Independent Relationship between 24-hour Blood Pressure and Carotid Intima–Media Thickness

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Abstract

Introduction: The changing socio-economic landscape in Africa has brought with it unique health challenges previously uncommon in people of African ancestry. Non-communicable diseases such as coronary artery disease and stroke have emerged as pressing public health concern highlighting the need to find more on-target diagnostic tools as well as therapeutic interventions. Although ambulatory blood pressure (AMBP) has in many studies conducted in the western world proved to be an independent predictor of carotid intima-media thickness (C-IMT), such results cannot outright be imputed to people of African ancestry living in Africa. That is because people of African ancestry living in Africa are not only of a different ethnicity but are still in the early phases of an epidemiological transition while people in the western countries who are mostly Caucasians, are believed to be in the middle to late phases of an epidemiological transition.

Methods: The relationship between the intima-media thickness of the common carotid artery (SonoCalc^{TM} IMT version 3.4) and AMBP (Space labs model 90207) was determined in 320 randomly selected participants of African descent living in an urban developing community in South Africa. Relationships were determined after adjustment for (clinic blood pressure) BPc, age, gender, alcohol and tobacco use, the presence or absence of diabetes mellitus or inappropriate blood glucose control measured by glycated hemoglobin (ghb), antihypertensive therapy and menopausal status.

Results: Mean age for the study population was 43.7± 16.0 years. Both BPc and AMBP parameters were strongly associated with C-IMT ($p<0.001$) in univariate analysis. In multivariate analysis with BPc. and AMBP entered into separate models and after adjusting for cofounders, BPc. and AMBP maintained significant associations with C-IMT. [BPc (partial $r=0.0648$, $p< 0.1612$), systolic blood pressure 24 (SBP24) (partial $r= 0.236$, $p< 0.001$), systolic blood pressure day (SBPd) (partial $r= 0.302$, $p<0.05$), systolic blood pressure night (SBPn) (partial $r= 0.0983$, $p<0.05$)]. When adjustments were made with BPc. and SBP24 entered into the same model, BPc lost its association with C-IMT, [SBP24 (partial $r=0.236$, $p<0.001$) SBPd (partial $r=0.149$, $p<0.05$), SBPn (partial $r=0.172$, $p<0.05$)]. Importantly the relationship between SBP24 and C-IMT persisted independent of body mass index (BMI), BPc and age. SBP24 had the highest significant association with C-IMT.

Conclusion: SBP24 independently predicts C-IMT even in a model that includes conventional systolic blood pressure (SBPc) leading to the conclusion that AMBP is a more effective tool at diagnosing C-IMT alterations while BPcdoes not have an independent relationship C-IMT.

Presentations arising from the thesis

Poster Presentations

Metsing LS, Sibiya M, Maseko MJ, Majane OHI, Night time systolic blood pressure determine Carotid intima media thickness in a community sample of African ancestry with a high prevalence of excess adiposity. The 38th congress of Physiology Society of Southern Africa. 2010.

Metsing LS, Sibiya M, Maseko MJ, Majane OHI Independent relation between 24-hour blood pressure and Carotid intima-media thickness. The 39th congress of Physiology Society of Southern Africa. 2011.

Declaration

I declare that this is my own unaided work. It is being submitted for the degree of Master of Science in Medicine in the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa. The work contained in this dissertation has not been submitted for any degree or examination in this University, or any other University.

..... ……..day of ……2012

Lebogang Stanley Metsing

I certify that the studies contained in this dissertation have the approval of the Committee for Research in Human Studies of the University of the Witwatersrand, Johannesburg. The ethics approval number is M02-04-72

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Abbreviations

ABPM …….Ambulatory Blood Pressure Monitoring

AII……...Angiotensin II

AP-1…….Activator protein-1

APOGH……...African Project on Genes in Hypertension

ARIC……...Atherosclerosis Risk in Communities

AT1……..Angiotensin II type 1

ATII……..Angiotensin II type 2

BH4**…….**Tetrahydrobiopterin

BMI……..Body mass index

BP……....Blood pressure

BPc……..Conventional blood pressure

CCA……Common Carotid Artery

C-IMT……..Carotid Intima Media thickness

CRP……..C-Reactive Protein

CVD……Cardiovascular disease

DBP24…….Diastolic Blood Pressure 24

DBPc……...Diastolic Blood Pressure conventional

DBPd……..Diastolic Blood Pressure day

DBPn……..Diastolic Blood Pressure night

eNOS……...endothelial Nitric Oxide Synthase

FLEMENGHO……Flemish Study on Environment, Genes and Health Outcomes

FSH……..Follicle Stimulating Hormone

ghb……...Glycated haemoglobin

IL-1……..Interleukin-1

IL-6……..Interleukin-6

ICAM-1……...Intercellular Cell Adhesion Molecule

LAAS……..Los Angeles Atherosclerosis study

LDL…….Low Density Lipoprotein

NADPH……..Nicotinamide Adenine Dinucleotide Phosphate

NF-κB ……Nuclear Factor kappaB

NO……..Nitric Oxide

PHYLLIS……..Plaque Hypertension Lowering Italian study.

PI-3…….Phosphatidylinositol-3

PP……Pulse Pressure

PWV……...Pulse Wave Velocity

ROS……Reactive Oxygen Species

SAA…….Serum Amyloid A

SBP24…….Systolic Blood Pressure 24

SBP…….Systolic Blood Pressure

SBPc……...Systolic Blood Pressure conventional

SBPd……...Systolic Blood Pressure day

SBPn……...Systolic Blood Pressure night

SD……...Standard Deviation

SNS…….Sympathetic Nervous System

SOWETO……South West Township

THUSA…….…...Transition and Health during Urbanisation of South Africans

TNF…….Tumour Necrosis Factor

UK……...United Kingdom

USA…….United States of America

VCAM-1……..Vascular Cellular Adhesion Molecule-1

VSMC ……..…Vascular Smooth Muscle Cell

WC……...Waist Circumference

WHR……....Waist-to-Hip Ratio

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Chapter 1

Introduction

1.1 Background

Blood pressure monitoring is an important component of assessing as well as determining an individual's cardiovascular disease profile. Studies have shown similarities as well as differences in the performance levels between different blood pressure (BP) monitoring tools. Low degree of correlation between systolic or diastolic conventional blood pressure (SBPc and DBPc respectively) and similar ambulatory blood pressure (AMBP) parameters has been reported in general population, untreated and treated hypertensive groups. This implies that for any given measurement, a conventional or clinic (BPc) value could be high when compared to that of AMBP or vice versa (Mancia et al., 2000).

Bliziotis *et al* suggests that while AMBP is as reliable as home blood pressure, the two are superior to BPc in their association with pre-clinical organ damage assessed by echocardiographic left ventricular mass index (Bliziotis et al., 2012). AMBP has demonstrated convincingly that it provides a more comprehensive picture and close representation of a patient's BP profile hence it is generally regarded as a more reliable tool at diagnosing target organ damage and cardiovascular events over and beyond BPc (Majane et al., 2007; Blioziotis et al., 2012; Dechering et al., 2009; Mancia et al., 2000; Hansen et al., 2007; Wang et.al., 2006; O'Brien et al., 2003).

Despite the demonstrated reliability of AMBP over BPc reported in studies from our laboratory and others elsewhere, we have also shown in another study that a high quality, single visit, systolic BPc independently associates just as well with multiple organ changes of the heart, kidney and large arteries as AMBP (Woodiwiss et al., 2009). This

investigation did not however include a measure of arterial wall thickness namely carotid intima-media thickness (C-IMT), a surrogate marker of atherosclerosis. The joint guidelines of the European Societies of Hypertension and Cardiology proposes C-IMT as an intermediate marker of target organ damage due to atherosclerosis (Mancia et al., 2007). This is consistent with a growing body of evidence that shows that C-IMT may predict cardiovascular events such as myocardial infarction and stroke, both atherosclerotic related conditions (Kotsis et al., 2005; Lorenz 2006).In the Cardiovascular Health Study involving about 4000 individuals, those with thicker common and internal carotid intima media thickness were found to have a 5-fold higher risk of developing stroke and a 46% higher risk of developing myocardial infarction (Polak 2009).

Investigations of associations between the blood pressure tools discussed above, AMBP and BPc with C-IMT would provide interesting and invaluable information to clinicians and researchers alike. Only a few studies looking at such associations have been conducted but the dimension of a different ethnicity as well as the phase of epidemiological transition the study population is in has not considered. Most studies directly comparing associations of C-IMT with AMBP and BPc were conducted on Caucasian populations in the middle to late phases of epidemiological transition. One of the few such studies explored the associations of intima-media thickness (IMT) at both the carotid and femoral arteries with AMBP and BPc in a general population. In that study AMBP predicted C-IMT over and beyond highly standardized SBPc in models that included AMBP components additionally adjusted for SBPc and other co-variables. The BP measurements in that study however preceded the arterial ultrasound examination by

a median of 26 months (Dechering et al., 2009). Other studies such as the Flemish Study on Environment, Genes and Health Outcomes (FLEMENGHO), OHASAMA and the Plaque Hypertension Lowering Italian study (PHYLLIS) also found ABPM to associate more closely with IMT than BPc (Dechering et al., 2009).

1.2 Hypertension

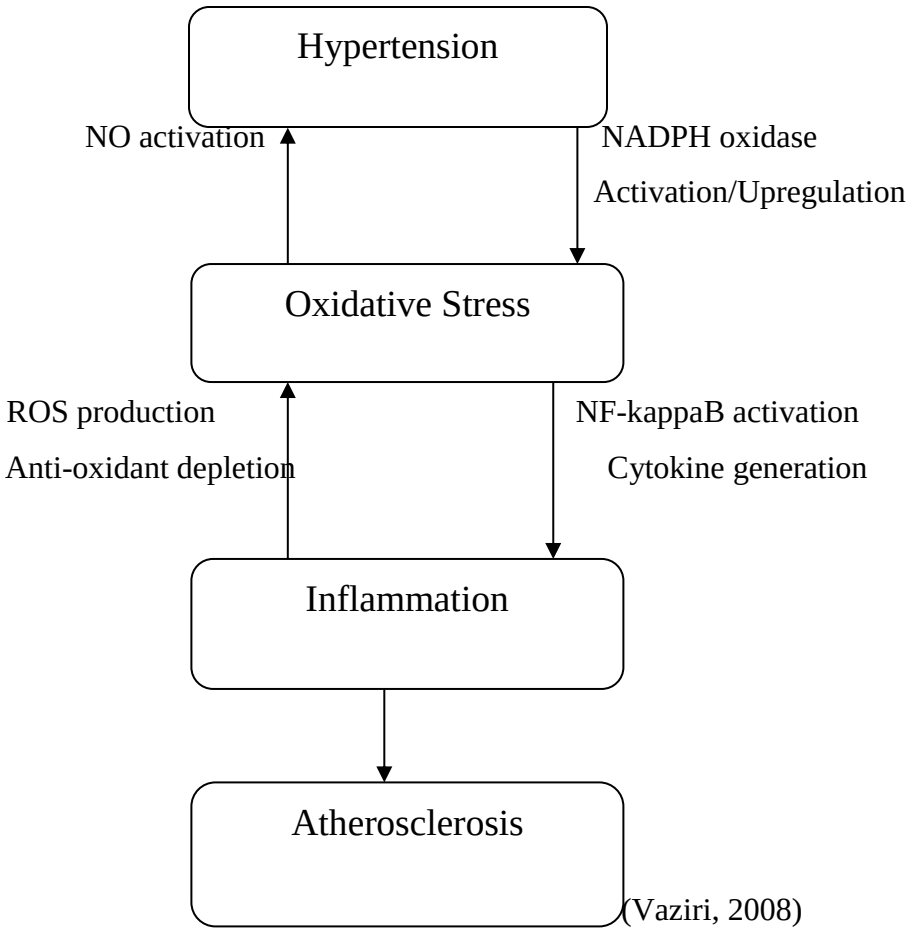
Hypertension is syndrome of persistent elevation of blood pressure which increases an individual's risk of cardiovascular disease (CVD) (Wood, 2007). In this study, it was considered the principal cardiovascular risk factor causing changes to C-IMT. This was in order considering that hypertension is one risk factor that has reached epidemic proportions and contributes significantly to the disease burden of many countries (Kearney et al., 2005).By 2005, World Health Organization (WHO) was reporting that the number of hypertensives worldwide has reached the 1 billion mark and that number was projected to continue rising (Kearney et al., 2005). In the non-western world such as in Africa, hypertension was almost non-existent as late as the 1940's but the adoption of a western lifestyle has changed that (Tibarazwa et al., 2008). One recent study on a population of black Africans living in South Africa noted that they had a high degree of hypertension of as much as 42% (Majane et al., 2007). Through its complications which include those atherosclerotic related, it claims as many as 4million human lives annually (Kearney et al., 2005).

1.2.1 Atherosclerosis in hypertension

There is a graded increase in the risk of atherosclerosis with increasing severity of hypertension. As the risk for atherosclerosis increases, that of IMT growth increases as well. Those individuals with severe hypertension have consequently been found to experience the greatest IMT growth (Su et al., 2001; Lande, 2006). Numerous studies have identified the interaction between several related events as being central to the development of the atherosclerotic process. Such events include oxidative stress, inflammation and endothelial dysfunction (Wang et al., 2010 ; Faxon et al., 2004).

1.2.1.1 Oxidative stress

Hypertension, oxidative stress and inflammation form a self-propagating cycle of interplay where it is not clear as to which process precedes and causes the other and such can lead to tissue injury as in atherosclerosis as Figure 1 below shows (Savoia et al., 2006; Vaziri 2008).



(Vaziri, 2008)

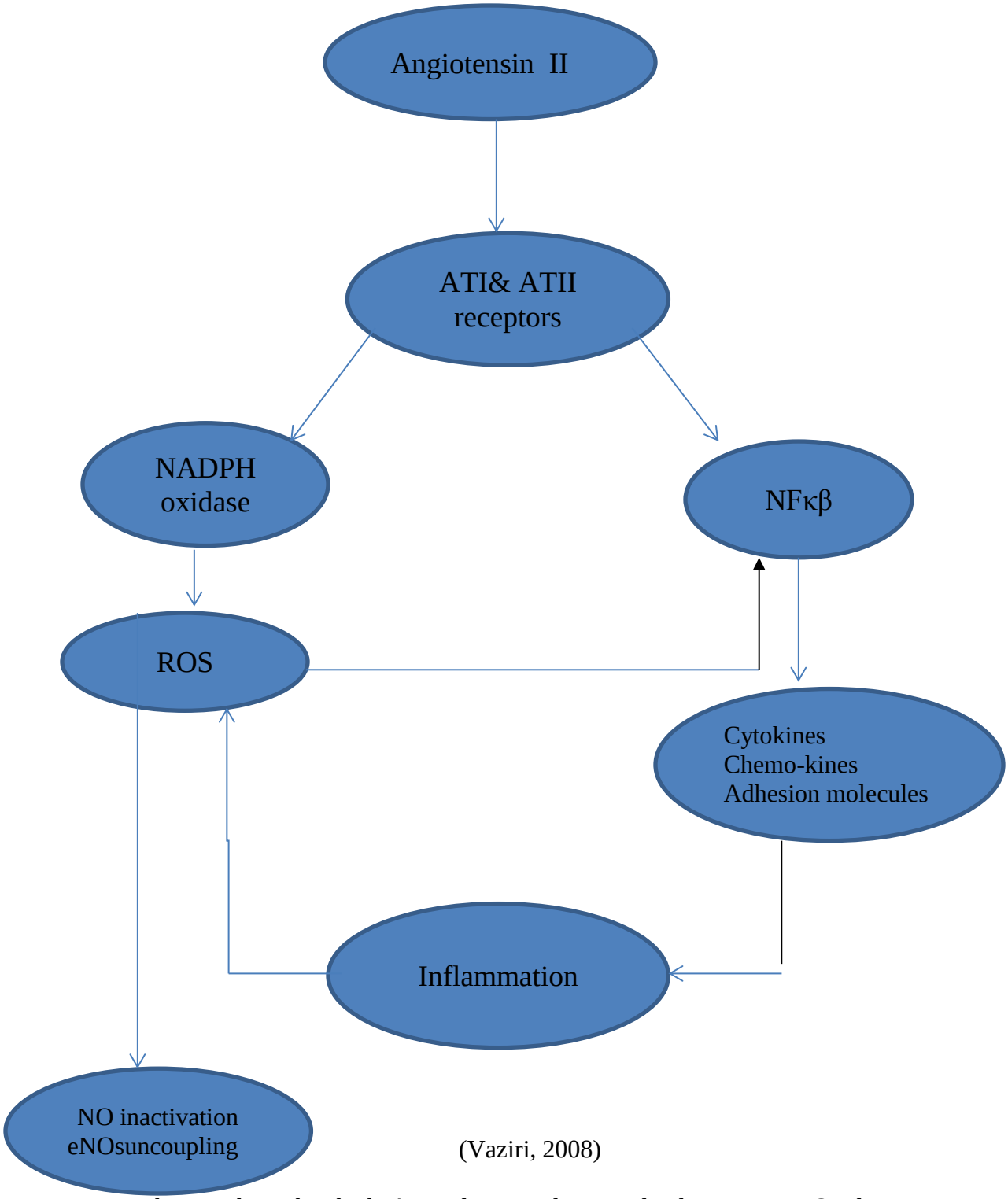
Figure 1.1 Inter-relations between hypertension, oxidative stress, inflammation and atherosclerosis. Nuclear Factor Kappa B (NF-kappaB); NO (Nitric Oxide); Nicotinamide Adenine Dinucleotide Phosphate (NADPH); ROS (Reactive Oxygen Species)

For purposes of this section, the discussion would be on hypertension as the causative agent of both oxidative stress and inflammation and consequently atherosclerosis (Savoia et al., 2006; Touyz, 2005). Oxidative stress is a state of excessive production of Reactive oxygen species (ROS) that overwhelms the defense capacity of the body's antioxidant systems. Besides an enhanced production of ROS, oxidative stress may result exclusively from an impaired antioxidant system or a co-existence of both a down-regulated antioxidant defense and an increased production of ROS (Vaziri, 2008). Levels of anti-

oxidant molecules have been found to be lower in some hypertensives (Cachofeiro et al., 2009).

The endothelium of the vasculature acts as the primary sensor to mechanical factors emanating from blood flow (Touyz, 2005). In hypertension, an increase in blood pressure stimulates endothelial mechano-receptors to activate transcription factors such as Nuclear factor kappa B (NF-kappaB) which in turn activates mechano-sensitive genes. This activation includes that of mechano-sensitive genes that will cause Nicotinamide Adenine Dinucleotide Phosphate (NADPH) oxidase to produce heightened levels of ROS and thereby lead to an onset of a state of oxidative stress (Cachofeiro et al., 2009). NADPH oxidases are groups of enzymes that are major sources of ROS in the vasculature (Vaziri, 2008).

