidence indicates that thyroid hormone promotes the acute phosphorylation of phospholamban and that this action attenuates the inhibitory effect of phospholamban on sarcoplasmic reticulum calcium-activated ATPase (Ojamaa *et al.*, 2002). Interestingly, the fact that this process is mediated at least in part by the activation of intracellular kinase pathways involved in signal transduction of the adrenergic stimulus (Ojamaa *et al.*, 2002) may help to explain

functional analogies between the cardiovascular effects of thyroid hormone and those promoted by the adrenergic system (Levey and Klein, 1990). Indeed, although most of the cardiovascular manifestations associated with hyperthyroidism and hypothyroidism mimic a condition of increased and reduced adrenergic activity, respectively, the sensitivity of the cardiovascular system to adrenergic stimulation does not seem to be substantially altered in these conditions (Hoit *et al.*, 1997; Ojamaa *et al.*, 2000).

Thyroid hormone also exerts an important effect on the vascular system. It acutely reduces peripheral vascular resistance by promoting relaxation in vascular smooth-muscle cells (Klemperer et al., 1995; Ojamaa et al., 1996a; Park et al., 1997).

2.5.2 THYROID HORMONES AND TRADITIONAL CARDIOVASCULAR RISK FACTORS

There is substantial evidence that overt hypothyroidism alters several of the traditional risk factors for cardiovascular disease. These studies support a biologically plausible role for hypothyroidism increasing the risk of atherosclerotic cardiovascular diseases, via increases in circulating levels of highly atherogenic low-density lipoprotein (LDL) cholesterol particles, induction of diastolic hypertension, altered coagulability, and direct effects on vascular smooth muscle. Furthermore, some evidence suggests that hypothyroidism may exacerbate the cardiovascular risks associated with cigarette smoking and insulin resistance.

Elevated levels of total cholesterol, LDL cholesterol, and apolipoprotein B are well documented features of overt hypothyroidism (Staub et al., 1992). Significant progress has been made in clarifying the mechanisms leading to these adverse changes in circulating lipid concentrations. Early studies in humans with hypothyroidism, using isotopically labeled LDL, demonstrated a prolonged half-life of LDL cholesterol because of decreased catabolism, an effect that was

reversible with T4 therapy (Walton et al., 1965). Additional data in human fibroblasts verified that the T3-induced increase in LDL degradation was mediated through an increase in LDL receptor number, without any change in the affinity of LDL for its receptor. A specific effect of thyroid hormone on the LDL receptor was suggested by a lack of T3 effect on LDL concentration in cultured cells without LDL receptors (Chait et al., 1979). These findings were supported by an in vivo study in a hypothyroid woman whose receptor-mediated LDL catabolism was reduced, compared with euthyroid controls, with significant improvement after T4 replacement therapy (Thompson et al., 1981). Further studies in rats with propylthiouracil-induced hypothyroidism showed a reduction in LDL receptor mRNA levels by 50% (Staels et al., 1990; Salter et al., 1991). Molecular mapping has revealed functional thyroid response elements in the promoter region of the LDL receptor. When the LDL receptor promoter was linked to a reporter gene and cotransfected with the ß1 isoform of the thyroid hormone receptor into a hepatic cell line, specific stimulation by T3 of this chimeric gene's activity was observed (Bakker et al., 1998). Furthermore, deletion of the upstream thyroid response elements in the LDL receptor promoter inhibited T3-mediated reporter gene activity.

Although T4 therapy in overt hypothyroidism is standard practice, controversy exists regarding the indications for therapy in subclinical hypothyroidism. One rationale for treating subclinical hypothyroidism is to lower levels of LDL cholesterol and thereby decrease atherosclerotic risk. Because the magnitude of the expected effect from treatment of subclinical hypothyroidism is smaller than that from overt hypothyroidism, larger sample sizes are required to detect a treatment effect in clinical trials. Multiple small, randomized trials have been performed examining the effect of T_4 treatment on lipid parameters in subclinical hypothyroidism, with the majority reporting a tendency toward beneficial effects, without achieving statistical significance. Danese et al. (2000) have performed a metaanalysis of these data using rigorous criteria to evaluate each study. Data from

247 patients with subclinical hypothyroidism, who were enrolled in 13 studies of T_4 therapy, were included for analysis. Overall, T_4 therapy decreased total cholesterol levels, with different degrees of cholesterol lowering in those with suboptimally treated overt hypothyroidism and those with previously untreated subclinical hypothyroidism. In those with suboptimally treated overt hypothyroidism, a decrease in total cholesterol of 0.44 mmol/liter (17 mg/dl) was seen, whereas those with previously untreated subclinical hypothyroidism demonstrated an average decrease of only 0.14 mmol/liter (5.6 mg/dl) after normalization of TSH levels. The largest treatment effect was evident in those with higher baseline TSH levels and those with higher pretreatment lipid levels. Overall, the average LDL cholesterol declined by 0.26 mmol/liter (10 mg/dl).

Studies have also shown that hypothyroidism causes qualitative changes in circulating lipoproteins that increase their atherogenicity. Two studies have shown that LDL is more susceptible to oxidation in patients with hypothyroidism, with normalization after restoration of the euthyroid state (Sundaram et al., 1997; Diekman et al., 1998).

The inverse relationships between atherosclerotic risk and concentrations of HDL cholesterol and its constituent apoprotein A1 are well known. Some studies have shown that hypothyroidism is associated with a lower HDL cholesterol level. In a report comparing 52 patients with subclinical hypothyroidism and 18 with overt hypothyroidism with 46 euthyroid controls matched for age, sex, and body mass index, Althaus et al. (1999) found a significantly lower HDL cholesterol fraction in even the subclinically hypothyroid patients. Caron et al. (1990) also reported that the HDL cholesterol level was significantly decreased among 29 women who had subclinical hypothyroidism, compared with 41 euthyroid women matched for age and metabolic parameters. Furthermore, Caron et al. (*1990*) observed a significant increase in the HDL cholesterol level with T_4 therapy, which normalized the serum TSH concentration. However, a controlled trial in which 66 women with subclinical hypothyroidism were randomly assigned to T_4 or placebo treatment found no

significant change in either HDL cholesterol or apolipoprotein A1 (Meier *et al.*, 2001).

Additional potentially atherogenic effects of hypothyroidism on lipid metabolism include a reversible reduction in clearance of chylomicron remnants (Weintraub et al., 1999); reduced activity of cholesteryl ester transfer protein, which is involved in reverse cholesterol transport pathway (Tan et al., 1998); and decreased activity of hepatic lipase and lipoprotein lipase (Lam et al., 1986).