Activation of the Renin-angiotensin-aldosterone system (RAAS) which occurs in hypertension is also implicated in the onset of hypertension associated oxidative stress. Angiotensin II (AII) is one of the central mediators of the system with strong associations with both oxidative stress and inflammatory processes (Savoia and Schiffrin, 2006). It promotes oxidative stress also through stimulating NADPH oxidase to produce more ROS cytokines as Figure 1.2 below shows (Touyz, 2005; Vogiatzi et al., 2009).



(Vaziri, 2008)

Figure 1.2 Pathways through which AII induces and is involved in causing Oxidative stress and Inflammation. AT1, angiotensin II type 1; AT11, angiotensin II type 2

When AII binds to angiotensin II type 1(AT1) receptors, NADPH oxidases in the vasculature are activated to produce ROS which ultimately raises the ROS levels and induces a state of oxidative stress (Vaziri, 2008). The involvement of AII in promoting a state of oxidative stress is confirmed by the findings that when hypertensive patients are treated with AT1 receptor blockers, levels of systemic markers of oxidative stress in their plasma is reduced (Savoia et al., 2006).

1.2.1.2 Inflammation

Besides the close association between oxidative stress and hypertension as discussed above, there also exists a relationship between oxidative stress, inflammation and atherosclerosis as Figure 1.1 shows. Both oxidative stress and inflammation are regarded as the link between hypertension and atherosclerosis (Vaziri 2008). Oxidative stress through a number of pathways promotes an inflammatory response while the same can also be induced directly by the same factors that were involved in the onset of oxidative stress. Atherosclerosis is believed to be an inflammatory condition with a strong oxidative stress link or component (Vogiatzi et al., 2009). One of the important pro-atherogenic stages to occur in oxidative stress is the oxidative modification of low density lipoprotein (LDL). Through hydrogen peroxide action on tyrosine kinases, oxidative stress alters the endothelium permeability making it more permeable to lipids (Vogiatzi et al., 2009). The lipids that had moved into the sub-endothelial space due to altered permeability of the endothelium get trapped by fibers produced by the vascular wall cells and eventually get oxidized (Berliner, 1995). Oxidized lipids in the sub-endothelial space are a powerful stimulant of an inflammatory response causing

expression of leukocyte adhesion molecules such as vascular cell adhesion molecule-1, intercellular adhesion molecule-1, endothelial-leukocyte adhesion molecule-1 (Hwang et al., 1997; Cachofeiro et al., 2009; Berliner 1995). Circulating monocytes are as a result bound at the arterial endothelial surface from where they migrate into the sub-endothelial space to become macrophages. These macrophages then engulf large amounts of oxidized LDL and transform into foam cells causing atherosclerotic changes to the vascular wall. Over time smooth muscles join in and start to proliferate and produce extracellular matrix (Cachofeiro et al., 2009).

Oxidative stress may also cause inflammation/atherosclerosis through activation of redox sensitive transcription factors such as activator protein 1 (AP-1) and NF-κB which enhances the production of pro-inflammatory cytokines and chemokines such as adhesion molecules Vascular Cellular Adhesion Molecule 1 (VCAM-1). Pro-inflammatory cytokines and chemokines will cause inflammation and consequently atherosclerosis by among others activating leukocytes and macrophages (Vaziri, 2008; Vogiatzi et al., 2009). It is believed that oxidative stress activates NK-κβ by degrading the inhibitory proteins bound to it and once it is activated, NK-κβ moves to the nucleus where it will activate inflammatory genes leading to production of cytokines and chemokines mentioned above (Cachofeiro et al., 2009).

AII is not only capable of causing vascular injury through inducing oxidative stress but can stimulate pro-inflammatory transcription factor NK-κB as Figure 1.2 shows. Activation of these transcription factors up-regulates expression of adhesion molecules

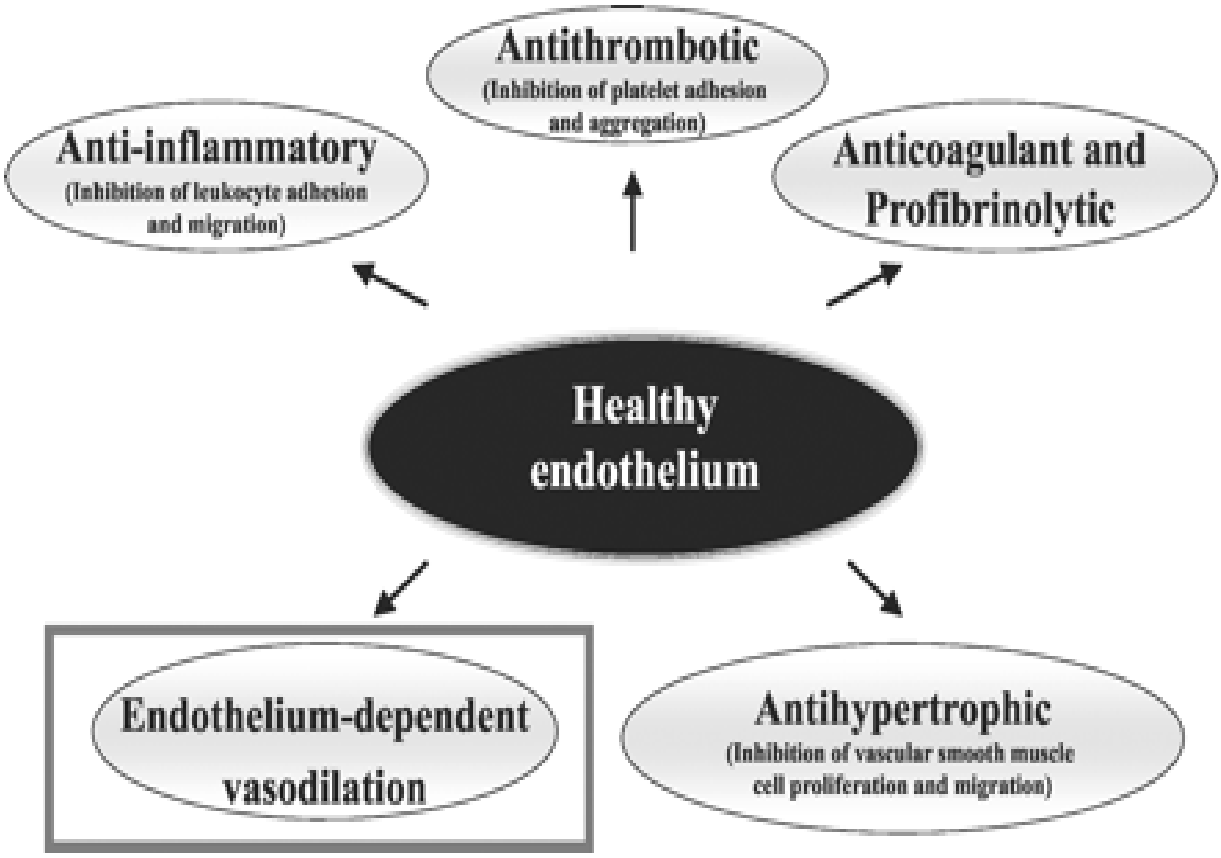
such as VCAM-1 and intercellular cell adhesion molecule-1 (ICAM-1) as well as pro-inflammatory cytokines all of which will induce inflammation. This activation also leads to deposition of extracellular matrix as well as hypertrophy and hyperplasia of vascular smooth muscle cells (VSMC) (Cachofeiro et al., 2009; Vaziri, 2008; Savoia & Schiffrin 2006). AII promotes inflammation also by regulating endothelin-1 production which itself plays an important role of inducing chronic inflammation in the vasculature (Savoia et al., 2006).

The role of inflammatory markers such as C-Reactive protein (CRP), Serum amyloid A (SAA) and Interleukin 6 (IL-6) in the development of atherosclerosis is confirmed by the findings of research (Wang et al 2010; Libby et al., 2002; Lee et al., 2010). In the Honolulu Heart Program, Physicians Health and Framingham Heart studies, the association between CRP and stroke was demonstrated (Elzahwy et al., 2010). One mechanism through which CRP exerts its pro-atherogenic effects is by promoting the recruitment and infiltration of monocytes into the vascular wall as well as the formation of foam cells. In vitro exposure of vascular endothelial cells culture to CRP was found to lead to a heightened expression of adhesion molecules in one study (Papazafiropoulou et al., 2008). In some studies, another inflammatory marker SAA was found in atherosclerotic lesion. SAA is believed to promote atherosclerosis through mostly associating with lipoproteins in blood plasma and subsequently promoting their transport as well as retention in the vascular wall (O'Brien et al., 2005; Hua et al., 2009). Studies have shown that SAA can influence the development of atherosclerosis through direct pathways (Hua et al., 2009). IL-6 is another inflammatory marker that is found elevated

in individuals with atherosclerosis. It has been detected in atherosclerotic lesions as well and is involved in causing endothelial dysfunction, recruitment of inflammatory cells and stimulation of adhesion to the endothelial layer by the monocytes, all of which are important steps in the development of atherosclerosis (Le et al., 2010). These studies demonstrate that inflammatory markers are indeed useful indicators of inflammation that are can be used in clinical settings and research.

1.2.1.3 Endothelial dysfunction

One of the changes to occur in the vasculature during hypertension related oxidative stress is endothelial dysfunction believed to be induced by excessive production of free oxidative radicals. This is another pathway through which oxidative stress contributes to the thickening of the vascular wall. Endothelial dysfunction is one of the events to occur in the early stages of atherosclerosis. Increased levels of ROS leads to them reacting with and inactivating Nitric Oxide (NO), thereby reducing NO bioavailability as shown in Figure 1.2 (Vaziri, 2008; Vogiatzi 2009; Cai et al.,2000). A healthy endothelium synthesizes and releases a number of vaso-active substances such as NO, prostaglandins, AII and endothelin. Among the vasodilators, NO is the most potent, the under-expression of which increases the risk to endothelial dysfunction and development of atherosclerosis.



(Landmesser*et al.*, 2004)

Figure 1.3.Anti-atherogenic properties of a healthy endothelium which includes anti-inflammatory and anti-hypertrophic functions

Besides its vasodilatory function, NO also prevents platelet activation, smooth muscle cell proliferation, production of chemokines and cytokines, leukocyte recruitment and adhesion to the endothelium and their subsequent migration into the vessel wall as Figure 1.3 above shows. These are important prevention steps in the development of the atherosclerotic process (Beckman et al., 2002; Zeiher et al., 1995; William et al., 2002). Endothelial dysfunction therefore abolishes the endothelium's anti-atherogenic properties. Indeed, the key features of endothelial dysfunction have been found to include platelet adhesion/aggregation, increased vascular permeability and leukocyte-endothelial

interactions (Savoia & Schiffrin 2007). An increase in arterial pressure does not only cause altered vascular wall thickness through inducing oxidative stress and its downstream effects as demonstrated above but can do so through VSMC hypertrophic remodeling as well (Berk, 2009; Touyz 2005). Hypertrophic remodeling occurs when the vascular wall due to hyperplasia of VSMC and altered production of extracellular matrix, as some of the factors, encroaches into the luminal space. The cross section of the vascular wall increases and luminal diameter decreases (Touyz, 2005).

1.2.2 Hypertension diagnosis

Hypertension is diagnosed when the arterial blood pressure is more than 140/90 mmHg on a clinic evaluation tool and above 135/85 mm Hg on AMBP (Marchiando and Elston, 2003). Obtaining correct BP measurements for appropriate classification can be a challenge, owing partly to the sensitivity of BP to a number of internal physiological influences. These include anger, excitement and anxiety (Giuseppe et al., 2005). BP, like several other biological factors has a circadian rhythm which involves a blood pressure fluctuation cycle that occurs repeatedly every 24 hours. Each individual has an internal master biological clock consisting of the supra-chiasmatic nucleus that orchestrates this rhythm. Blood pressure regulation is achieved in part through this circadian pattern which rises during the day to help us to prepare in anticipation to day-time challenges (Young, 2006; Kitamura et al., 2002; Shea et al., 2011). In good health, this rhythm exhibits a dipper pattern where BP is high during the day and drops to a low at night (≥10% drop of daytime BP). It rises again in the morning and then gradually declines as the day progresses. Certain abnormal conditions, however eliminate or attenuate this drop in BP,

making individuals susceptible to developing disease. This sub-group of individuals who do not experience low night-time BP, is referred to as non-dippers (Kitamura, et al., 2002; Shea et al., 2011). External stresses such as activity also contribute to the blood pressure variation found in this BP cycle. Human beings are generally more active during the day than at night, hence they record higher BP readings during the day. This pattern of observed difference between day-time and night-time measurements is known as a diurnal pattern. It is largely determined by activity and sleep patterns (Marchiando et al., 2003).

1.2.2.1 Ambulatory versus Conventional (clinic) BP

Clinic or office based blood pressure measurements are BP tools conducted in a medical setting and have been used for a long time to determine the blood pressure classification of patients. Controversy however surrounds their reliability in correctly classifying patients according to their true BP status when compared with other BP evaluation tools. In some studies, clinic based blood pressure measurements have been unequivocally found to provide values that are higher than those of self- measured and ambulatory measurements (Apel and Stason, 1993; Head et al., 2010). This translates into differences in the prediction of target organ damage. Other studies however have not found such a difference. Woodiwiss *et al* found out that high quality clinic BP may have the same prognostic value as AMBP in predicting target organ damage in a sample of people of African ancestry (Woodiwiss et al., 2009).

AMBP is a diagnostic tool that automatically measures a patient's blood pressure at set intervals over a 24-h or 12-h period (Head et al., 2010; Majane et al., 2007). It involves the use of a portable device which is worn and works even as the patient goes about his daily activities, outside of a medical environment. Since it repeatedly measures patient's blood pressure, even when he/she is sleeping, it is viewed as providing a more comprehensive, reliable and consistent analysis of a patient's blood pressure status. AMBP is a more reliable tool in detecting target organ damage and cardiovascular events than conventional blood pressure as it reduces chances of mis-diagnosis and misclassification of BP status (Head et al., 2010; Majane et al., 2007). It captures key events in the BP circadian cycle which includes BP variability which itself covers day/night BP transition periods. Key features of BP variability monitoring include morning BP surge and nighttime BP dipping. The increased risk for stroke and sudden death in the mornings is attributed to a sudden rise in BP in the early hours of the day (Kaplan, 2003). Evidence exists that those who experience a blunted nocturnal decline in BP have significantly increased incidences of thickening of the common and internal carotid intima media layers among others when compared to those with dipping BP (Routledge et al., 2007; Dolan et al., 2005). The ability of AMBP to monitor events such as this and to track any changes to BP and establish patterns makes it a tool of choice.

One of the most important differences between conventional BP and AMBP is the white coat effect. The white coat effect can lead to an overestimation of an individual's BP and is suspected when a patient records a raised BP in a medical environment while they experience a reduced or normal BP on a home or AMBP device. Those whose BP is

raised by the white coat effect into the hypertensive range have white coat hypertension. This group of patients is normotensive on AMBP and hypertensive on clinic BP (Head et al., 2010; Pickering et al., 2002). Alternatively they are labeled as a group that has an abnormal clinic blood pressure and a normal AMBP. The white coat phenomena is believed to be a positive pressor effect on an alarmed patient's blood pressure who is faced with stern doctors in white coats (Chung and Lip, 2003; Gustavsen et al., 2003). To minimize overestimation due to white coat effect and improve on clinic BP quality, several measurements conducted at different hours of the day provide a better assessment of a patient's true blood pressure status than single BP measurements. The white coat hypertensive group falls within those BP groups where there are differences between AMBP and clinic BP (Staessen et al., 2000). While the impression is that white coat hypertensives should not suffer any organ alterations, some studies have proved that they do experience organ damage while others have not. Owens *et al* showed higher cardiac mass in white coat hypertensives which was attributed to hypertension related left ventricular hypertrophy (Owens et al., 1998).

Another BP group which like the white coat hypertensives has varying results between clinic and AMBP is the masked hypertensives. Masked hypertension alternatively is referred to as reverse white coat hypertension because it is the opposite of white coat hypertension in its detection using the two BP tools. It is missed by clinic BP which detects hypertension in white coat hypertension but detected by AMBP that detects no type of hypertension in white coat hypertensives. In some scientific circles, it is termed Undetected Ambulatory Hypertension. This is a group of subjects who are normotensive

by clinic BP but hypertensive by AMBP. Masked hypertension diagnosis causes the most confusion to clinicians than any other BP diagnosis as patients appear normal through routine BP evaluation which obliterates the need for further assessment specifically target organ damage. This type of hypertension is real and was first detected in the OHASAMA study where 10.2% of the clinically normotensive individuals, were either found to be borderline hypertensives or true hypertensives on AMBP. On assessing and comparing the target organ damage of this group with the normotensives, one study by Pickering et al found that masked hypertensives had a higher left ventricular mass and carotid atherosclerosis (Pickering et al., 2002).

A third BP group emanating from the use of both the two BP evaluation tools discussed above is that of patients who are classified hypertensive by both AMBP and clinic BP. This group is known as the true or sustained hypertension group and there is no discrepancy in the diagnosis reached using different tools. The fourth BP group which like the true hypertension group demonstrates consistency in terms of classification by AMBP and clinic evaluation tools is the normotensive group with subjects being normotensive on both clinic and AMBP. Some researchers have more confidence in these kind of results where the same classification of an individual's BP status is arrived at by both AMBP and clinic BP (Pickering et al., 2002).

1.3 Co-Variables

For purposes of this study hypertension was regarded as the main risk factor inducing changes on C-IMT which makes the rest of the conventional risk factors confounders. It

must be noted that the prevalence, treatment, patho-physiology as well as contribution to atherosclerosis of these risk factors will differ compared to those found in similar investigations. One explanation for such differences is the fact that such studies were conducted on caucasians. In a systemic review of published population based studies that had one or more ethnic comparisons on cardiovascular risk factors, Kurian *et al* concluded that indeed significant ethnic differences existed in the incidence and prevalence of these risk factors (Kurian and Cardarelli, 2007;Ferdinand et al., 2007; August et al., 2000). Such risk factors include obesity, arterial stiffness, age, smoking, diabetes mellitus and alcohol and will henceforth be adjusted for (Tierney et al., 2000; Meydani, 2000; Kiechl et al., 1998).

1.3.1 Obesity

Obesity is one cardiovascular risk factor that significantly contributes to the global disease burden owing to its high prevalence. In 2000, about 115 million people in developing countries which include those in the continent of Africa, were reported to be suffering from obesity related disorders (Rutengwe et al., 2001). In South Africa the prevalence of obesity has increased dramatically over the years with one study reporting approximately 65% of the population of African ancestry as being overweight or obese (Majane et al., 2007).

In assessing the risk emanating from excessive fat accumulation, fat distribution is an important factor. Central or abdominal obesity is a type that is more common in males and is characterized by upper body fat accumulation in areas that include around the

viscera. In pre-menopausal women, peripheral obesity is more common. Of the two types, abdominal obesity carries more risk owing to high lipolytic activity of visceral fat (Tariq et al., 2005; Fredrik et al., 1997). Lipolysis readily produces large amounts of free fatty acids and glycerol into portal venous circulation causing ultimately an increased production of very low density lipoprotein, the dangerous cholesterol which promotes atherosclerosis (Fredrik et al., 1997). Evidence exists that suggests that visceral fat is a more active source of adipokines than sub-cutaneous fat (Ibrahim, 2010; Wang et al., 2010*).* A cross sectional study performed in a population with a wide age range (20-75 years) showed measures of abdominal obesity to correlate with sub-clinical atherosclerosis evaluated by C-IMT better than body fat percentage and Body Mass Index(BMI), an index of generalized obesity, independently of diabetes or hypertension (Fredrik et al.,1997).

Men have hence forth been found to demonstrate a higher free fatty acid mobilization from visceral fat than women. A man who has the same amount of excess fat as a woman is likely to be at an increased risk of developing cardiovascular disease due to the preferential deposition of fat around the viscera that occurs in males as well as the high lipolytic activity of such fat (Frederick et al., 1997; Karastergiou et al., 2012.). Among the females, menopausal status is the determining factor in the distribution of fat in the body. In pre-menopausal women, peripheral obesity predominates while in post-menopausal, fat deposition resembles that of males (Tariq et al., 2005).

Obesity is not only a cosmetic problem that affects an individual's self esteem but a complex chronic medical one as well. These include atherosclerosis and hypertension (Poirier et al., 2006). The mechanisms though which obesity causes atherosclerosis include inducement of inflammation, of which its atherosclerotic causing mechanisms have already been discussed. The adipocytes of obese people undergo molecular and cellular changes that render them functionally distinct from adipocytes of people of normal weight. They release high amounts of pro-inflammatory markers which are known to cause cell apoptosis. Cell apoptosis attracts macrophages to the adipose tissue where they participate in the clean-up campaign. These activated macrophages together with the local adipocytes will in the process secrete substances that promote the local inflammatory process. In a study by Esposito et al, it was found that the expression of vascular inflammatory markers was directly proportional to the level of obesity. The resulting local inflammation soon spreads out and induces inflammation at a systemic level. The high serum levels of pro-inflammatory molecules such as Interleukin-1 (IL-1) and IL-6 being components of systemic inflammation, will at a vascular level cause inflammation of the wall leading ultimately to onset of the atherosclerotic process (Wang et al., 2010).

Obesity also promotes oxidative stress in the vasculature. One route through which a state of oxidative stress is promoted in obesity is by the depletion of antioxidant molecules in the body. In one study done to assess the serum levels of antioxidant substances, tocopherol and β-carotene, in obese and non-obese children, the tocopherol and β-carotene levels were lower in the obese group (Khan et al., 2006). As the antioxidant

defense is compromised, certain processes such as the increased myocardial oxygen consumption produces increased amounts of ROS. Obesity places a higher mechanical demand on the heart leading to increased oxygen consumption. Elevated oxygen consumption leads to excess production of ROS such as superoxide and it is this imbalance between decreased antioxidants levels and elevated ROS production that will cause oxidative stress and contribute to the development of atherosclerosis (Khan et al., 2006). Obesity can also cause atherosclerosis and thickening of the vascular wall through inducing hypertension which has already been shown to cause atherosclerosis. Even though obesity is in this paper discussed separately from hypertension, the two have a strong relationship and often co-exist. The Framingham Health Study reported 70% of new essential hypertension cases to be linked to excess adiposity (Kotsis et al., 2006).

In the Framingham Heart Study Multidetector Computed Tomography study, systolic blood pressure (SBP) in men increased by 3.3mmHg for each 1 standard deviation (SD) increase of visceral adipose tissue and 2.3mmHg for each SD increase in sub-cutaneous adipose tissue (Fox et al., 2007). In another study by Majane *et al* conducted on people of African ancestry living in South Africa and investigating the association of waist circumference (an index of abdominal obesity) with AMBP independent of alternative adiposity indices every 1 SD (15cm) increase in waist circumference corresponded to 4.04 mmHg increase in 24-h systolic and 4.33 mmHg in 24-h diastolic BP after adjustments for potential confounders (Majane et al., 2007).The association between obesity and atherosclerotic causing mechanisms discussed above is further confirmed by findings of studies that found correlations between obesity and C-IMT independent of

blood pressure (Ciccone et al., 2001; de Michelle et al., 2002; Lo et al., 2006; Koskinen et al., 2009; Maher et al., 2009; Skilton et al., 2009). Ciccone *et al* found associations between C-IMT and leptin concentrations, a marker of obesity (Ciccone et al., 2001).

1.3.2 Arterial stiffness

One important property of the arteries is their compliance, the ability to expand and recoil in response to pressure changes during the cardiac cycle. Arteries with a reduced distensibility or cushioning capacity to blood pressure are said to be stiff (Olivier and Webb, 2003). A stiff artery is not protected against systolic blood pressure which makes the artery more susceptible to assaults from shear stress and increased flow velocity (Olivier and Webb, 2003). Increase in shear stress and flow velocity potentially will promote atherosclerosis through the pathways already discussed. Blood pressure increases as it flows through stiffer, narrower vessels. Arterial stiffening may result from aging or be disease related. Conditions such as hypertension and obesity are known to cause arterial stiffness and will only be discussed in that context in this section (Savoia & Schiffrin 2006).

Obesity has negative effects on the compliance of both large and medium sized arteries (Acree et al., 2007; Safar et al,. 2006). In one study comprising of subjects of normal weight, over-weights and the obese, it was found that the level of arterial stiffness was highest in the obese group for both the large and medium sized arteries. This was followed by the over-weights and lastly the normal weights. Confounding factors on arterial stiffness were eliminated by excluding diabetics, smokers and also by adjusting

for hypertension (Acree et al., 2007). Plausible explanation for arterial stiffness in obesity is the presence of endothelial dysfunction that results from the action on the endothelium of factors such as CRP and IL-6 produced by excess adipose tissue (Acree et al., 2007). Hypertension however can be a cause or a patho-physiological consequence of arterial stiffness (Sasamura et al., 2005). One mechanism through which hypertension causes arterial stiffness is by causing an overproduction of collagen fibers (Zieman et al., 2005). Collagen fibers found in all the layers of the vasculature including the intima and media layers provide the necessary strength to prevent overexpansion and their enhanced production as happens in hypertension is one of the important mechanisms through which the vascular wall is thickened (Sasamura et al., 2005; Cernes et al., 2008). Hypertension also triggers eutrophic remodeling which involves the reorganization of the vascular wall components around a reduced lumen diameter. The vessel loses its elasticity and becomes stiff (Touyz, 2005).Stiff arteries result in widened pulse pressures (PP) and isolated hypertension. Isolated systolic hypertension would through flow velocity and shear stress worsen atherosclerosis. Pulse pressure is the difference between systolic and diastolic blood pressures. A widened PP is characterized by low diastole and high systole and if systole is above the threshold for normal then the patient has isolated hypertension (Olivier and Webb, 2003).

Lastly arterial stiffening may be a part of the normal aging process and not due to any pathology. Aging of the arterial wall may cause structural changes such as splitting and degeneration of the elastic fibers, VSMC proliferation, increase in collagen fiber production with arteries losing their distensibility in the process (Carallo et al., 1999; Lee

and Oh, 2010). Aging in the elderly can also be caused by accumulated calcium deposits in the vasculature. Calcium deposition in the blood vessels increases with age especially in those individuals who are older than 50 years (Lee and Oh, 2010).

1.3.3 Age

The atherogenic nature of ageing is usually explained by what is termed the "free radical theory of aging". This theory states that with aging, there is a shift in the oxidant-antioxidant balance in the body leading to a state of oxidative stress, whose association with atherosclerosis has already been discussed. Humans experience a reduction in the efficiency of their antioxidant defense systems with aging. The body fails to deal with any new oxidative stress challenges as well as the accumulated free radicals from the past. The individual becomes vulnerable to developing oxidative stress, atherosclerosis and other age related conditions (Meydani, 2000). Aging also enhances production of NADPH oxidase-derived ROS which will also contribute the progression to oxidative stress (Collins et al., 2009). The rate of C-IMT growth in aging humans is reported to be in the range of 0.008 to 0.0147 mm/yr such that 0.1 mm translates into an expected change over 7 to 10 years (Polak, 2009).

1.3.4 Smoking

Research has shown the existence of an association between active cigarette smoking and atherosclerosis (Ambrose and Barua 2004; Fan and Dwyer, 2009; Jiang et al., 2010). In one study, passive and active smoking were both found to associate with a consistent increase of the carotid artery intima-media thickness. With regard to passive smoking,

this proves the dangers of environmental exposure to cigarette or tobacco smoke (Ambrose and Barua, 2004). One pathway through which tobacco use causes atherosclerosis is its effects on the vascular endothelium cells. Endothelial cells exposed to smoke undergo necrosis which is cell death. The dying cells release pro-inflammatory factors, which will trigger an inflammatory process. As the endothelial cells die, they change morphology making the endothelium leaky and thus allowing direct access to the vascular smooth muscle cells below by the plasma. Factors in blood plasma stimulate the smooth muscle cells to proliferate and grow the vessel wall (Fan and Dwyer, 2009).

The effect of cigarette smoke on the endothelium leads to a reduced bio-availability of NO and consequently endothelial dysfunction. Cigarette smoke comes out in two phases, the tar and gas phases. Both phases are active sources of free radicals that promote the onset of a state of oxidative stress. Cigarette smoking would therefore increase the oxidation of LDL, a powerful pro-inflammatory event. Cigarette smoke was found to be associated with high levels of inflammatory markers such as C-reactive protein and interleukin-6 in in-vivo studies (Ambrose and Barua 2004). The abolished protection of NO together with increased levels of pro-inflammatory molecules would lead to leukocyte recruitment to the vascular wall (Ambrose and Barua 2004).

The pro-atherogenic effects of smoking on the IMT complex however seem dependent on gender. In the Los Angeles Atherosclerosis Study (LAAS), the carotid intima media thickness (C-IMT) of the never smoked, current and former smokers groups was compared. Amongst men, current smokers had the highest C-IMT followed by former

smokers and lastly the never smoked group. This was however not the pattern amongst the women where no significant differences in C-IMT could be noted between the three categories of smoking classification. The explanation for this is that cigarette smoking in women is not only associated with carotid intima media thickening alone but also with medial atrophy. Medial atrophy would of-course counteract the increase in wall thickness caused by atherosclerosis, with the over-all effect being no significant change in the wall thickness of the smokers group (Fan and Dwyer, 2009). This was the same finding in another study where current smoking in men associated with a thicker wall of the common carotid artery while in women the thickening of the vascular wall was counteracted by the thinning of the same resulting in no association between current smoking status in women and an increased common carotid artery wall (Fan et al., 2006).

1.3.5 Diabetes mellitus

The mechanisms through which diabetes causes atherosclerosis and vascular wall thickening include inducement of hyperglycemia and insulin resistance. Diabetes mellitus is one condition that amplifies the risk for cardiovascular disease especially those of an atheromatous nature (Beckman et al., 2002). Seventy five percent of diabetics die from myocardial infarction (Maiti et al., 2007). Diabetics also 150-400% increased risk for stroke (Beckman et al., 2002). In the Atherosclerosis Risk in Communities (ARIC) study a positive relationship was established between C-IMT and two markers of diabetes, abnormal glucose metabolism and fasting plasma insulin (O'Neal et al., 2003).

Hyperglycemia can lead to high BP and atherosclerosis through mechanisms that include fluid retention, changes in insulin uptake by cells and reduced ability distensibility of the vasculature. Amongst the diabetics those not on treatment or any good glycemic control regimen, have a poor prognosis and will go on to develop complications such as atherosclerotic changes (O'Neal et al., 2003). Markers of diabetes, insulin resistance and hyperglycemia are heavily involved in blocking NO synthesis and bioavailability (Beckman et al., 2002; Creager and Luscher 2003). Hyperglycemia causes endothelial dysfunction by preventing eNOS (endothelial Nitric Oxide synthase) activation. It also stimulates endothelial and vascular smooth muscle cells to produce more reactive oxygen species leading to oxidative stress as discussed in the preceding sections of the current report. Through these mechanisms, hyperglycemia induces an increased stimulation of activator protein-1 and NK-κB which in turn activates genes that secrete leukocytes attracting chemokines and leukocyte cell adhesion factors. In this regard the atherosclerotic progress is initiated or enhanced (Beckman et al., 2002).

Insulin resistance promotes atherosclerosis by stimulating lipolysis leading to release of lots of free fatty acids. Excess free fatty acids pro-atherogenic effects include enhanced production of ROS and inhibition of phosphatidylinositol-3(PI-3) kinase which promotes the activity of eNOS. Through fatty acid production insulin resistance does not only promote vasoconstriction through reduced levels of NO but also increased production of vasoconstrictors such as Endothelin-1. Endothelin-1 in addition to its vaso-constrictory properties stimulates VSMC hypertrophy as well (Beckman et al., 2002).

1.3.6 Alcohol

Alcohol is more notorious as a source of social ills and its deleterious effects on body structures such as the liver but not much is known of its effects on the vasculature. Alcohol consumption per se does not always cause vascular injury but its effects are dose dependent. Scientific studies have demonstrated that light and regular use of alcohol actually confers some cardio-protective benefits (daLuz and Coimbra, 2004; Kiechel et al., 1998). In a 5 year study by Kiechl *et al*, the anti-atherogenic or pro-atherogenic effects of alcohol on the carotid artery were demonstrated where alcohol was consumed in varying amounts and frequencies. In a study that involved abstainers and occasional drinkers, heavy drinkers and light but regular drinkers, the atherogenic changes were studied. The abstainers and the occasional drinkers groups did not show any atherogenic changes and scored less on the risk scale. On the extreme end on the risk scale were heavy drinkers who demonstrated the highest risk for atherosclerosis development from alcohol consumption (Kiechl et al., 1998).

Heavy drinkers experience LDL oxidation which is a powerful pro-atherogenic event (Sacanelle and Estruch 2003). Indeed in another study heavy drinkers without any cardiovascular risk factors were found to have developed higher serum levels of inflammatory molecules such as ICAM-1 (Sacanelle and Estruch, 2003). In Kiechl *et al* study, light but regular drinkers was the only group in which alcohol intake was found to have had some beneficial impact on either the onset or progression of the atherosclerotic process. The transition from benefits associated with light but regular alcohol intake to injury of heavy drinking is explained either wholly or in part by the onset of alcohol

induced hypertension as the drinking pattern becomes heavier. Alcohol consumption influence on atherosclerosis is therefore dependent on the pattern and manner of its consumption (Kiechl et al., 1998; Sacanella and Estruch, 2003). In this regard in multivariate analysis, when adjusting for alcohol consumption, I will do so for heavy drinkers and not for any other alcohol consumption group.

1.4 C-IMT as a Marker of Atherosclerosis

The blood vessel wall consists of three layers or tunics namely tunica adventitia, tunica media and tunica intima. The adventitia is the outer covering of arteries and veins comprising of elastic fibers and collagen fibers. Elastic fibers allow the vessels to stretch and absorb blood pressure as it changes in the system while collagen fibers prevent over-expansion. The middle layer, the media is composed of elastic fibers and smooth muscles and is stretched as well with pressure increases. The inner layer, the intima tunica, is an elastic membrane lining composed of smooth endothelium at its interface with the lumen. C-IMT measurements therefore refer to the combined thickness of the intima and media layers of carotid arteries (Bauer et al., 2012).

C-IMT is an established tool for the detection and progression of atherosclerosis which is linked to a number of cardiovascular conditions. It does not only provide information on the atherosclerotic related risk in the carotid artery alone but also in a number of other territories in the body. C-IMT increases with the development of atherosclerosis in other body regions making it possible to make inferences about other unexamined and perhaps

hard to reach blood vessels such as coronary, supra-aortic and renal arteries (Kablak-Ziembicka et al., 2007).

The significance and sensitivity of using C-IMT changes as a marker to detect disease was demonstrated in one study which assessed the C-IMT in about 100 young patients with no evidence of cardiovascular disease. Of these, 75% had a coronary artery calcium score of 0 which suggested absence of sub-clinical vascular disease. On further assessment with another tool, this supposedly coronary artery disease free subjects, recorded an annualized cardiovascular event rate score of <1% on the Framingham Risk Score test. However, when evaluated even further using C-IMT, 34% had carotid plaques and 13% had C-IMT of more than 75th percentile which represents a high risk status (Cobble and Bale, 2010).

Since C-IMT can detect asymptomatic arterial wall changes, it is an excellent tool for discovering non-obstructing atherosclerosis (Spence et al., 2006). This is of great clinical significance as a large portion of cardiovascular events involve non-stenotic arterial plaques (Little et al., 1988). The use of B-mode ultrasound for measurement of carotid atherosclerosis is widely preferred for its simple, non-invasive and reliable detection of pre-clinical atherosclerosis (Chambless et al., 1997; O'Leary et al., 1999; Su et al., 2001; Sharma et al., 2009).

Some studies have however found that in certain groups, the use of C-IMT without carotid plaque significantly reduces predictive power of C-IMT which means that they

may be need for proper patient selection if C-IMT alone is to be used (Nambi et al., 2010; Stein et al., 2008). While C-IMT development in men is fairly common, that is not the case with women. In women, the addition of C-IMT to multivariate analysis models that include plaque presence, does not change classification of individuals according to risk level. Since C-IMT is generally lower in women, those that it classifies as being in the seventy fifth percentile are infact misclassified as high risk while they are not. The presence of plaques in women instead signifies a high atherosclerotic development. Conversely, C-IMT is fairly common in men and addition of C-IMT to a model that already has plaque presence would be beneficial. Addition of carotid plaque to a model of C-IMT would not cause re-stratification (Nambi et al., 2010). In this regard, the use of C-IMT alone without consideration for factors such as gender, seem controversial. The use of both C-IMT and carotid plaque is perceived to improve CVD risk assessment (Nambi et al., 2010; Stein et al., 2008).

While cardiovascular events between groups of different ethnicities are usually attributed to either the prevalence or resulting patho-physiology of conventional risk factors, some C-IMT growth seem inherent and cannot be explained by such risk factors (Ferdinand et al., 2007; August et al., 2000). In one study in the United Kingdom (UK) black African-Carribean adults were found to have increased C-IMTs than their caucasian counterparts. Interestingly, these ethnic differences in C-IMT were actually apparent even in childhood which implied that they could not be explained by exposure to conventional cardiovascular risk factors (Whincup et al.,2012).

1.5 Problem statement

Urban developing communities of African ancestry living in Africa are undergoing the early phases of an epidemiological transition brought about by the adoption of new lifestyles. Diseases of an atheromatous nature, previously unknown are now common. Finding the best risk assessment methods is therefore imperative. Although in caucasians in developed countries, AMBPis by far the best BP tool to assess target organ changes that include C-IMT, recent data from our laboratory shows that high quality clinic BP is as closely associated with a number of target organ changes that do not include C-IMT as AMBP.