Hypothyroidism can also increase cardiovascular risk by causing diastolic hypertension. In one study of 169 women with overt hypothyroidism, the prevalence of hypertension was nearly 3 times higher than in a euthyroid control group (14.8% vs. 5.5%) (Saito & Saruta, 1994). Euthyroid normotensive patients in another report had an increase in diastolic blood pressure after thyroidectomy-induced hypothyroidism (Fommei & Iervasi, 2002), and hypertension was reversed by T4 treatment. There is less published evidence regarding subclinical hypothyroidism and hypertension. Luboshitzky et al. (2002) did observe that mean diastolic blood pressure was higher in 57 women with subclinical hypothyroidism than in 34 euthyroid controls (82 vs. 75 mm Hg; $P < 0.01$). Potential mechanisms for reversible diastolic and systolic hypertension in hypothyroidism include increases in peripheral vascular resistance and arterial stiffness (Obuobie et al., 2002), respectively. Vasoconstriction may, in turn, reflect the absence of demonstrated vasodilatory T3 effects on vascular smooth muscle (Ojamaa et al., 1996) or be the result of a higher circulating noradrenaline level and a decrease in the number of vascular ß-adrenergic receptors mediating vasodilatation in skeletal muscle (Saito & Saruta, 1994). In addition, type II iodothyronine deiodinase has been found in cultured human coronary artery smooth muscle cells and human aortic smooth muscle cells, suggesting a potential direct role of local T3 on vascular smooth muscle (Mizuma et al., 2001).

Synergistic effects between smoking and hypothyroidism have been reported. Smokers with overt hypothyroidism have been shown to have higher serum concentrations of total and LDL cholesterol, higher clinical symptom scores, more prolonged ankle-reflex times, and higher creatine kinase concentrations than nonsmokers with hypothyroidism. These differences were noted despite similar concentrations of TSH, free T_4, and triiodothyronine, suggesting that cigarette smoking may impair thyroid hormone action in target tissues (Muller *et al.,* 1995).

Whether to treat subclinical hypothyroidism to reduce risk of future cardiovascular events is controversial. As described above, the strongest evidence for a salutary effect of thyroid hormone therapy is the considerable, if imperfect, demonstration that TSH-normalizing T4 therapy can lower the LDL cholesterol concentration in many patients with subclinical hypothyroidism, especially those with a serum TSH concentration greater than 10–12 mU/liter and/or those with suboptimally treated overt hypothyroidism. Practically speaking, it seems advisable to institute a 3-month trial of T_4 therapy in most subclinically hypothyroid patients with coexisting hypercholesterolemia to determine whether their hyperlipidemia can be corrected, and statin therapy, with its more common adverse reactions and greater expense, can be avoided. In contrast, for normocholesterolemic patients with subclinical hypothyroidism, there is currently insufficient epidemiological evidence to recommend thyroid hormone treatment for the sole purpose of reducing cardiovascular risk.

Hyperthyroidism is accompanied by systolic hypertension in up to one-third of patients, especially in the elderly (Saito & Saruta, 1994). For instance, in patients with hyperthyroidism, cardiac output is 50 to 300 percent higher than in normal subjects. The increase is due to the combined effects of a decrease in systemic vascular resistance, an increase in resting heart rate, increases in left ventricular contractility and ejection fraction, and an increase in blood volume (Klein & Ojamaa, 1998).

Patients with endogenous subclinical hyperthyroidism have also been shown to be at an increased risk of developing cardiovascular disease and dysrhythmia (Vadiveloo et al., 2011).

Chapter 3

MATERIALS AND METHODS

3.1 STUDY POPULATION AND SETTING

The study was conducted at the Out-patient hypertension clinic of Komfo Anokye Teaching hospital (KATH), Kumasi, Ghana. The Committee on Human Research, Publications and Ethics, KNUST, School of Medical Sciences and KATH, Kumasi, Ghana approved the protocol for the study. Informed consent was obtained from 300 participants consisting of 200 hypertensives (diagnosed by a Consultant Physician based on WHO – International Society of Hypertension Guideline of blood pressure ≥140/90 mmHg or use of antihypertensive) and 100 apparently healthy normotensives served as control.

3.2 SAMPLING, BIOCHEMICAL ANALYSIS AND DATA COLLECTION

Blood samples were collected in the morning after an overnight fast of at least 12 hours. Serum and plasma were stored at -80^{o}C after centrifugation at 2000g for 5 minutes until assayed. Fasting blood glucose (FBG), Apolipoprotein A-1 (Apo A-1), Apolipoprotein B (Apo B), Total Cholesterol (TC), Triglyceride (TG) and High Density Lipoprotein (HDL) were measured on an Auto-Analyzer (Flexor junior, Vital Scientific N.V., The Netherland) with reagents from ELITech Group company, SEPPIM S.A.S,France. Low Density Lipoprotein Cholesterol was calculated using the Friedewald equation.

Serum Thyroid Stimulating Hormone (TSH) and Free Thyroxine (fT4) were determined by Quantitative ELISA obtained from (AUTOBIO Diagnostics Ltd, China). Reference range was: TSH 0.3-4.8 mIU/l, fT4 10.3-24.5 pmol/l.

The following information was obtained from participants medical folders and standardized survey questionnaire;

Social and demographic characteristics (Age, sex and marital status)

Lifestyle characteristics (physical activities and smoking habits)

Clinical aspects (use of antihypertensive and lipid lowering medications)

3.3 ASSAY PRINCIPLES

3.3.1 Total Cholesterol

The method for this assay is based on that described by Trinder, (1969). Cholesterol esterase hydrolyses esters to free cholesterol and fatty acids. The free cholesterol produced plus the preformed cholesterol is then oxidized in the presence of cholesterol oxidase to cholest-4-en-3-one and hydrogen peroxide. The quinoneimine chromogen, with absorption maximum at 500 nm, is produced when phenol is oxidatively coupled with 4-aminophenazone in the presence of peroxidase with hydrogen peroxide. The intensity of the final red colour is directly proportional to the total cholesterol concentration.

Cholesterol ester + H_2O $\xrightarrow{\text{Cholesterol esterase}}$ Cholesterol + Fatty acids

Cholesterol +O_2 $\xrightarrow{\text{Cholesterol oxidase}}$ Cholest-4-en-3-one +H_2O_2

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

3.3.2 Triglycerides

The method for this assay is based on a modified Trinder (Barham & Trinder, 1972) colour reaction to produce a fast linear endpoint reaction (McGowan *et al.*, 1983). Triglycerides in the sample are hydrolyzed by lipase to glycerol and fatty acids. Glycerol is then phosphorylated by adenosine-5-triphosphate (ATP) to glycerol-3-phosphate and adenosine-5-diphosphate (ADP) in a reaction catalyzed by glycerol kinase. Glycerol-3-phosphate is then converted to dihydroxyacetone phosphate (DHAP) and hydrogen peroxide (H_2O_2) by glycerophosphate oxidase. The hydrogen peroxide then reacts with 4-aminoantipyrine and 3, 5 dichloro-2-hydroxybenzene (Chlorophenol) in a reaction catalyzed by peroxidase to yield a

red coloured quinoneimine dye. The intensity of the colour produced is directly proportional to the concentration of triglycerides in the sample.

Triglycerides + H_2O $\xrightarrow{\text{lipoprotein lipase}}$ Glycerol + fatty acid

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

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

H_2O_2 + 4-Aminoantipyrine $\xrightarrow{\text{Peroxidase}}$ Quinoneimine

3.3.3 HDL Cholesterol

Anti human ß-lipoprotein antibody in reagent R1 binds to lipoproteins (LDL, VLDL and chylomicrons) other than HDL. The antigen-antibody complexes formed block enzyme reactions when reagent R2 is added. Cholesterol esterase and cholesterol oxidase in reagent R2 react only with HDL-C. Hydrogen peroxide produced by the enzyme reactions with HDL-C yields a blue colour complex upon oxidative condensation of F-DAOS (N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxy-4-fluoroaniline, sodium salt) and 4-aminoantipyrine in the presence of peroxidase. By measuring the absorbance of the blue colour complex produced, at the average wavelength of 600 nm, the HDL-C concentration in the sample can be calculated when compared with the absorbance of the HDL-C calibrator.