To my knowledge no population based study has provided answers on the relationships between AMBP and BPc with C-IMT in a population of people of African ancestry that is in the early phase of an epidemiological transition.

1.6 Research Aim:

The aim of the study was as follows;

a). To investigate whether blood pressure tools, AMBP and clinic BP associate with C-IMT

b). To investigate independent relationships of AMBP and clinic BP with C-IMT

CHAPTER 2

MATERIALS AND METHOD

2.1 Ethical clearance

Ethical approval for the study was granted by the University of Witwatersrand Committee for Research in Human Subjects (approval number: M02-04-72) and the study was conducted according to the principles outlined in the declaration of Helsinki. This study is a component of an on-going African Project on Genes in Hypertension (APOGH) which was initiated in 2003. The aim of the APOGH study is to identify the environmental, phenotypic and genetic determinants of cardiovascular risk factors and target organ damage on subjects of African ancestry.

2.2 Study participants

A total of 446 participants of African ancestry were randomly recruited from families living in South West Township (SOWETO), in Johannesburg, South Africa. SOWETO is a cosmopolitan township with the Sotho, Zulu and Venda tribes being the most predominant. Of the 446 participants, 320 had ambulatory and conventional blood pressures. Since ambulatory blood pressure was the limiting parameter the total number of participants that were statistically analysed was 320. Prior to the commencement of the study, each participant voluntarily gave a written informed consent for participation. In preparation for the glucose tolerance test, participants fasted for at least 8 hours while smokers refrained from smoking until after the tests.

2.3 Site of Study

Most of the measurements were performed at the Cardiovascular Patho-physiology and Genomics Research Unit of the School of Physiology, University of the Witwatersrand,

Medical school campus in Johannesburg. Only the clinical work was performed elsewhere at Lynsfield laboratories, an accredited clinical laboratory in Johannesburg.

2.4 Demographic and Anthropometric data

A standardized questionnaire was administered to obtain information on the clinical history and family history, current and past treatments for diabetes mellitus and hypertension, current and past pregnancies, menopausal status, tobacco use and alcohol intake. Trained study assistants explained and assisted the participants with completion of the form.

General obesity was measured as BMI which was calculated as weight in kilograms divided by the square of height in meters. Height measurements were performed with the participants standing, wearing indoor clothes (gowns) with no shoes. The following BMI categories were used: below 20 kg/m^2 were categorised as being underweight, 20-24.9 kg/m^2 as being normal weight, 25-29.9 kg/m^2 as overweight, 30 kg/m^2 and above as being obese. Sub-cutaneous obesity was determined from the skin-fold thickness measurements. Briefly a Harpenden skin-fold callipers was used to measure skin-fold thickness at the triceps and sub-scapular regions and the mean of these values obtained for statistical analyses.

Central adiposity was measured as waist circumference (WC) and waist-to-hip ratio (WHR). Measurements of WC were conducted at the end of a gentle expiration, at a point midway between the lowest rib and the iliac crest with the subject in a standing position.

WC measures of more than 102cm and 88cm for men and women respectively confirmed presence of obesity. WHR was calculated from the hip circumference values, measured at the widest part of the hips with the participant standing and the WC values using the same approach previously described in WC. The values thus obtained were used to calculate the waist-to-hip ratio calculation. WHR values of more than 1.0 for men and 0.8 for women were interpreted to indicate presence of obesity.

2.5 Clinical laboratory measurements

A trained technician obtained blood samples from participants, appropriately preserved them to maintain their integrity and sent them to an accredited laboratory for clinical analysis. The use of insulin or oral hypoglycaemic agents or values of percentage glycated haemoglobin (ghb or HbA_{1C}) (Roche Diagnostics, Mannheim Germany) over 6.1%confirmed presence of diabetes mellitus or inappropriate blood glucose control, considered as a single covariate. Menopause status was confirmed with measurements of follicle stimulating hormone (FSH) concentrations (Bayer, Leverkusen, Germany).

2.6 Blood pressure measurements

Conventional blood pressure-A trained nurse using a sphygmomanometer measured brachial artery systolic and diastolic blood pressure using the standard protocol (O'Brien et al., 2003).Each participant was allowed to sit with legs uncrossed and fitted with an appropriate sized cuff that has an inflatable bladder on the left arm (Figure 2.1). Care was taken to prevent cuff hypertension by not choosing a cuff of a smaller size. Inflatable bladders measuring 22 x 12cm were used on those with smaller arm circumferences

while those with larger ones cuffs with bladders of 31 x 15cm were preferred. The patient's arm was made to rest on an object to prevent isometric exercise at the level of the heart or nearer (O'Brien et al., 2003) After 5 minutes of rest 5 successive measurements were carried out and their average deemed as the patient's systolic and diastolic BP readings.

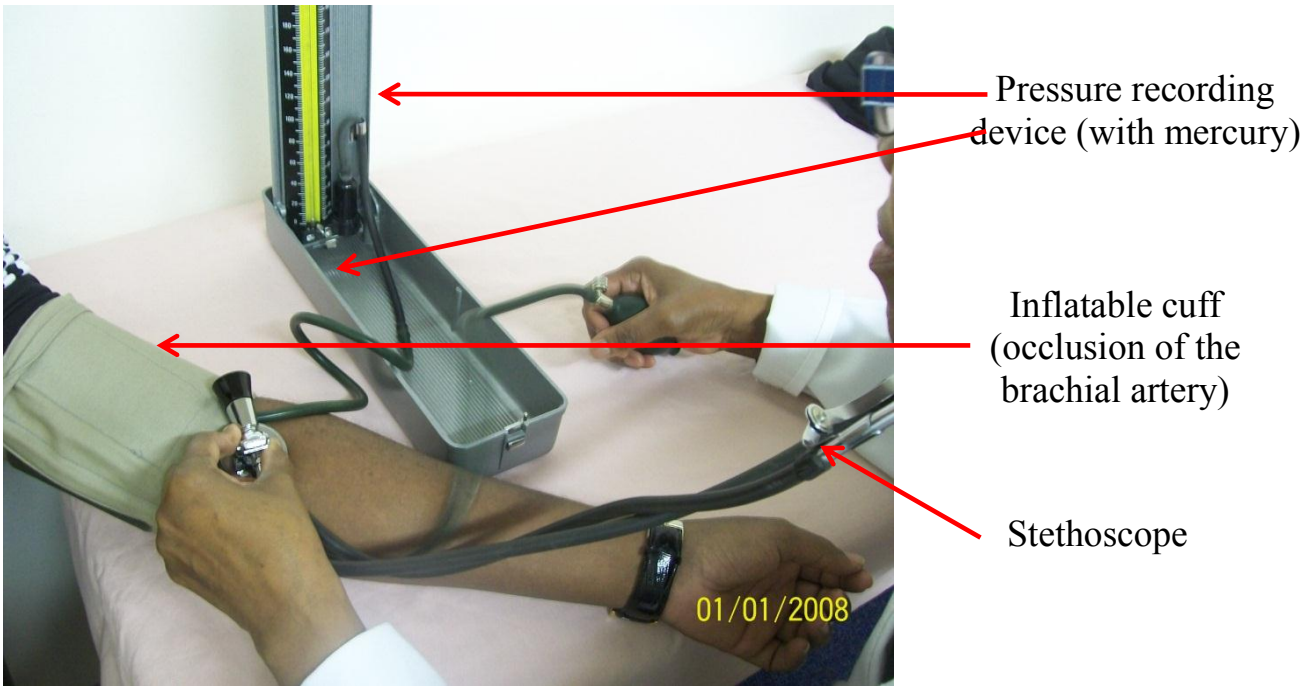


Figure 2.1.Demonstration of a clinic or conventional BP measurement showing a sphygmomanometer, recording device, inflatable cuff, stethoscope. Note the position of the cuff, over the brachial artery and the patient's arm resting on a table at the level near the heart's.

Ambulatory blood pressure- AMBP was recorded on the same day as the conventional blood pressure using oscillometric monitors (SpaceLabs, model 90207; Spacelabs, Redmond, Washington, USA).The monitor's calibration had been checked against a

mercury manometer and this was done monthly. The size of the cuff used was the same as that for BPc. Since patients went home with the oscillometric monitors they were given instructions on how to operate them for the 24-hour period that they wore them. The monitors were programmed to measure BP at 15-minute intervals between 06:00 to 22:00 and then 30-minute intervals from 22:00 to 06:00. Subjects were issued a diary card for the duration of the recordings to note the time of going to bed in the evening and the time of getting up in the morning and this was then used to determine the awake and sleeping periods of the patient. The day- and night-time periods were set as time intervals between 09:00 h to 19:00 h and from 23:00 h to 05:00 h respectively based on the activity pattern of an average participant. The breaks between the day- and night-time intervals are so defined in order to eliminate the transition periods (evening and morning) during which there are significant BP variations in most subjects (Staessen et al., 1997). AMBP data was expressed as 24-hour, daytime and night-time average systolic and diastolic BP. Hypertension was defined as SBP24 $\geq$ 135mmHg and/or DBP24 $\geq$ 85 mmHg and/or hypertension treatment.

2.7 Arterial Stiffness Measurements

These measurements were carried out using a SphygmoCor Pulse wave velocity System (AtCor Medical Holdings, Sydney, Australia). This is a computerised non-invasive system that measures pulse wave velocity in a segment of the arterial tree and henceforth gives the stiffness of that particular segment. A patient was made to rest on a bed in a supine position and a radial artery and its pulsations felt by palpations before a lead probe

was placed on the same spot (spotted radial artery) and measurements taken and displayed on the machine screen.

2.8 Carotid intima-media thickness

Intima media thickness of the common carotid artery was determined using a SonoSite (SonoCalc^{TM} IMT) version 3.4 using doppler imaging (B-mode ultrasonography) with a HFL38X/13-6 MHz (Figure 2.2).The patient's data and images were captured and saved on the ultrasound system (SonoSite MicroMaxx, USA) then transferred to a personal computer using SiteLink Image Manager for manual examination and confirmation of the C-IMT at a later stage.

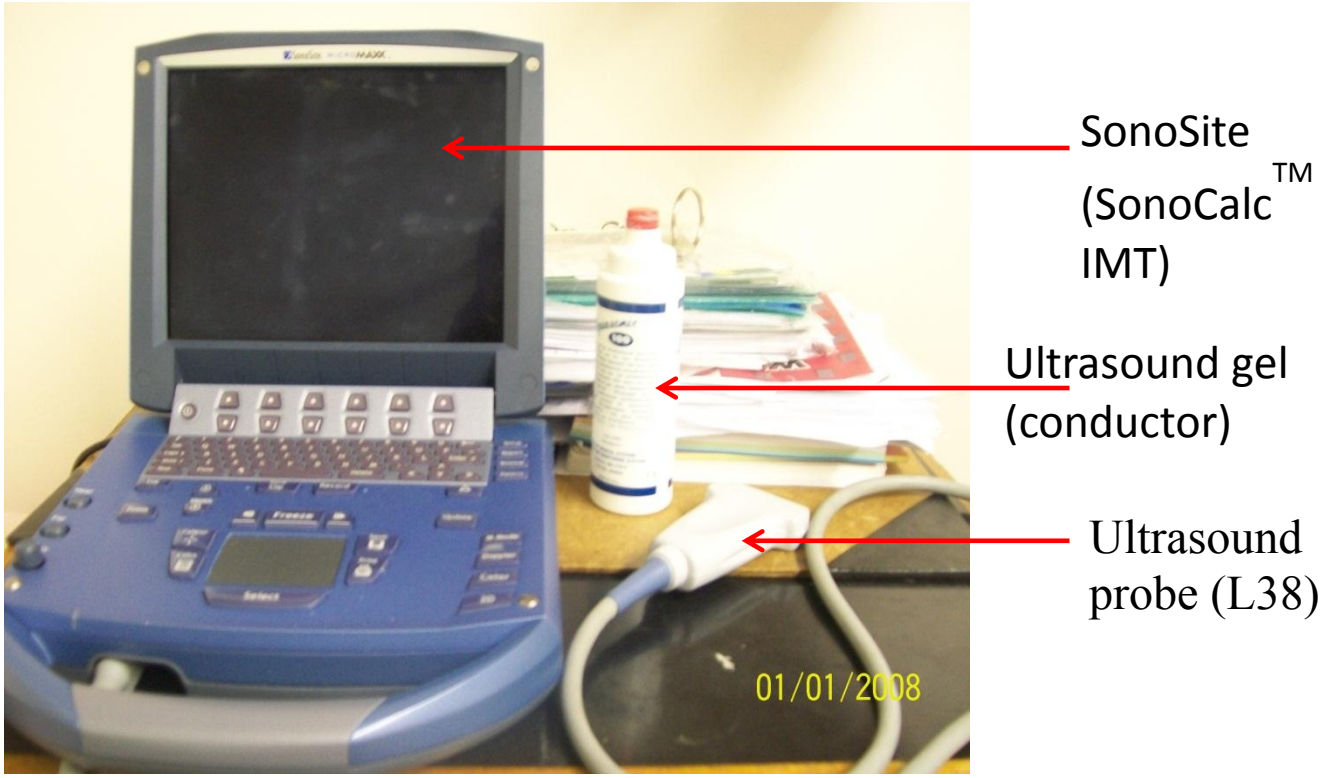


Figure 2.2 The SonoSite (SonoCalc™ IMT) version 3.4ultrasound MicroMaxx system with linear probe and ultrasound gel**.**

2.8.1 Scanning procedure – C-IMT exam

After changing into a patient gown, a participant was asked to lie on an examination table (or bed) in a supine position, while the patient's identification data including participation number, age, gender, ethnicity, BP was entered on the ultrasound system and a file set up. The participant was then asked to tilt his/her head in the direction opposite that of the side being examined. A warm ultrasound gel (SSEM Mthembu Medical [Pty] Ltd, South Africa) was then applied to the linear ultrasound probe (L38) to act as a conductor, hence enhancing image quality and also eliminates air bubbles that could be trapped between the participant's skin and the ultrasound probe.

The carotid artery was first located by placing the transducer low in the neck in a transverse orientation to the vessel's longitudinal axis and with the orientation marker facing posteriorly. The probe was then moved slowly up the neck until the anatomical landmarks of interest namely the common carotid artery (CCA), thyroid gland and the jugular vein were clearly in view. This generally allowed for the vessel's identification and overview of the surrounding structures. The probe was then placed on the carotid artery and turned to a horizontal orientation until the vessel's longitudinal motion visuals with clear contrast showing different layers of the wall appeared on the screen (Figure 2.4). Slight pressure was applied on the vessel so as not to obliterate the vessel's pulsations as systole and diastole alternate. Motion visuals were frozen in diastole and not systole because the vessel wall thins out in systole and images saved and analysed.

C-IMT measurements were carried out on the left carotid artery as opposed to the right or bilaterally (both left and right carotid arteries). A recent review by Lee *et al* suggests no significant difference between C-IMT measured from the left and right carotid arteries (Lee et al., 2011).The left carotid was preferred because C-IMT measurements on the left carotid arteries in general and hypertensive populations are slightly higher than in the right probably due to the left carotid artery's proximity to the heart (Denarie et al., 2000).

Of the three carotid artery's segments, the bifurcation, common and internal carotids, the common carotid was preferred as the site of measurements. To examine the common carotid segment the transducer was placed at least 10mm away from the bifurcation or bulb (see Figure.2.3). The common carotid artery segment has the highest visualization of

the three which is attributed to its relative linear or tubular shape and superficial location which makes universal accessibility easier (Urbina et al., 2009; Roman et al., 2006).Most importantly is that the common carotid artery has significant correlations with CVD risk factors as well as stroke compared to other arterial segments (Urbina et al., 2009; Roman et al., 2006).

Measurements were also conducted on the CCA's far and not the near wall. To obtain the carotid artery far wall's images, the transducer's rotation that occurred above was such that the orientation marker faced superiorly (towards the head of the patient), to be in line with both the neck's and carotid artery's longitudinal axis. Once the carotid artery far wall was in focus the measurement of the IMT complex was carried out using the computerized edge detecting system (SonoSite MicroMaxx)that uses algorithms to analyze ultrasound (doppler) images (one at a time)and calculate the IMT value. The wall was viewed from lateral, posterior and anterior angles andapproximately three to six measurements were taken (Urbina et al., 2009). The average IMT of these measurements was taken as the subject's C-IMT and then compared to results of large reference populations (Stein et al., 2008), Table 2.1, and interpreted for cardiovascular disease risk assessment based on percentile analysis. Measurements of the far wall are more accurate and demonstrate less variability than those of the near wall (Urbina et al., 2009).