Reagents composition

R1- Good's buffer,pH 7.0, 4-aminoantipyrine, peroxidase, ascorbate oxidase, anti human ß-lipoprotein antibody.

R2- Good's buffer, pH 7.0, cholesterol esterase, cholesterol oxidase and F-DAOS

3.3.4 LDL Cholesterol

The LDL-Cholesterol concentration (LDL-C) is calculated from the total cholesterol concentration (TC), HDL-Cholesterol concentration (HDL-C) and the triglycerides concentration (TG) according to Friedewald equation (Friedewald *et al.,* 1972)

$$LDL - C = TC - [\frac{TG}{2.2} + HDL - C]$$

3.3.5 Apolipoprotein A1 (APO A1)

The formation of ApoA1-anti-ApoA1 antibody complexes, started by the addition of the antiserum to the sample, is accelerated in presence of polyethylene glycol. These complexes precipitate leading to an increase of turbidity measured at 340 nm. The ApoA1 concentration is determined by means of a non linear calibration curve.

3.3.6 Apolipoprotein B (APO B)

The formation of ApoB-anti-ApoB antibody complexes, started by the addition of the antiserum to the sample, is accelerated in presence of polyethylene glycol. These complexes precipitate leading to an increase of turbidity measured at 340 nm. The ApoB concentration is determined by means of a non linear calibration curve.

3.3.7 Fasting Blood Glucose

Glucose concentration in the samples was estimated with the glucose oxidase method according to the following reactions:

$$Glucose + O_2 \xrightarrow{\text{Glucose oxidase}} \text{Gluconic acid} + H_20_2$$

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

$$2H_2O_2 + \text{4-aminoantipyrine} + \text{N-Ethyl-N-(2-Hydroxy-3-Sulfopropyl) m-Toluidine} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + 4H_2O$$

3.3.8 Free Thyroxine (fT4) Enzyme linked Immunosorbent Assay (ELISA)

In the fT4 Enzyme Immunosorbent Assay (EIA), a certain amount of T4 analog is coated on microtiter wells. A measured amount of test serum and a constant amount of anti-T4 antibody conjugated with horseradish peroxidase are added to the microtiter wells. During the incubation, T4 analogue on microtiter wells and fT4 present in the samples and reference standards compete for binding to the anti-T4 monoclonal antibody-horseradish peroxidase conjugate. After 60 minutes incubation at 37^{o}C, the wells are washed by wash solution. Then substrate solution and chromgen solution are added and incubated for 20 minutes, resulting in the development of a blue colour. The colour development is terminated with the addition of stop solution, and the colour is changed to yellow and absorbance is measured spectrophotometrically at 450 nm. The colour intensity is inversely related to the concentration of fT4 in the test sample.

3.3.9 Thyroid Stimulating Hormone (TSH) ELISA

The TSH ELISA test is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a specific monoclonal antibody directly against a distinct antigenic determinant on the intact TSH molecule. Monoclonal anti-TSH antibody is used for solid phase immobilization and another anti –TSH antibody is in the antibody –enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the two antibodies, resulting in the TSH molecules being sandwiched between the solid phase and enzyme-linked antibodies. After incubating 60 minutes at 37 ^{o}C, the wells are washed with wash solution to remove unbound labeled antibodies. Substrate solution and Chromogen solution is added and incubated for 20 minutes, resulting in the development of a blue colour. The colour development is stopped with the addition of Stop solution, and the colour is changed to yellow and the colour intensity is directly proportional to the concentration of TSH in the test sample.

3.4 ANTHROPOMETRIC VARIABLES

Height to the nearest metres without shoes and weight to the nearest 0.1 kg in light clothing were measured using a standard stadiometer (RGZ-160 Health Scale, China). The body mass index (BMI) was calculated by dividing weight (kg) by the height squared (m^2). Waist circumference (to the nearest centimetre) was measured with a Gulick II spring-loaded measuring tape (Gay Mill, WI) midway between the inferior angle of the ribs and the suprailiac crest. Hip circumference was measured as the maximal circumference over the buttocks in centimetres and the waist to hip ratio (WHR) calculated by dividing the waist circumference (cm) by the hip circumference (cm).

3.5 BLOOD PRESSURE

Blood pressure was taken by trained Nurses using mercury sphygmomanometer and stethoscope. Measurements were taken from the left upper arm after subjects had been sitting more than 5 minutes in accordance with the recommendation of the American Heart Association (Kirkendall *et al.*, 1967). Duplicate measurements were taken with a 5 minute rest interval between measurements and the mean value was recorded to the nearest 2.0 mmHg.

3.6 CLASSIFICATION OF METABOLIC SYNDROME

Three of the competing definitions of metabolic syndrome generally referred to in medical writings were used in the study as follows:

3.6.1 National Cholesterol Education Program, Adult Treatment Panel III (NCEP ATP III) Criteria:

The NCEP ATP III criteria mandates that individuals with metabolic syndrome should have three or more of the following five components of metabolic syndrome: (1) Abdominal obesity (waist circumference >102 cm for men or >88 cm for women); (2) Raised triglyceride (≥1.7 mmol L-1); (3) Low HDL-cholesterol (<0.9

mmol L-1 in men or <1.0 mmol L-1 in women); (4) High Blood Pressure (systolic BP ≥130 mmHg or diastolic BP ≥85 mmHg or treatment of hypertension) and (5) Raised fasting glucose (≥6.1 mmol L-1) (NCEP, 2002).

3.6.2 International Diabetes Federation (IDF) Criteria

The IDF criteria mandates that metabolic syndrome be diagnosed if Central obesity (waist circumference >90 cm for men or >80 cm for women) is accompanied by any two (2) of the following four (4) factors: (1) Triglyceride level ≥1.7 mmol L-1; (2) HDL cholesterol <1.03 mmol L-1 for men or <1.29 mmol L-1 for women; (3) Blood pressure ≥130/85 mmHg or treatment of previously diagnosed hypertension and (4) Fasting blood glucose (FBG) ≥5.6 mmol L-1 or previously diagnosed type 2 diabetes (Alberti et al., 2006).

3.6.3 World Health Organization (WHO) Criteria

The WHO criteria mandates the presence of diabetes mellitus, impaired glucose tolerance or insulin resistance and any two (2) of the following: (1) Body mass index (BMI) ≥30 kg m-2 and/or waist to hip ratio >0.90 for males or >0.85 for females; (2) Blood pressure ≥140/90 mmHg or on medication; (3) Triglyceride ≥1.7 mmol L-1 and (4) HDL cholesterol <0.91 mmol L-1 in males or <1.01 mmol L-1 in females (World Health Organization, 1999).

3.7 STATISTICAL ANALYSIS

Results were presented as Means ± SD. Unpaired t-test was used to compare the means of all continuous variables. The Chi-square test statistic (Fisher's exact test) was used to assess the statistical significance of categorical variables. Logistic regression test statistic was used to estimate the crude odds ratio (cOR) and adjusted odds ratio (aOR) for risk factors of hypertension. A p-value < 0.05 was considered to be statistically significant. All statistical analyses were performed using GraphPad Prism 5 and SigmaStat 3.5.