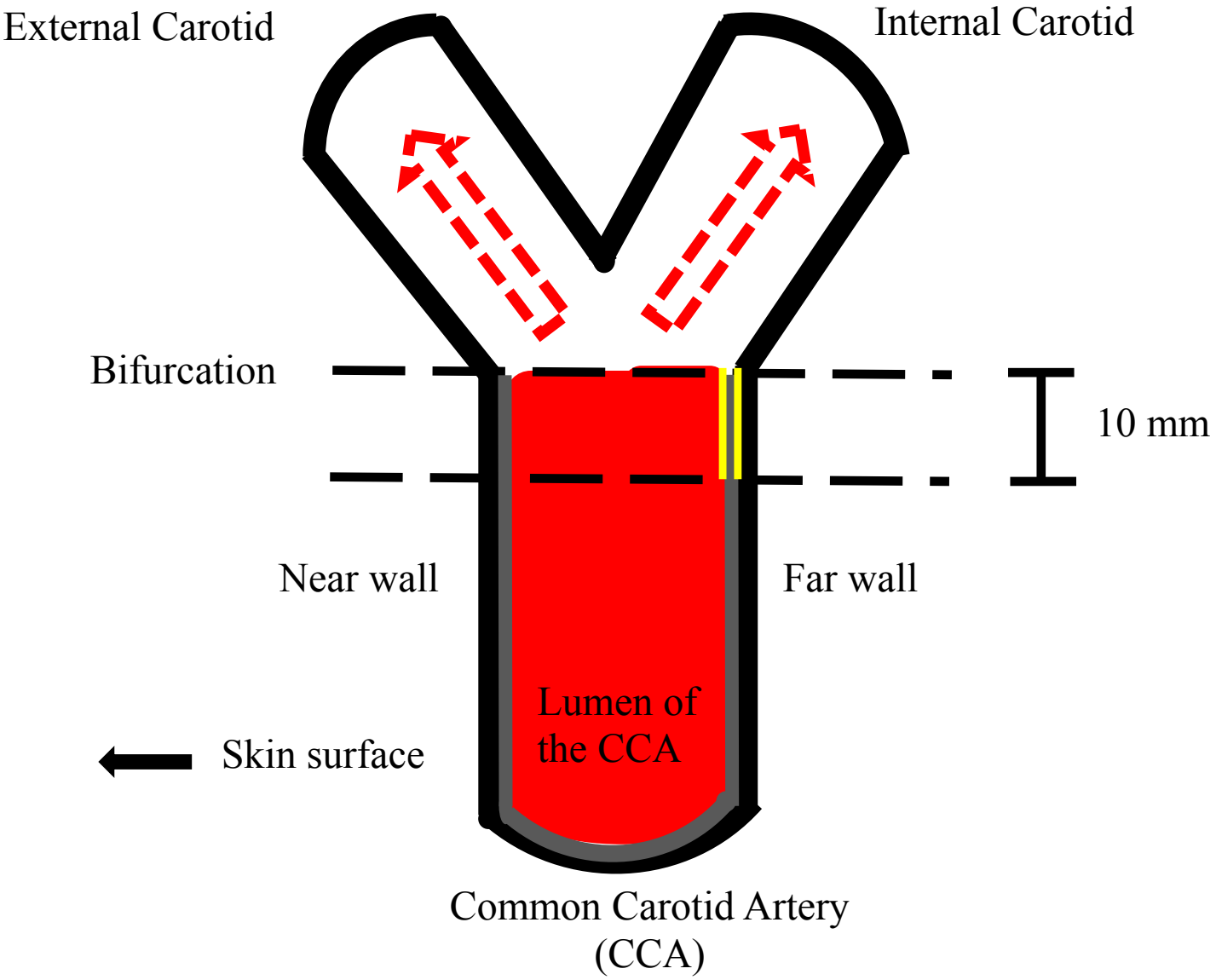


Figure 2.3. Carotid artery showing the common carotid, internal and external carotid segments, point of bifurcation as well as the near and far walls. Note also the portion of the CCA, 10mm away from the bifurcation or bulb, where measurements on the segment are preferably carried out. CCA, Common carotid artery

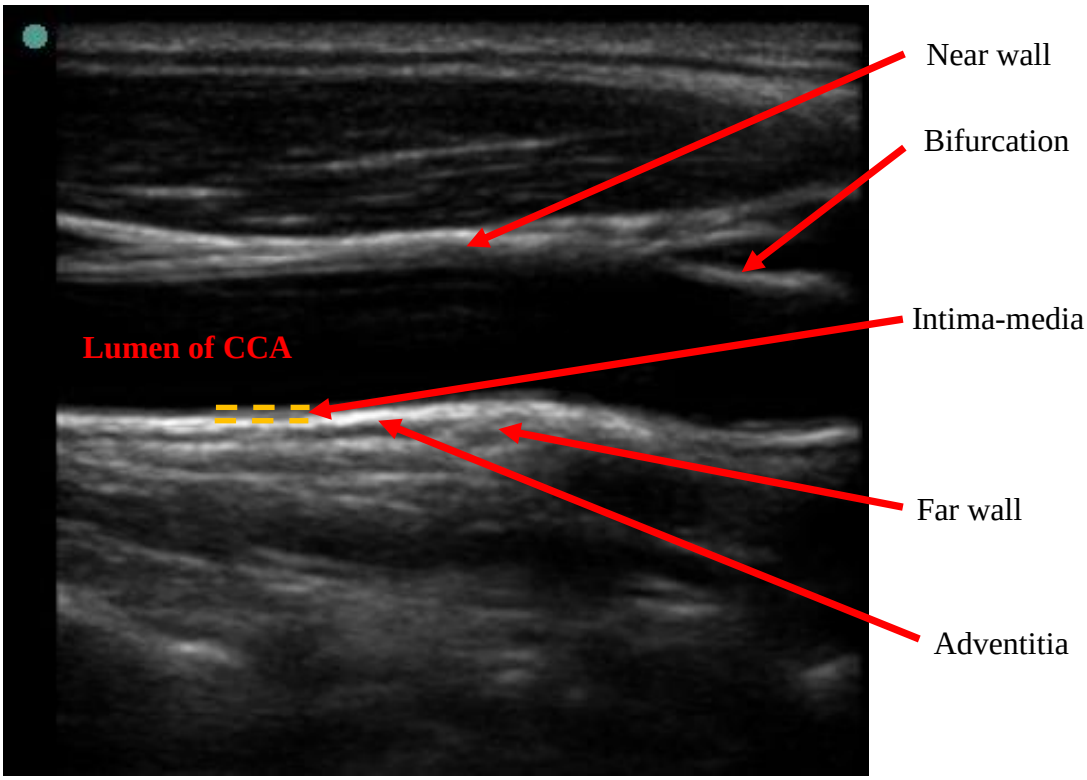
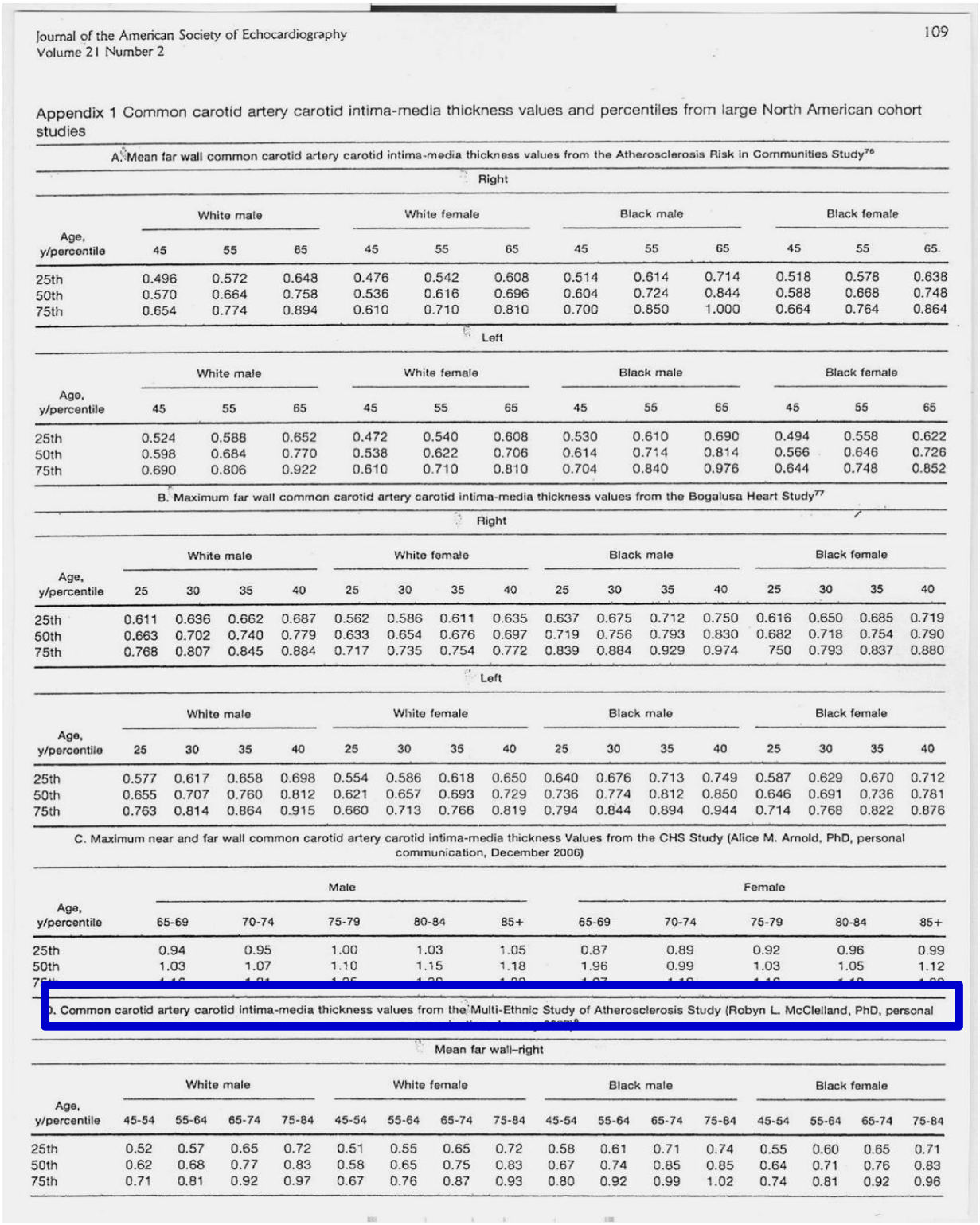


Figure 2.4.Ultrasound image of common carotid artery segment showing the lumen, near and far walls, the intima- media complex (between the dotted yellow lines) as well as the adventitia layers.

Table 2.1 The reference chart for C-IMT values of various population groups indicating the percentile ranges for ethnicity, sex and age (Stein et al., 2008).



Appendix 1 Common carotid artery carotid intima-media thickness values and percentiles from large North American cohort studies

A. Mean far wall common carotid artery carotid intima-media thickness values from the Atherosclerosis Risk in Communities Study[76]

Right

Age, y/percentile	White male			White female			Black male			Black female		
	45	55	65	45	55	65	45	55	65	45	55	65
25th	0.496	0.572	0.648	0.476	0.542	0.608	0.514	0.614	0.714	0.518	0.578	0.638
50th	0.570	0.664	0.758	0.536	0.616	0.696	0.604	0.724	0.844	0.588	0.668	0.748
75th	0.654	0.774	0.894	0.610	0.710	0.810	0.700	0.850	1.000	0.664	0.764	0.864

Left

Age, y/percentile	White male			White female			Black male			Black female		
	45	55	65	45	55	65	45	55	65	45	55	65
25th	0.524	0.588	0.652	0.472	0.540	0.608	0.530	0.610	0.690	0.494	0.558	0.622
50th	0.598	0.684	0.770	0.538	0.622	0.706	0.614	0.714	0.814	0.566	0.646	0.726
75th	0.690	0.806	0.922	0.610	0.710	0.810	0.704	0.840	0.976	0.644	0.748	0.852

B. Maximum far wall common carotid artery carotid intima-media thickness values from the Bogalusa Heart Study[77]

Right

Age, y/percentile	White male				White female				Black male				Black female			
	25	30	35	40	25	30	35	40	25	30	35	40	25	30	35	40
25th	0.611	0.636	0.662	0.687	0.562	0.586	0.611	0.635	0.637	0.675	0.712	0.750	0.616	0.650	0.685	0.719
50th	0.663	0.702	0.740	0.779	0.633	0.654	0.676	0.697	0.719	0.756	0.793	0.830	0.682	0.718	0.754	0.790
75th	0.768	0.807	0.845	0.884	0.717	0.735	0.754	0.772	0.839	0.884	0.929	0.974	750	0.793	0.837	0.880

Left

Age, y/percentile	White male				White female				Black male				Black female			
	25	30	35	40	25	30	35	40	25	30	35	40	25	30	35	40
25th	0.577	0.617	0.658	0.698	0.554	0.586	0.618	0.650	0.640	0.676	0.713	0.749	0.587	0.629	0.670	0.712
50th	0.655	0.707	0.760	0.812	0.621	0.657	0.693	0.729	0.736	0.774	0.812	0.850	0.646	0.691	0.736	0.781
75th	0.763	0.814	0.864	0.915	0.660	0.713	0.766	0.819	0.794	0.844	0.894	0.944	0.714	0.768	0.822	0.876

C. Maximum near and far wall common carotid artery carotid intima-media thickness Values from the CHS Study (Alice M. Arnold, PhD, personal communication, December 2006)

Age, y/percentile	Male					Female				
	65-69	70-74	75-79	80-84	85+	65-69	70-74	75-79	80-84	85+
25th	0.94	0.95	1.00	1.03	1.05	0.87	0.89	0.92	0.96	0.99
50th	1.03	1.07	1.10	1.15	1.18	1.96	0.99	1.03	1.05	1.12
75th										

D. Common carotid artery carotid intima-media thickness values from the Multi-Ethnic Study of Atherosclerosis Study (Robyn L. McClelland, PhD, personal

Mean far wall–right

Age, y/percentile	White male				White female				Black male				Black female			
	45-54	55-64	65-74	75-84	45-54	55-64	65-74	75-84	45-54	55-64	65-74	75-84	45-54	55-64	65-74	75-84
25th	0.52	0.57	0.65	0.72	0.51	0.55	0.65	0.72	0.58	0.61	0.71	0.74	0.55	0.60	0.65	0.71
50th	0.62	0.68	0.77	0.83	0.58	0.65	0.75	0.83	0.67	0.74	0.85	0.85	0.64	0.71	0.76	0.83
75th	0.71	0.81	0.92	0.97	0.67	0.76	0.87	0.93	0.80	0.92	0.99	1.02	0.74	0.81	0.92	0.96

Table 2.1 continued, showing reference values for Black males and females (highlighted by blue box).

Appendix 1 Continued

Age, y/percentile	Chinese male				Chinese female				Hispanic male				Hispanic female			
	45-54	55-64	65-74	75-84	45-54	55-64	65-74	75-84	45-54	55-64	65-74	75-84	45-54	55-64	65-74	75-84
25th	0.54	0.56	0.62	0.66	0.55	0.54	0.59	0.67	0.53	0.60	0.65	0.71	0.51	0.57	0.65	0.63
50th	0.64	0.70	0.73	0.79	0.60	0.63	0.71	0.77	0.62	0.67	0.78	0.81	0.58	0.69	0.76	0.78
75th	0.73	0.83	0.92	0.98	0.70	0.77	0.84	0.96	0.73	0.82	0.90	0.92	0.67	0.77	0.87	0.92

Mean far wall–left

Age, y/percentile	Chinese male				Chinese female				Hispanic male				Hispanic female			
	45-54	55-64	65-74	75-84	45-54	55-64	65-74	75-84	45-54	55-64	65-74	75-84	45-54	55-64	65-74	75-84
25th	0.55	0.57	0.62	0.69	0.49	0.52	0.58	0.64	0.55	0.61	0.68	0.72	0.51	0.58	0.62	0.68
50th	0.63	0.70	0.72	0.84	0.58	0.63	0.71	0.76	0.64	0.72	0.80	0.86	0.58	0.68	0.72	0.77
75th	0.73	0.84	0.86	0.97	0.67	0.72	0.87	0.94	0.75	0.85	0.98	0.97	0.68	0.79	0.86	0.91

Age, y/percentile	White male				White female				Black male				Black female			
	45-54	55-64	65-74	75-84	45-54	55-64	65-74	75-84	45-54	55-64	65-74	75-84	45-54	55-64	65-74	75-84
25th	0.54	0.57	0.67	0.71	0.50	0.55	0.63	0.70	0.56	0.63	0.69	0.72	0.54	0.59	0.63	0.68
50th	0.63	0.69	0.81	0.85	0.58	0.64	0.73	0.80	0.69	0.75	0.82	0.85	0.63	0.67	0.76	0.78
75th	0.78	0.82	0.95	1.00	0.67	0.75	0.85	0.94	0.81	0.92	0.99	1.02	0.73	0.80	0.90	0.91

Maximum far wall–right

Age, y/percentile	White male				White female				Black male				Black female			
	45-54	55-64	65-74	75-84	45-54	55-64	65-74	75-84	45-54	55-64	65-74	75-84	45-54	55-64	65-74	75-84
25th	0.61	0.66	0.73	0.83	0.59	0.66	0.77	0.82	0.66	0.72	0.79	0.83	0.63	0.72	0.72	0.79
50th	0.72	0.79	0.89	0.94	0.67	0.74	0.88	0.94	0.77	0.83	0.94	0.96	0.74	0.83	0.87	0.94
75th	0.87	0.94	1.05	1.11	0.79	0.88	1.00	1.07	0.89	1.05	1.11	1.13	0.87	0.94	1.05	1.10

Age, y/percentile	Chinese male				Chinese female				Hispanic male				Hispanic female			
	45-54	55-64	65-74	75-84	45-54	55-64	65-74	75-84	45-54	55-64	65-74	75-84	45-54	55-64	65-74	75-84
25th	0.66	0.63	0.66	0.72	0.62	0.61	0.66	0.72	0.61	0.67	0.72	0.78	0.61	0.67	0.72	0.72
50th	0.75	0.79	0.83	0.90	0.72	0.72	0.80	0.88	0.74	0.82	0.88	0.89	0.67	0.77	0.87	0.88
75th	0.86	0.94	1.05	1.07	0.83	0.82	0.94	1.05	0.87	0.95	1.05	1.05	0.78	0.91	1.00	1.03

Maximum far wall–left

Age, y/percentile	White male				White female				Black male				Black female			
	45-54	55-64	65-74	75-84	45-54	55-64	65-74	75-84	45-54	55-64	65-74	75-84	45-54	55-64	65-74	75-84
25th	0.64	0.68	0.77	0.77	0.61	0.66	0.72	0.82	0.66	0.72	0.82	0.83	0.62	0.66	0.72	0.77
50th	0.73	0.79	0.90	0.97	0.67	0.77	0.84	0.94	0.79	0.86	0.93	0.95	0.72	0.78	0.84	0.89
75th	0.89	0.94	1.09	1.12	0.78	0.88	1.00	1.11	0.94	1.04	1.11	1.11	0.86	0.94	1.03	1.00

Age, y/percentile	Chinese male				Chinese female				Hispanic male				Hispanic female			
	45-54	55-64	65-74	75-84	45-54	55-64	65-74	75-84	45-54	55-64	65-74	75-84	45-54	55-64	65-74	75-84
25th	0.65	0.64	0.72	0.77	0.61	0.61	0.66	0.72	0.62	0.72	0.77	0.77	0.61	0.66	0.72	0.77
50th	0.75	0.79	0.81	0.94	0.72	0.73	0.82	0.83	0.72	0.83	0.94	0.94	0.66	0.77	0.83	0.88
75th	0.88	0.95	1.00	1.06	0.80	0.83	0.96	1.05	0.88	0.97	1.11	1.11	0.78	0.89	0.97	1.02

Y, years. All values are in mm.

2.9 Statistical analysis.

For database management and statistical analysis, SAS software, version 9.1 (SAS Institute Inc., Cary, NC) was employed. Independent relationships were determined by multivariate regression analysis with adjustments for confounders such as age, sex, DM or diabetic treatment, BMI, antihypertensive treatment, regular tobacco or alcohol intake, and pulse rate. The probability values will be further adjusted for non-independence of family members in non-linear models (Mixed procedure as defined in the SAS package). Data will be expressed as mean ± SEM. A p-value of less that 0.05 will be considered significant ($p<0.05$).

CHAPTER 3

RESULTS

Characteristics of the participants. Table 3.1 gives the demographic and clinical characteristics of the participants who numbered 320. More women (202) than men (118) participated. In general the population sample had a high BMI, with ~63% of subjects being either overweight (25%) or obese (~37%) and ~37% having central obesity by both WC and WHR. Excessive adiposity was noted more frequently in women than in men and with women having greater values for all indices of adiposity, Table 3.1**.** A greater proportion of women than men were hypertensive and diabetic and also had central obesity. On average a very low proportion of participants reported smoking (regular smoker) or a regular intake of alcoholic beverages, but the percentages for regular smokers and drinkers were far greater in men than in women. No differences were noted in AMBP between all participants recruited and those with high quality 24-hr BP profile. Furthermore, no differences in AMBP were noted between gender groups, Table 3.2.