Chapter 4

RESULTS

4.1 GENERAL CHARACTERISTICS

General characteristics of the studied population indicated that, out of the total 300 study participants only 4(1.3%) were smokers. About half of the participants are physically inactive 144(48.0%). According to the 2003 ESH/ESC guideline, blood pressure was controlled in about 33% of the hypertensive patients (65/200), 92% (184/200) of the hypertensive patients were on at least one antihypertensive medication and only 3(1.5%) were on lipid lowering medication. The mean age of the hypertensive patients (50.30±11.58 years) was similar to the mean age of the normotensive control (49.32±10.10 years). The hypertensive patients had broader waist circumference, higher waist-to-hip ratio, reduced HDL-cholesterol, higher FBG level, reduced apolipoprotein A1 and higher apolipoprotein B as compared to the normotensive control as shown in table 4.1. When both hypertensive and normotensive subjects were stratified based on gender, the male as well as the female hypertensive patients had broader waist circumference, higher waist-to-hip ratio, reduced HDL-cholesterol, higher FBS level, reduced apolipoprotein A1 and higher apolipoprotein B as compared to their male and female normotensive control (Table 4.1).

Table 4.1General characteristics of the study population stratified by gender

Parameters	Hypertensive	Normotensive	Hypertensive male	Normotensive male	Hypertensive female	Normotensive female
Age (yrs)	50.30±11.58	49.32±10.10	50.38±11.33	48.85±10.90	50.26±11.73	49.89±9.12
BMI (kg m^{-2})	27.04±6.42	26.95±4.97	26.55±5.72	26.52±4.49	27.19±6.75	27.47±5.50
WC (cm)	93.29±13.91	85.43±11.33***	87.17±11.25	81.31±8.49††	95.86±14.19	90.47±12.38‡
WHR	0.92±0.06	0.87±0.06***	0.90±0.05	0.86±0.05†††	0.93±0.06	0.87±0.07‡‡
TC (mmol L^{-1})	4.54±1.70	4.36±0.82	4.69±2.06	4.36±0.85	4.48±1.50	4.36±0.79
TG (mmol L^{-1})	1.19±0.57	1.17±0.52	1.25±0.67	1.17±0.47	1.16±0.51	1.16±0.58
HDL-C (mmol L^{-1})	1.04±0.30	1.26±0.39***	0.97±0.29	1.26±0.37†††	1.07±0.31	1.28±0.41‡‡
LDL-C (mmol L^{-1})	3.06±1.76	2.91±0.83	3.28±2.15	2.90±0.95	2.95±1.52	2.92±0.66
FBG (mmol L^{-1})	7.59±3.83	5.23±1.24***	7.14±3.46	5.43±1.47†††	7.79±3.98	4.98±0.82‡‡
APOA1 (g L^{-1})	1.36±0.52	1.92 ±0.36***	1.41±0.45	1.94±0.35†††	1.33±0.56	1.89±0.37‡‡
APO B (g L^{-1})	2.13±0.80	1.74±0.77***	2.22±0.76	1.68±0.77†††	2.08±0.82	1.81±0.77

Data are presented as mean±SD and compared using unpaired t-test. ***Significant at the 0.001 level (2-tailed) when the hypertensive subject were compare to the normotensive control. ††Significant at the 0.01 level (2-tailed) and †††Significant at the 0.001 level (2-tailed) when the hypertensive male were compared to normotensive male. ‡Significant at the 0.05 level (2-tailed) and ‡‡Significant at the 0.001 level (2-tailed) when the hypertensive female were compared to the normotensive female.

4.2 CARDIOVASCULAR RISK FACTORS

Among the hypertensive patients, the highest prevalence of cardiovascular risk factor was abdominal obesity as measured by WHR (77.0%), followed by reduced HDL-cholesterol (74.0%), central obesity as measured by WC (69.5%). The least was cigarette smoking (1.5%) (Table 4.2). Among the normotensive control group, the highest prevalence of cardiovascular risk factors was reduced HDL-cholesterol (47.0%), followed by central obesity as measured by WC (42.0%), abdominal obesity as measured by WHR (33.0%) and general obesity as measured by BMI (26.0%). The least was cigarette smoking (1.0%) (Table 4.2). The prevalence of diabetes (34.5%), reduced HDL-cholesterol (74.0%), high LDL-cholesterol (13.0%), abdominal obesity (77.0%) and central obesity (69.5%) were significantly higher among the hypertensive patients as compared to the normotensive control (6.0%, 47.0%, 4.0%, 33.0% and 42.0% respectively) using Fischer's exact test (Table 4.2).

Whereas the highest cardiovascular risk factors among hypertensive male was reduced HDL-cholesterol (61.9%), among the female, it was central obesity (92.0%). The highest among the normotensive control male was reduced HDL-cholesterol (34.5%) and among the female control it was central obesity (82.2%). Cigarette smoking was the lowest cardiovascular risk factor among both hypertensive and normotensive subjects stratified by gender (Table 4.2). Generally, the hypertensive male and female had significantly higher prevalence of diabetes, reduced HDL-cholesterol, raised LDL-cholesterol, physical inactivity and higher abdominal obesity as compared to their normotensive male and female control group. However, general obesity as measured by BMI was significantly higher among the normotensive female as compared to the hypertensive female as shown in table 4.2.

Table 4.2 Percentage prevalence of cardiovascular risk factors among the study population stratified by gender

Variables	Hypertensive (n=200)	Normotensive (n=100)	Hypertensive Male (n=63)	Normotensive Male (n=55)	Hypertensive Female (n=137)	Normotensive Female (n=45)
Diabetes	69(34.5)	6(6.0)***	19(30.2)	5(9.1)††	50(36.5)	1(2.2)##
Hypercholesterolaemia	47(23.5)	14(14.0)	14(22.2)	7(12.7)	33(24.1)	7(15.6)
Hypertriglyceridaemia	25(12.5)	11(11.0)	12(19.0)	4(7.3)	13(9.5)	7(15.6)
Low HDL	148(74.0)	47(47.0)***	39(61.9)	19(34.5)††	109(79.6)	28(62.2)‡
High LDL	26(13)	4(4.0)*	11(17.5)	2(3.6)†	15(10.9)	2(4.4)
Cigarette smoking	3(1.5)	1(1.0)	3(4.8)	1(1.8)	0(0)	1(2.2)
Physical inactivity	103(51.5)	41(41)	20(31.7)	10(18.2)	83(60.6)	0(0)##
Obesity-BMI	57(28.5)	26(26.0)	16(25.4)	13(23.6)	41(29.9)	31(68.9)##
Obesity-WHR	154(77)	33(33.0)***	31(49.2)	7(12.7)†††	123(89.8)	26(57.7)##
Obesity-WC	139(69.5)	42(42.0)***	13(20.6)	5(9.1)	126(92.0)	37(82.2)