Table 3.1 Demographic, anthropometric and clinical characteristics of participants

	ALL N=320	MALE N=118	FEMALE N=202
AGE(years)	43.7±16.0	41.2±17.7	45.1±14.7*
BODY MASS INDEX (kg/m^2)	28.9±13.6	24.8±4.26	31.4±6.61***
WAIST CIRCUMFERENCE (cm)	89.4±13.6	83.3±11.9	93.0±14.0***
WAIST HIP RATIO	0.841±0.1	0.867±0.1	0.826±0.1**
SKIN FOLD THICKNESS(cm)	2.000±0.9	1.30±0.7	2.41±0.8***
% OVERWEIGHT or OBESE	62.5%	42.7%	74.2%
% CENTRAL OBESITY	37.3%	8%	55%
% HYPERTENSION	39.1%	36.4%	41.1%

Table 3.1 (continued)

% TREATMENT HYPERTENSION	25%	17.8%	29.2%
% DIABETES MELLITUS or ghb>6.1	12.5%	8.47%	14.4%
% SMOKING	12.5%	21.2%	7.4%
% ALCOHOL	16.6%	28.8%	9.4%
PWV	6.88±2.1	6.76±2.0	6.95±2.2

Values expressed as mean (± standard deviation) or percentages. *p<0.05,**p<0.01, ***p<0.001, male's means (± standard deviation) versus women's. PWV, Pulse wave velocity

Table 3.2 Blood Pressure Status of Patients

	ALL N=320	MALE N=118	FEMALE N=202
SBPc(mmHg)	128 ± 17	128 ± 17	130±16
DBPc (mmHg)	83 ± 9	83 ± 10	84±8
SBP24(mmHg)	118 ±10	121 ±14	122±12
DBP24 (mmHg)	75 ±9	75 ± 10	75±8
SBPn(mmHg)	111 ±12	109 ±12*	113=11
DBPn (mmHg)	64 ±8	64 ±8	64 ±8
SBPd(mmHg)	122 ±11	121 ±11	124±10
DBPd(mmHg)	77 ± 8	78 ±8	76±7

Values expressed as mean (± standard deviation) *p<0.05, **p<0.01, male's means (± standard deviation) versus women's. SBPc, conventional systolic blood pressure; DBPc, conventional diastolic blood pressure; SBP24, systolic blood pressure 24;

Table 3.2 (continued)

DBP24 diastolic blood pressure 24; SBPn, systolic blood pressure night; DBPn, diastolic blood pressure night; SBPd, systolic blood pressure day, DBPd, diastolic blood pressure day.

In bivariate analysis, systolic blood pressure conventional (SBPc), systolic blood pressure 24h (SBP24), systolic blood pressure day (SBPd) and systolic blood pressure night (SBPn) (Figures 3.7, 3.1, 3.5, and 3.3 respectively) were significantly associated with C-IMT. Other parameters that significantly associated with C-IMT were age ($r = 0.642$, $p < 0.0001$), BMI ($r = 0.269$, $p < 0.0001$), DBPn ($r = 0.339$, $p < 0.0001$) and WC ($r = 0.35$, $p < 0.0001$).