Data are presented as proportion and compare using Fischer's exact test.*Significant at the 0.05 level (2-tailed) and ***Significant at the 0.001 level (2-tailed) when the hypertensive subject were compare to the normotensive control. †Significant at the 0.05 level (2-tailed), ††***Significant at the 0.01 level (2-tailed) and*** †††***Significant at the 0.001 level (2-tailed) when the hypertensive male were compared to normotensive male.*** ‡***Significant at the 0.05 level (2-tailed) and*** ‡‡***Significant at the 0.001 level (2-tailed) when the hypertensive female were compared to the normotensive female.***

Table 4.3Prevalence of metabolic syndrome and its components among the study population stratified by gender

Condition	Hypertensive (n=200)	Normotensive (n=100)	Hypertensive Male (n=63)	Normotensive Male (n=55)	Hypertensive Female (n=137)	Normotensive Female (n=45)
National Cholesterol Education Programme – Adult Treatment Panel III Criteria						
MetS	113(56.5)	9(9.0)***	24(38.1)	1(1.8)††	89(65.0)	8(17.8)##
WC >102 , >88	104(52.0)	21(21.0)***	5(7.9)	0(0)	99(72.3)	21(46.7)#
TG ≥ 1.7	33(16.5)	13(13.0)	14(22.2)	6(10.9)	19(13.9)	7(15.6)
HDL < 1.03,<1.3	148(74.0)	47(47.0)***	39(61.9)	19(34.5)††	109(79.6)	28(62.2)‡
FBG ≥ 6.1	110(55.0)	12(12.0)***	37(36.4)	8(14.5)††	73(53.3)	4(8.9)##
BP ≥130/85	200(100.0)	1(1)***	44(69.8)	0(0)††	79(57.7)	1(2.2)##
World Health Organization Criteria						
MetS	109(54.5)	5(5.0)***	39(61.9)	1(1.8)††	70(51.1)	4(8.9)##
WHR >0.90, >0.85	154(77.0)	33(33.0)***	31(49.2)	7(12.7)††	123(89.8)	26(57.8)##
TG ≥1.7	33(16.5)	11(11.0)	14(22.2)	4(7.3)†	19(13.9)	7(15.6)
HDL < 1.03, < 0.90	87(43.5)	19(19.0)***	28(44.4)	5(9.1)††	59(43.1)	14(31.1)
FBG ≥6.1	110(55.0)	12(12.0)***	37(58.7)	8(14.5)††	73(53.3)	4(8.9)##
BP ≥140/90	200(100.0)	0(0)***	63(100.0)	0(0)††	137(100.0)	0(0)##
International Diabetes Federation Criteria						
MetS	131(65.5)	15(15.0)***	13(20.6)	3(5.5)†	118(86.1)	12(26.7)##
WC ≥80 , ≥94	139(69.5)	42(42.0)***	13(20.6)	5(9.1)	126(92.0)	37(8.2)
TG ≥1.7	33(16.5)	11(11.0)	14(22.2)	4(7.3)†	19(13.9)	7(15.6)
HDL < 1.03, <1.3	148(74.0)	47(47.0)***	39(61.9)	19(34.5)††	109(79.6)	28(62.2)‡
FBG ≥ 5.6	143(71.5)	21(21.0)***	45(71.4)	14(25.5)††	98(71.5)	7(15.6)##
BP ≥130 or ≥85	200(100.0)	15(15.0)***	63(100.0)	10(18.2)††	137(100.0)	5(11.1)##

Data are presented as proportion and compare using Fischer's exact test. ***Significant at the 0.001 level (2-tailed) when the hypertensive subject were compare to the normotensive control. †Significant at the 0.05 level (2-tailed), ***††Significant at the 0.01 level (2-tailed) and †††Significant at the 0.001 level (2-tailed) when the hypertensive male were compared to normotensive male. ‡Significant at the 0.05 level (2-tailed), #Significant at the 0.01 level (2-tailed) and ##Significant at the 0.001 level (2-tailed) when the hypertensive female were compared to the normotensive female.***

4.3 METABOLIC SYNDROME AND ITS COMPONENTS

The prevalence of MetS among the hypertensive patients was significantly higher than the normotensive control (56.5% vrs 9.0%, 54.5% vrs 5.0% and 65.5%vrs15.0%) using NCEP ATP III, WHO and IDF criteria. Irrespective of the criteria, all the components of MetS were significantly higher among the hypertensive patients as compared to the normotensive control. Using NCEP ATP III criteria, the highest prevalence of components of MetS among the hypertensive patients was raised BP (100.0% vrs 1.0% in normotensive control), reduced HDL-C (i.e. 74.0% vrs 47.0% in normotensive control), followed by raised FBG (55.0% vrs 12.0% in normotensive control), central obesity (52.0% vrs 21.0% in normotensive control) and raised TG (16.5% vrs 11.0% in normotensive control) (Table 4.3). From the WHO criteria, the highest prevalence of components of MetS among the hypertensive patients was raised BP (100.0%vrs 0.0% in normotensive control), abdominal obesity (77.0% vrs 33.0% in normotensive control), followed by raised FBG (55.0%vrs 12.0% in normotensive control), reduced HDL-C (i.e. 43.5% *vrs* 19.0% in normotensive control) and raised TG (16.5% *vrs* 11.0% in normotensive control) (Table 4.3). Among hypertensive patients, raised BP had the highest prevalence rate (100.0%*vrs* 15.0% in normotensive control), followed by reduced HDL-C (74.0% *vrs* 47.0% in normotensive control), raised FBG (71.5.0% *vrs* 21.0% in normotensive control), central obesity (i.e. 69.5% *vrs* 42.0% in normotensive control) and raised TG (16.5% *vrs* 11.0% in normotensive control) using the IDF criteria (Table 4.3).

The prevalence of MetS was significantly higher among the hypertensive male patients as compared to the normotensive male control using NCEP ATP III (38.1% vrs 1.8%), WHO (61.9% vrs 1.8%) and IDF criteria (20.6% vrs 5.5%). Also, the prevalence of MetS was significantly higher among the hypertensive female patients as compared to the normotensive female control using NCEP ATP III (65.0% vrs 17.8%), WHO (51.1% vrs 8.9%) and IDF criteria (86.1% vrs 26.7%) (Table 3). Apart from that, irrespective of the criteria used, the prevalence of the components of MetS was significantly higher when the male and female

hypertensive patients were compared to the male and female normotensive control (Table 4.3).

4.4 PREVALENCE OF METABOLIC SCORE

The percentage of hypertensive patients with zero and one metabolic score were significantly lower as compared to the normotensive control group using NCEP ATP III (2.0% vrs 35.0% and 10.0% vrs 43.0% for zero and one respectively), WHO (0.0% vrs 44.0% and 6.0% vrs 27.0% for zero and one respectively) and IDF criteria (0.0% vrs 23.0% and 4.5% 35.0% for zero and one respectively) (Table 4.4). Using the NCEP ATP III criteria, 33.0% of the hypertensive patients had metabolic score of 2 whereas 15.0% of the normotensive control had metabolic score of 2. The difference was significant ($p = 0.0009$). The prevalence of individuals who had metabolic score of 2 was similar between the hypertensive and normotensive subjects (i.e. about 15%) using WHO criteria. However, using IDF criteria, higher percentage of the normotensive control group had metabolic score of 2 (i.e. 35.0%) as compared to the hypertensive patients (i.e. 4.5%). Virtually, none of the normotensive control group had metabolic score of 4 and 5 whereas significant proportion of the hypertensive patients had metabolic score of 4 and 5 as shown in Table 4.4. Generally, these trends are the same when the hypertensive and normotensive subjects were stratified based on gender (Table 4.4).

Table 4.4Proportion of the study population with various metabolic score

Variables	Hypertensive (n=200)	Normotensive (n=100)	P value	Hypertensive Male (n=63)	Normotensive Male (n=55)	P value	Hypertensive female (n=137)	Normotensive female (n=45)	P value
Metabolic score as determine by NCEP ATP III criteria									
5	10(5.0)	0(0.0)	0.0340	1(1.6)	0(0.0)	1.0000	9(6.6)	0(0.0)	0.1151
4	32(16.0)	0(0.0)	<0.0001	9(14.3)	0(0.0)	0.0034	23(16.8)	0(0.0)	0.0013
3	59(29.5)	7(7.0)	<0.0001	12(19.0)	1(1.8)	0.0027	47(34.3)	6(13.3)	0.0077
2	66(33.0)	15(15.0)	0.0009	23(36.5)	3(5.5)	<0.0001	43(31.4)	12(26.7)	0.5812
1	20(10.0)	43(43.0)	<0.0001	6(9.5)	24(43.6)	<0.0001	14(10.2)	19(42.2)	<0.0001
0	4(2.0)	35(35.0)	<0.0001	3(4.8)	27(49.1)	<0.0001	1(0.7)	8(17.8)	<0.0001
Metabolic score as determine by WHO criteria									
5	26(13.0)	0(0.0)	<0.0001	9(14.3)	0(0.0)	0.0034	17(12.4)	0(0.0)	0.0078
4	39(19.5)	1(1)	<0.0001	10(15.9)	0(0.0)	0.0016	29(21.2)	1(2.2)	0.0020
3	72(36.0)	13(13.0)	<0.0001	15(23.8)	3(5.5)	0.0088	57(41.6)	10(22.2)	0.0210
2	31(15.5)	15(15.0)	1.0000	2(3.2)	7(12.7)	0.0801	29(21.2)	8(17.8)	0.6763
1	12(6.0)	27(27.0)	<0.0001	7(11.1)	13(23.6)	0.0873	5(3.6)	14(31.1)	0.6763
0	0(0)	44(44.0)	<0.0001	0(0.0)	32(58.2)	<0.0001	0(0.0)	12(26.7)	<0.0001
Metabolic score as determine by IDF criteria									
5	21(10.5)	0(0.0)	0.0002	6(9.5)	0(0.0)	0.0294	15(10.9)	0(0.0)	<0.0001
4	64(32.0)	1(1)	<0.0001	5(7.9)	1(1.8)	0.2132	59(43.1)	0(0.0)	<0.0001
3	74(37.0)	15(15)	<0.0001	26(41.3)	3(5.5)	<0.0001	48(35.0)	12(26.7)	0.3625
2	32(16.0)	26(26)	0.0444	17(27.0)	8(14.5)	0.1174	15(10.9)	18(40.0)	<0.0001
1	9(4.5)	35(35.0)	<0.0001	9(14.3)	23(41.8)	0.0009	0(0.0)	12(26.7)	<0.0001
0	0(0)	23(23.0)	<0.0001	0(0.0)	20(36.4)	<0.0001	0(0.0)	36.7)	0.0144

4.5 RISK FACTORS FOR HYPERTENSION

From the univariate analysis, the female participants were at about 3 times at risk of developing hypertension as compared to their male counterparts (OR = 2.7; 95% CI = 1.6-4.4; p = 0.0000). Physical inactivity also poses about 2 times risk to developing hypertension as compared to those who exercise (OR = 1.6; 95% CI = 1.0-2.5; p = 0.0430) whereas hypercholesterolaemia poses about 3 times risk of developing hypertension. Reduced HDL-cholesterol poses about 2 times risk of developing hypertension. Individuals with low levels of apolipoprotein A1 are at about 13 times at risk of developing hypertension as compared to those with normal apolipoprotein A1. However, individuals with high apolipoprotein A1 are protected from developing hypertension as compared to those individuals presenting with normal apolipoprotein A1 as shown in Table 4.5. High apolipoprotein B, impaired fasting glucose and diabetes poses about 3, 7 and 12 times risk of developing hypertension respectively as compared to the normal subjects (Table 4.5).

After adjusting for gender, exercise, total cholesterol, HDL-cholesterol, fasting glucose, apolipoprotein A1 and B in the multivariate logistic regression analysis, the female participants were about 5 times at risk of developing hypertension as compared to the male(aOR = 5.3; 95% CI = 2.3-12.4; p = 0.0000). Reduced apolipoprotein A1 served as a risk factor (aOR = 13.4; 95% CI = 1.5-121.4; p = 0.0210) whilst high apolipoprotein A1 protects persons from developing hypertension (aOR = 0.1; 95% CI = 0.0-0.2; p = 0.0000). High apolipoprotein B poses about 9 times risk of developing hypertension as compared to the normal subjects (aOR = 9.3; 95% CI = 4.2-20.9; p = 0.0000). Impaired fasting glucose as well as diabetes poses more than 10 times risk each of developing hypertension as compared to normoglycaemia as shown in Table 4.5. The effect of exercise, hypercholesterolaemia and reduced HDL-cholesterol resolved after adjusting for the confounding factors (Table 4.5).

Table 4.5 Logistic regression analysis of risk factors for hypertension

Variable	OR(95% CI)	P value	aOR(95% CI)	P value
Age (yrs)				
20-30	2.3(0.2-21.5)	0.474	0.7(0.1-8.9)	0.7610
31-40*	1		1	
41-50	1.3(0.6-2.4)	0.498	0.5(0.2-1.6)	0.2690
51-60	0.6(0.3-1.2)	0.18	0.4(0.2-1.3)	0.1260
>60	1.8(0.9-3.8)	0.115	1.2(0.4-3.5)	0.8070
Gender				
Male*	1		1	
Female	2.7(1.6-4.4)	0.0000	5.3(2.3-12.4)	0.0000
Smoking				
Yes	1.5(0.2-14.7)	0.724	1.5(0.0-82.6)	0.8450
No*	1		1	
Exercise				
Yes*	1		1	
No	1.6(1.0-2.5)	0.0430	0.6(0.3-1.4)	0.2320
BMI				
Underweight	1.6(0.4-6.0)	0.504	1.1(0.1-8.9)	0.9340
Normal*	1		1	
Overweight	0.6(0.3-1.1)	0.103	0.8(0.3-1.9)	0.5660
Obese	0.9(0.5-1.7)	0.853	0.7(0.3-2.0)	0.5670
Total cholesterol				
Low	4.4(2.2-8.7)	0.0000	3.8(1.5-9.7)	0.006
Normocholesterolaemia*	1		1	
Hypercholesterolaemia	2.5(1.3-4.9)	0.0090	0.9(0.3-2.5)	0.827
Triglyceride				
Normal*	1			
Hypertriglyceridaemia	1.2(0.6-2.3)	0.5840	0.8(0.2-2.8)	0.745
HDL-C				
Low	1.9(1.2-3.2)	0.0120	0.9(0.4-1.9)	0.7200
Normal*	1		1	
LDL-C				
Normal*	1		1	
High	1.3(0.7-2.4)	0.47	0.4(0.1-2.0)	0.273
Apolipoprotein A1				
Low	13.3(1.8-99.4)	0.012	13.4(1.5-121.4)	0.0210
Normal*	1		1	
High	0.1(0.1-0.3)	0.0000	0.1(0.0-0.2)	0.0000
Apolipoprotein B				
Low	1.0(0.1-15.8)	0.981	0.5(0.0-28.5)	0.9450
Normal*	1			
High	3.2(2.0-5.3)	0.0000	9.3(4.2-20.9)	0.0000
Glucose				
Hypoglycaemia	5.1(0.6-44.2)	0.143	6.7(0.5-89.4)	0.1530
Normal*	1		1	
Impaired fasting glucose	6.7(2.7-16.7)	0.0000	19.7(4.8-81.0)	0.0000
Diabetes Mellitus	11.6(4.8-28.2)	0.0000	13.1(3.9-43.6)	0.0000