Bivariate Graphs

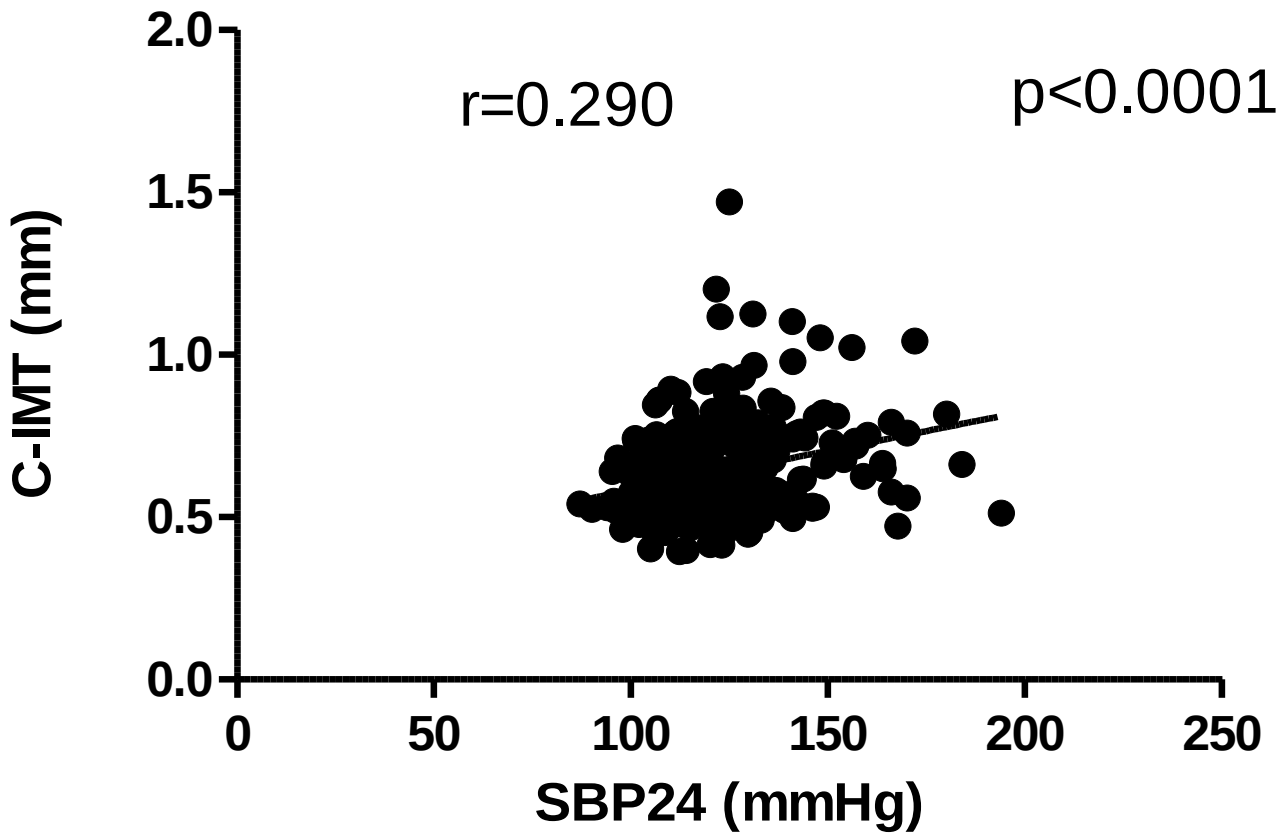


Figure 3.1 Relationship between SBP24 and C-IMT

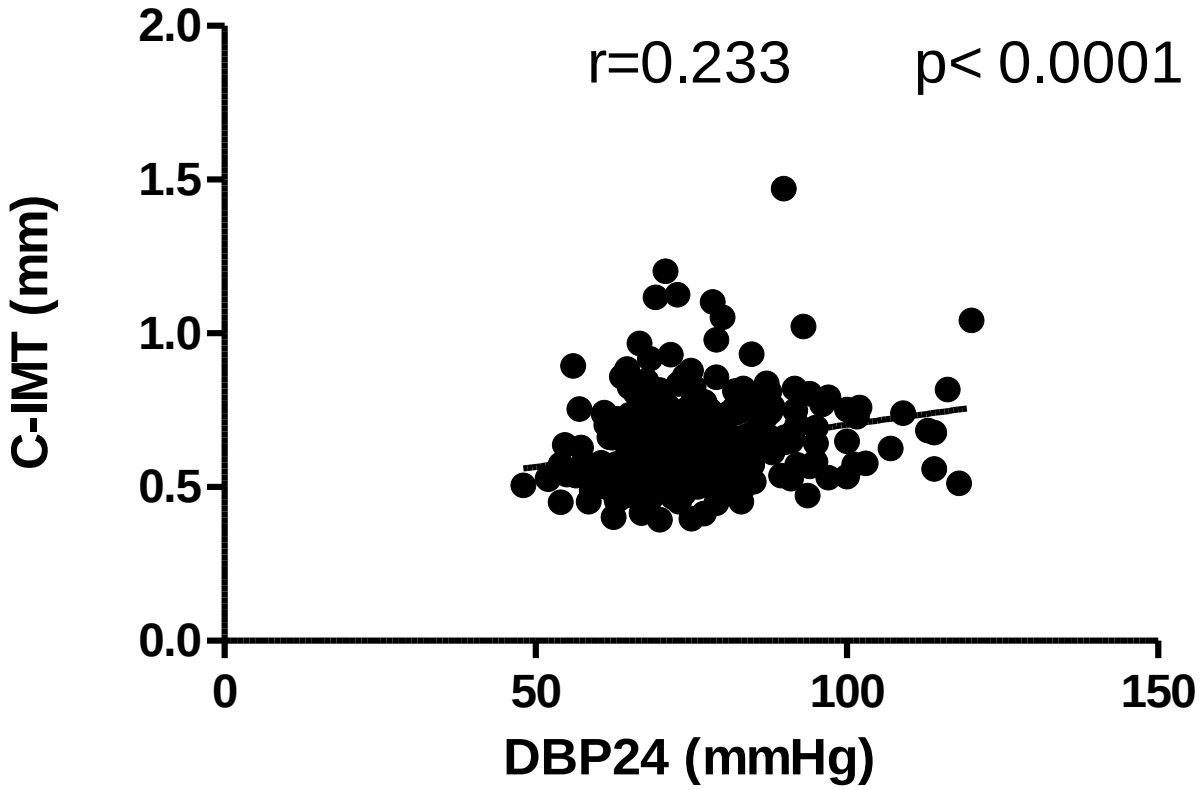


Figure 3.2 Relationship between DBP24 and C-IMT

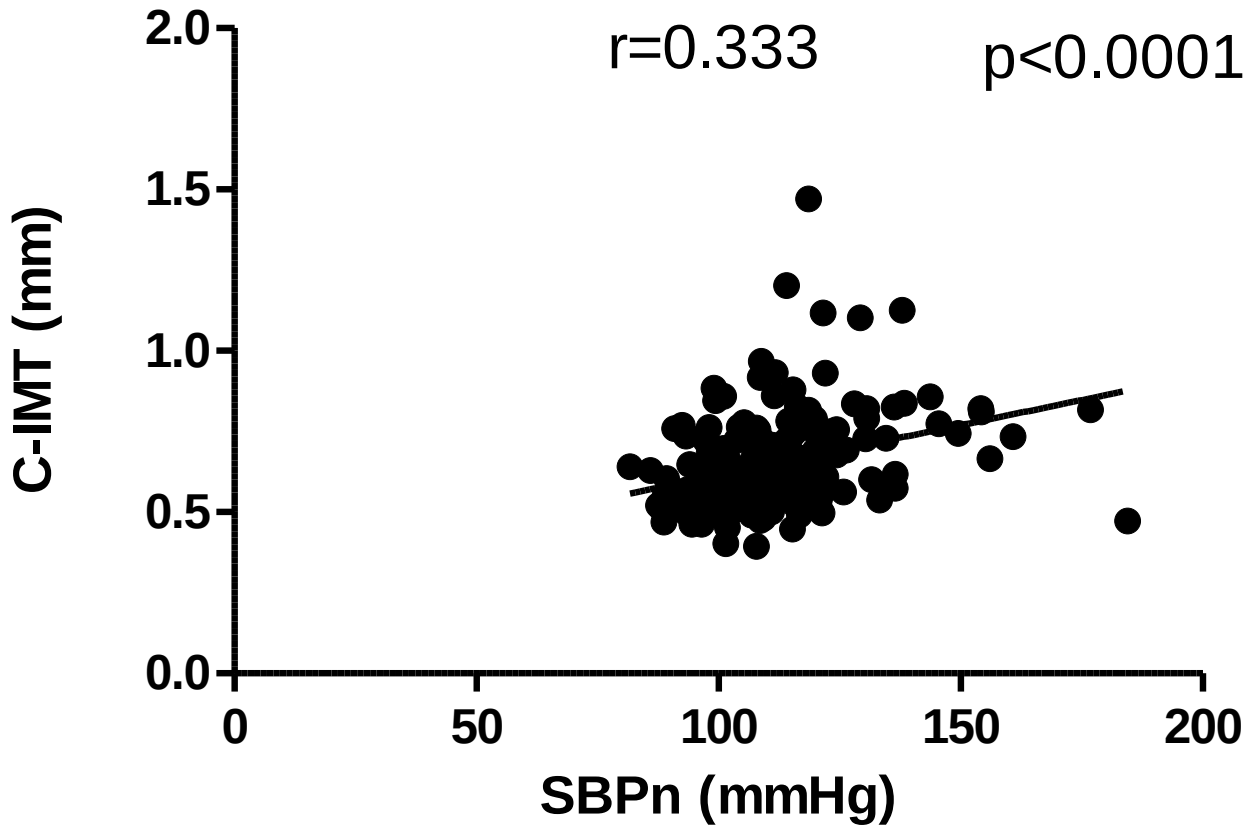


Figure 3.3 Relationship between SBPN and C-IMT

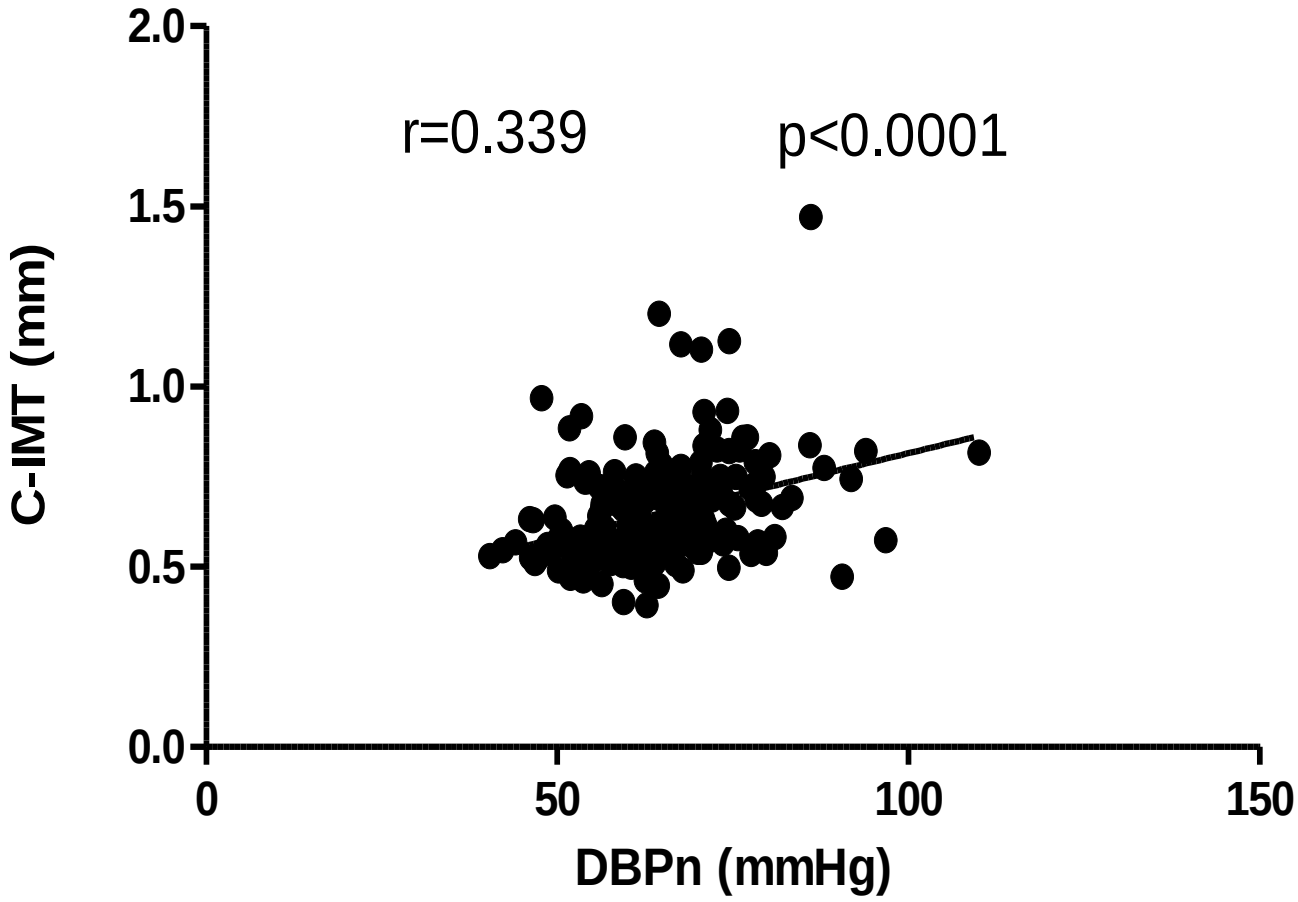


Figure 3.4 Relationship between DBPn and C-IMT

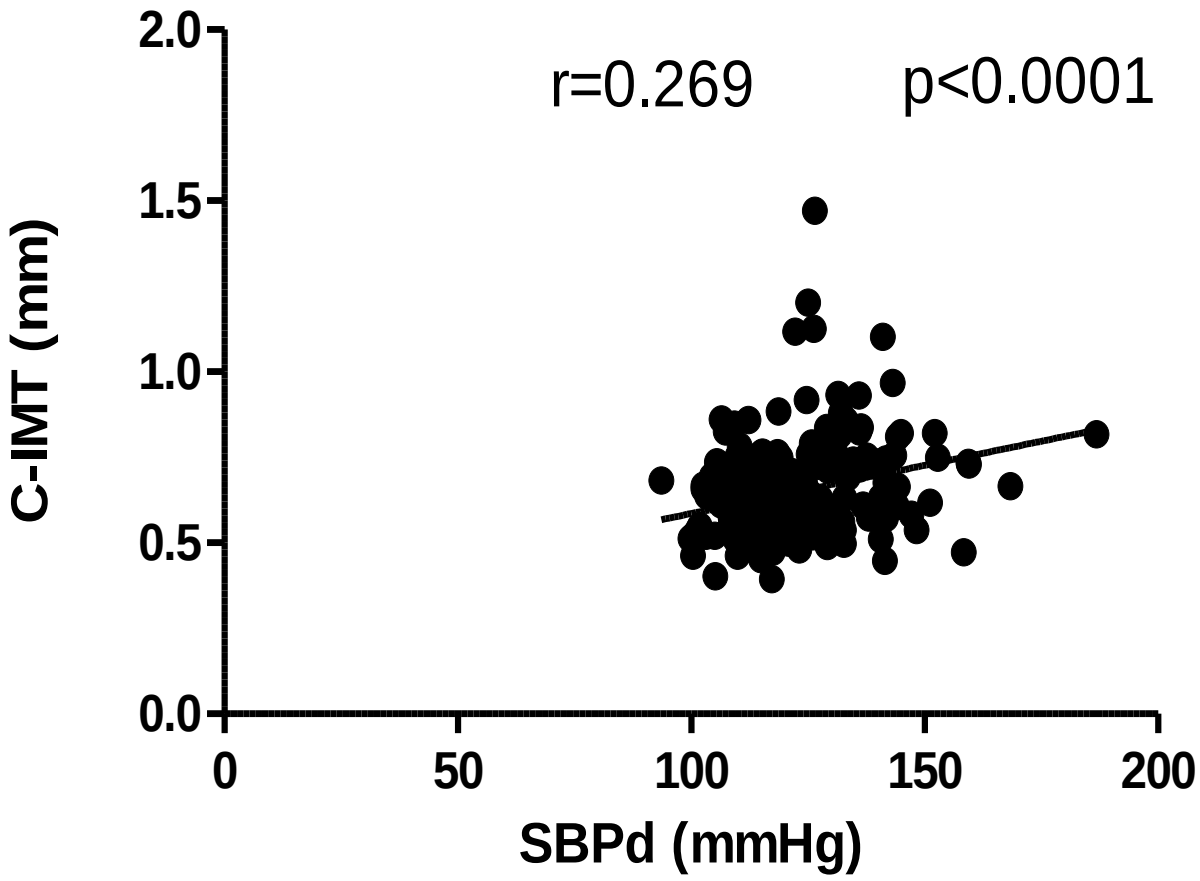


Figure 3.5 Relationship between SBPd and C-IMT

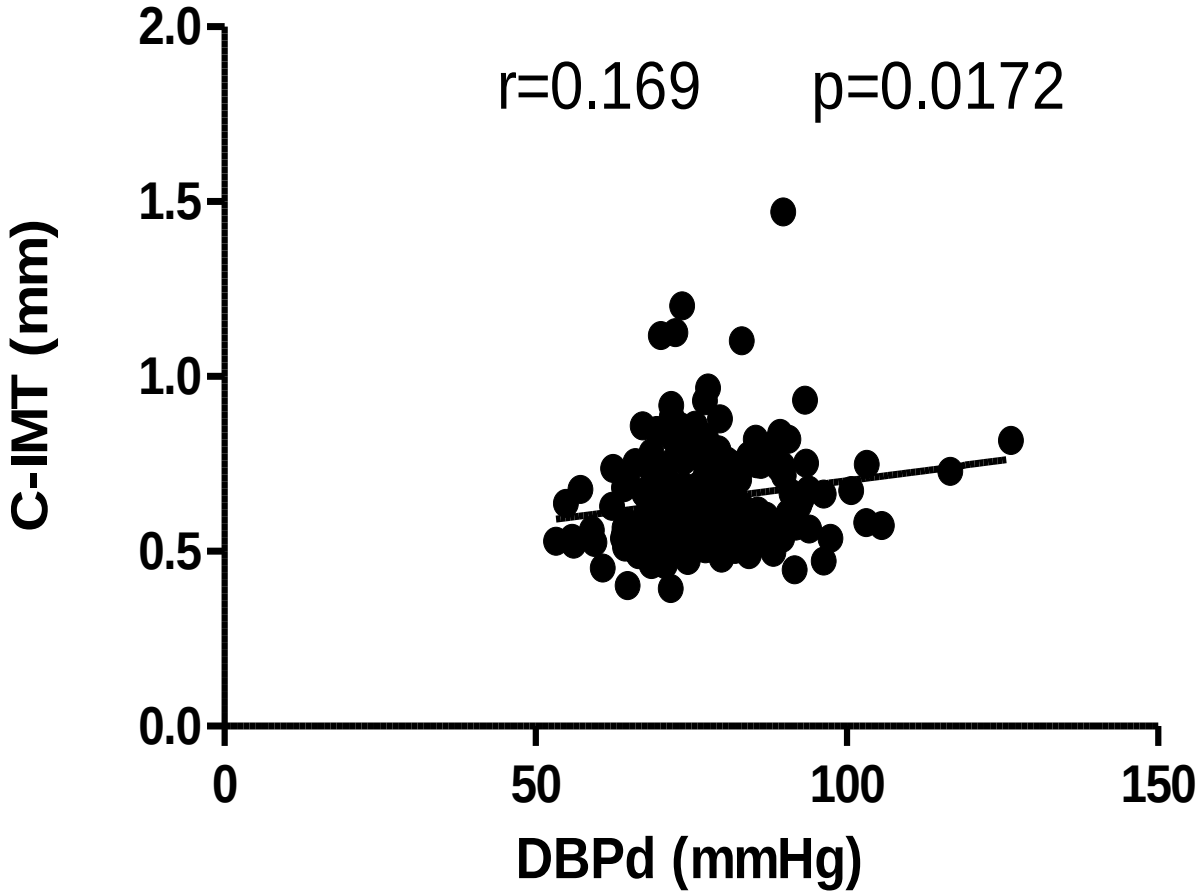


Figure 3.6 Relationship between DBPd and C-IMT

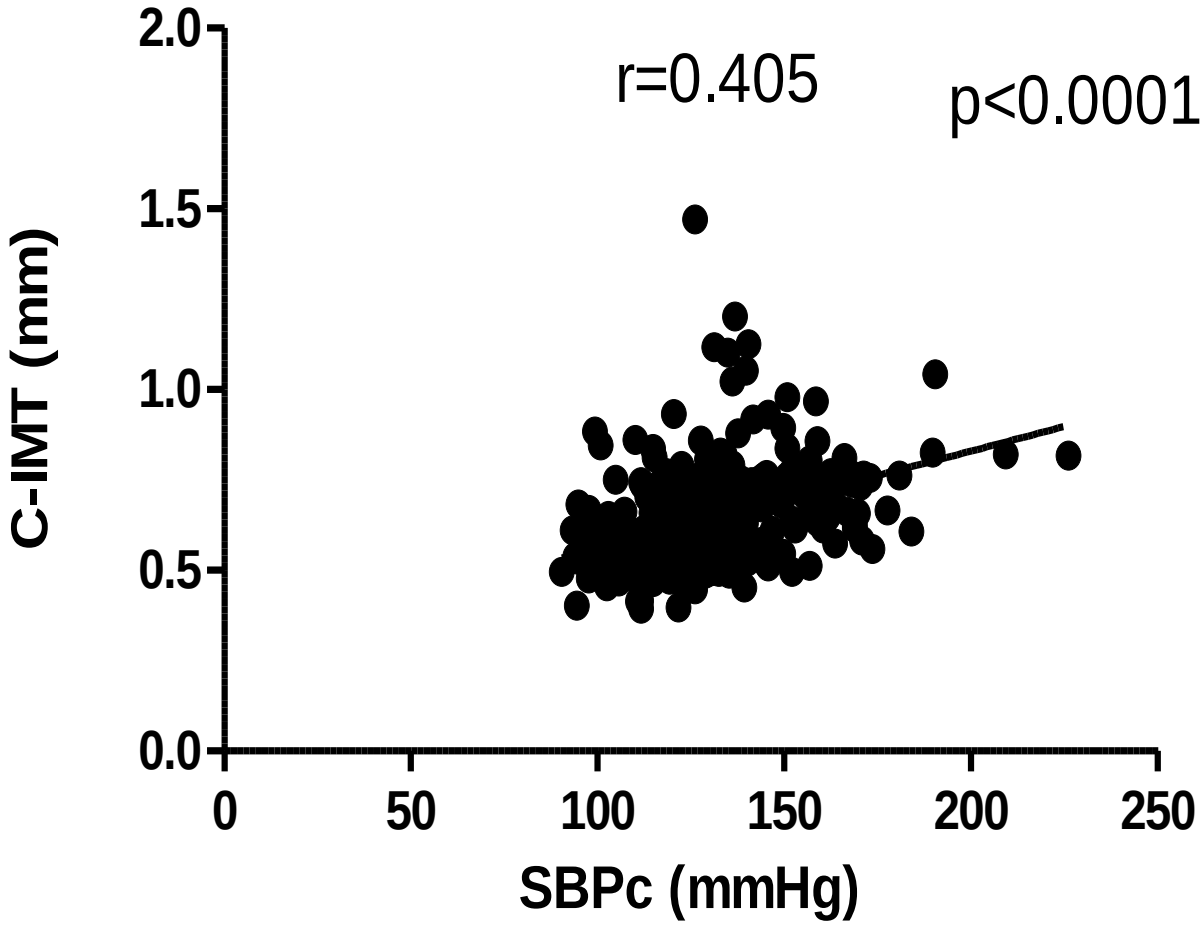


Figure 3.7 Relationship between SBPc and C-IMT

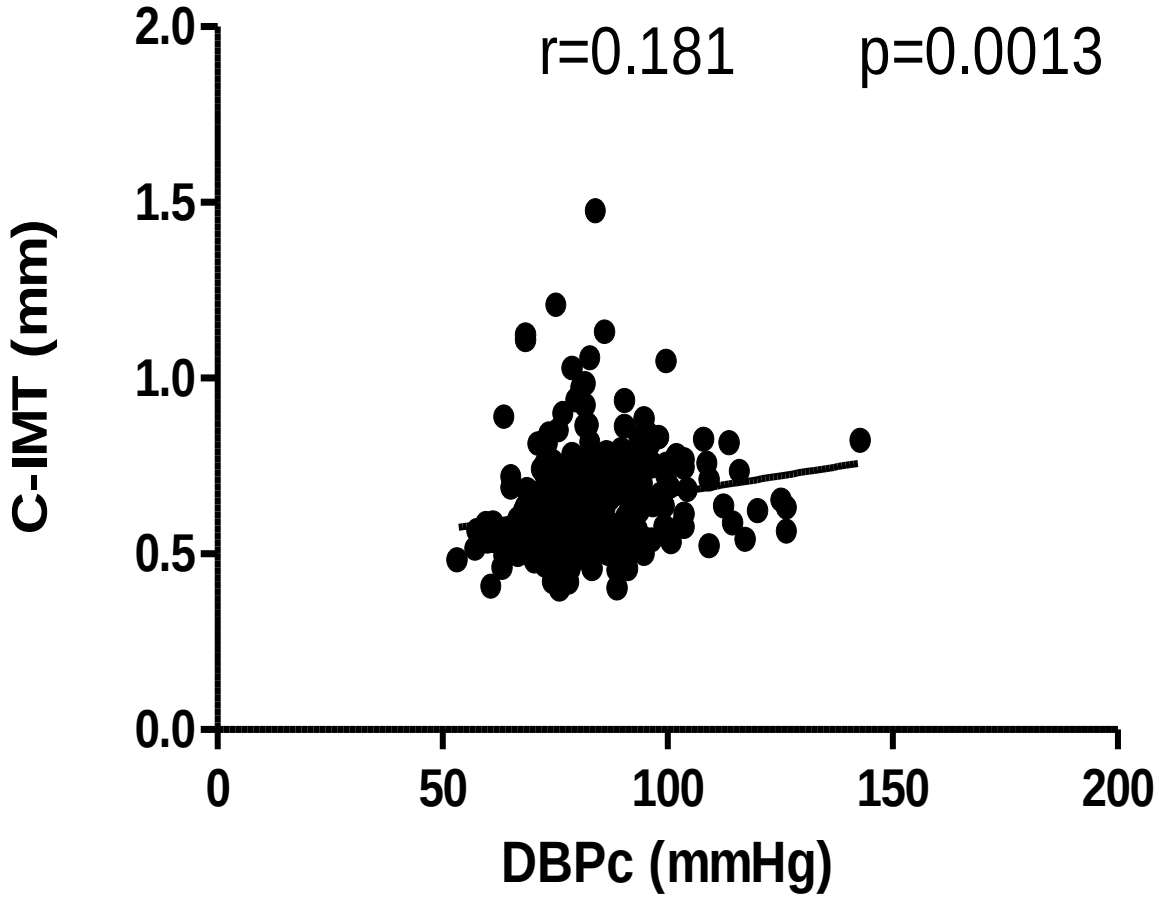


Figure 3.8 Relationship between DBPc and C-IMT

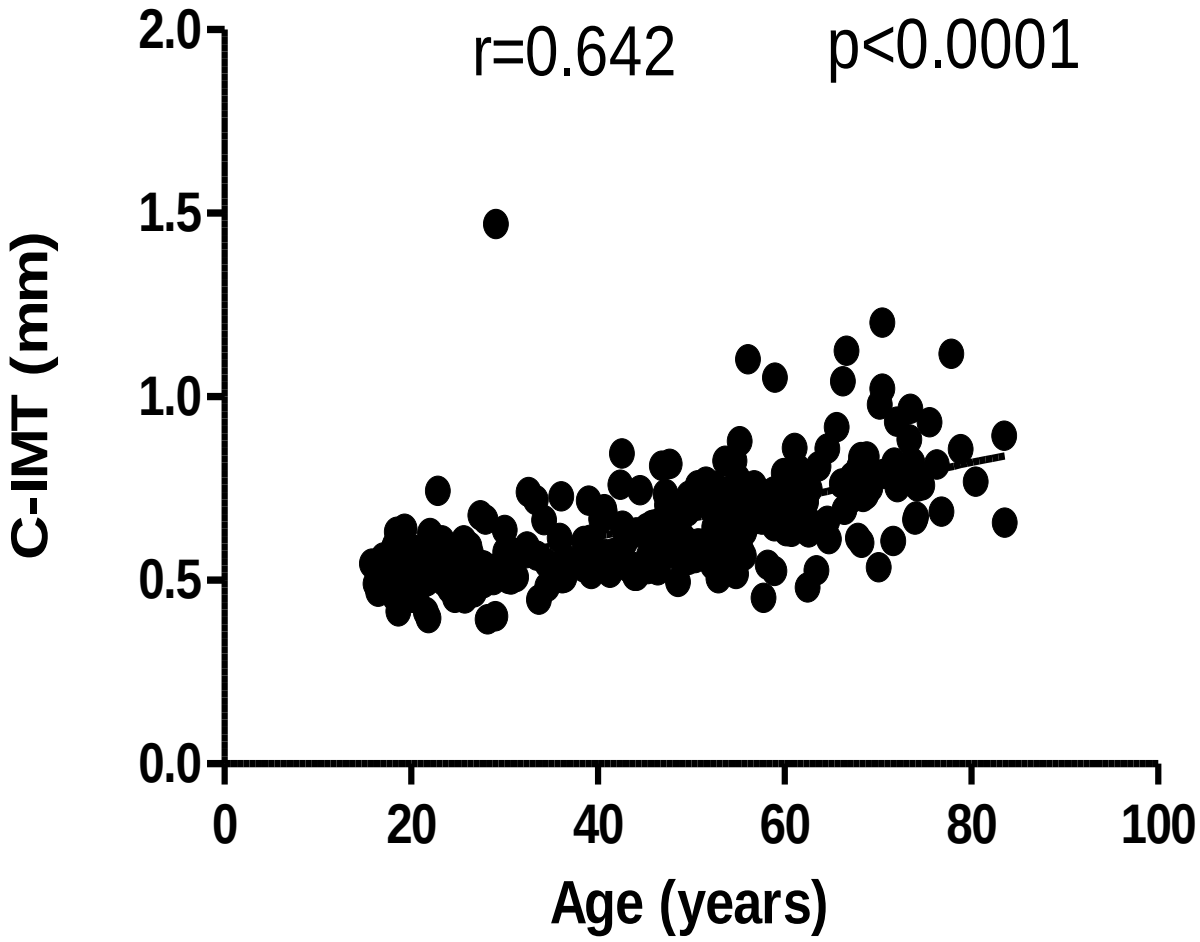


Figure 3.9 Relationship between Age and C-IMT

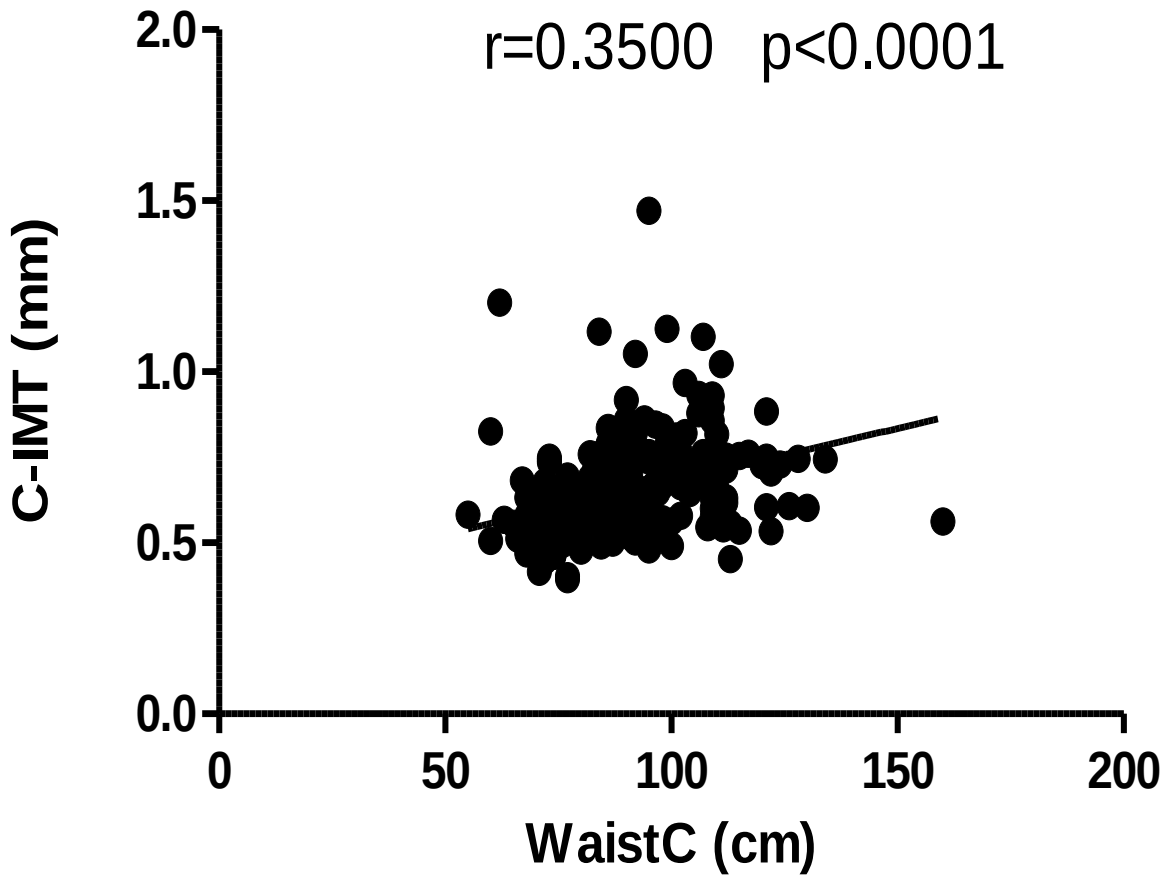


Figure 3.10 Relationship between WC and C-IMT

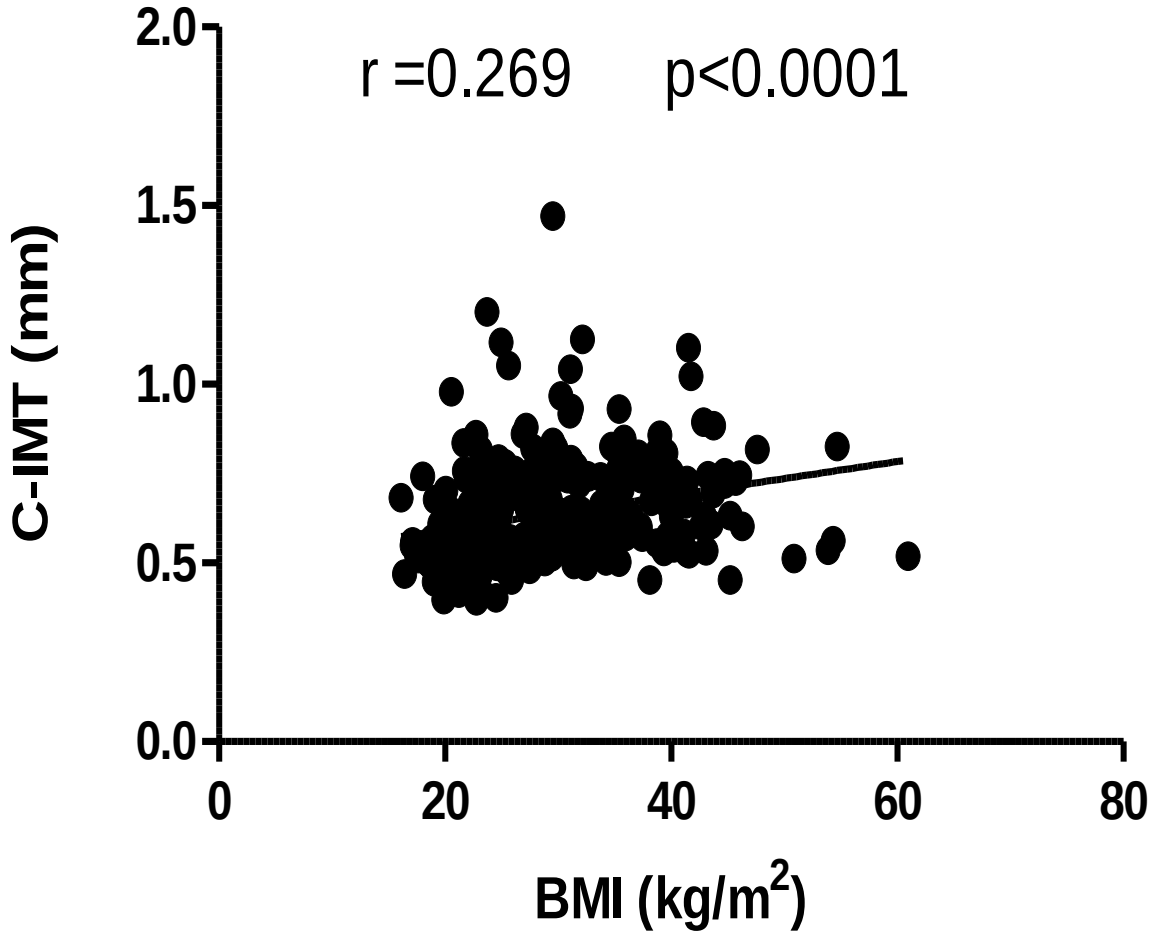


Figure 3.11 Relationship between BMI and C-IMT

Multivariate regression associations between BPc and C-IMT are shown in Table 3.3. With adjustment for confounders, neither systolic nor diastolic BPc correlated with C-IMT. When multivariate regression analysis was carried out on AMBP with adjustment for confounders that did not include SBPc (Table3.4), SBP24, SBPd and SBPn associated with C-IMT**,** with SBP24 ($r = 0.229$, $p = 0.0084$) demonstrating the strongest association. Most importantly the association between ambulatory parameters SBP24, SBPd and SBPn persisted after inclusion of SBPc in the same regression model (Table 3.5). The strongest AMBP relationship with C-IMT was observed with SPB24 ($r=0.236$, $p=0.001$) and SBPn ($r=0.172$, $p=0.0031$).

Table 3.3 Correlation coefficients (adjusted) for the relationship between C-IMT and BPc

	Partial correlation coefficient (r)	p-value
SBPc	0.0648	0.1612
DBPc	0.0265	0.5808

Adjustments for sex, BMI, diabetes mellitus, alcohol intake, regular tobacco use, heart rate, post-menopausal status, treatment hypertension

Table 3.4 Correlation coefficients (adjusted without SBPc) for the relationship between C-IMT and AMBP

	Partial correlation coefficient (r)	p-value
SBP24	0.229	0.0084**
DBP24	0.0812	0.3343
SBPd	0.302	0.0255*
DBPd	0.204	0.1302
SBPn	0.312	0.0203*
DBPn	0.230	0.0927

Adjustments for age, sex, BMI, diabetes mellitus, alcohol intake, regular tobacco use, heart rate, postmenopausal status, treatment hypertension.Each of the measures of blood pressure were analysed in separate models.

*p<0.05, **p<0.1, p<0.001

Table 3.5 Correlation coefficients (adjusted additionally with SBPc) for the relationship between C-IMT and AMBP

	Partial correlation coefficient (r)	p-value
SBP24	0.236	0.001**
DBP24	0.0480	0.5582
SBPd	0.149	0.0109*
DBPd	0.101	0.0819
SBPn	0.172	0.0031**
DBPn	0.0352	0.7260

Adjustments for age, BMI, diabetes mellitus, regular alcohol intake, regular tobacco use, heart rate, postmenopausal status, treatment hypertension and systolic blood pressure conventional. Each of the measures of blood pressure were analysed in separate models.

*p<0.05, **p<0.1, p<0.001

CHAPTER 4

DISCUSSION

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4.1 Primary outcomes of the current study

The main finding of the present study is that in an economically emerging community of black Africans living in Africa (age range 16-86 years), ABPM independently associates with C-IMT above and beyond BPc. Moreover, SBPn and SBP24 are more strongly associated with C-IMT independent of SBPc and measures of other cardiovascular risk factors including those of central adiposity, age and diabetes mellitus when compared to SBPd. SBPc also influenced SBPd, SBP24 and SBPn (Tables 3.4 and 3.5) but did so the least on SBP24 as the values for these changed when adjustment for SBPc was performed in Table 3.4. The present study provides more insight into the relationship between blood pressure and atheroma with specific focus on black populations in the early phase of the epidemiological transition. We did not however carry out gender specific analysis due to the small sample size.

4.2 Possible mechanisms

In this population the impact of hypertension to the growth of C-IMT and subsequent predisposing to cardiovascular events was significant owing to the high prevalence of hypertension (39.1%) in it (the population). The prevalence of hypertension was second only to that of obesity which is after all intimately associated with hypertension and would probably with time increase hypertension cases in the population. Plausible explanation for the observed independent relations between hypertension as measured by AMBP and C-IMT are the presence of factors in the population that specifically influences either blood pressure, inflammation or oxidative stress. As Figure 1.1 shows

any changes to any of the above mentioned processes, could trigger a cycle that potentially will cause changes to both hypertension and atherosclerosis.

Since the mechanisms through which changes in BP, oxidative stress and inflammation cause atherosclerosis have already been elucidated in the introduction, the focus on this section should therefore be on how such changes (of oxidative stress and inflammation) cause hypertension. Firstly, I will focus on those factors that separately influence rise in blood pressure, oxidative stress and inflammation in this population.

One factor that I propose as explaining the high incidence of hypertension in this group is urbanization as these were urban dwellers. Taking note of the findings of another study conducted on the same population grouping (urban black South Africans) in South Africa, a positive relationship was indeed found between hypertension prevalence and urbanization (Seedat et al., 1982). This rise in blood pressure occurs as a physiological response to changes in lifestyle. In the THUSA study, increases in BP were highest amongst those who had recently adopted an urban lifestyle which suggests that populations such as ours which are in the early phases of a lifestyle change, would experience the highest prevalence of hypertension owing to physiological stress emanating from that lifestyle change (van Rooyen et al., 2003). Specific factors within urbanization that caused a rise in hypertension rates include obesity which indeed had a high prevalence (~63%) in our study sample. There is a high Sympathetic Nervous System (SNS) activity to the kidneys in obese people caused by an inappropriate activation of the system (Ali and Crowther, 2005). This activation will lead ultimately to

AII production which promotes rise in BP through stimulating aldosterone release and vasoconstriction (Takahashi et al., 2011). Other previously identified factors that may cause hypertension in populations of African ancestry include the presence of genetic defects in renal sodium handling (Fuch, 2011). People of African ancestry have a problem of sodium retention which promotes fluid retention and an increase in blood pressure. Their blood pressure is most responsive to salt loading as their kidneys are quite effective at retaining it (Lopes 2002; Fusch 2011). Black people are also known to excrete sodium much more slowly. In one study, black people were found to record a greater response to diuretics than their white counterparts which further confirms the greater salt retention in this group. This salt retention is believed to be due to the high sodium channel activity in their kidneys' nephrons leading ultimately to cytosolic sodium accumulation (Baker et al., 2001; Lane and Lip 2001; Touyz et al., 1993)

On exploring the inflammation factor in this population, we know that possible triggers of inflammation in human beings include communicable diseases which are highly prevalent in Africa. Aikins *et al* in their study noted that Africa is still grappling with a high rate of communicable diseases which account for as much as 69% of deaths in the continent (Aikins et al., 2010). HIV/AIDS which is highly prevalent in Africa is regarded as one of the main contributors of inflammation in this community. It is a chronic inflammatory condition that directly or indirectly causes accelerated atherosclerosis. Furthermore the use of combination retroviral therapies has also proved to be atherogenic (Bryer et al., 2012). Inflammation as has already been mentioned can influence both hypertension and atherosclerosis and hence contribute to the association. In inflammation

activation of NF-kappa B, which produces pro-inflammatory molecules, occurs (Vaziri 2008). Some of these inflammatory markers have been shown to be independent risk factors for cardiovascular disease (Cachofeiro, 2009; Lakoski et al., 2005)One such marker CRP is believed to cause hypertension through causing endothelial dysfunction (Wang et al., 2010). Endothelial dysfunction can result in arterial stiffness, rendering the artery incapable of absorbing pressure through expanding as the cardiac cycle enters systole hence a rise in blood pressure. Arterial stiffness is a known risk factor for widened pulse pressure which is associated with isolated systolic hypertension (Olivier and Webb, 2003). The causation effect between inflammation and hypertension was proved in some animal studies where hypertension was reduced when inflammation blocking drugs such as NF-kappaB activation inhibitor were administered (Vaziri, 2008).