****Reference group, OR = odds ratio, aOR = adjusted odds ratio and CI = confidence interval***

4.6 ASSOCIATIONS OF THYROID FUNCTION WITH CARDIOVASCULAR RISK FACTORS IN EUTHYROID HYPERTENSIVES

TSH levels were negatively associated to WHR, an association that remained significant after adjustment for age.

fT4 levels were positively associated to BMI and Apo A1 after adjustment for age. It was however negatively associated to TC (β= -0.275; p<0.05), LDL-C (β= -0.337; p<0.05) and FBG (-0.121; p<0.05) as shown in Table 4.6.

Table 4.6 Associations of thyroid function with serum lipid concentrations and the components of the metabolic syndrome among euthyroid hypertensives.

PARAMETERS		TSH		fT4	
	MODEL	B	P -value	β	P -value
WC(cm)	1	-0.008	0.13	-0.019	0.222
	2	-0.008	0.143	-0.019	0.228
BMI(kg/m²)	1	-0.001	0.93	-0.072	0.033
	2	0.002	0.858	0.075	0.034
WHR	1	-2.762	0.028	0.582	0.87
	2	-2.94	0.02	0.476	0.894
SBP(mmHg)	1	-0.001	0.808	0.004	0.745
	2	-0.002	1.018	0.004	0.772
DBP(mmHg)	1	-0.007	0.386	0.016	0.463
	2	-0.007	0.361	0.015	0.471
TC(mmol L^{-1})	1	0.038	0.417	-0.275	0.033
	2	0.038	0.412	-0.275	0.033
TG(mmol L^{-1})	1	-0.011	0.936	-0.68	0.079
	2	-0.009	0.947	-0.679	0.08
HDL-C(mmol L^{-1})	1	-0.452	0.081	0.223	0.759
	2	-0.436	0.094	0.238	0.744
LDL-C(mmol L^{-1})	1	0.064	0.156	-0.337	0.007
	2	0.065	0.15	-0.336	0.007
APO A1(g L^{-1})	1	0.121	0.419	1.537	<0.001
	2	0.103	0.495	1.549	<0.001
APO B(g L^{-1})	1	0.134	0.17	0.515	0.059
	2	0.131	0.18	0.513	0.061
FBG(mmol L^{-1})	1	-0.036	0.079	-0.121	0.034
	2	-0.036	0.079	-0.121	0.034

Values of β are standardized regression coefficients: model 1, crude; model 2, after adjustment for age.

Chapter 5

DISCUSSION

5.1 PREVALENCE OF METABOLIC SYNDROME

The prevalence of chronic diseases is increasing worldwide. In many developing countries, the growing epidemic of chronic disease disrupts health planning and overwhelms the already under-resourced health care systems (WHO, 2002). While the definition of metabolic syndrome continues to evolve, it is generally recognized as a cluster of risk factors that includes abdominal obesity, glucose intolerance, hypertension, dyslipidaemia and abnormalities in peripheral glucose and fatty acid utilization (Czernichow, 2005). The three most commonly used sets of criteria for the identification of metabolic syndrome are the WHO, ATP-III and IDF guidelines (Yasein, 2005).

Metabolic syndrome may amplify hypertension-related cardiac and renal changes over and above the potential risk of each risk factor in isolation (Mule, 2005). Metabolic syndrome has been shown to be significantly associated with a higher risk of death or major cardiovascular event (Kip, 2004).

In the present study, irrespective of the criteria applied, more than half of the hypertensive subjects were identified with metabolic syndrome (NCEP ATP III-56.5%, WHO-54.5.0% and IDF-65.5%) and was more prevalent in female hypertensives. This finding is consistent with other studies revealing that hypertension tends to coexist with metabolic risk factors and that about half of hypertensives are insulin-resistant (Reaven et al., 1996; Zavaroni et al.,1992; Lind et al., 1995; Kelishadi et al., 2005). It also confirms the existing evidence of gender differences in the relationship between blood pressure and insulin resistance (Chen et al., 2000; Haffner et al., 1992). On the contrary, in the factor analysis by Choi et al. (2003), blood pressure was not closely aggregated with other CVD risk factors.

The frequency of metabolic syndrome was significantly higher than the prevalence of 34.0% (NCEP ATP III) observed in Kuwait (Sorkhou *et al.*, 2004), 51.6% in Iran (Kelishadi *et al.*, 2005) and other studies elsewhere but slightly lower than 62.5% observed in Jordan (Yasein, 2005) in which the patients were much older. A possible explanation may be that our subjects were exclusively hypertensives. Another plausible explanation is the high frequency of multiple risk factors such as increased waist circumference, diabetes and reduced HDL-C. This assertion is supported by the interrelationship between age and obesity, particularly central obesity, and the expected risk of diabetes, hypertension and dyslipidaemia, which are the features of metabolic syndrome (Cameron *et al.*, 2004). Studies have shown that in women, waist circumference correlates strongly with hypertension (Després *et al.*, 2001; NCEP ATP III, 2001; Yasein, 2005). Increased waist circumference played a major role in the contribution to metabolic syndrome in our hypertensive subjects, particularly among females. This observation may be attributed in part to their sedentary lifestyle.

5.2 RISK ASSOCIATED WITH HIGH METABOLIC SCORE

When the percentage risk score of the hypertensive subjects was analyzed according to the NCEP ATP III classification, female hypertensive patients did have higher MetS score (all 5 components, 6.6% versus 1.6%), which had been related to more severe coronary angiographic alterations and higher frequencies of unstable angina and myocardial infarction (Solymoss *et al.*, 2004). This was higher than 2.9% and 2.2% found by Ford *et al.* (2002) in the US, and comparable to 4.6 and 1.7 observed by Kelishadi *et al.* (2005) in Iran.

5.3 SERUM LIPIDS AND HYPERTENSION

The higher serum plasma total cholesterol, triglyceride and LDL-C in the hypertensives compared to the control subjects in the present study were in

corroboration with other studies conducted in Nigeria (Ahaneku *et al,* 1999; Jarikre *et al.,* 1996). Again, studies in non-blacks have demonstrated similar trends of increased cholesterol in hypertensives compared to normotensive controls (Jovanovic *et al.,* 2000; Krisela *et al.,* 1987).

The finding of a statistically significant difference in the HDL-C levels between the hypertensive and control subjects was not in agreement with studies conducted elsewhere (Idemudia & Ugwuja, 2009; Timothy et al., 1986). This highlights the difference in the distribution of cardiovascular risk factors in different populations which may be due to genetic and environmental factors. The finding also means that, our hypertensive subjects may not be protected from the good effects of high HDL-C level.

5.4 RISK FACTORS ASSOCIATED WITH HYPERTENSION

It is noteworthy that, Apo B was significantly high in our hypertensive subjects and high levels were shown to significantly predict hypertension (aOR=9.3, CI 4.2-20.9) while LDL-C was a relatively weaker predictor (aOR=0.4, CI 0.1-2.0). Although LDL-C is well established as a predictor of coronary artery disease (CAD), it may not be the best circulatory marker (Chan & Watts, 2006). Four prospective epidemiologic studies: Quebec Cardiovascular Study (Lamarche et al., 1998), Moss Heart Study (Moss et al., 1999), AMORIS study (Walldius et al., 2001) and Northwick Park Heart Study (Talmud et al., 2002), have shown that apoB is superior to total cholesterol or LDL-C as an index of the risk of vascular disease. In 2006, thirty-person/ten country panel recommended the use of apoB for estimating cardiovascular risk and guiding therapy. More recently, in 2008, the same apoB targets were recommended in a consensus statement from the American Diabetes Association (ADA) and the American College of Cardiology (ACC) Foundation to guide the therapy of patients with high cardiometabolic risk (Brunzell et al., 2008). The biological mechanisms by which lipids may play a role in the development of hypertension remain poorly understood. Atherogenic lipid

abnormalities clearly cause endothelial dysfunction. A dysfunctional endothelium, possibly through impaired nitric oxide production and activity, as well as alterations in endothelin-1 and endothelin A and B receptor expression, cannot respond to changes in intravascular conditions to constrict and dilate as needed. This vasodysregulation could lead to an inability or difficulty in vasodilatation to appropriate stimuli and eventually to increased resting BP. Because atherosclerosis can be a diffuse process, it is possible that hypertension is a manifestation of a diffuse atherosclerotic process in large conduit arteries, as well as smaller resistance vessels.

This study showed that impaired fasting glucose and diabetes are strongly associated with hypertension. This is consistent with the study of Sowers & Bakris (2000) who showed that persons with hypertension have a high prevalence of insulin resistance and are at substantially higher risk of developing type 2 diabetes mellitus. Verdecchaia et al.'s (2004) data also supports prior observations that certain antihypertensive drug classes (diuretics and ß-blockers) may increase this propensity of patients with hypertension to develop type 2 diabetes.

5.5 USE OF CARDIOVASCULAR MEDICATIONS AND BLOOD PRESSURE CONTROL AMONG THE HYPERTENSIVES

In the present study, although, almost all the hypertensive subjects were under medical treatment with at least one antihypertensive drug, blood pressure control was effective in only one-third (32.5%) of the study participants. This observation is slightly higher than the finding in the GOOD survey (Kjeldsen *et al.*, 2008) that, high BP control in treated hypertensives was achieved in less than 30% of patients. This highlights the role of cardiometabolic risk factor management in BP control. This finding also confirms the well-known difficulty in controlling BP in patients with diabetes and metabolic syndrome, recognized as a high-added risk in the ESH/ESC guidelines (Mancia *et al.*, 2007)

It is noteworthy that, lipid lowering agents were used in only 1.5% of the hypertensive subjects despite the existence of atherogenic dyslipidaemia in more than half of the study participants. Our finding therefore supports the conclusion of the global GOOD survey (Kjeldsen et al., 2008) which emphasizes the need to consider the overall cardiometabolic profile of a patient, rather than BP per se, while determining optimal management regimen for hypertensives.

5.6 THYROID FUNCTION AND CARDIOVASCULAR RISK FACTORS

In the present study, a significant negative correlation between fT4 and total cholesterol as well as atherogenic LDL-C was found in the hypertensives. This finding is in agreement with the well-known association of subclinical hypothyroidism with elevated levels of TC and LDL-C (Duntas, 2002). Moreover, fT4 levels were significantly related to three of the cardiovascular risk factors. These findings suggest that hypertensives with fT4 abnormalities are already at increased cardiovascular risk. This shows that the influence of thyroid function on lipid metabolism extends into the euthyroid range.

The pathophysiological process behind the influence of thyroid function on lipid metabolism is known from subjects with overt thyroid dysfunction. Hypercholesterolemia in hypothyroidism, characterized by elevated levels of LDL-C and Apo B, is caused by a decreased catabolism of LDL due to a reduction in the number of LDL receptors on liver cell surfaces (Duntas, 2002; Diekman *et al.,* 1998). Moreover, changes in plasma LDL-C in the transition from hypothyroidism or hyperthyroidism to euthyroidism were found to correlate with changes in fT4 (Diekman *et al.,* 2000). Our results thus showed that these pathophysiological mechanisms are already operative in the euthyroid state.

Our results showed that, in the euthyroid hypertensives, fT4 rather than TSH is related to cardiovascular risk factors. This finding is consistent with a study by Roos *et al.* (2006) who demonstrated a similar trend among euthyroid subjects in

the general population. A possible explanation is that, at least in the euthyroid range, a discrepancy between effects that thyroid hormone has on peripheral tissues and the effect that the hormone has on central feedback inhibition of TSH release. Such a discrepancy may, for instance, originate from differences between the central and peripheral tissues in expression of thyroid hormone receptor isoforms, and in expression of type 1 and type 2 iodothyronine deiodinase, with different catalytic properties (Croteau *et al.,* 1996; Flamant & Samarut, 2003; Zhang & Lazar, 2000). Polymorphisms in the TSH receptor have influenced ratios of plasma TSH and thyroid hormones, and can, therefore, also play a role in inducing a discrepancy between central and peripheral tissues (Peeters *et al.,* 2003).

Chapter 6

CONCLUSIONS

6.1 CONCLUSION

In conclusion, the study demonstrated that, hypertension is more than just elevated blood pressure; it is intimately associated with the metabolic syndrome.

The study also demonstrated that, low normal fT4 levels were significantly associated with three of the cardiovascular risk factors. These findings are consistent with an increased cardiovascular risk in subjects with low normal thyroid function.

High prevalence of major CVD risk factors (obesity-WHR, low HDL and diabetes) was found in the study population. The cluster of several cardiovascular risk factors, especially in the hypertensives, leads to an increased relative risk of cardiovascular diseases.

There is therefore the need for metabolic screening of all hypertensives and increased awareness creation on the critical importance of public health strategies aimed at reducing risk factors in the entire population. Early detection and treatment of the global risk profile (Multi-target approach) should thus become a priority.

The study also proved that Apolipoproteins A1 and Apo B which are not measured in our clinical setting were significantly associated with hypertension. The possible role of Apo B as a quantitative atherogenic marker in hypertensives should be further investigated.

6.2 RECOMMENDATION

- The possible role of Apo B as a quantitative atherogenic marker in hypertensives should be further investigated.

- Further studies on metabolic side effects of various classes of antihypertensive drugs must be carried out.
- A prospective study has to be performed to assess a possible role for the treatment of thyroid dysfunction at an earlier stage.
- Relationship between metabolic syndrome and target-organ damage should be investigated.

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APPENDIX