Oxidative stress can cause hypertension through a number of pathways which includes increasing the concentration of intracellular calcium ions. An increase in free calcium ions will increase vascular smooth muscle contractile activity and consequently an increase in vascular tone and blood pressure (Grossman, 2008). Another pathway is to affect Nitric oxide (NO) synthesis by depleting NO synthase co-factor tertrahydrobiopterin (BH4) (Grossman 2008). Reduced NO synthesis and bio-availability would cause endothelial dysfunction, arterial stiffness and hypertension(Olivier and Webb, 2003).

4.3 Comparison with other studies

Consistent with existing literature, we found that both clinic and ambulatory BP were associated with C-IMT on bivariate analysis (Zanchetti et al., 1998; Zanchetti et al., 2001; Nyström et al., 2005; Kotsis et al., 2006; Dechering et al., 2009; Schutte et al. 2012). However, on multivariate analyses we were unable to show an independent relationship between conventional or clinic BP and C-IMT. With regard to AMBP, we did however find that on multivariate analysis, AMBP independently associated with C-IMT. This is consistent with several other studies including one by Dechering *et al* who included femoral intima media thickness in their investigation (Dechering et al., 2009: (Kamarck et al., 2002). In addition AMBP was an independent correlate of ultrasound measures of atherosclerosis after controlling for BPc, even after methodological differences between conventional and ambulatory measures had been ruled out as an explanation for this effect (Kamarck et al., 2002). Even though the focus of the current study was not on plaque formation, previous data suggest that the odds of the presence of detectable plaque may triple or quadruple among those with high AMBP (Kamarck et al., 2002), illustrating the clinical significance of this approach as a standard method for the evaluation of hypertension and to assess the effect of antihypertensive treatment in clinical practice.

There are however studies with results at variance to our findings. Data from Nyström, *et al* suggest that the BPc recordings are at least as good a predictor of intima-media thickness as 24-hr-, day and night-time BP (Nystrom et al., 2005). Karmack *et al* study also found AMBP to significantly associate with IMT of the common carotid, carotid

bulb, internal carotid artery segments as well as the mean of the segments previously mentioned (Kamarck et al., 2002).Our data showed that both BPc and AMBP significantly associated with C-IMT in bivariate analysis but only the relationship between C-IMT and SBP24**,** DBP24,SBPd and SBPn survived multivariate analysis. The discrepancies between our study and Nyström's and others could be attributed to the fact that their study was designed prospectively to compare the designated variables and no adjustment was made for multiple comparisons. In addition their sample size (123) was entirely on patients with mild-to-moderate hypertension and LV hypertrophy and their average C-IMT values were calculated and indexed by height to assess vascular hypertrophy. In contrast, no screening criteria was employed in the recruitment of participants for our study. Woodiwiss *et al* had also found BPc to associate just as well with target organ changes as ambulatory BP but their study was not focused on atheroma nor did it include assessment of the carotid artery but the heart predominantly (Woodiwiss et al., 2009).

Focusing on AMBP parameters, we showed that SBPn strongly associated with C-IMT before and after adjustments for SBPc when compared to SBPd. A similar observation was made in the OHASAMA study where SBPn was strongly associated with C-IMT (Shintani et al., 2007). The potential explanation for this nighttime superiority in associating with target organ changes is that nighttime BP recording are more stable than daytime pressures. Daytime BP inherently has great fluctuations caused by factors that include mental stress, smoking and physical activity that occur during the day (Shintani et al., 2007). BP like several other physiological parameters is regulated in a circadian

fashion characterized by fluctuations. During the day, humans engage in physical activity which raises BP but does not cause a corresponding pathological effect on C-IMT hence the attenuation in the relationship between SBPd and C-IMT.

With regard to the strong association between SBPn and C-IMT, one explanation is that people of African ancestry experience a blunted nocturnal decline resulting in high nighttime blood pressure which in turn subjects them to an increased cardiovascular disease risk. In a 15 year prospective study in the United States, black people had a high incidence of blunted nocturnal BP and consequently a high nighttime BP (Wang et al., 2006). Studies have shown that those who experience a blunted nocturnal decline in BP have significantly increased incidences of thickening of the common and internal carotid intima media layers when compared to those with dipping BP (Routledge et al., 2007). Indeed, AMBP values in this population may be higher than for other populations for a given level of BPc because of attenuated decreases in BP at night (Profant et al., 1999; Wang et al., 2006). Thus, BPc measurements in this population may not closely reflect the extent of cardiovascular risk attributed to increases in BP when compared to other populations.

In one study by Maseko *et al* on the same population of urban black individuals in Johannesburg, it was found that in all participants, treated hypertensives, or all hypertensives, the proportion of participants with uncontrolled night BP was higher than the proportion with an uncontrolled day BP. These differences in control rates were noted in men and women, obese and non-obese, in smokers and in non-smokers as well as for systole and diastole (Maseko et al., 2011). Our data therefore suggests the use of SBPn

in assessing risk associated with atheroma, at least in people of African descent living in Africa.

Chapter 5

Conclusion

We conclude that SBP24 is more strongly associated with C-IMT above and beyond SBPc in people of African ancestry living in Africa. This is a group of people who are considered to be in the early phase of an epidemiological transition and conclusions of studies conducted elsewhere on people of the same ancestry, may not necessarily apply to our study population. We also conclude that the performance of AMBP versus clinic with regard to diagnosis of target organ changes in this population, are organ specific. This is so because previous work in our laboratory investigating a different set of organs arrived at a different conclusion. As SBPn associated with C-IMT more strongly than SBPd, we recommend the use of long acting (24 h) BP control medication or treating nighttime BP more aggressively. Such a treatment protocol can include treatment of those factors such as sleep apnea that are known to cause elevated nighttime BP.

Our results should be interpreted with caution as mainly women participated in the study. However despite the higher sample size of women (202) compared to men (118) in this study, the AMBP and PWV were similar in both genders, suggesting that the observed increases in indices of obesity did not translate into significant changes in BP. Moreover indices of central adiposity were significantly higher in men when compared to women. A higher sample size would have given us a statistical power to perform gender specific assessments in multivariate analysis. However, in our study, no gender differences in C-IMT and in BP were evident. Our study was also cross sectional in design and we also could not carry out any direct measurements of fat which would have been a more reliable measure of levels of adiposity in the study sample. Other important limitations are not carrying out and including measures of HIV infection, LDL cholesterol,

inflammatory markers and oxidative stress. Given the high prevalence of HIV infection and associated inflammations in South Africa, the inclusion of HIV status and use of antiretroviral drugs would have been an important component of the study. We also did not include the arterial cross sectional area in our correlation investigations which would have provided additional information of clinical relevance. While some studies have shown that common carotid artery's wall thickness was associated with lumen narrowing, others have provided contrasting results where the CCA dilated as its walls thickened, that being a compensatory mechanism to prevent lumen narrowing (Crouse et al., 1994; Polak et al., 1996). Both these conclusions have important implications on the relationship between CCA and CVD and requires investigation in our population.

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Appendix

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

COMMITTEE FOR RESEARCH ON HUMAN SUBJECTS (MEDICAL)
Ref: R14/49 Woodiwiss/Norton et al

CLEARANCE CERTIFICATE **PROTOCOL NUMBER** M02-04-72

PROJECT Gene Candidates As Determinants of Blood Pressure And Intermediary Phenotypes In Pathogenesis of Hypertension In Black South Africans

INVESTIGATORS Prof's AJ/G et al Woodiwiss/Norton et al

DEPARTMENT School of Physiology, Wits Medical School

DATE CONSIDERED 02-04-26

UNIVERSITY OF THE WITWATERSRAND
PROF PE CLEATON-JONES
HREC (MEDICAL)
2007 -05- 09
JOHANNESBURG
This clearance is valid and within the next 5 year validity

DECISION OF THE COMMITTEE *

Approved unconditionally

DATE 02-05-14 **CHAIRMAN** .....(Professor P E Cleaton-Jones)

* Guidelines for written "informed consent" attached where applicable.

c c Supervisor: Prof AJ Woodiwiss
Dept of School of Physiology, Wits Medical School

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DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10001, 10th Floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES