ARTERIAL STIFFNESS IN TYPE 2 DIABETES PATIENTS IN GHANA

KWAME YEBOAH
(Student's ID: 103310161)

THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF PHD PHYSIOLOGY DEGREE

JULY 2013

Declaration

I, Kwame Yeboah, hereby declare that this thesis is my own work. It is being submitted to the University of Ghana, Legon, for the award of PhD Physiology degree. This thesis has not been submitted before, as part or whole requirement, for any degree or examination to this or any other university or institution.

Student: Kwame Yeboah

Signature:……

Date: ____ July, 2013.

Supervisor: Dr. Daniel A. Antwi

Signature:……

Date: ____ July, 2013.

Co-supervisor: Prof. Albert G.B. Amoah

Signature:……..

Date: ____ July, 2013.

Co-supervisor: Prof. Ben Gyan

Signature:……

Date: ____ July, 2013.

Abstract

Diabetes mellitus is associated with increased risk of cardiovascular diseases (CVDs) and mortality. Type 2 diabetes (T2DM) exerts it deleterious effects through the blood vessel which may lead to hypoperfusion and end organ damage. The ability to characterize the arterial system and derive related biomarkers that can predict the occurrence of CVDs and discriminate high risk subject for management would be of immense importance. Assessment of arterial stiffness and central hemodynamic indices had been shown to predict CVD events in healthy and diseased Caucasian and Asian population; few studies had been reported of the utility of arterial stiffness assessment in sub-Saharan Africa. This study measured the levels of arterial stiffness and central hemodynamic indices in Ghanaian T2DM subjects with and without hypertension, and compared to the levels in age and gender matched nondiabetes subjects with and without hypertension; in order to assess the impact of arterial stiffness in diabetes and hypertension conditions. Also, the associations of vibration perception threshold (VPT) and vascular growth factors, angiopoietin (ang)-1, ang-2 and vascular endothelial growth factors (VEGF), to the levels of arterial stiffness were investigated.

In this study, 197 individual, composed of 68 hypertensive T2DM subjects, 46 nonhypertensive T2DM, 48 nondiabetes hypertensive subjects and 35 nondiabetes normotensive subjects volunteered to participate. Anthropometric characteristic and blood pressure measurement were performed. VPT was measured with Horwell Neurothesiometer and arterial stiffness was assessed using Tensiomed Arteriograph and Vasera. Fasting blood samples were collected from all the participants and nondiabetes subjects were screened for diabetes using the oral glucose tolerance test.

Plasma glucose and lipid profile were assayed whereas vascular growth factors were assayed with ELISA.

The study showed that aortic pulse wave velocity (PWV), heart-ankle (ha) PWV and aortic pressure indices were highest in hypertensive T2DM subjects, followed by nondiabetes hypertensive subjects, then the nonhypertensive T2DM subjects, and the control subjects with the lowest levels. However, cardio-ankle vascular indices (CAVI) was highest in hypertensive T2DM subjects, followed by nonhypertensive T2DM subjects, and then the nondiabetes hypertensive subject, with the control subjects having the least levels. The study also showed that female subjects had higher levels of aortic stiffness than the male counterpart, though no difference in CAVI and ha-PWV was observed. Arterial stiffness indices were found to generally increase with increasing age, duration of diabetes and body composition indices. Subjects with peripheral sensory neuropathy (PSN) were found to have higher levels of arterial stiffness indices than those without PSN. Of vascular growth factors, ang-2 was found to be associated with central aortic pressure and indices ang-1 was found to be associated with VPT.

The findings of this study showed that diabetes and hypertension are associated with arterial stiffness in Ghanaians. Arterial stiffness is associated with impaired angiogenesis in Ghanaians. Arterial stiffness might also contribute to the development of PSN in Ghanaians.

Dedication

To my dear mum, ***Loveline****.*

Acknowledgement

My profound gratitude goes to my study volunteers who left their busy schedule and participated in this research for the advancement of science.

I would like to express my deepest gratitude to my supervisor and mentor, Prof. Albert G.B. Amoah of the Department of Medicine and Therapeutics, for his excellent guidance, care, patience, and provision of excellent equipment and atmosphere for doing this research; directing me to the right research path to take and sticking with me in the adverse circumstances. I would like to thank my supervisors Dr. Daniel A. Antwi, head of Physiology Department and Prof. Ben Gyan of Noguchi Memorial Institute for Medical Research, not only for their good advice, support and friendship, but also, for providing me reagents, laboratory experience and academic opportunities throughout this research.

I would like to express my sincere gratitude to Prof. Kennedy Cruickshank and Virginia Govoni of King's College London, for the provision of equipment and training in arterial stiffness assessment. Also, my appreciation goes to all the staff of Clinical Research Facility at St. Thomas Hospital, London.

I would also thank the staff at the National Diabetes Management and Research Centre and Diabetes Research and Chronic Disease Reference Laboratory, especially Ben, Dickson and David for their immense assistance; also to the staff of Department of Physiology, especially Mr. Clottey for his assistance in subject recruitment. My deepest gratitude goes to my good friends Elizabeth Afua Gyamfi and Efua Adjei Bontu for their immense assistance.

The greatest thanks go to Jehovah God for helping me to complete this thesis.

Table of Content

List of Figures

List of Tables

List of Abbreviations

ACCORD	Action to Control Cardiovascular Risk in Diabetes
ADA	American Diabetes Association
ADVANDE	Action in Diabetes and Vascular Disease – Preterax and Diamicron Modified Release – Controlled Trial
AII	Angiotensin II
AIx	Augmentation index
ALDO	Aldosterone
Ang-1	Angiopoietin-1
Ang-2	Angiopoietin-2
ARIC	Atherosclerosis Risk in Community
ba-PWV	Brachial-ankle pulse wave velocity
BMI	Body mass index
BP	Blood pressure
CAD	Coronary arterial disease
CAVI	Cardio-ankle vascular index
CV	Cardiovascular
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
ECM	Extracellular cell matrix
E_{inc}	Incremental Young's modulus
ELISA	Enzyme-linked immunoabsorbent assay
FHS	Framingham Heart Study
GDM	Gestational diabetes mellitus

ha-PWV	heart-ankle pulse wave velocity
HDL	High density lipoprotein cholesterol
HOPE	Heart Outcomes Prevention Evaluation
IDDM	Insulin-dependent diabetes mellitus
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
INVEST	International Virapamil – SR/Trandolapril Study
LADA	Latent autoimmune diabetes of adults
LDL	Low density lipoprotein cholesterol
MAP	Mean arterial pressure
MMP	Matrix Metalloprotease
NCEP/ATP III	National Cholesterol Education Programme/Adult Treatment Panel III
NF-κB	Nuclear factor κB
NIDDM	Noninsulin dependent diabetes mellitus
NO	Nitric oxide
NOS	Nitric oxide synthase
OGTT	Oral glucose tolerance test
PP	Pulse pressure
PPAR	Peroxisome proliferator activator receptor
PPG	Photoplesthysmography
PSN	Peripheral sensory neuropathy
PWV	Pulse wave velocity
PWVc	Characteristic pulse wave velocity
R	Luminal radius

RAAS	Renin-angiotensin-aldosterone system
REASON	Regression of Arterial Stiffness in a Controlled double blind Study
ROS	Reactive oxygen species
SBP	Systolic blood pressure
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 Diabetes Mellitus
TChol	Total cholesterol
TG	Triglycerides
TGF	Transforming growth factor
TIMP	Tissue inhibitor of matrix metalloprotease
TNF	Tumour necrosis factor
UKPDS	United Kingdom Prospective Diabetes Study
VADT	Veteran Affairs Diabetes Trial
VEGF	Vascular endothelial growth factor
VLDL	Very low density lipoprotein cholesterol
VPT	Vibration perception threshold
VSMC	Vascular smooth muscle cell
WHO	World Health Organisation
WHR	Waist-to-hip ratio
WSR	Waist-to-stature ratio

CHAPTER ONE

INTRODUCTION

1.1 Background

Vascular diseases, including arteriosclerosis and atherosclerosis, are prevalent in patients with type 2 diabetes mellitus (T2DM) and are the principal causes of death and disability in these individuals [1]. Vascular diseases have been categorised into microvascular diseases which affects the small blood vessels, as in the case of nephropathy, retinopathy and neuropathy; and macrovascular diseases which affects the large-to-medium sized arteries as in the case of heart disease (coronary arterial disease), stroke (cerebrovascular diseases) and peripheral arterial disease. Atherosclerosis and arteriosclerosis, predominantly affect large-to-medium size arteries, occur earlier in T2DM patients, frequently with greater severity and more diffuse distribution [2]. Atherosclerosis is viewed as lipid-laden arterial condition, that occurs as a result of lipid peroxidation, subclinical inflammation, endothelial dysfunction and subsequent plaque formation, which culminate in hypoperfusion of tissues distal to the plaque[3].

Hyperglycaemia, a cardinal feature of T2DM, has been intuitively thought to be the possible cause of macrovascular diseases in T2DM. However, evidence from well-designed observational studies and multi-centre clinical trials indicate otherwise. Macrovascular diseases begin at the prediabetes stage of T2DM prior to development of overt hyperglycaemia[4]. It has been demonstrated that, at the time of diagnosis, about 50% of T2DM patients have already developed macrovascular abnormalities, though microvascular abnormalities might not yet be present [5]. This observation may

imply that, hyperglycaemia *per se* might not be the sole cause of macrovascular damage. It had been demonstrated that hyperinsulinemia, as a result of insulin resistance, might be responsible for initiation of macrovascular damage in prediabetes period [6].

In addition, several research studies and clinical trials have reported that the reduction of plasma glucose, as measured by glycated haemoglobin levels, yielded no effect on the incidence of macrovascular diseases. The Diabetes Control and Complications Trial (DCCT) reported of a trend towards reduction in cardiovascular (CV) events in the intensive glycaemic control arm, but the number of events were small and non-significant [7]. The UK Diabetes Prospective studies (UKDPS) observed 16% reduction of CV events in the intensive glycaemic arm but this was also non-significant [8].

Because of on-going debate regarding whether intensive glycaemic control can reduce the increased risk of cardiovascular diseases (CVD) in T2DM patients, several large long-term trials were designed to investigate the effects of intensive glycaemic control on CVD outcomes in T2DM subjects. Two of these trials, Action in Diabetes and Vascular Disease-Preterax and Diamicron Modified Release-Controlled Evaluation (ADVANCE) [9] and the Veterans Affairs Diabetes Trial (VADT) [10] showed no significant reduction in cardiovascular outcomes with intensive glycaemic control. A third trial, Action to Control Cardiovascular Risk in Diabetes (ACCORD) terminated its intensive glycaemic control arm prematurely due to the excess mortality in participants randomized to a strategy of very intensive glycaemic control[11]. Nonetheless, several meta-analyses [12, 13] and follow-up studies [14] on subjects in the intensive glycaemic control arm of the UKDPS indicate that glycaemic control may be beneficial, in the long-term, reducing CVD events; the “legacy effect”.

The benefits of blood pressure (BP) control in reducing CV events in T2DM patients have never been questioned in any clinical trial. It was demonstrated in the UKPDS trial[15] that diabetes related end point was reduced by 24% with tight BP control, while diabetes related all-cause mortality was reduced by 32%. The risk of stroke was reduced by 44%, retinopathy progression by 34% and microvascular disease 37%[15]. The UKPDS study and several other well-design randomised trials such as the International Verapamil-SR/trandolapril (INVEST) study [16], ACCORD study [17], VADT study [18] and ADVANCE study [19] reported similar benefits of blood pressure control on CVD outcomes in diabetes and high risk subjects. The consensus now in diabetes management is the combined glucose and BP control[20].

As highlighted by the above clinical studies, BP is an important prognostic factor in CVD and the ability to characterise arterial properties would be of immense benefit in combating the deleterious effects of diabetes and hypertension. Traditionally, BP has been measured and described by systolic BP (SBP) and diastolic BP (DBP). In 1971, investigators with the Framingham Heart Study (FHS) [21] emphasized the greater risks of an elevated SBP compared with the DBP in patients over the age of 55 years, but pulse pressure (PP) was not mentioned. Two large prospective studies by Madhavan et al [22] and Fang et al [23] in 1994 and 1995 respectively, established and confirmed that PP was the only index of brachial BP, significantly and independently, related to the in-treatment incidence of myocardial infarction in hypertensive subject.

Other clinical trials about the effectiveness of antihypertensive medication in large cohorts of patients in the Regression of Arterial Stiffness in a Controlled Double Blind Study (REASON) [24] trial and the Heart Outcomes Prevention Evaluation (HOPE) [25] trial showed the limitations of conventional brachial blood pressure indices and emphasized the usefulness of arterial stiffness and central haemodynamic indices

in predicting CV events in high risk subjects. Therefore, in 2006, the European Society of Cardiologist [26] recommended inclusion of arterial stiffness assessment and measurement of central haemodynamics as part of clinical care of hypertensive patients.

Arterial wall properties such as distensibility and its converse stiffness, are physiological concepts that explain the mechanistic link between CVDs and its risk factors [27]. A large body of evidence associates aortic stiffness with an increased risk of cardiovascular events[27-30]. Stiffened arteries interrupt the "Windkessel" effect of the arteries; the ability of the arteries to convert pulsatile ejection from the heart into steady flow of blood in distal vessels [30]. This function is made possible not only because arteries are compliant and are readily able to expand due to pressure, but also because they also possess the ability to recoil [28]. Stiffened arteries require a greater amount of force to expand and take up the blood ejected from the heart. This increased force requirement is provided by the heart, which must contract more forcefully to accommodate the arteries [28, 30]. Over time, this increased load placed on the heart causes left ventricular hypertrophy and, eventually, left ventricular failure. In addition, the increased time required for systole and the reduction in diastole cause additional cardiac damage. This reduction in both time and pressure during diastole decreases the amount of perfusion for cardiac tissue, Coronary blood flow, that occurs during diastole [30]. Thus, the now hypertrophic heart is starved of oxygen and nutrition, adding to cardiac damage [28, 30].

Microvascular complications of diabetes, which include retinopathy, neuropathy and nephropathy, are major contributors to morbidity and mortality [31].

Peripheral sensory neuropathy (PSN) is a major cause of foot ulcers and amputation in diabetes patients; however, few studies in sub-Saharan Africa have utilized the quantitative sensory testing for assessment of neuropathy [31]. Although neuropathy severity is related to duration and degree of glycaemic control, individual subjects may have widely disparate clinical presentations despite similar risk factors [32]. Also, the characteristic anatomic feature of vascular supply to peripheral nerves may account for the unique presentation of PNS but fails to explain why hyperglycaemia and duration of diabetes are associated with occurrence and severity of PSN [33]. It is known that the vasa nervorum lacks autoregulation[34]. Therefore, it is reasonable to hypothesize that arterial stiffness, which limits the cushioning effect of the large arteries might cause the damage of the vasa nervorum leading to neuropathy in T2DM. The association between PNS and cardiovascular risk factors specifically associated with diabetes such as hypertension, dyslipidaemia, and body composition are incompletely elucidated.

Vascular growth factors such as vascular endothelial growth factor (VEGF) and angiopoietins have been found to be associated with diabetes and hypertension[35-38]. However, studies of the effects of vascular growth factors on arterial stiffness and central haemodynamic are uncommon. Few studies have examined the relationship between arterial stiffness and central haemodynamics[39, 40]. The finding of association of the central haemodynamics and other indices of arterial stiffness with vascular growth factors would be very beneficial in the development of therapeutic options for arterial stiffness.

1.2 Problem statement

T2DM patients have 2- to 4-fold higher risk of CVDs than individuals without diabetes at any level of standard risk factors [1, 27]. Hypertension is a leading risk factor for mortality in both developing and developed countries [41] and a well-established risk factor for CVD in patients with diabetes [42]. The co-existence of hypertension and diabetes, as has been shown in large observational studies, increases drastically the risk of CVD mortality[43]. The increasing prevalence of CVD risk factors such as diabetes [44, 45] and hypertension[44] in Ghana implies that, unless holistic steps are taken, deaths as result of CV complications might also increase dramatically[46, 47]. Among the immediate steps to be taken is the precise characterisation of CVD risks in diabetes and hypertension patients in Ghana.

CVDs were considered, in the past, to be rare in sub-Saharan Africa.[46] However, both population-based[48] and hospital-based[49] studies now provide evidence for an increasing burden of cardiovascular disease in sub-Saharan Africa, with diabetes mellitus and hypertension as major contributors [31]. In Ghana, Amoah [49] reported that coronary artery disease (CAD) was the fifth commonest form of CVDs, with a prevalence of 11.3%, in patients with heart failure at the National Cardio-thoracic Referral Centre. The subjects with CAD were relatively older and most of them had hypertension (66.3%) and diabetes (22.5%)[49]. This is different to the trend seen in western countries where CAD is the leading cause CVDs [42]. In a recent study in South Africa, Connor *et al*., (2009)[48] reported that black subjects presenting with their first ever stroke event, with similar severity compared to the whites but 10 years younger. Hypertension and diabetes were equally common in black and white stroke patients, but mean cholesterol levels were lower in blacks. These studies in Ghana and South

Africa emphasize the peculiarity of CVDs (heart failure and stroke) in sub-Saharan Africans.

Epidemiological studies have shown that in Western countries, black subjects of Africa origin have increased risk of CVDs compared with whites subjects [50]. This may be explained, in part, by increased prevalence and severity in blacks of some risk factors for arteriosclerosis and atherosclerosis. In particular, in black subjects, essential hypertension has a higher prevalence, earlier onset, and is associated with more severe end-organ damage, including left ventricular hypertrophy, renal failure and stroke [50, 51].

The UKPDS study [52] showed that UK Afro-Caribbean subjects with diabetes had a lower risk for myocardial infarction than white subjects after adjustment for conventional cardiovascular risk factors. In the London cohort of the World Health Organization (WHO) study of vascular disease in subjects with diabetes in the United Kingdom, Afro-Caribbean ethnicity was associated with a lower risk of death of ischemic heart disease relative to white subjects when adjusted for sex, but not when also adjusted for smoking[53]. In the United States, the Atherosclerosis Risk in Communities (ARIC) study showed that diabetes in black subjects conferred a lower relative risk of coronary heart disease than it did in nonblack subjects [54].

The above studies show that black individuals of Africa descent, irrespective of their geographic location, are exposed to similar CVDs risk which has a pattern of presentation different from the Caucasian population.

Most of the risk factors of CVDs initiate their debilitating effects via the vascular system; making assessment of vascular function critical and quantification of vascular health useful in predicting of CVD burden [55].

Precise assessment of cardiovascular risk factors is a necessary step for the identification of diabetes and hypertensive patients at high risk of developing cardiovascular events. When formulating the algorithm for CV risk determination in the Framingham Heart Study (FHS) [56] and the UKDPS [57], measurement of blood glucose and lipids, estimation of blood pressure levels, identification of smoking status, and assessment of existing organ damage represent the main determinants of the tendency to develop cardiac and vascular problems. Each of these classical risk factors included in the FHS risk function algorithm was specific to the population on which the studies were performed and aimed at yielding absolute quantitative values of cardiovascular risk [29, 58].

Arterial stiffness has been estimated from the pulse pressure and mean arterial pressure in conventional BP measurement; but this has failed to precisely estimate arterial distensibility[30]. Also, regional variation in the arterial tree stiffness cannot be accounted for by conventional arm BP measurement [28]. Oscillometric and tonometric methods of assessing arterial compressibility provide valid, repeatable detection function via flow and pressure-related waveforms, from which indices of arterial stiffness is derived [29]. Non-invasive central and peripheral BP measurements using tonometric and oscillometric methods, from which indices of arterial stiffness can be derived, are now used in Asia, Europe and United States [27, 29]. Few studies had been conducted in sub-Saharan African on the application of arterial stiffness in a population with low CV risk [59], AIDS [60, 61] and in hypertension [62, 63]. The utility of arterial stiffness assessment and their advantage over the conventional methods of assessing the arterial physiology of diabetes and hypertensive subjects in Ghana are yet to be explored.

1.3 Relevance of the study

The arterial system is a major part of the overall circulatory system, playing an important role in cardiovascular physiology and pathophysiology [28]. Arteries expand and contract passively with arterial pulsations [30]. This property is associated with different physiological [28] and clinical factors [27-29, 50], through alterations of the structure and properties of the arterial wall. Changes in arterial pressure and other haemodynamic properties in diabetes and hypertension cause major changes in arterial wall properties [27].

The link between CVDs and its risk factors require the accurate assessment of the vascular function, based on universally acceptable measures of vascular assessment [64]. Arterial stiffness assessment is based on a fundamental concept of cardiovascular physiology, which has been proven to be universal to human populations and co-vary with the presence and severity of CVD as well as its risk factors. The above argument makes it imperative to apply this form of assessment to high CV risk population like diabetes and hypertension patients in Ghana.

Furthermore, because of increased longevity of Ghanaians in recent years, dissociation of arteriosclerosis from the effects of disease and aging *per se* need to be made. It is now accepted that arteriosclerosis may not just a normal aging process, but a disease state [65]. To dissociate and understand the mechanisms of diabetes and/or hypertensive associated vascular disease from the normal aging process, the structural and functional changes of the blood vessels, especially the arteries, of each condition must be recognized as clearly as possible with respect to their global impact on the functions of the arterial and arteriolar system. In contrast to atherosclerosis, arterial stiffness is predominant in vessels such as the elastic aorta, where non-fibrous and non-calcified plaques rarely form. Arterial stiffness can exist independently or in

association to atherosclerosis, and its assessment in disease conditions and different age groups can help to delineate physiology from pathophysiology.

The ability to quantify accurately, changes in arterial wall properties with pressure is clinically important in screening and discriminating individual at risk of CVD event [27, 66]. This will assist in understanding the haemodynamic features of diabetes and hypertension and predict how their impact culminates in various target organ damage. This will be beneficial in developing therapeutic and management strategies to ameliorate debilitating the effects of these conditions.

1.4 Aim of the study

The aim of the study was to assess the impact of T2DM and hypertension on arterial stiffness and peripheral sensory neuropathy in Ghanaians.

1.5 Objectives and hypotheses of the study

1. To measure and compare the level of various indices of arterial stiffness in various strata of study subjects.
    - Hypothesis: Diabetes and hypertension increase arterial stiffness in synergistic manner.
2. To investigate the relationship between indices of body composition and arterial stiffness in various strata of study subjects.
    - Hypothesis: Obesity is associated with increased arterial stiffness in Ghanaians.

3. To investigate the association between the glycaemic status, lipid and arterial stiffness in various strata of study subjects.
4. To compare the level of PSN in various strata of study subjects and assess the contribution of arterial stiffness and lipid profile on peripheral sensory nerve lesion.
    - Hypothesis: Increased level of arterial stiffness is associated with PSN in Ghanaians.
5. To investigate the association between vascular growth factors and indices of arterial stiffness in various strata of study subjects.
    - Hypothesis: Arterial stiffness is associated with impaired angiogenesis, measured by the plasma levels of vascular growth factors.
6. To assess the relationship between vascular growth factors and VPT in various strata of study subjects.
    - Hypothesis: Impaired angiogenesis, as measured by plasma levels of vascular growth factors, is associated with PSN.

CHAPTER TWO

LITERATURE REVIEW

2.1 Definition of Diabetes

Diabetes mellitus refers to constellation of complex metabolic abnormalities characterised by persistent hyperglycaemia resulting from insulin deficiency, insulin inaction or both[67]. Various biochemical aberrations are present in diabetes mellitus, but the fundamental defects to which most of the abnormalities can be traced are reduced entry of glucose into various "peripheral" tissues (insulin resistance) and increased synthesis of glucose into the general circulation from the liver (gluconeogenesis and glycogenolysis)[68]. These events culminate in high level of plasma glucose (hyperglycaemia) with the cells “starving” due to low level of intracellular glucose. In diabetes therefore, the body tissues have to metabolize protein and fat reserve for energy[69].

In addition to carbohydrate dysmetabolism, diabetes is also associated with aberration in protein and lipid metabolism. In diabetes, amino acids catabolism increases, with the liver converting amino acids into glucose under stimulation of glucagon[68]. The principal abnormalities of fat metabolism in diabetes are acceleration of lipid catabolism, with increased formation of ketone bodies, and decreased synthesis of fatty acids and triglycerides[70].

2.1.2 Classification of diabetes mellitus

Before the late 1970s, there was no consensus on the diagnostic criteria for diabetes. This led to much confusion and precluded any meaningful comparison of the

prevalence of diabetes within or between populations. As noted by West[71], diabetologists classified as normal more than half of the one and two-hour values of glucose tolerance test considered to be abnormal by other well-qualified diabetologists. In 1965 the WHO presented its first expert report on the diagnosis of diabetes, and these diagnostic criteria were subsequently modified and simplified by the WHO and the National Diabetes Group in the United States in 1979, 1980 and 1985 [72, 73]. The 1980 WHO Expert Committee proposed two major classes of diabetes mellitus, referred to as insulin-dependent diabetes mellitus (IDDM) or type 1 and non-insulin-dependent diabetes mellitus (NIDDM) or type 2. In the 1985 Study Group Report, the terms type 1 and type 2 were omitted, but the classes IDDM and NIDDM were retained, and a class, Malnutrition Related Diabetes Mellitus (MRDM) was introduced. In both the 1980 and 1985 reports, other classes of diabetes mellitus included "other types" and impaired glucose tolerance (IGT) as well as gestational diabetes mellitus (GDM)[72-74].

A revision of classification of diabetes based on both clinical stages and aetiopathogenesis of diabetes mellitus and other categories of hyperglycaemia was proposed [75]. It was argued that the terms "insulin-dependent diabetes mellitus" and "non-insulin-dependent diabetes mellitus" and their acronyms "IDDM" and "NIDDM", should no longer be used; these terms were confusing and often resulted in patients being classified based on treatment rather than on pathogenesis. Therefore, terms type 1 and type 2 were reintroduced. The diabetes class named type 1 encompasses those cases attributable to an autoimmune process, as well as those with beta-cell destruction and who are prone to ketoacidosis for which neither the aetiology nor pathogenesis is known (idiopathic). It does not include those forms of beta-cell destruction or failure to which specific causes can be assigned such as cystic fibrosis,

mitochondrial defects, etc. The type named type 2 includes the common major form of diabetes mellitus which results from defect(s) in insulin secretion, almost always with a major contribution from insulin resistance [75].

A subsequent international workshop reviewed the evidence for, and characteristics of, diabetes mellitus seen in undernourished populations [76, 77]. Whilst it appeared that malnutrition may influence the expression of several types of diabetes, the evidence that diabetes mellitus can be caused by malnutrition or protein deficiency *per se* was not convincing. Therefore it was recommended that the class, "malnutrition-related diabetes mellitus" (MRDM) be removed. The former subtype of MRDM, protein-deficient pancreatic diabetes, may be considered as malnutrition modulated or modified form of diabetes mellitus for which more studies were needed. The other former subtype of MRDM, fibrocalculous pancreatic diabetes, is now classified as a disease of the exocrine pancreas, fibrocalculous pancreatopathy, which may lead to diabetes mellitus [78]. The class "impaired glucose tolerance (IGT)" is now classified as a stage of impaired glucose regulation, since it can be observed in any hyperglycaemic disorder and is itself not diabetes. A clinical stage of impaired fasting glycaemia (IFG) was introduced to classify individuals who have fasting glucose values above the normal range but below those diagnostic of diabetes mellitus.

GDM was retained but now encompasses the groups formerly classified as gestational impaired glucose tolerance and gestational diabetes mellitus. Thus the new classification system identifies four major types of diabetes mellitus: type 1, type 2, "other specific types" and GDM [74].

GDM is carbohydrate intolerance resulting in hyperglycaemia of variable severity with onset or first recognition during pregnancy. It does not exclude the possibility

that the glucose intolerance may occur before pregnancy, but has been previously unrecognized. The definition applies irrespective of whether or not insulin is used for treatment or the condition persists after pregnancy [79]. Individuals at high risk for gestational diabetes include older women, those with previous history of glucose intolerance, those with history of large for gestational age babies, women from certain high risk ethnic groups, and any pregnant woman who has elevated fasting or casual blood glucose levels [80]. It may be appropriate to screen pregnant women belonging to high-risk populations during the first trimester of pregnancy in order to detect previously undiagnosed diabetes mellitus. Formal systematic testing for gestational diabetes is usually done between 24 and 28 weeks of gestation [79].

The aetiological classification of diabetes mellitus refers to physiological processes, defects or disorders which often result in chronic hyperglycaemia. The main aetiological classes of diabetes mellitus are type 1 diabetes mellitus (T1DM), which in turn is sub-classified into type 1 autoimmune and type 1 idiopathic diabetes mellitus, type 2 diabetes mellitus (T2DM) and "other types" diabetes mellitus. However the major classes of diabetes mellitus are the type 1 and type 2. Type 2 diabetes comprises approximately 90 – 95% of all cases of diagnosed diabetes mellitus, whilst type 1 comprises about 5 – 10% [74, 81]. In both sub-classes of type 1 diabetes mellitus there is absolute deficiency of insulin. The onset of symptoms is abrupt. Insulin is often required for survival to prevent the development of ketoacidosis, coma and death [78].

Autoimmune T1DM results from an inflammatory autoimmune and T-cells mediated destruction of the insulin-producing beta-cells of the pancreas, usually leading to absolute insulin deficiency. Insulin resistance does not play a major role in its pathogenesis [81]. Majority of individuals are lean, young and with autoimmune

markers associated with diabetes mellitus and most have susceptibility HLA haplotypes with linkage to the DQA and DQB genes, and is influenced by the DRB genes [82]. Individuals with this subclass of diabetes mellitus often become dependent on insulin for survival eventually and are at risk for ketoacidosis [83]. At this stage of the disease there is little or no insulin secretion as manifested by low or undetectable levels of plasma C-peptide. The rate of destruction of beta-cells is quite variable, being rapid in some individuals and slow in others [74]. The rapidly progressive form is commonly observed in children, but also may occur in adults [81]. The slowly progressive form generally occurs in adults and is sometimes referred to as latent autoimmune diabetes in adults (LADA). Some patients, particularly children and adolescents, may present with unprovoked ketoacidosis as the first manifestation of the disease [78]. Others have modest fasting hyperglycaemia that can rapidly change to severe hyperglycaemia and/or ketoacidosis in the presence of infection or other stress. Still others, particularly adults, may retain residual beta-cell function, sufficient to prevent ketoacidosis, for many years [74].

Idiopathic T1DM is a subclass of T1DM with an unknown aetiology, but it is likely related to insulin resistance and transient β-cell dysfunction, perhaps because of glucose desensitization [84]. Patients with idiopathic T1DM have permanent insulinopenia, and they are prone to ketoacidosis, but have no evidence of autoimmunity and it is not HLA associated [83]. Idiopathic T1DM has been described mostly in African-Americans (African origin) and Asians as well as other ethnic groups [82]. In most patients with idiopathic T1DM, insulin therapy is better in terms of glycaemic control than either oral hypoglycaemic agents or diet therapy alone and that long-term glycaemic control is better maintained with insulin treatment [81].

T2DM refers to condition of chronic hyperglycaemia as result of insulin resistance with a background of gradual beta-cell destruction. Insulin levels may be normal, decreased or increased. There are two identifiable defects in T2DM. There is predominantly insulin resistance, which is decreased ability of insulin to act on peripheral tissues [85]. Thus initially, and usually throughout their lifetime, these individuals may not need insulin treatment for survival. There may also be predominantly impaired insulin secretion due to β-cell dysfunction. However insulin resistance is the primary defect, preceding the derangement in insulin secretion and clinical diabetes by about 20 years [84]. The specific aetiologies of this class of diabetes mellitus are unknown, however autoimmune destruction of β-cells of the pancreas does not occur and patients do not have other known specific causes of diabetes mellitus listed under "other specific types" diabetes mellitus.

"Other specific types" diabetes mellitus is currently a less common class of diabetes mellitus, but includes those in which the underlying defect or disease process can be identified in a relatively specific manner. They may be due to genetic defects of beta-cell function, genetic defects in insulin action, disease of the exocrine pancreas, endocrinopathies, drug- or chemical-induced and infections [81]. Uncommon forms of immune-mediated diabetes and other genetic syndromes sometimes associated with diabetes mellitus also belong to this group [74].

2.2 Epidemiology of diabetes in sub-Saharan Africa

Diabetes affects people worldwide and poses major public health and socioeconomic challenges. The disorder was previously thought to be rare or undocumented in rural Africa, but over the past few decades it has emerged as an important non-

communicable disease in sub-Saharan Africa [86-88]. After the introduction of standardised diagnostic criteria [72, 73, 89], King and Rewers [90] showed in 1993, that diabetes in adults was a global disorder and that populations of developing countries, minority groups around the world and disadvantaged communities in industrialised nations faced the greatest risk. Subsequently, several reports on global estimates and projections [91] confirmed the diabetes epidemic and indicated that the numbers of people with diabetes and prevalence of both diabetes and impaired glucose tolerance will rise. The increases are expected to be largest in developing regions of the world because of population ageing and urbanisation [92].

Estimates by the International Diabetes Federation [92] suggest that the number of adults with diabetes in the world will expand by 54%, from 284.6 million in 2010 to 438.4 million in 2030. The projected growth for sub-Saharan Africa is 98%, from 12.1 million in 2010 to 23.9 million in 2030. Impaired glucose tolerance in sub-Saharan Africa is expected to rise by 75.8%, from 26.9 million in 2010 to 47.3 million in 2030. This proportion is more than double the predicted global increase of 37%. The report also highlighted the paucity of data from Africa [93]. Mortality attributable to diabetes in sub-Saharan Africa is estimated in 2010 at 6% of total mortality, an increase from 2.2–2.5% in 2000 [91].

2.2.1 Type 1 Diabetes

In Africa, the presence of atypical forms of diabetes makes it difficult to classify patients, based on usual clinical criteria, as having T1DM and T2DM [94, 95]. This atypical form of diabetes has been suggested by the ‘accelerator hypothesis’ to be overlay, rather than overlap, between T1DM and T2DM; implying that T1DM and

T2DM may share some common aetiologies [96]. The difficulty in classifying and diagnosing some forms of diabetes in Africa may explain why approximately half (42–64%) of African patients initially treated with insulin do not have classical T1DM and may enter prolonged remission [97, 98]. It is widely believed that classical T1DM is less common in Africa than in Europe or North America, and this is certainly common clinical experience. However, early out-of-hospital mortality may confound this impression, and the question remains open [93].

Epidemiology studies of T1DM in sub-Saharan Africa are difficult to conduct because of problems in finding cases as well as enumeration of the background population; few reports are available in literature [87]. In published studies, prevalence of T1DM is low: 0.33 per 1000 in Nigerian and 0.95 per 1000 in Sudanese school children [99]. Incidence of T1DM has been reported to be as low as 1.5 per 100 000 per year in Tanzania and high as 10.1 per 100 000 per year Sudan [100]. This large difference could be attributable to methodological discrepancies between studies, or true ethnic dissimilarities, because Tanzanian people are predominantly of African origin, whereas Sudanese populations are of mixed Arab and African heritage. However, the prevalence of T1DM is lower than that of western countries [91].

Findings of clinical studies from South Africa, Tanzania and Ethiopia suggest that the characteristics of T1DM in people from sub-Saharan Africa differ from typical European populations [91]. As expected, insulinopenia is a prominent feature [87]. However, the age at onset in African communities (age 22–29 years) is about 10 years later than in populations from Europe, with peak age at onset of 15–19 years in Tanzania, 22–23 years or 21–30 years in South Africa, and 20–25 years in Ethiopia.[87] A female preponderance was reported in affected people from South Africa, Ethiopia, Sudan, Nigeria, and Libya [99].

2.2.2 Type 2 diabetes

Most of the studies done between 1960s and early 1980s reported the prevalence of T2DM, using urine analysis in localized settings in countries such as Ethiopia, Ghana, Lesotho, Uganda and Malawi, to be lower than 1%; except the prevalence in South Africa (0. 6 - 3. 6%) and Cote d'Ivoire (5. 7%) [86]. However, T2DM is presently the most common form of diabetes in sub-Saharan Africa, similar to other regions of the world. Most of the available, albeit still scarce, data for diabetes prevalence in sub-Saharan Africa are based on standardised 1985 WHO criteria [73], with several reports from west, east and northeast Africa, and South Africa [86, 88, 91]. These published data show that, contrary to previous notion, diabetes is not rare in sub-Saharan Africa. Although rates of less than 3% have been recorded in rural and urban communities in west and east Africa, frequencies of 3‑10% are noted in urban and peri-urban populations in South Africa and in Sudanese communities of African origin, comparable with rates in developed countries [100, 101].

Few epidemiological studies in sub-Saharan Africa have been published in which 1998/2003 American Diabetes Association [81] and 1998 WHO [74] criteria were used to determine the prevalence of diabetes. The prevalence of diabetes ranges from 3.9% in rural South Africa to 6.7% in urban Guinea [91]. Although low diabetes prevalence has been noted in some rural communities, in most studies, moderate rates are recorded in both rural and urban populations. The high prevalence reported in people from urban areas of Kenya [102] could be attributable to non-probability sampling in that study, so these results should be interpreted with caution.

The WHO STEPwise chronic disease risk factor surveillance programme (STEPS) undertaken in many African countries aims to clarify the burden of diabetes in sub-

Saharan Africa. Reported prevalence varies widely (Benin 3%; Mauritania 6%; Cameroon 6. 1%; Congo 7. 1%; Zimbabwe 10. 2%; Democratic Republic of Congo 14. 5%) [101]. In most of these studies, diagnosis is based on fasting blood glucose concentration, measured in either capillary whole blood or venous plasma. The high rates noted in some studies are inconsistent with other reports in indigenous African people. Whether this inconsistency is an indication of a sudden upsurge of diabetes in sub-Saharan Africa or is related to study methods (sampling strategy, true fasting blood samples) needs further assessment [91].

2.2.3 Epidemiology of Diabetes in Ghana

Up to date, there is no reported study on the prevalence of diabetes in Ghana based on representative national sample. Few authors have reported on the prevalence of diabetes in specific locations in Ghana. For instance, Dodu in 1958 used urinalysis for diabetes screening and reported the prevalence of diabetes to be 0.4% among 4000 urban outpatient population in Accra [103]. Intrigued by the results, Dodu, together with de Heer, moved the study from clinic based to community based sampling; this time recruiting 5000 subjects from a provincial capital in Ghana. They reported prevalence of diabetes to be 0.2% [104]. The results from these studies created the impression that diabetes is a rare disease and in a country, like Ghana, plunged with high level of communicable diseases, maternal and infant mortality, a disease like diabetes deserves little or no attention.

However, analysis of admissions to the largest hospital in the country, Korle-Bu Teaching Hospital revealed that diabetes accounts for 6.8% of all adult admissions. In the same hospital diabetes was responsible for 7.3% of adult deaths, and 22% of

people diagnosed with diabetes died from diabetes-related complications [105]. These clinical observations, coupled with the change in diagnostic criteria for diabetes, necessitated revision of epidemiological studies of diabetes to reflect the present trends. In 1998, a non-communicable diseases survey was initiated to determine the prevalence of diabetes, hypertension, obesity, ischaemic heart disease and cardiovascular risk factors in selected communities in this area. This study provided the first community-based prevalence of diabetes in Ghana using internationally accepted diagnostic criteria by ADA and WHO [45, 106].

Amoah et al [45] recruited 4733 subjects aged 25 years or more from the Nation's capital and reported the crude prevalence of diabetes, using fasting plasma glucose (FPG) and oral glucose tolerance test (OGTT), to be 6.3%. In this same study, the age-adjusted prevalence of diabetes, IFG and IGT, were 6.4%, 6.0% and 10.7% respectively. Diabetes was found to be more common in males than females (7.7 vs. 5.5%), and worsening of glycaemic status tended to be associated with increase in age, body mass index, systolic and diastolic blood pressures. FPG and OGTT criteria for glucose homeostasis were compared in a community study in Accra, Ghana, using the ADA and WHO diagnostic criteria. The prevalence of undiagnosed diabetes mellitus by FPG (3.2%) and OGTT (3.1%) criteria were similar [106], and both giving an overall prevalence of undiagnosed diabetes mellitus of 4.5%. In this same study, the prevalence of IGT (15.8%) was higher than that of IFG (10.7%). Thus, 42% subjects with diabetes by OGTT had normal or impaired fasting glucose, showing poor agreement between the two diagnostic criteria [106].

2.3 Diabetes and Cardiovascular diseases: Epidemiological perspective

CVDs have been classified into two groups based on presentations: macrovascular diseases that affect the large-to-medium blood vessels and microvascular diseases that affect the small blood vessels. Macrovascular diseases that are found in diabetes patients include coronary heart disease, cerebrovascular disease peripheral arterial disease. Microvascular diseases include diabetic retinopathy, nephropathy and neuropathy [46]. The relationship between diabetes and CVDs has been proved through various interventional and non-interventional longitudinal studies conducted since half a century ago. Beginning from a landmark study the FSH, several other hallmark studies have been conducted to study the effect of diabetes and CV risk factors on the cardiovascular system and possible interventions than can yield the most health benefits. The participants for FHS were mostly white and of European ancestry, their health experience mostly reflects the health experience Caucasian populations [107].

2.3.1 Diabetes and Hypertension

High blood pressure had been reported in over two-thirds of patients with T2DM, and its development coincides with the development of hyperglycaemia [108]. The most relevant physiological factors affecting blood pressure, and their effect size, can be extracted from the follow-up data of a cohort of carefully phenotyped patients without hypertension [109]. Blood pressure is consistently lower in women than in men, but menopause, family history of hypertension, age, BMI, waist girth, heart rate, diabetes or impaired glucose tolerance, and smoking are all independently associated with an increase in mean blood pressure [108]. As populations have become urban and westernised in culture, particularly their nutritional habits and lifestyle, hypertension has become increasingly prevalent in low-income and middle-income countries [110]

In people without diabetes, the prevalence of untreated hypertension in women is less than that of men until they reach 55–64 years of age, when the prevalence reaches that of men and then begins to increase at a faster rate. In people with diabetes, the prevalence of untreated hypertension in women reaches that of men about one decade earlier (between 45 and 54 years) [109]. 20% or more of people with hypertension have diabetes, and 80% or more of people with diabetes have hypertension [111].

In patients with T2DM, gender (male), family history, age, and BMI continue to be independent risk factors for the high blood pressure; additionally, duration of diabetes adds to risk, whereas good renal function (measured by estimated glomerular filtration rate) is protective [112]. Both microvascular (retinopathy and albuminuria) and macrovascular (myocardial infarction and stroke) complications are significantly more prevalent in patients with diabetes who also have hypertension than in those without hypertension [108].

Findings from epidemiological surveys consistently suggest that excess hypertension in patients with diabetes decreases with age, is greater in women than in men, and tends to disappear in men older than 70 years [113-115] These findings might be attributable to two concurrent events: some patients with diabetes and hypertension, particularly men, would have died by the time screening took place (survival bias), and later onset of diabetes is generally indicative of a weaker predisposition to diabetes than is an early onset of diabetes [115].

Plenty of data show that blood pressure and blood glucose concentration covary, both in children [116] (in whom the confounding effects of alcohol and drugs are negligible) and in adults [117], although the correlation coefficients are generally low. Because blood pressure and blood glucose concentration are both variables that tend to remain

constant over time in an individual and can therefore be predicted, they should be able to be used to cross-predict each other. In a long-term observational study in Finland in which men without hypertension were stratified by tertiles of blood glucose concentration measured in 1968, more individuals in the upper two tertiles had hypertension in 1986 than did those in the lower tertile, even after adjustment for age, adiposity, alcohol consumption, and initial blood pressure [118]. Conversely, in a 5 year follow-up of about 10 000 men in Israel, systolic blood pressure was a significant predictor of T2DM (particularly in people aged 40‑49 years) independently of age, obesity, and presence of peripheral vascular disease [119]. The extent of such cross-prediction can be gauged in the cohort of people without hypertension or diabetes in the RISC (Relationship between Insulin Sensitivity and Cardiovascular disease) study, in which 12% of participants progressed to either hypertension or dysglycaemia over 3 years, 2% developed both disorders, and about 18% of people with either confirmed prehypertension or confirmed prediabetes had a 50% increased risk of both diabetes and hypertension [120].

2.3.2 The Framingham Heart Study

Both diabetes and hypertension are strong risk factors for cardiovascular disease morbidity and mortality, and are terms of the Framingham equation [121]. In the original Framingham cohort of 1952‑74, the hazard ratio for cardiovascular disease in people with diabetes was 3.0 and 8.1% of people with diabetes also has cardiovascular disease, yielding a population-attributable risk of 5.4% [122].

In the Framingham off spring cohort of 1975‑98, the hazard ratio of cardiovascular disease in people with diabetes had dropped to 2.5, but the prevalence of

cardiovascular disease had risen to 14.6% within this group, and population-attributable risk had increased by 60% [122, 123]. Thus, throughout the past four decades, the individual cardiovascular disease risk of people with diabetes has decreased, but the total cardiovascular disease burden in the population that is attributable to diabetes has increased. Furthermore, analysis of both Framingham cohorts showed that participants with hypertension at the time of diabetes diagnosis had higher rates of all-cause mortality and cardiovascular disease events than did people with diabetes without hypertension. In fact, after adjustment for demographic and clinical covariates, hypertension was associated with a 72% increase in the risk of all-cause death and a 57% increase in the risk of any cardiovascular disease event in individuals with diabetes [124]. Thus, a sizeable portion of the excess cardiovascular disease risk in people with diabetes is attributable to coexistent hypertension, especially in men, even after controlling for various risk factors.

The prevalence of obesity, defined as body mass index (BMI) $\geq$ 30 kg/m^2, in FHS has rose inexorably in both men and women within 30 years period, from just a few percentile in the 1970s to 25–30% among men in the 1990s. Over the same time frame, the prevalence of T2DM in FHS rose steadily [125]. Alarmingly, virtually the entire rise in T2DM has occurred in individuals with obesity, from about 6% in the 1970s to over 12% in the 1990s [126]. This association clearly demonstrates that the widespread rise in obesity is pushing a rising tide of T2DM. Although the absolute risk of CVD in FHS, defined by fatal and nonfatal myocardial infarction, stroke, and intermittent claudication, has declined between the 1950s and the 1990s by 35% in people without diabetes and by 49% in those with diabetes, the relative risk among those with diabetes to develop CVD has persistently remained about twofold higher relative to those without diabetes [127]. The rising prevalence of T2DM, combined with

a constant relative risk for CVD, has translated into a 60% increase in the attributable risk ratio for CVD associated with diabetes, even while the attributable risk for CVD associated with other risk factors like hypertension and smoking has held constant or fallen [123]. The experience of FHS shows that the rising tide of obesity is the driving force for rising tide of T2DM, which in turn is pushing a rising tide of CVD and death.

From a clinical perspective, CVD is often viewed as a consequence of diagnosed T2DM. However, the experience of FHS and other cohorts has shown that pre-diabetes - defined by blood glucose levels below the diagnostic threshold for diabetes, but nonetheless not normal - is also associated with risk for cardiovascular disease [128]. This observation has given rise to the notion that T2DM and CVD may share a common pathogenesis. Indeed, T2DM and CVD share many common risk factors [126], including obesity (especially central obesity), hyperinsulinemia (reflecting, in part, insulin resistance), hyperglycaemia, a dyslipidaemia characterized by low levels of HDL cholesterol and elevated levels of triglycerides, and elevated blood pressure. These measurable clinical traits and biomarkers are intercorrelated, co-occur to a far greater degree than would be expected by chance alone, and cluster together in an identifiable pattern linked by obesity and insulin resistance. This phenomenon of risk factor clustering is now called metabolic syndrome [129]. There are a variety of enthusiastically contested definitions of metabolic syndrome; in FHS, the definition proposed by the National Cholesterol Education Program Adult Treatment Panel III (NCEP/ATPIII) is the one most often studied [126].

A fundamental hypothesis raised by the concept of metabolic syndrome is that its presence increases future risk for both T2DM and CVD, even after accounting for other disease-specific risk factors [129]. In FHS, metabolic syndrome increases the 7- to

11-year risk for CVD about threefold relative to those without metabolic syndrome, and for T2DM, the increase is about sevenfold [130]. CVD risk increases steadily in a dose-response relationship as the number of component traits of metabolic syndrome increases. Further, risk is increased regardless of which of the various heterogeneous combinations of specific traits are present and even in the absence of impaired glycaemia. For instance, in individuals with the combination of low HDL cholesterol and elevated triglycerides and blood pressure but without impaired glycaemia, the relative risk for CVD is about twofold increased relative to those without this trait combination, and for T2DM, the relative risk is 3.5-fold increased [129]. The data suggest that beyond the specific risk factors that may be present, it is something about the phenomenon of risk factor clustering itself that appears to account, at least in part, for subsequent disease risk. Metabolic syndrome also accounts for some of the heterogeneity for future disease risk observed in individuals with obesity [130, 131]. Among FHS individuals with BMI <25 kg/m^2 who meet the criteria for metabolic syndrome, the 7-year cumulative incidence of T2DM was about 7%, while among those with BMI ≥ 30 kg/m^2 but without metabolic syndrome, the rate was only about 3% [125]. These individuals can be considered to represent "metabolically obese, normal weight" and "metabolically healthy obese" sub-phenotypes, respectively. Similar albeit less dramatic patterns were seen for risk of CVD [126]. One obvious lesson that can be drawn from the studies of metabolic syndrome in FHS is that metabolic syndrome is a far more powerful risk factor for T2DM than for CVD.

2.4 Diabetes and CVDs: mechanistic perspective

Several mechanisms are likely to contribute to the accelerated atherosclerosis and increased CVD risk noted in patients with T2DM. Several studies have assessed

pathophysiological linkage between hyperglycaemia, diabetic dyslipidaemia and inflammation to the accelerated vascular injury and CVD risk in T2DM [70, 132]. The roles of hyperglycaemia and hyperlipidaemia in atherogenesis have been difficult to separate in animal models of diabetes. Hyperlipidaemia is usually exacerbated by the onset of hyperglycaemia, as in mouse models of LDL-receptor deficiency and apolipoprotein-E deficiency, thereby confounding the effect of hyperglycaemia. However, in two animal models, hyperglycaemia seems to have an independent role [133, 134].

First, fat-fed diabetic pigs had more atherosclerosis than equally dyslipidaemic fat-fed animals without diabetes [134]. Second, consumption of a cholesterol-free diet by LDL-receptor-deficient mice with a novel form of diabetes induced by a β-cell-directed viral antigen resulted in hyperglycaemia without changes in lipids and lipoproteins [133]. Hyperglycaemia was associated with lesion initiation. Addition of increasing amounts of dietary cholesterol led to dyslipidaemia, which was the major factor in atherosclerosis progression, independent of hyperglycaemia [133].

2.4.1 Hyperglycaemia and the vessel wall

Hyperglycaemia can lead to vascular complications by several mechanisms. First, high glucose concentrations can activate nuclear factor κB (NF-κB) [135], which in turn can increase the expression of various genes in the endothelial cells, monocyte-derived macrophages, and vascular smooth-muscle cells. Advanced glycation end-products (AGEs) – including protein cross-links, fluorophors, and other low molecular-weight residues – are formed by sustained exposure of proteins and lipids to high concentrations of glucose, which can generate reactive oxygen species [136].

Ligation of AGEs to specific cell-surface receptors can regulate gene expression in vessel-wall cells. Glucose increases oxidative stress, which has several possible harmful effects on the artery wall; for instance, auto-oxidation of glucose leads to the formation of several reactive oxygen species, such as the superoxide anion, which can promote LDL oxidation in vitro [137].

Indirect observational evidence suggests that lipoprotein oxidation might be increased in patients with T2DM [138] and is related to glycaemic control [139]. However, many of the studies relied on non-specific assays of oxidative stress. The absence of highly specific markers in collagen, plasma, or urine from individuals with diabetes does not support a generalised increase in oxidative stress in diabetes [70]. Glycoxidation reactions are thought to contribute to macrovascular disease in diabetes by damaging tissues in the local microenvironment of the arterial wall [140]. The pathways leading to these reactions include the generation of superoxide in the mitochondria, NADPH generation by monocyte-derived macrophages, or a redox-sensitive mechanism that generates hydroxyl radicals. Accumulation of the products of hydroxyl radicals locally in arterial tissue of diabetic monkeys is consistent with a redox-sensitive mechanism [141].

Postprandial hyperglycaemia as an important index of glycaemic exposure and potential oxidative stress has had a resurgence in interest. 24-hour excretion of 8-iso-prostaglandin F2 – an indicator of free radical production derived from arachidonic acid in cell membranes – was increased in patients with diabetes compared with that in non-diabetic controls [142]. The concentrations of this prostaglandin were highest in patients with the greatest glycaemic variability. Moreover, this variability was a strong predictor of total free radical production, whereas postprandial blood glucose concentrations were not. Indeed, fluctuations in blood glucose concentrations

accelerated atherosclerosis in apolipoprotein-E-deficient mice [143]. Further studies are needed to assess the importance of oxidative stress that results from glycaemic variability.

2.4.2 Glucose and the endothelium

An important initial event in the pathogenesis of atherosclerosis is the adhesion of circulating monocytes to arterial endothelial cells, followed by their transmigration into the sub-endothelial space along a chemotactic gradient (figure 1). Hyperglycaemia enhances monocyte adhesion to cultured aortic endothelial cells by activation of NF-κB, which increases the expression of several inflammatory genes, including adhesion molecules that promote monocyte adhesion to the endothelial cells (figure 1) [135]. Expression of adhesion molecules might result from impaired nitric oxide production, since agents that increase the production of nitric oxide reduce the expression of adhesion molecules[144]. Glucose-mediated and AGE-mediated inhibition of nitric oxide production by endothelial cells is associated with impaired endothelial-dependent relaxation, an early marker of vascular injury [145]. In addition to substantial impairment of endothelium-dependent relaxation, diabetic mice show evidence of increased peroxynitrite generation, nitrotyrosine expression, and lipid peroxidation in the aortic tissues [146]. Hyperglycaemia and AGEs stimulate the production of superoxide by endothelial cells, partly by activation of NADPH oxidase, thereby providing a link between hyperglycaemia, AGEs, and oxidative stress [147].

2.4.3 Glucose and monocyte-derived macrophages

Both high glucose concentrations and AGEs are associated with an increased state of activation of circulating monocytes *in vitro* and *in vivo* [148]. Monocytes grown in the presence of high glucose concentrations or isolated from individuals with poorly controlled diabetes are in an activated and inflammatory state, as shown, for example, by the increased expression of cytokines − interleukin 1β, and interleukin– and expression of CD36 and monocyte chemoattractant protein 1 [149]. These inflammatory changes are associated with induction of protein-kinase C, NF-κB activation, and increased release of superoxide, and all three could play a part in the oxidative stress that occurs in the presence of hyperglycaemia [149]. Monocytes entering the endothelial space in response to chemotactic factors proliferate and differentiate into intimal macrophages, which accumulate in the artery wall in diabetes (figure 1). Hyperglycaemia is not sufficient to stimulate macrophage proliferation in lesions of atherosclerosis or in isolated murine macrophages; in combination with hyperlipidaemia, it stimulates macrophage proliferation by a pathway that might include glucose-dependent oxidation of LDL [148].

Arterial wall macrophages can accumulate lipid from modified forms of LDL, which are taken up by scavenger receptors. The modifications include LDL that has become oxidised as a result of glucose-mediated oxidative stress and AGE-modified LDL [150]. Additionally, AGE-modified albumin can inhibit the selective uptake of cholesteryl esters from HDL, an essential step in reverse cholesterol transport [151]. Thus, modification of lipoproteins and other proteins resulting from an increased exposure to high glucose concentrations can change the delivery and removal of lipids from macrophages in a way that is likely to promote atherosclerosis.

2.4.4 Glucose and vascular smooth-muscle cells

High glucose concentrations can stimulate the proliferation of vascular smooth-muscle cells (VSMC) *in vitro* [152]. As atherosclerotic lesions progresses, VSMCs migrate from the media to the intima, in which they proliferate, generate growth factors, and participate in the formation of a fibrous cap. Similar findings were noted after exposure of cells to AGEs and high insulin concentrations, which often accompany hyperglycaemia in T2DM [153].

VSMC generate several matrix molecules that are implicated in atherogenesis. Vascular proteoglycans bind atherogenic lipoproteins, leading to their retention in the sub-endothelial space [154]. At post-mortem examination, the expression of chondroitin sulphate and dermatan sulphate is increased and that of heparan sulphate proteoglycans is reduced in the atherosclerotic lesions of patients with diabetes compared with lesions from non-diabetic individuals [155]. The increase in chondroitin and dermatan sulphate proteoglycans might contribute to the increased atherosclerosis in patients with diabetes by increasing LDL retention in the artery wall.[154] In the mammalian models, diabetes is associated with a loss of intimal elastin content and increased elastin fragmentation. Reduced intimal elastin content, whether through reduced production or increased breakdown, seems to promote atherosclerosis by mechanisms that are unclear [156]. Therefore, elastin fragmentation might be another mechanism by which hyperglycaemia increases atherosclerosis in diabetes.

Collagen – synthesised by VSMC – accumulates in atherosclerosis. In the presence of hyperglycaemia, collagen undergoes increased nonenzymatic glycation that increases its ability to bind LDL, which could result in increased LDL retention in the vessel

wall. This increased retention could promote vessel wall accumulation and inflammation [143].

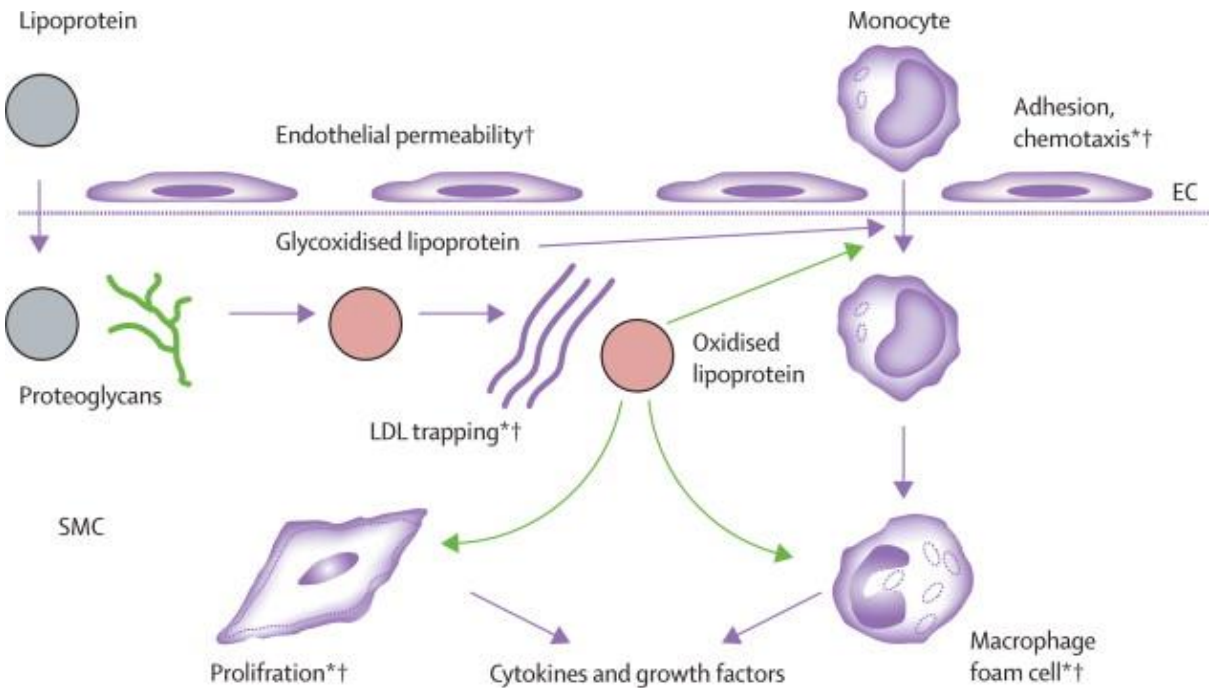


Figure 1. Possible mechanism linking hyperglycaemia to vascular damage (Adapted from Mazzone et al, 2008).

2.4.5 Diabetes dyslipidaemia and the vessel wall

Diabetic dyslipidaemia is strongly related to atherosclerosis. Even though patients with T2DM might not have substantially increased concentrations of LDL-cholesterol compared with matched individuals without diabetes, a cornerstone of the management of cardiovascular disease risk in diabetes is the use of LDL-cholesterol-lowering drugs, statins. These drugs generally reduce incidence and recurrence of cardiovascular disease events by 25‐50%, but the excess residual cardiovascular disease risk remains for treated patients with diabetes compared with those without

diabetes [157]. Some of this residual risk could be attributed to lipoprotein abnormalities in patients with T2DM that are not adequately managed by statin treatment. T2DM is characterised by reduced HDL-cholesterol concentrations, increased triglyceride-rich lipoprotein concentrations, and abnormalities in the composition of HDL, LDL, and triglyceride-rich lipoprotein particles [70].

2.4.5.1 Triglyceride-rich lipoproteins

The triglyceride-rich lipoproteins – which can be increased in the fasting or postprandial state – in patients with T2DM are very low density lipoprotein cholesterol (VLDL) and metabolites of VLDL, and chylomicron remnants. The role of these lipoproteins in diabetic atherosclerosis remains controversial [70]. Triglyceride concentrations vary inversely with HDL-cholesterol concentrations, confounding interpretations related to increases in concentrations of triglyceride-rich lipoproteins to atherosclerosis [158]. Postprandial triglyceride concentrations might be a better predictor of cardiovascular disease events than fasting triglyceride concentrations, independently of HDL cholesterol concentrations [70]. A proatherogenic effect of triglyceride-rich lipoproteins in the vessel wall is supported by substantial *in vitro* evidence (figure 2). Triglyceride-rich lipoproteins enhance the proinflammatory phenotype of endothelial cells and macrophages and produce apoptosis in endothelial cells [159]. They increase expression of tumour necrosis factor α (TNFα) and adhesion receptors in macrophages, resulting in increased adherence of monocytes and monocyte-derived macrophages to endothelial cells [70]. Apolipoprotein CIII – a component of triglyceride-rich lipoproteins and an inhibitor of lipoprotein lipase – increases adhesion of monocytic cells to endothelial cells [159].

Chylomicron remnants and triglyceride-rich lipoproteins produce lipid accumulation in macrophages [160]. Uptake of the lipid-rich VLDL particles is favoured by macrophages, promoting lipid accumulation. Disruption of the VLDL receptor expression in macrophages reduces atherosclerosis in cholesterol-fed mice, whereas VLDL receptor expression in VLDL receptor-deficient mice increases atherosclerosis [70]. Reduction in triglyceride-rich lipoprotein concentrations and hyperlipidaemia prevented disruption of atherosclerotic plaques in a mouse model of T1DM [158]. Increased concentrations of postprandial remnant lipoprotein particles have been proven to contribute to impaired arterial compliance [157].

The fatty-acid composition of chylomicron remnants affects their uptake and the induction of lipid accumulation in macrophages. The ability of triglyceride-rich lipoprotein particles to induce an inflammatory phenotype in macrophages might be enhanced by lipolytic release of fatty acids from VLDL [160]. Increased concentrations of free fatty acids are another component of diabetes dyslipidaemia and accompany increased triglyceride concentrations [70]. Fatty acids can directly lead to changes in the composition of the extracellular matrix produced by arterial smooth-muscle cells in a manner that favours increased immobilisation and retention of lipoproteins [157]. Excess free-fatty-acid delivery to peripheral tissues can worsen insulin resistance and might play a part in activation of the inflammatory processes through activation of toll-like receptors [149]. Free fatty acids are proven to impair endothelium-dependent vasodilation and disrupt the function of cellular sterol transporters that are important for reverse cholesterol transport [158].

However, data suggest that in some circumstances physiological lipolysis of triglyceride-rich lipoproteins might have beneficial anti-inflammatory effects. In some model systems, the lipolytic release of fatty acids can provide a ligand for nuclear

hormone receptors, such as the peroxisome-proliferator-activated receptor (PPAR) γ, which are implicated in the inhibition of inflammation [70, 158]. Taken together, these results suggest that inappropriate generation or handling of fatty acids, or both, might represent a fundamental abnormality in diabetes, leading to accelerated atherosclerosis.

2.4.5.2 LDL

Patients with T2DM might not have substantially higher concentrations of LDL cholesterol than matched individuals without diabetes, but for any LDL-cholesterol concentration, those with diabetes generally have an increase in LDL particles due to accumulation of small, dense lipid-poor LDL particles in the circulation [158]. Each LDL particle contains one apolipoprotein-B molecule and therefore patients with T2DM will also have a parallel increase in concentrations of apolipoprotein B. An increased number of LDL particles, measured directly or indirectly by concentrations of apolipoprotein B, might contribute to atherogenesis and cardiovascular disease risk [161]. An increase in the number of LDL particles in diabetes can be treated by statins [157]. However, a separate issue is whether or not small, dense LDL particles are inherently more atherogenic on a per-particle basis than the larger buoyant particles. An increased atherogenicity of small, dense LDL particles is supported by results of *in vitro* studies, showing that small LDL particles rapidly enter the arterial wall and can be toxic to endothelial cells (Figure 2), cause greater production of procoagulant factors, be oxidised more readily, and be more readily immobilised by proteoglycans present in the arterial wall than can the large buoyant LDL particles [70]. The small particles do not bind very well to the LDL receptor, which might lead to impaired clearance by the liver. How these *in vitro* results translate to the *in vivo* milieu,

however, remains unclear. A satisfactory *in vivo* model for testing atherogenicity of small, dense LDL particles on a per-particle basis compared with large particles is needed [143]. In non-human primates fed fat-modified diets, LDL-particle size was not independently atherogenic [162]. Results from studies of healthy individuals and those with coronary heart disease showed that both large and small LDL particles are related to atherosclerosis and cardiovascular disease [158].

2.4.5.3 HDL

Individuals with T2DM have reduced HDL cholesterol and circulating apolipoprotein AI, the major apolipoprotein in HDL cholesterol [158]. Abnormalities in the size and composition of the HDL particle have also been noted in diabetes patients [70, 162]. HDL and apolipoprotein AI remove excess cholesterol from atherosclerotic plaque cells, and their reduced concentrations in diabetes would be expected to have a detrimental effect on cholesterol content in vessel walls (figure 2) [158]. The cell type of most interest is the monocyte-derived macrophage because cholesterol-ester-engorged macrophages, often referred to as foam cells, are hallmarks of the atherosclerotic plaque. Removal of cholesterol from macrophages is thought to be an important first step in the process of reverse cholesterol transport, and might be important for the prevention of progression and for regression of atherosclerotic plaques [163]. The HDL particle and its apolipoprotein AI component might act through distinct cellular sterol transporters for removal of cholesterol from cells. The HDL particle seems to rely mainly on the ATP-binding cassette transporter G1 to facilitate sterol efflux, and expression of this transporter in cells can be inhibited by exposure to glycosylated proteins [164]. Additionally, glycation of apolipoprotein AI, which acts mainly through the ATP-binding cassette transporter A1, suppresses its ability to remove cholesterol

from cells [165]. HDL has anti-inflammatory and antioxidant properties in cells of the vessel wall. Monocyte-derived macrophages isolated from individuals with low HDL cholesterol concentrations manifest a proinflammatory phenotype [158].

In addition to changes in HDL-cholesterol and apolipoprotein AI concentrations, patients with T2DM have changes in HDL composition. HDL is perhaps the most heterogeneous and complex of all lipoprotein particles, and changes in its composition might affect HDL atheroprotective properties (figure 2) [158]. In isolated cells, HDL particles of different sizes and composition show different abilities to remove cholesterol from cells [163]. Changes in the content of many proteins associated with HDL such as paroxonase, which opposes oxidation of lipoprotein lipid, might change its atheroprotective properties [70]. Compositional abnormalities of HDL isolated from patients with T2DM have been linked to impaired antiatherogenic properties [166]. Cholesterol-ester transfer protein inhibition with torcetrapib did not protect against cardiovascular disease events, underscoring the notion that HDL-particle composition might be more important than HDL-cholesterol concentrations for reduction of cardiovascular disease risk [167].

Mice without apolipoprotein AI and with very low HDL cholesterol concentrations have increased rates of atherosclerosis because of both reduced cholesterol transport and increased inflammation [70]. Conversely, increased expression of apolipoprotein AI with high HDL-cholesterol concentrations reduces the amount of atherosclerosis in the apolipoprotein-$E^{-/-}$ mouse, a model of accelerated and progressive atherosclerosis [167]. An increase in HDL-cholesterol concentrations in patients with T2DM has been linked to reduced carotid atherosclerosis [158]. HDL has been proven to improve

mobilisation and function of endothelial precursor cells and to protect the myocardium from ischemia and reperfusion injury [164].

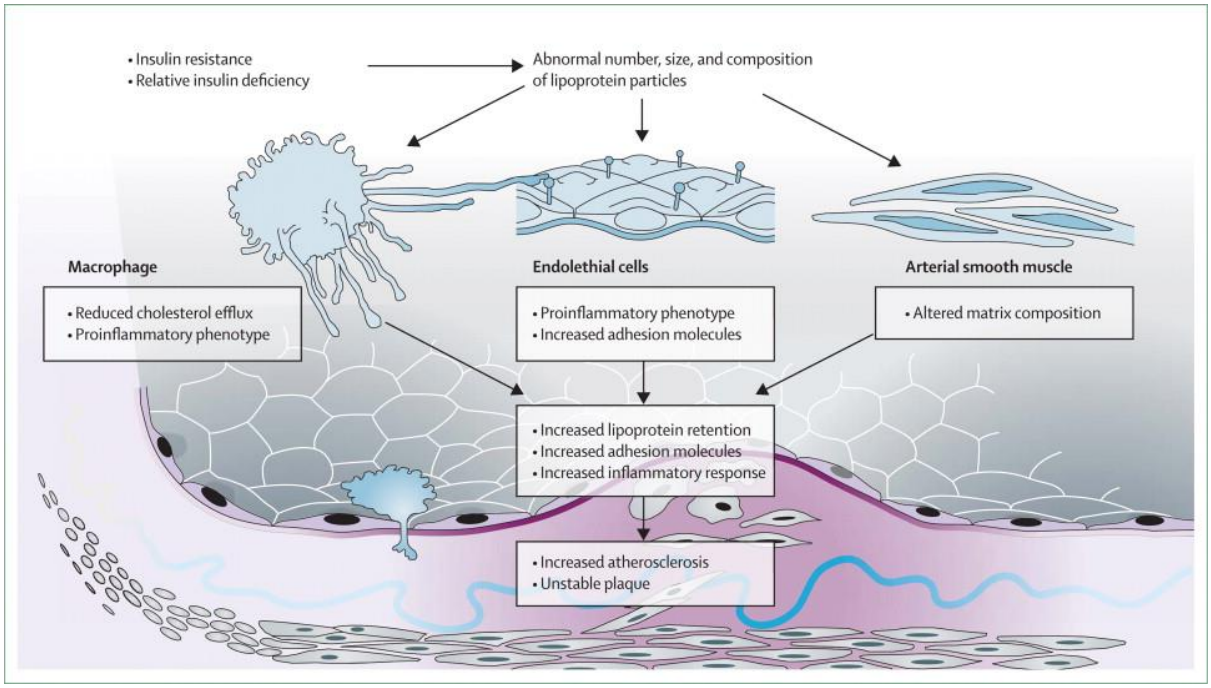


Figure 2. Diabetes Dyslipidaemia and the vessel wall (Adapted from Mazzone et al (2008)

2.4.6. Chronic subclinical inflammation and the vessel wall

Evidence ranging from pathological studies in people to *in vivo* mouse models has established the role of inflammatory cells, such as macrophages and T lymphocytes, and inflammatory mechanisms in the pathogenesis of atherosclerosis [143, 160]. Because T2DM and atherosclerosis are chronic conditions that take decades to arise, the cause and effect are difficult to discern (figure 3) [70]. Inflammation is implicated in the pathogenesis of T2DM and atherosclerosis. Since diabetes promotes atherosclerosis and increases cardiovascular events, a distinction might exist between inflammation that fosters diabetes and inflammation that arises after the T2DM and promotes atherosclerosis directly [70]. Most of the inflammatory mechanisms discussed also seem

to be implicated in the atherosclerosis seen in prediabetes and non-diabetes states [143]. Although the evidence implicating inflammation in atherosclerosis and T2DM is wide-ranging, a specific mechanism or an integrated framework has not been identified to explain precisely why patients with diabetes are at increased risk of inflammation or atherosclerosis [70, 134].

2.4.7 Mechanisms of inflammation in diabetes atherosclerosis

The endothelium – as the cellular interface between the circulation and hyperglycaemia and dyslipidaemia that characterise T2DM – responds to hyperglycaemia and dyslipidaemia by showing an inflammatory response [168]. Most of the responses induced in atherosclerosis are common to both diabetes and non-diabetes atherosclerosis. Classic proatherosclerotic endothelial responses - adhesion molecule expression, secretion of chemokines, and coagulation proteins (plasminogen activator inhibitor 1, total plasminogen activator, and tissue factor), release of vasoactive mediators (endothelial nitric oxide and bradykinin) - are induced or regulated by inflammatory stimuli in diabetes models *in vitro* or *in vivo*, or both [168].

Lymphocytes provide crucial proinflammatory signals to monocyte-derived macrophages and vascular smooth muscle cells, and are activated by metabolic stimuli [168]. Macrophages directly respond to the common abnormalities in T2DM such as glucose, free fatty acids and hypertriglyceridemia, by augmentation of the inflammatory responses [160]. Several stimuli and cellular pathways are implicated in the effects of macrophages (figure 4), including increased foam cell formation, release of matrix metalloproteinases, and secretion of growth factors and cytokines [160]. These effects emphasise the important link between insulin resistance, inflammation, and

atherosclerosis. When bone marrow from insulin-receptor-deficient mice was transplanted into LDL-receptor-deficient mice, lesions of increased complexity were noted [161]. Macrophage-specific deficiency of the nuclear receptor PPARγ in mice worsens insulin resistance, suggesting that the presence of this ligand-activated transcription factor in macrophages regulates insulin sensitivity, which could be related to PPARγ-mediated inhibition of inflammation [168]. Similar issues apply to retinoid signalling through the retinoid-X receptor, the essential partner of PPARγ and many other nuclear receptors [70].

The available data suggests cellular responses to injury, inflammation, and metabolism might converge on control points that are important in atherosclerosis. A central regulator of inflammation is NF-κB, a transcriptional complex activated by various stimuli, including cytokines, oxidised LDL, lipopolysaccharide, and oxidative stress (Figure 4)[168, 169]. NF-κB is reported to regulate LDL oxidative modification, chemokine and cytokine expression, macrophage growth and differentiation, apoptosis, and vascular smooth muscle cell proliferation. NF-κB, its regulatory proteins such as inhibitor κB, and distal targets such as c-Jun N-terminal kinase have all been strongly implicated in insulin sensitivity and in atherosclerosis (figure 4) [170]. NF-κB might have a role in the common pathway, linking many inputs that are activated in T2DM to atherosclerotic responses [168]. It is activated by factors commonly abnormal in T2DM, including fatty acids, glucose, AGE pathways, and some toll like receptors – a family of pattern recognition receptors expressed in various inflammatory cells [70]. Several NF-κB-regulated targets are implicated in diabetes atherosclerosis, including TNFα, which increases insulin resistance, toll-like receptors, and resistin [158].

Several mechanistic pathways have been proposed for how glucose brings about cellular injury and subsequent inflammation. Cells that do not have the ability to counter the increase in intracellular glucose concentrations might activate pathways of cellular injury and inflammation [168]. These mechanisms include activation of protein-kinase C, formation of polyols, which promotes intracellular oxidative stress, and increased hexosamine activation, with subsequent increases in reactive oxidant species and mitochondrial stress [171]. Although much of this evidence was linked to diabetes microvascular disease, increased flux of free fatty acids into the endothelium might cause macrovascular disease through similar pathways, inducing inflammation.

All secretory and membrane proteins, many nutrients, and many pathogens pass through the endoplasmic reticulum. Several lines of study implicate endoplasmic reticulum stress in the promotion of inflammation [168]. Hypoxia, hyperglycaemia, and increased fatty-acid concentrations can all induce endoplasmic reticulum stress and a specific cellular process known as the unfolded protein response, which is a homoeostatic mechanism that restores normal endoplasmic reticulum function [70]. Endoplasmic reticulum stress, present in the liver and adipose tissue, can activate pathways leading to oxidation and inflammation and has been implicated in both diabetes and atherosclerosis [171].

2.6 Anatomy of the Human arterial system

The arterial system is responsible for the transport of blood to the body tissues. The pressure is kept at a relatively high value because the distal end of the arterial system bifurcates into many vessels with small diameters (arterioles) and hereby forms a large peripheral resistance [172]. The Aorta is the largest artery in the human body; it is

the root systemic arterial tree. It originates in the left ventricle at the aortic valve, and curved at 180º, forming the aortic arch and giving branches off to the head and upper limbs (Figure 3). The Aorta receives blood directly from the left ventricle of the heart through the aortic valve, it branches into large arteries. The aortic arch gives off, from right to left, the innominate (brachiocephalic trunk), the left common carotid, and the subclavian arteries [173]. The innominate artery passes beneath the left innominate vein before it branches into the right subclavian and the right common carotid arteries. The vertebral arteries branch off the subclavian arteries 2 or 3 cm from the arch, but many variations may occur [174]. The left common carotid artery may arise from the innominate (bovine arch) in 16% of patients and cross to a relatively normal position on the left side. The left vertebral artery may arise directly from the aortic arch instead of from the left subclavian arteries. The right vertebral artery may arise as part of a trifurcation of the brachiocephalic trunk into subclavian, common carotid, and vertebral arteries. Occasionally, both subclavian arteries originate together as a single trunk off of the arch, or the right subclavian may arise distal to the left subclavian artery and cross to the right side [175].

The common carotid arteries on each side travel in the carotid sheath up to the neck, before branching into internal and external carotid arteries just below the level of the mandible [175]. The external carotid artery supplies the face. Important branches of the external carotid artery include the superior thyroid, which can actually arise from the common carotid artery, and is important in that it accompanies the external branch of the superior laryngeal nerve, the ascending pharyngeal, and the lingual and occipital arteries that have a close association with the hypoglossal nerve. No branches of the internal carotid artery occur in the neck [173].

The innominate artery arises from the aortic arch on the right and behind the sternoclavicular joint divides into the subclavian and common carotid arteries [173]. On the left, the subclavian artery arises directly from the aortic arch as does the common carotid artery at a more proximal site. Along its course, the subclavian artery first gives off the vertebral artery cranially. Further along the course, the thyrocervical trunk arises, likewise from the posterior aspect, and at once divides into a branch supplying the thyroid and other branches supplying the skin and soft tissue. Together with the brachial plexus, the subclavian artery passes through the scalene triangle, between the anterior and medial scalene muscles and cranial to the first rib, and arches over the pleural dome, crossing under the clavicle, to continue as the axillary artery. In individuals with a cervical rib, the subclavian artery is displaced cranially and anteriorly [176]. The branches of the axillary artery have extensive collateral connections to the branches of the subclavian artery and supply the region of the shoulder girdle. The axillary artery courses along the lower border of the pectoralis muscle through the axilla and continue as the brachial artery. The latter runs through the medial bicipital groove near the humerus to the elbow and divides into the radial and ulnar arteries at the level of the joint space [177]. There are anatomic variants in which the brachial artery already gives off the radial artery in the upper arm (in about 15%) or arises directly from the distal axillary artery (1–3%). The ulnar artery may likewise originate from the axillary artery (in about 1%) [176]. The radial artery continues through the forearm on the ulnar side of the radius to the wrist where it unites with the deep branch of the ulnar artery to form the deep palmar arch. The radial artery primarily feeds the deep arch and the ulnar artery the superficial arch [173].

The abdominal aorta divides into the two common iliac arteries (Figure 3). They descend into the true pelvis taking an arched course along which they bifurcate at

about the most posterior point. The common and external iliac veins course behind the respective arteries. The internal iliac artery arises at the level of the sacroiliac joint, coursing in a posterior direction to supply the pelvic organs, pelvic wall, and buttocks. The external iliac artery is the continuation of the common iliac artery and arches into the lacuna vasorum under the inguinal ligament [173]. It runs medial to the iliopsoas muscle and gives off the inferior epigastric and deep circumflex iliac arteries shortly before it reaches the inguinal ligament. These two arteries can serve as collaterals in pelvic artery occlusion. Diameters range from 0.6 to 1.4 cm in the common iliac artery, 0.5–1.0 cm in the external iliac artery, and 0.4–0.8 cm in the internal iliac artery [176].

The common femoral artery is about 2–4 cm long and, below the inguinal ligament, divides into the profunda femoris artery, which typically arises from the posterolateral aspect, and the superficial femoral artery [173]. The origin of the profunda femoris artery is variable, and several branches may arise directly from the common femoral artery. Typically, one branch arises posteriorly, while the main branch arises posterolaterally or, in rare cases, posteromedially. The profunda femoris artery is the most important collateral route in occlusion of the femoropopliteal segment [177].

The superficial femoral artery continues inferiorly to become the popliteal artery at its point of entry into the adductor canal. The popliteal artery then continues below the knee, where the anterior tibial artery branches, piercing the interosseous membrane to supply the anterior compartment of the lower leg [173]. The tibioperoneal trunk then continues briefly, where the posterior tibial artery branches to course in a plane deep to the soleus muscle. The vessel then continues inferiorly as the peroneal artery. The posterior tibial artery is divided into lateral and medial plantar arteries below the medial malleolus to supply the sole of the foot [177]. Ultimately, the anterior tibial artery

continues on to the dorsum of the foot, where it becomes the dorsalis pedis artery. Here it anastomoses with branches of the posterior tibial and peroneal arteries to form the plantar arch [176].

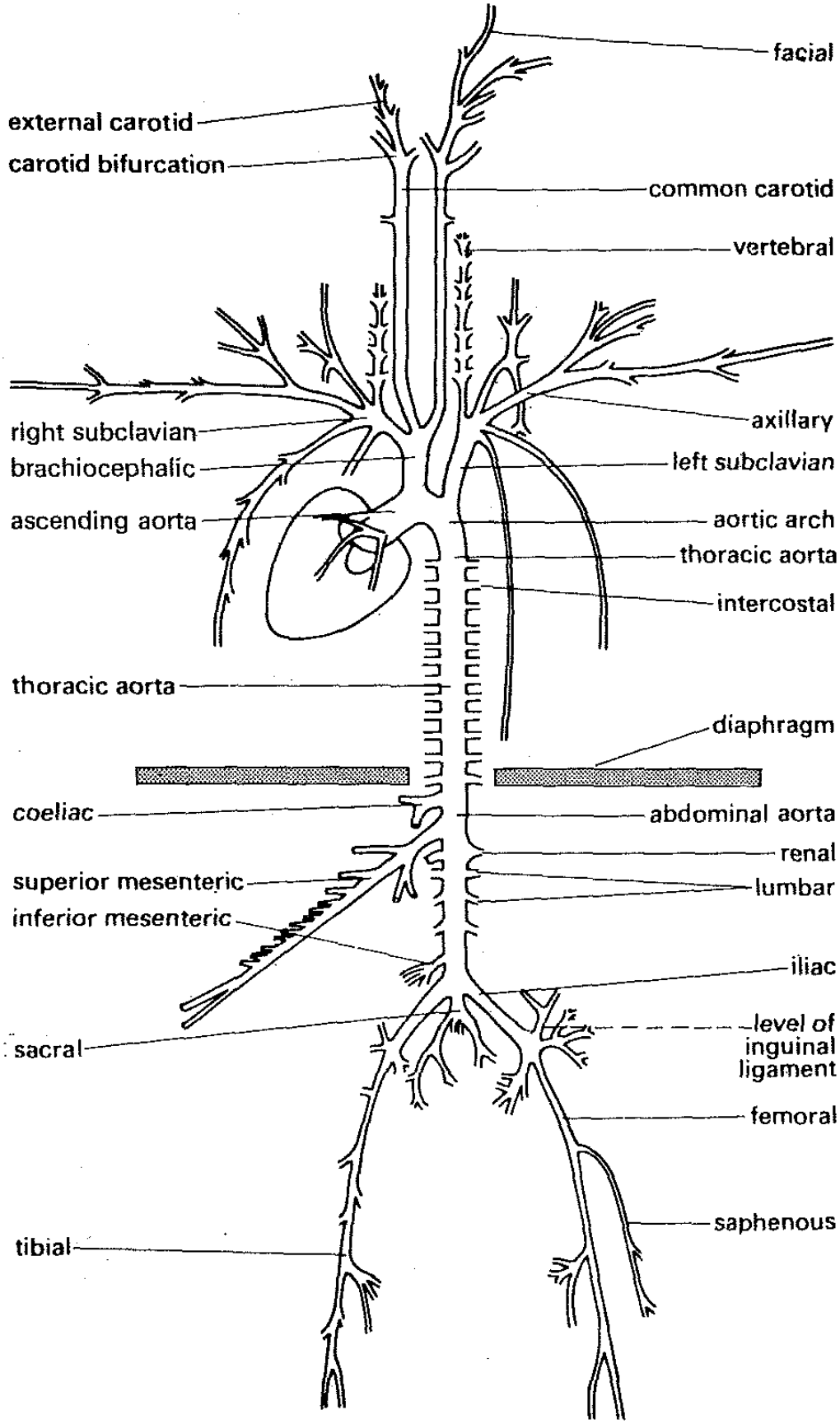


Figure 3. Schematic representation of the arterial tree. Adapted from Ballal, 2011 [178].

2.6.1 Cross-section of arterial wall

The arterial wall normally consists of three well-defined concentric layers that surround the arterial lumen, each of which has a distinctive composition of cells and extracellular matrix. The layer immediately adjacent to the lumen is called the intima, the middle layer is known as the media, and the outermost layer comprises the arterial adventitia (Figure 4). These three layers are demarcated by concentric layers of elastin, known as the internal elastic lamina that separates the intima from the media, and the external elastic lamina that separates the media from the adventitia [176].

A single contiguous layer of endothelial cells lines the luminal surface of arteries. These cells sit on a basement membrane of extracellular matrix and proteoglycans that is bordered by the internal elastic lamina. Although smooth muscle cells are occasionally found in the intima, endothelial cells are the principal cellular component of this anatomic layer and form a physical and functional barrier between flowing blood and the stroma of the arterial wall. Endothelial cells regulate a wide array of processes including thrombosis, vascular tone, and leukocyte trafficking among others [179].

Progressing outwards from the internal elastic lamina, the media consists principally of smooth muscle cells arranged in layers, the number of layers depending on the arterial size. An extracellular matrix consisting largely of elastic fibres and collagen with a lesser content of proteoglycan holds the smooth muscle cells together. An increasing content of elastin is typical of larger arteries that need to provide for considerable elastic recoil during diastole, the time period between ejections of blood from the heart [180].

The adventitia is the outermost layer of the artery and typically consists of a loose matrix of elastin, smooth muscle cells, fibroblasts, and collagen. Most of the neural input into blood vessels also traverses through the adventitia. At one time, the adventitia was considered inactive with respect to vascular homeostasis; however, recently it has become clear that the adventitia, through the production of reactive oxygen species (ROS), may play an important role in controlling vascular remodelling and nitric oxide (NO) bioactivity [181].

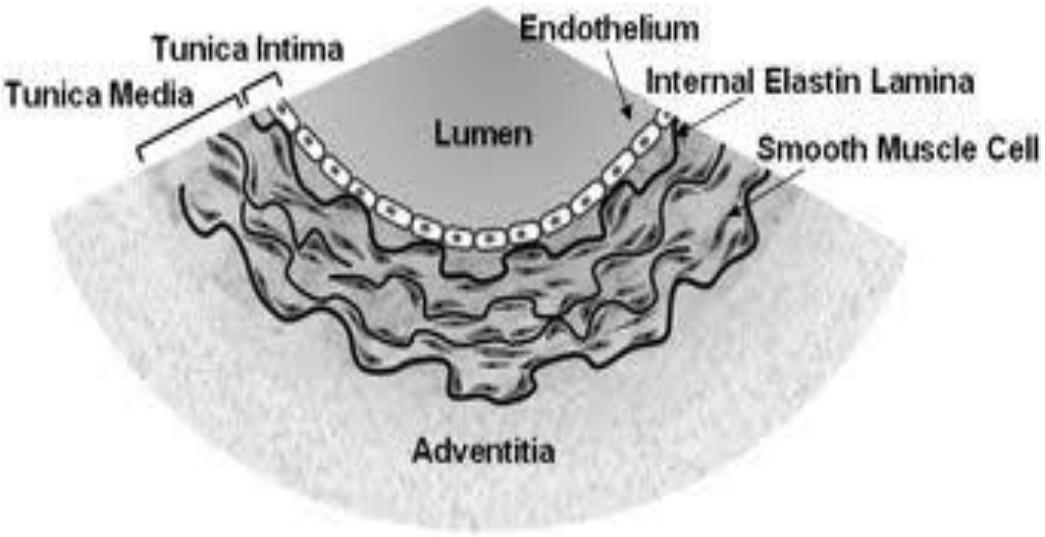


Figure 4. Cross-section of elastic artery (Accessed from sciencedirect.com on 30th July, 2013).

2.6.2 Functions of the arterial system

Arteries act as a conduit system to transport blood through the body, and dampen oscillations from the pulsatile ejection of blood form the heart, in order to provide steady flow throughout the arterial tree. There are three separate anatomical arterial regions addressing these functions [182]. First, large elastic arteries such as the aorta provide the predominant cushioning reservoir for blood flow. Second, large muscular

arteries act as the conduit for blood to the periphery and actively modify wave propagation through smooth muscle tone regulation. Finally, arterioles function to alter peripheral artery resistance, and subsequently aid in the maintenance of mean arterial pressure and delivery of a continual flow to required systems and subsequent capillary beds [55].

The composition of the arterial wall, in particular the elastin and collagen content, changes from central to peripheral arteries. Starting in the proximal aorta, elastin is the dominant component. At the abdominal aorta the content of collagen and elastin appears similar, and by the periphery collagen becomes dominant [183, 184]. As collagen is 300 times stiffer than elastin (elastic modulus 1000 x 10^6 dyne/cm^2 vs. 5 x 10^6), the altering arterial wall composition causes an increasing 'stiffness gradient' down the arterial tree [185]. For example, in the central arteries, up to 50% of the stroke volume ejected from the heart is momentarily stored in the aorta and large elastic arteries. Approximately 10% of the energy produced by the heart is used to distend the arteries during systole. The elastic walls of the artery store the energy, and subsequently use it to recoil the vessel wall during diastole [186], thus ensuring continuous flow to the stiffer, more collagen based, peripheral arteries. For the dampening effect in the central arteries to be more efficient, the energy needed to distend the arterial wall needs to be as low as possible, which not only depends on the wall's elasticity and high elastin content, but also the geometry of the vessel walls [187].

Elastin and collagen contents of the arterial wall cause the pressure–diameter relationship at any specific area on the arterial tree to be non-linear [185]. At low distensions, pressure is mainly governed by elastin fibres, which are quite compliant and the resulting curve is more linear, where at higher tensions it is governed by the

supporting latticework of collagen content, which is much stiffer, resulting in a steeper slope; a greater pressure required for a given diameter change [188].

2.7 Basic concepts of arterial haemodynamics

Although blood flow is subject to specific conditions due to the solid components in plasma and the elasticity of the vessel wall, it basically follows the laws of hemodynamics [182]. The laws of haemodynamics govern the flow of a fluid in tubes and apply to watery or oily solutions of a constant viscosity (Newtonian fluid) and assume that flow velocity under these conditions is primarily a function of the pressure difference that exists between the two ends of the tube. These ideal conditions for continuous laminar flow are typically not met in a living organism because various factors like elasticity of the vessel wall, pulsatility resulting from cardiac activity, curving of vessels, and branching affect blood flow, resulting in changing velocity distributions in the different layers [55].

Moreover, blood is not a watery or oily solution of constant viscosity (non-Newtonian fluid) but a suspension of solid blood cells in plasma. The viscosity of blood is primarily dependent on its hematocrit content and is only constant at haematocrit levels below 10, increasing exponentially at higher values. Other factors affecting blood viscosity are plasma viscosity and vessel diameter. In the terminal capillary bed, viscosity is additionally influenced by the deformation of red blood cells [55]. Despite these specific features of blood flow, some basic hemodynamic terms and laws are useful and make it easier to understand normal and abnormal flow in arteries and veins. In addition, *in vitro* experiments and *in vivo* blood flow measurements using duplex scanning have provided new insights into the flow behaviour in specific

vessels under normal and abnormal conditions as well as under the influence of pharmacologic agents [182].

The blood volume ejected from the left ventricle is partially transferred to large arteries extensible under increasing pressure. During systole, about a half of the ejection volume is directed to peripheral tissues, whereas the remaining 50–60% dilates aorta and large arteries. Part of the energy produced by the heart (10%) is used to dilate and accumulate blood within the walls of large arteries [182]. During the following diastole, when the aortic valve is closed and no blood flow from the heart, the elastic recoil of the aorta and large arteries causes the progression of blood flow towards the distal peripheral arteries. This results in the cushioning process, transforming the blood flow from pulsatile in the proximal aorta to continuous, steady flow in distal arteries [187]. The lesser the energy required to dilate the aorta and central arteries during systole and recoil them in diastole, the more effective the dampening function [186]. This function of the large arteries is strongly affected by stiffening of arterial vessels, a phenomenon due mainly to the physiological and pathophysiological processes [182]. The relationship between distension and changes in volume is described as distensibility or compliance [187]. On the contrary, arterial stiffness is the variation of transmural pressure for any given change in the vessel inner volume. Arterial stiffness influences systolic blood pressure, diastolic blood pressure and pulse pressure through both a direct and an indirect mechanism [189]. By a direct mechanism, stiffer arterial walls give rise to a higher systolic pressure wave from the left ventricle resulting in an increased SBP and a decreased relaxation of large arteries [30]. The indirect mechanism involves the effects of stiffness on pulse wave velocity and on timing of pulse waves [182]. During systole, the left ventricle generates a pulse wave travelling along the arterial tree at a given speed. Such

velocity is labelled pulse wave velocity (PWV) and is influenced mainly by the degree of arterial stiffness: the stiffer the arterial tree, the higher the PWV. Therefore, PWV may be considered as an indirect index of arterial stiffness [190]. Its value is rather elevated, reaching 5–7 m/sec under normal conditions, whereas with stiffer arterial walls it may be even higher [26]. With the increasing PWV value, the timing of incident and reflected waves changes as compared with physiology. The forward wave reaches peripheral sites earlier, thus the reflected wave returns to the heart during systole rather than diastole and, as a result, a lower DBP and a higher SBP and PP can be observed [190, 191].

2.8 Hemodynamic Consequences of Arterial Stiffness

The stiffness on various arterial segments have complex effects on ventricular afterload, through their effects on the early aortic systolic pressure rise, the total compliance of the arterial system and the velocity at which the pulse waves travel forward in the arteries and reflected waves travel backward toward the heart [187, 188]. At the beginning of each cardiac cycle, the heart creates a forward-traveling energy pulse that results in increased pressure and forward flow in the proximal aorta during early systole. If proximal aortic characteristic impedance is high due to a stiff wall, a small aortic calibre, or both, the amount of pressure intensification is relatively large for any given early systolic flow [192]. The energy wave generated by the left ventricles (incident wave) is conducted by conduit vessels and partially reflected at sites of impedance mismatch, such as points of bifurcation or modification in wall diameter or material properties along the arterial tree [190]. Multiple reflections are conducted back to the heart and merge into an effective reflected wave, composed of the contributions of the scattered backward reflections. The time of arrival of the reflected wave to the

proximal aorta depends on the location of reflection sites and on the PWV of conduit vessels, particularly the aorta, which transmits both the forward and backward traveling waves [193].

Aortic PWV is directly related to the stiffness of the aortic wall (square root of its elastic modulus) but it is also affected by aortic geometry [193]. Stiffer aorta conducts the forward and backward traveling waves at greater velocity and therefore promotes an earlier arrival of the reflected wave for any given distance to reflection sites. The interaction between the stiffness of large and muscular arteries also impacts the magnitude and location of reflection sites. Reflected waves that arrive during left ventricular ejection increase the mid-to-late systolic workload of the left ventricle [189].

Remarkably, although the timing of arrival of reflected waves to the heart is influenced by aortic stiffness, the relationship between aortic stiffness and reflected wave transit time is poor, presumably given the wide variability in the distance to wave reflection sites [182]. Furthermore, there is not a direct correlation between aortic stiffness and the magnitude of wave reflections. It has been proposed that aortic stiffening promotes "impedance matching" with more distal muscular arteries, therefore decreasing the magnitude of wave reflections as the pulse travels from the aorta to more distal muscular arteries [194, 195].

Another imperative aspect of increased large artery stiffness is its proposed role in promoting an unwarranted penetration of pulsatility into smaller vascular beds in target organs such as the brain and kidneys [189]. Both an increase in absolute pulse pressure in the large arteries as well as "impedance matching" with distal vascular beds, allowing forward waves to penetrate further into smaller vascular beds, may play a role in target organ damage [193, 195].

2.9 Elastic behaviour of arteries

Arterial stiffness denotes the capacity of the arterial wall to accommodate fluctuations in pressure by corresponding changes in dimension of luminal diameter. Large arteries are elastic and stretch in response to gradual changes in pressure; either induced by left ventricular contraction or sustained blood pressure changes [196]. This vascular property, called 'stiffness' as well as its inverse form 'elasticity', are purely descriptive term that cannot be measured and quantified [182]. Compliance or distensibility are more practical with precise meaning, since they can be measured and quantified as changes in volume related to changes in pressure, either raw or normalized to initial volume, respectively [55]. A more complex definition of arterial stiffness corresponds to the properties of the arterial wall, approached through the elastic modulus, defined as the percentage of increase in wall stress for 100% elongation [196].

The ability to describe and compute the elastic behaviour of arteries has progressively become important, because its application has widened from basic physiology to clinical domains and the prediction of cardiovascular risk [197]. Despite the growing interest in this field, the physical principles and nomenclature of the multiple indices that may be used to quantify arterial behaviour may be perplexing in clinical practice and research [198]. Although terms such as elastic modulus, distensibility, compliance, elastance, PWV, and characteristic impedance are all related to arterial stiffness, they are not interchangeable [182, 197].

2.9.1 Stress – strain relationship of arteries

Stiffness refers to the resistance offered by an elastic body to deformation. All measures of stiffness ultimately deal with the relations between forces applied to an elastic body, which result in mechanical stress and deformation, referred to as strain, in that body. Stress is the intensity of the force applied across a given plane on a body per unit area, whereas strain is the relative increase in length in a given plane produced by that force [182]. The stiffness of a material in a given direction can be expressed by the Young's elastic modulus, which is the ratio of stress to strain expressed in units of force per unit area [30]. Practically, the Young's modulus is obtained from the slope of the stress–strain relationship from the origin, "unstressed" state, to any point of the graph. The slope can also be computed using any two points on the stress-strain relationship, other than the unstressed state to obtain the incremental elastic modulus, also called the Young's incremental elastic modulus [55].

Young's moduli express multidimensional stiffness in elastic substances. Elastic materials are said to be isotropic if they demonstrate material properties that are independent of direction in space for which the force was applied; Young's modulus is the same regardless of the direction from which the force is applied [199]. Conversely, for anisotropic materials, Young's modulus will change depending on the direction from which the force is applied [55]. Therefore, the mathematical expression of multidimensional stress–strain relations is greatly simplified for isotropic materials. In vivo, measurements of arterial stiffness and formulae for the most commonly used indices assume arterial wall is isotropic and are therefore true material properties are estimates by these algorithms. In reality however, arterial wall is anisotropic [182].

Hooke's law states that, within certain limits, strain of an elastic material is linearly related to stress applied [193, 200]. Materials that demonstrate such linear relationship are

said to be linear-elastic or "Hookean"; the stress-strain relation is straight graph and Young's modulus is therefore constant throughout the range of stress and strain. Similarly, incremental slopes are always identical to the slope from any point in the stress-strain line to the unstressed state [200].

In arterial vessel of constant length with intraluminal pressure exceeding extraluminal pressure, the transmural gradient in pressure creates lengthening (positive strain) and tension (stress) in the circumferential direction referred to as tensile stress [201]. According to the law of Laplace, the circumferential wall stress is directly proportional to the tube radius and the luminal pressure and inversely proportional to the wall thickness [182]. Therefore, at greater distending luminal pressures, the arterial wall progressively become thin as diameter progressively increases, and changes in stress become progressively larger for any given change in luminal pressure [55]. Under assumptions of perfectly cylindrical shape and no deformation in the longitudinal direction of arteries, simplified models of isotropic thick-walled tubes can be used to derive relatively simple formulae based on changes in luminal pressure and diameter, or cross-sectional area, that provide descriptions of the mechanical properties of the arteries and their relationships to the stiffness of the wall material [55, 202]. The incremental elastic modulus of the wall material, which represents the stiffness of the wall material at operating conditions, can thus be computed as long as arterial wall thickness and at least two diameter measurements and corresponding pressure points are known [30].

2.9.2 Pressure–Volume and Pressure–Area Relations

The local mechanical properties of arteries can also be expressed using indices that relate the local relation between changes in pressure and changes in volume, diameter, or area. Pressure–volume and pressure–area relations are influenced not only by the stiffness of the wall material, but also by vessel geometry [55]. When intra-arterial pressure increases, the artery distends and its volume increases. Arterial compliance (C) expresses the change in volume (V) relative to the change in pressure (P) [30]. For arteries, the pressure–volume relation, as in elastic modulus, is not straight graph. Therefore, compliance will be different when measured around different points of the non-linear pressure–volume relationship [191]. Within relatively narrow ranges of pressure, the non-linearity of the pressure–volume curve is minimized and the pressure-volume relationship can be approximated with linear equations. Compliance can therefore be expressed as a local linear slope of the pressure-volume curve [188]. The inverse of compliance is the elastance; this is given as the change in arterial pressure relative to the change in arterial volume [197]. Notably, compliance and elastance, although influenced by the material properties of the arterial wall, are not direct indices of the stiffness of the wall material because they are prominently affected by arterial size and wall thickness. Arteries with thicker walls will have lower compliance than those with thinner walls, irrespective of identical elastic properties of the wall material. Similarly, larger arteries will have larger volume compliance than smaller arteries for identical wall material properties and relative wall thickness-to-luminal diameter ratio [182]. Hence, to compare arteries of different sizes, compliance should be controlled for arterial volume. This normalized value is called distensibility, which is the ratio of fractional volume change over pressure change [55].

Due to the difficulty in measuring volume of arteries in vivo, diameter or cross-sectional area of vessel measured and utilised in computations instead, with the assumption that the vascular cross-section is perfectly circular and volumetric increase is due to radial vascular distension rather than axial elongation [26]. In this case, the cross-sectional compliance coefficient, which is the ratio of area change to pressure change, can be computed. Like volume compliance, the compliance coefficient is dependent of vessel cross-sectional area and should be normalized to compute the cross-sectional distensibility coefficient, which is the fractional area change divided by the pressure change [55]. Although distensibility and distensibility coefficient eliminate the influence of vessel calibre, distensibility is not only influenced by the stiffness of the wall, but also by the relative ratio between wall thickness and lumen diameter of a vessel [182].

In 1960, Peterson *et al.*,[203] suggested a definition of arterial mechanical properties based on the linear ratio of pressure and diameter, which is now known as the Peterson's modulus (E_P), or pressure–strain modulus. E_P is the arterial pressure change for a given fractional diameter change [$E_p = \Delta P/(\Delta D/D)$]. This relationship shows a striking similarities between E_P and the distensibility coefficient, which is the fractional volume change for a given pressure change. Like distensibility, E_P is not a direct indicator of the stiffness of the wall material because it is also affected by the relative vessel geometry such as wall thickness-to-lumen ratio. In the mid-1970's, Gosling [204] suggested calling the inverse of E_p the arterial compliance [where $C = (\Delta D/D)/\Delta P$].

A closely related parameter is the β stiffness index, which is computed as the logarithm of systolic/diastolic pressure over the fractional diameter change [205]. The mathematical expression of the β stiffness index may better account for non-linearity

in the relationship between pressure change and diameter change and as a result of this mathematical property, it appears to demonstrate much less within-subject variations in response to blood pressure changes induced by nitroprusside infusions [206]. Like the distensibility coefficient and E_P, the β stiffness index is not only affected by the elastic modulus of the wall material but also by the vessel wall thickness [191].

The pressure-strain relation is neither similar nor is it linearly related to the stress–strain relation within a single artery. Also, variability between arteries in terms of arterial size and wall thickness influence pressure–strain relations, pressure–volume, and pressure–diameter relationships for any given stiffness of the wall material [182]. Regarding pressure–strain relations, it is the transmural gradient in pressure, and not just intraluminal pressure that produces tensile stress and circumferential stretch. Extraluminal pressure is typically small and can be neglected, although it may become quantitatively important in specific circumstances [191].

2.9.3 Arterial resistance in terms of pressure-flow relationship

In electric circuit theory, the terminology "resistance" applies to signals, such as direct current, that do not vary in time. Ohm's law states that resistance is the ratio of the potential difference (voltage) over a conductor and the electric current [55]. To describe resistance to electric current that fluctuates over time, as the case of alternating current, the term impedance is used. Impedance is a more general formulation than resistance, which it actually includes, but varies with the frequency of fluctuations in the electric signal; implying that resistance is the value of impedance at zero-frequency. These terms have been applied to describe hemodynamic phenomena [192]. Analogous to the dampening of electric flow by electric

devices, resistance and impedance in haemodynamics refer to dampening of blood flow by blood vessels, expressing the relation between pressure (voltage) and flow (current). By convention, the term "resistance" is typically used to describe non-oscillatory opposition to flow, whereas the term "impedance" is used for opposition to fluctuating (pulsatile) flow [182].

The properties of intermittent flow injection into a single elastic tube of isotropic wall material and infinite length illustrate the pressure–flow relations in the absence of reflected waves. Under such conditions, pulsatile energy imparted from one end of the tube promotes forward flow and increases pressure within the tube [187]. The change in pressure (ΔP) in relationship to change in flow (ΔQ) for any given amount of imparted energy is a linear function, determined by the characteristic impedance (Zc) of the tube (therefore, $Zc = \Delta P/\Delta Q$), which is the slope of the pressure-flow relation in a plot of instantaneous pressure versus instantaneous flow [188]. The characteristic impedance of an artery can be more generally defined as the slope of the pulsatile pressure–flow relation, in which pressure and flow are measured in the same point within the artery, in the absence of reflected waves [30]. Zc can be approximated as $\rho \times PWV/A$, where ρ is blood density, PWV is the "local" propagation speed of the pulse through that vessel segment, and A is its cross-sectional area [55]. Assuming a constant blood density, arterial Zc is therefore linearly related to PWV, which in turn is proportional to the square root of the elastic modulus of the wall material and the arterial "relative" cross-sectional geometry such as wall thickness-to-lumen ratio [191]. However, Zc is additionally linearly related to luminal cross-sectional area. Therefore, for any given luminal cross-sectional area, relative geometry and wall stiffness, smaller vessels will definitely have proportionally greater Zc [188].

2.10 Pulse wave analysis

The periodic characteristics of cardiac ejection can be analysed by means of Fourier analysis of oscillatory time-dependent functions of arterial pressure and flow, and relationships expressed in the frequency domain [55]. Fourier analysis involves decomposing recorded pressure and flow waveforms into a spectrum of single frequency components, each having its own associated amplitude and phase. The modulus at each harmonic is the ratio of the pressure modulus to the flow modulus at that harmonic, and the phase at each harmonic is the difference between pressure phase and flow phase at the same harmonic. The inherent assumptions in the concept of impedance and wave transmission are that the system is linear and in steady state oscillation [182, 207]. This means that, a specific component of pressure is uniquely related to the same harmonic component of flow determined by the vascular impedance at that frequency. Similarly, a specific harmonic component of distal pressure is uniquely related to the same harmonic component of proximal pressure as determined by the vascular transmission values of modulus and phase at that frequency [208].

Although blood flow in elastic arteries does not strictly obey the properties of linearity and steady state oscillation, the contribution of nonlinearities under normal operating conditions is relatively small [182]. In the canine system, using intermodulation frequency methods, the contribution of nonlinearities to pressure and flow total power was of the order of 1% and 2% respectively [209]. These effects were also measured in conscious animals to quantify wall viscoelasticity [210, 211]. *In vitro* studies of the human aorta showed a pressure-dependent compliance of the thoracic aorta over the cardiac cycle [212], although aortic pressure and diameter wave-shapes show a large degree of similarity [213]. In terms of energy transfer, the major energy

component responsible for flow is potential energy. Kinetic energy provides a much smaller component, approximately 5% of total energy [182]. Thus, the effect of factors that contribute to nonlinearity is relatively small and so the assumption of linearity greatly facilitates the analysis by enabling the derivation of closed-form relations [55].

Other methods of characterising the arterial pulse waveform include wavelet transforms [214] and wave intensity analysis [215]. Wave intensity analysis does not require the assumption of steady state oscillation. It considers the pressure wave as a series of wave fronts of solitary waves [216, 217]. This analysis is entirely in the time domain and uses the concept of compression and expansion waves [215]. For normal operating conditions, the frequency and time analysis methodologies give complementary information [218]. However, there is recent interest in recasting the Windkessel concept so as to provide an alternative interpretation of the quantification of the principal factors that contribute to the morphology of the arterial pulse [217].

2.10.1 Pulse waveform features

The time course of the pressure pulse waveform at the aortic root is determined by the pattern of ventricular ejection and the elastic and geometric properties of the arterial tree [55]. During the cardiac cycle, the waveform exhibits prominent features that can be used as descriptors of the pressure pulse. In the ascending aorta, peak pressure (P_s) occurs after peak flow due to the capacitive effects of the ascending aortic segment [208]. The first inflection generally coincides with the time of peak flow velocity at approximately 30% of the ejection duration at time T_i and pressure value of P_i [55]. The ratio of the augmented component of pressure described as $(Ps-Pi)/(Ps-Pd)$ is defined as the augmentation index (AIx). From figure 5, it can be inferred that if Pi is

at the peak, AIx is zero, and if Pi is after SBP, AIx is negative. From simultaneous measurements of pressure and flow in the ascending aorta, peak flow generally coincides with the location of Pi. Hence, if AIx is zero or negative, peak pressure is essentially due to the cardiac ejection into the compliant aorta. If AIx is positive, peak pressure is due to secondary phenomena emanating from the arterial load, such as wave refection, since pressure rises while flow decreases [219]. Clearly, AIx is related to cardiac and arterial function. AIx has been found to have a significant heritability factor and shows changes with age [190]. It is generally negative in young individuals and increases to positive values with increasing age due to significant changes in the waveform morphology brought about by changes in structural components of the arterial system affecting pulse wave propagation [186] (figure 5). AIx has been conceived as a parameter associated with ventricular–vascular coupling. Modelling studies have also assessed the contribution of ventricular ejection to systolic pressure augmentation [208, 220].

The radial pulse is also characterized by a number of waveform features (figure 5). The minimum point in the pressure waveform corresponding to diastolic pressure (P_d) is often equated to that in the aorta or in the carotid artery, and together with mean pressure is used as a calibration reference [221]. The first peak in the waveform of radial pulse corresponds to systolic pressure (P_s) and is different to that in the central pulse, with a large variation in the difference (figure 5). An inflection (P_i) occurs late in systole and is usually lower than P_s but may form a local maximum higher than P_s in cases of high arterial stiffness, as seen with advancing age [208]. It is also related to systolic peak in the aorta [222]. A local minimum near the end of systole, the dichrotic notch, (often incorrectly called the incisura) strongly correlates with the timing of the

incisura obtained from the aortic or carotid pressure pulse, and therefore corresponds to aortic valve closure, and can be used to obtain systolic duration [208, 221].

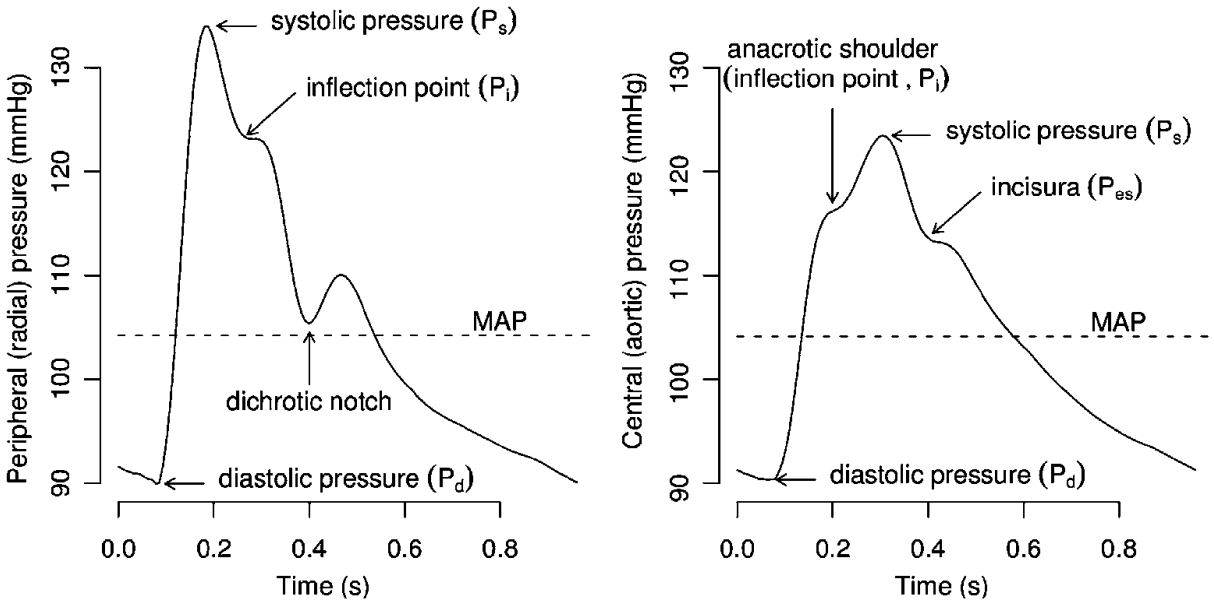


Figure 5. Features of the radial pulse wave and corresponding central aortic pressure wave (Adapted from Avolio et al, 2010)

2.10.2 The wave transfer function in arterial system

Transfer functions are mathematical entities used to describe a system in terms of the relationship between input and output signals. In arterial system, if the input signal is blood flow and the output is blood pressure, the transfer function between flow and pressure measured at the same location and at the same time is the impedance of the system, described as the ratio of the frequency components of pressure and flow [55]. If the input is a blood pressure signal (P_0) and the output is another blood pressure signal (P_L) measured at the same time but at a different location separated by a finite distance (L), the transfer function is the ratio of the frequency (ω) components of the output and input pressure signals ($TF(\omega) = P_L(\omega)/P_0(\omega)$). The presence of impedance

mismatch due to arterial branching and geometry will alter the relationship between the input and output pressure pulse and ultimately wave propagation. according to the theory of wave propagation [207], the presence of changes in arterial elasticity and dimensions along the arterial tree will produce changes in the magnitude and phase of the frequency components of the pressure pulse between two points, with the result that, for the same mean pressure, the pulse pressure and the wave shape will be different. This has implications for the measurement of peripheral pressure and in its use as a surrogate measure of central aortic pressure [208]. The transfer function is related to the arterial length, the propagation coefficient, which depends on arterial geometric and elastic properties defining pulse wave velocity, and the reflection coefficient. Like impedance, transfer function is a complex quantity expressed in modulus and phase as a function of frequency. The high frequency phase delay is related to the pulse wave velocity between the two measuring sites [182]. Transfer functions are described in the frequency domain or as autoregressive functions [55]. They can also be developed as closed form filter functions such as neural networks and Chebychev filters [223], in the time domain using peripheral pressure and wave velocity [221] or multichannel adaptive algorithms [224].

A relevant characteristic of the pressure pulse is that it changes shape as it travels away from the heart. In large arteries, mean pressure is essentially constant. Hence, shape changes are such that the total waveform area over one cardiac period is constant, and this generally results in a change of pulse height [223, 225]. These studies are significant as they illustrate the different time delay and the marked difference in pulse pressure at different anatomical sites. The importance of these observations is in the large differences in peak peripheral pressure as surrogate values of aortic pressure and thus the peak load on the ejecting ventricle [208]. The difference is exaggerated in

exercise where radial pulse pressure can be more than double the aortic pulse pressure [226].

Studies employing direct intra-arterial measurements of blood pressure such as those of Kroeker and Wood (1955)[225] and Rowell *et al* (1968)[226] highlighted the importance of understanding the fundamental physiological mechanisms to explain the difference between central and peripheral systolic pressure and pulse contour. This, of course, is significant in relating the conventional cuff measurements in the arm to cardiovascular function. Many modelling studies, involving simple tube models, showed the change in arterial pulse and pulse amplitude amplification due to elastic and geometric non-uniformity, such that pulse amplification can occur with progressive stiffening [223]. O'Rourke (1970) [227] investigated the influence of ventricular ejection on the transmission ratio of pulse pressure between the aorta and brachial pulse. The transmission ratio is, in effect, related to the modulus of the transfer function of the brachial system. Subsequent studies by Lasance *et al*., (1976)[228], although principally aimed at using the peripheral pulse for estimation of stroke volume, showed a frequency dependence of transfer function modulus and phase delay.

The development of pulse-sensing techniques has made it possible to detect the peripheral pulse with a high degree of accuracy. Although many devices use the finger as the site for pulse detection, the assumption is that there is an association between the volume pulse usually detected in the finger and the pressure pulse in the large arteries [229]. The most reliable registration of the peripheral arterial pulse is where it can be palpated and readily accessible, usually the wrist. Thus, from the early systems which employed mechanical levers to register the pulse at the wrist, the resurgence of the recording of the radial pulse through the application of the

technique of arterial tonometry has made a significant contribution in advancing the field of blood pressure measurement by providing a reliable signal that can be analysed to estimate central aortic pressure non-invasively [208].

Tonometry of the radial artery has enabled the application of the brachial transfer function, determined by invasive catheter measurements, for non-invasive determination of the aortic pressure pulse and, in combination with the brachial cuff sphygmomanometer, to provide calibrated values. The study by Karamanoglu *et al*., (1993)[64], where intra-arterial pressures were measured directly by high-fidelity catheter-tipped transducers, showed that the modulus and phase of the brachial transfer function was relatively constant under control conditions and following administration of sublingual glycerol trinitrate (GTN). Although GTN resulted in a reduction in systemic mean pressure, the overall frequency did not change markedly, especially at low frequencies (below 4 Hz), the bandwidth containing most of the energy of the arterial pulse [208]. In this bandwidth, the combined results in both aortic-brachial and aortic-radial transfer showed a close correspondence with results from other previous studies [225, 226].

The study by Karamanoglu *et al*., (1993) formed the basis for the development of a generalized transfer function where it was proposed that it could be applied to adults for the non-invasive estimation of central aortic pressure [64]. Since its early use and initial prospective validation using invasive measurements at rest [230, 231] and during exercise [226], there has been substantial debate on the generalized transfer function concept regarding the use of essentially a constant mathematical filter in all adult subjects, irrespective of age and gender [208, 232]. There have been attempts at individualization and development of group-specific transfer functions [233]. While it is recognized that there are necessary individual differences, the constancy of the

transfer function at low frequencies makes the transfer function essentially a robust low pass filter, with relatively low mean differences between estimated and derived pulse pressure [230]. Furthermore, the transfer function validation procedures included considerable changes in vascular haemodynamics, as occur in the Valsalva manoeuvre [214]. As with the vasodilatory effects of GTN [64], the generalized transfer function tracked changes in central systolic and diastolic pressure with marked decreases in systemic mean pressure due to altered intra-thoracic pressure during forced expiration against a closed glottis as occurs during a Valsalva manoeuvre [208].

Transfer functions derived for specific groups such as diabetes patients [234] or submaximal exercise [226] have shown average differences of less than 5% for systolic pressure estimates when compared to the generalized transfer function. These findings are in agreement with recent studies analysing pulse wave propagation in the human arm which have shown that individualization of the transfer function would produce minimal effect in adults [231]. Other techniques have been proposed for the non-invasive estimation of central aortic pressure from the analysis of the pressure signal in the brachial artery, but these have not gained widespread use [208].

2.10.3 Central aortic pressure

Since the early development and validation of transfer function models as non-invasive means of obtaining central aortic pressure from the peripheral arterial pulse [64, 208, 230], there has been a surge of activity in the use of pulse wave analysis for the determination of vascular parameters to complement the conventional blood pressure measurement by the cuff sphygmomanometer [208].

Since the publication of recent large trials on anti-hypertensive treatment and specific endpoints such as the 'Losartan Intervention For Endpoint reduction in hypertension' (LIFE) study [235], the Conduit Artery Function Evaluation (CAFÉ) study [236], the Strong Heart study [237] and the REASON study [24, 238], there has been broad acceptance that central aortic pressure is a parameter that can improve cardiovascular risk stratification. Although there is an increasing number of methodologies [208], the principal techniques involve the use of the brachial generalized transfer function [64] or the calibration of the tonometric carotid pulse using brachial cuff mean and diastolic pressures [231]. A third method is emerging which equates the pressure at the inflection point of the radial artery to the aortic systolic pressure [182]. A modification of this method is now being incorporated in devices using a multi-sensor array for detection of the radial pulse. Whereas the generalized transfer function and the carotid method would always provide a value of central systolic pressure, the inflection point method relies on the actual presence and detection of the inflection, a feature that is not always present, especially at high heart rates and estimates may have increased error at low systolic pressure [55].

2.10.4 Central aortic pressure and pulse wave amplification

The general increase in the wall elastic modulus and relative wall thickness with respect to vessel calibre of the arterial tree with distance from the aorta results in increasing arterial stiffness and so pulse wave velocity [223]. This has the property of producing frequency-dependent transfer functions of modulus and phase, where the modulus is essentially unity at very low frequencies and increases to a peak, then decreases at higher frequencies [64]. For given values of pulse wave velocities, the frequency of the peak modulus depends on the length of the arterial segment [182]. In

adults, the mean length of the arm does not have large variations with age, since the wingspan is proportional to height and mean height varies little with age [239]. Brachial pulse wave velocity also changes little with age compared to pulse wave velocity in the aortic trunk. Hence, the peak of the modulus of brachial transfer function in adults occurs at a relatively constant frequency of mean value around 4 Hz [231]. This means that different frequency components will be amplified or attenuated to different degrees [208]. Hence, the amplification of the propagated pressure pulse depends on the spectral content of the aortic pulse, and so the shape of the pulse wave. These considerations, therefore, become important when associating the pressure as measured by the brachial cuff sphygmomanometer to central properties such as peak load on the ejecting ventricle.

2.10.5 Central aortic pressure and effect of heart rate

Inherent in the frequency spectrum of the brachial transfer function is that amplification of the arterial pulse between proximal and distal sites depends on heart rate. This is due to the fact that the first harmonic of the pulse waveform contains the bulk (60–75%) of the total energy [55]. Hence, the first harmonic, and therefore heart rate, is the major contributor to amplification of the pressure pulse. This implies that the association of central and peripheral pulse pressure depends on heart rate [223, 240]. Since diastolic pressure is essentially similar in central and peripheral large arteries, the variability in amplification is manifest as differences in systolic pressures [231]. Hence, similar systolic and diastolic pressures as measured with the brachial cuff at different heart rates would be associated with different systolic pressure in the central aorta. This effect can be detected by use of information available from the arterial pressure pulse waveform, in addition to the sphygmomanometer cuff measurements.

The amplification ratio to peripheral sites is also age dependent due to changes in waveform morphology with age. Studies using a brachial transfer function and atrial pacing showed that pulse amplification (A) between the derived central aorta and radial artery was estimated as 39% for a heart rate of 65 beats min^{-1} increasing to 95% for 120 beats min^{-1}, giving a relationship between A and heart rate (HR, beats min^{-1}) of $A = 0.01HR + 0.76$ [240].

The heart rate effect on pulse amplification becomes an important factor in assessing ventricular load, especially peak load due to systolic pressure, for conditions where there are large changes in heart rate, such as exercise [226], or large scale studies where anti-hypertensive agents also affect heart rate and where small differences become highly statistically significant. Results of the LIFE study in over 9000 hypertensive subjects show that an angiotensin receptor-blocking agent (losartan) has virtually identical effects on brachial blood pressure reduction as a beta-blocking agent (atenolol) for a follow-up period of 5 years [235]. For a similar reduction in blood pressure, losartan produced additional beneficial and pressure-independent effects such as improved regression of left-ventricular hypertrophy. However, the reduction in heart rate with atenolol was of the order of some 6 beats min^{-1}. This means that although brachial pulse pressure was identical for both agents, pulse amplification was greater with losartan; hence, aortic pulse pressure would be lower [235]. Central aortic pressure was not measured in the LIFE study. For a given diastolic pressure, and using the regression relation between pulse amplification and heart rate [240], the increase in central systolic pressure associated with beta-blocker treatment is estimated at approximately 3 mmHg [208]. In the subsequent CAFE study, where central aortic pressure was estimated from the radial pulse wave in over 2000 subjects [236], central systolic pressure was found to be an average of 4.3 mmHg higher for the beta-

blocker (atenolol) treatment compared to the calcium channel blocker treatment (amlodipine), despite similar brachial cuff pressures over a 4 year follow-up. Further analysis of the LIFE data has shown that in patients with hypertension and left ventricular hypertrophy, there were significantly higher risks for the primary composite end point, stroke and total mortality in the highest quartile of pulse pressure with atenolol-based treatment compared to the lowest quartile. The risks increased with pulse pressure for both losartan and atenolol with a trend of lower values of risk for losartan [241].

The observations from the LIFE [235, 241] and CAFE [236] studies, large long-term follow-up studies addressing the efficacy of anti-hypertensive treatments, are important in terms of highlighting the information obtained from the conventional brachial cuff measurements and that obtained from the combination of the cuff measurements with the pulse waveform. The conclusions drawn from the similar cuff measurements in the LIFE study would be that the end-organ effects such as regression of left ventricular hypertrophy are pressure-independent effects and are essentially due to the treatment [235]. However, with the inclusion of the arterial pulse and estimation of central pressures, it is seen that with treatments that alter heart rate, similar brachial cuff systolic pressure is associated with a different central aortic systolic pressure, and so with different peak systolic load on the left ventricle. In these studies, these effects persisted for 4–5 years; hence, conditions such as regression of left ventricular hypertrophy may not necessarily be pressure independent [208].

2.11 Mechanisms of Arterial Stiffness

Arterial stiffening develops from a multifaceted interaction between stable and dynamic alterations involving structural and cellular elements of the vessel wall (Figure 6). These vascular modifications are influenced by hemodynamic forces as well as by "extrinsic factors" such as hormones, salt, and glucose regulation [184]. Stiffness is not homogeneously disseminated throughout the vascular tree but is often patchy, occurring in central and conduit vessels while sparing more peripheral arteries [65]. This phenomenon contributes to impedance matching, in which the distensibility of elastic central arteries becomes similar to the collagenous peripheral arteries. Common diseases, such as hypertension and diabetes mellitus, or simply aging itself, amplify the vascular changes that result in artery stiffening and can do so in different, yet synergistic, ways [196].

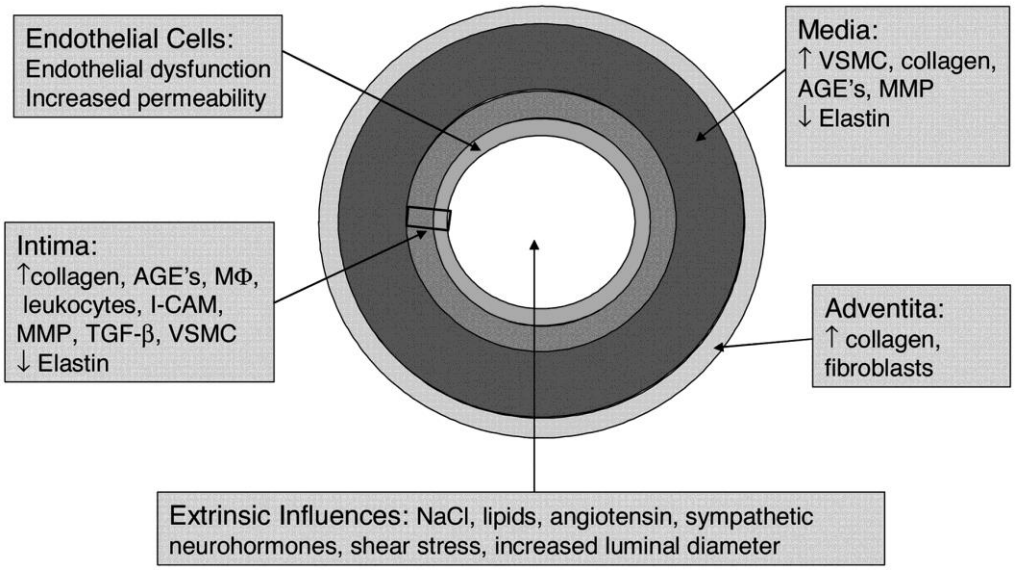


Figure 6. Summary of the multiple causes and locations of arterial stiffness (Adapted from Zieman S J et al. 2005).

2.11.1 Structural Components of Arterial Stiffening

The stability, resilience, and compliance of the arterial wall are dependent on the relative contribution of its two prominent scaffolding proteins: collagen and elastin. The relative content of these molecules is normally held stable by a slow, but dynamic, process of production and degradation [185]. Dysregulation of this balance, mainly by stimulation of an inflammatory milieu, leads to overproduction of abnormal collagen and diminished quantities of normal elastin, which contribute to vascular stiffness [242]. Increased luminal pressure, or hypertension, also stimulates excessive collagen production [243]. On gross pathologic vascular specimens, these molecular changes manifest as a doubling to tripling of intima-medial thickness between ages 20 to 90 years, as well as a hypertrophied vascular smooth muscle layer [182]. Histological examination of the intima of stiffened arteries reveals abnormal and disarrayed endothelial cells, increased collagen, frayed and broken elastin molecules, infiltration of vascular smooth muscle cells, macrophages and mononuclear cells, and increased matrix metalloproteinases, transforming growth factor (TGF)-β, intracellular cell adhesion molecules, and cytokines [55, 244]. In addition to vessel wall thickening, aging is associated with a gradual increase in central artery lumen diameter (9% per decade from 20 to 60 years in the ascending aorta) [245], although some recent studies have suggested this does not occur [246].

The extracellular matrix (ECM) of the vessel wall is comprised of collagen, elastin, glycoproteins and proteoglycans. Collagen and elastin provide structural integrity and elasticity, and are potently regulated by catabolic matrix metalloproteases (MMPs). Through their collagenolytic and elastinolytic effects, MMPs degrade the ECM by creating uncoiled, less effective collagen and broken and frayed elastin molecules, respectively (Figure 7). Vascular cells, as well as inflammatory cells such as

macrophages and polymorphonuclear neutrophils, produce collagenases (MMP-1, MMP-8, MMP-13) and elastases (MMP-7 and serine proteases) [247, 248]. Further degradation of the basement membrane ECM and stimulation of chemotactic agents occur through gelatinase activation (MMP-2 and MMP-9). Enzyme activity is regulated by augmented gene expression, post-translational activation by cleavage of pro-MMP protein, by MMP–MMP interactions, and by plasmin, thrombin, and reactive oxygen species (ROS) [249]. Tissue inhibitors of MMPs counter this response, and MMP–tissue inhibitors of MMPs balance are central in controlling remodelling. Deposition of chondroitin sulphate, heparin sulphate, proteoglycans, and fibronectin can also thicken and stiffen the ECM of vessel walls [248].

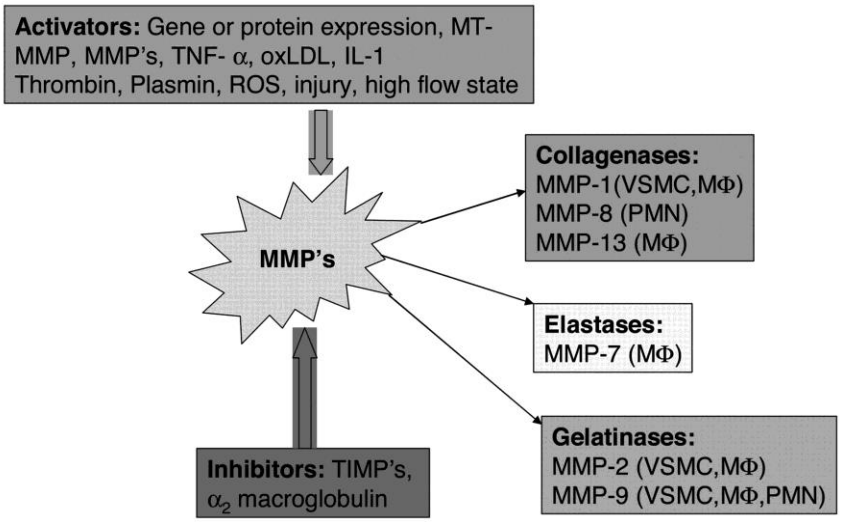


Figure 7. Matrix metalloproteinases affect collagen and elastin balance and are regulated by various activators and inhibitors (Adapted from Zieman S J et al. 2005).

Collagen molecules provide the tensile strength of the vessel wall and are enzymatically cross-linked soon after their formation to render them insoluble to hydrolytic enzymes [247]. Breaks in the integrity of these intermolecular bonds cause unravelling of the collagen matrix. Moreover, because of their slow hydrolytic turnover rate, collagen is particularly susceptible to nonenzymatic glycation cross-

linking. This leads to increased collagen content, often with a more unorganized and dysfunctional fibre distribution [242]. Elastin molecules are also stabilized by cross-linking (by LOX) to form desmosine and isodesmosine. Disruption of these crosslinks contributes to weakening of the elastin array with predisposition to mineralization by calcium and phosphorous, together increasing arterial stiffness [243]. Moreover, activation of various serine and metalloproteases generate broken and frayed elastin molecules. Alterations in elastin production and molecular repair mechanisms additionally contribute to the loss of vascular elasticity [247].

Arterial stiffness is also caused by AGEs, which result from nonenzymatic protein glycation to form irreversible cross-links between long-lived proteins such as collagen [250]. AGE-linked collagen is stiffer and less susceptible to hydrolytic turnover. This results in an accumulation of structurally inadequate collagen molecules [247]. Similarly, elastin molecules are susceptible to AGE crosslinking reducing the elastic matrix of the wall [251]. AGE may also affect endothelial cell function by quenching nitric oxide and increasing the generation of oxidant species such as peroxinitrite [137]. Through their immunoglobulin superfamily receptors (RAGE), AGE stimulates stress signalling and inflammatory responses, increasing the expression of p12(ras), NF-κB, oxidant radical formation, proinflammatory cytokines, growth factors, and vascular adhesion molecules [243]. Such mediators can increase arterial stiffness via MMPs, contribute to endothelial dysfunction that elevates smooth muscle tone, depress endothelial flow-mediated dilation, worsen the response to vascular injury, affect angiogenesis, and promote atherosclerotic plaque formation [137, 243]. A profibrotic response can be also triggered independently from a TGF-β pathway by the interaction of RAGE with AGE ligands [251].

It remains less clear whether the deposition of lipids in the vascular wall and development of atherosclerotic lesions alone contribute to vessel stiffness. Young subjects with isolated hypercholesterolemia have normal or even increased arterial compliance [252]. With progressing age, the relationship between arterial compliance and LDL cholesterol becomes negative, as a result of more pronounced endothelial dysfunction [253]. Clearly, the pathophysiology of atherosclerosis involves many similar inflammatory, protease, and oxidase-mediated stress/remodelling cascades that can lead to vessel remodelling and altered collagen and elastin structure [243]. However, because stiffness and atherosclerosis often coexist, causality remains uncertain.

2.11.2 Cellular Role in Vascular Stiffening

In addition to structural changes, arterial stiffness is strongly affected by endothelial cell signalling and VSMC tone. VSMC tone can be modified by mechano-stimulation, in part because of cell stretch and changes in calcium signalling, and by paracrine mediators such as angiotensin II, endothelin, oxidant stress, and nitric oxide [243]. Endothelial dysfunction is evidenced clinically by an impaired vasodilatory response to acetylcholine. This stems, in part, from an imbalance between nitric oxide and endothelial-derived hyperpolarizing factor and constricting hormones, and oxygenases such as cyclooxygenase, NADPH, and xanthine oxidase [146]. Nitric oxide expression may itself be reduced, and increased expression of a natural nitric oxide synthase (NOS) inhibitor, asymmetrical dimethylarginine, has been linked to arterial stiffening [146, 182]. Bioavailability of nitric oxide is also reduced by activation of reactive oxygen species caused by stress, hormones, and likely AGEs [251]. The formation of peroxynitrite and other highly reactive species results in abnormal vascular tone.

Although many studies have established a role of endothelial dysfunction in arterial stiffening, recent studies have suggested the opposite holds as well; that is, structural stiffening could alter endothelial function and thereby worsen stiffening [28]. When endothelial cells cultured in distensible silastic tubes are exposed to realistic pulsatile perfusion, the combined phasic shear and stretch results in greatly augmented phosphorylation of the serine-threonine kinase Akt and subsequent stimulation of endothelial NOS [254]. However, neither phosphorylation nor endothelial NOS expression are stimulated in cells cultured in stiff tubes and exposed to identical pulsatile perfusion. These data suggest that the ability of the vessel wall to stretch impacts endothelial mechanotransduction far more than a pulsatile stimulus and this lack of compliance may promote a decline in NOS activity, leading to further arterial stiffness [243].

2.11.3 Neuroendocrine Signalling and Salt

Many hormones are known to modulate vascular stiffness. Angiotensin II (AII) stimulates collagen formation, triggers matrix remodelling and vascular hypertrophy, depresses nitric oxide-dependent signalling, increases oxidant stress, and reduces elastin synthesis. In addition, AII stimulates cytokines and growth factors in the matrix that contribute to an increased inflammatory response [255]. Many of these changes are transduced by AII-stimulated NADPH oxidase and NOS uncoupling [256]. Aldosterone (ALDO) synthesis is primarily controlled by the action of AII on the angiotensin type I receptor, and also promotes vascular stiffness and hypertension by stimulating VSMC hypertrophy, fibrosis, and fibronectin [257]. The action of ALDO is closely tied to endothelin-1; infusion of ALDO increases endothelin-1 production, which has vasoconstrictive and fibrotic effects on the vasculature itself [247].

Dietary salt augments vascular stiffness with increasing age, and low-sodium diets consumed by older adults improve arterial compliance [258]. In response to NaCl, VSMC tone is stimulated and vascular wall composition altered with a marked increase in the medial layer with VSMC hypertrophy and abundant collagen and elastin production [259]. Salt intake interacts with genetic polymorphisms for genes such as angiotensin type I receptors, nitric oxide, and ALDO synthase [260]. Sodium also impairs endothelial function by reducing the production of nitric oxide by NOS, thereby diminishing nitric oxide bioavailability and by stimulating NOS inhibitor asymmetrical dimethylarginine and enhancing NADPH oxidase activity. This results in enhanced ROS stimulation as a common mechanism for arterial stiffening [258].

2.11.4 Glucose, Insulin, and Vascular Stiffening

In patients with diabetes and metabolic syndrome, arterial stiffening is consistently observed across all age groups. For example, increased arterial stiffness and abnormal endothelial reactivity is already present in obese children with metabolic syndrome [27, 261]. A core feature appears to be insulin resistance, because central arterial stiffness and insulin resistance are positively correlated [262]. Furthermore, the extent of metabolic changes predicts arterial stiffness in a dose-dependent fashion [263]. Chronic hyperglycaemia and hyperinsulinemia increases the local activity of renin-angiotensin-aldosterone system (RAAS) and expression of angiotensin type I receptor in vascular tissue, promoting development of wall hypertrophy and fibrosis [250]. Hyperinsulinemia itself has proliferative effects, because insulin resistance impairs PI3-kinase– dependent signalling responsible for the acute metabolic effects of insulin, yet activity of growth-promoting mitogen activated kinase pathways remains relatively preserved [247].

Impaired glucose tolerance also enhances nonenzymatic glycation of proteins with covalent cross-linking of collagen (AGEs) and alters the mechanical properties of interstitial tissue of the arterial wall [250]. Stiffness is further increased by endothelial dysfunction caused by high LDLs, free fatty acids, endothelin-l, inadequate vasodilatory effects of insulin, or decreased levels of adiponectin and natriuretic peptides [243]. Importantly, increased arterial stiffness in the metabolic syndrome is not the consequence of fully established diabetes, but rather caused by subtle hormonal and metabolic abnormalities present from the very beginning of an insulin-resistant state [264].

2.12 Assessment of Pulse waveform

Arterial stiffness can be evaluated at systemic, regional or local level with different methods and implications. Regional and local arterial stiffness can be evaluated directly and noninvasively at different sites along the arterial tree [26]. Evaluation of arterial stiffness as PWV could provide an integrated description of arterial alterations generated by the action in the time of several different pathological noxae [65]. Recently, a systematic review of studies analysing the correlation between carotid-femoral PWV and cardiovascular risk factors has confirmed a strong association of arterial stiffening with aging and arterial hypertension, but a weak association with diabetes, dyslipidaemia, body mass index and smoking [265].

2.12.1 Applanation tonometry

Of all the diverse techniques employed for non-invasive detection of the arterial pressure pulse, applanation tonometry has the widest application in devices that perform pulse wave analysis. The strict definition of a tonometer is essentially any

instrument that measures pressure or tension. The specific application of 'applanation' tonometry, however, is one where a curved surface is flattened, such that the wall tension is effectively reduced to zero and there is transmission of the internal force to the external transducer [266]. This found specific application in the field of ophthalmology, where intraocular pressure can be determined by a force transducer applanating the cornea [267]. The governing relationships are based on the Imbert–Fick principle which states that the internal pressure (Pi) in a spherical body consisting of an infinitely thin, dry and elastic membrane wall is the ratio of the applied force (Fa) and the area of the applanated surface (A) [Pi = Fa/A]. Although the Imbert–Fick principle requires ideal conditions such as thin wall, with correct applanation of the corneal surface and with accurate calibration, it is possible to determine IOP and quantify changes [268].

The theoretical basis for arterial applanation tonometry which was developed from the ocular application was also applied specifically to arteries. The models developed by Pressman and Newgard [268, 269] included properties of the transducer elements and the overlying tissues where the uniform compressible tissue surrounding the elastic artery can be represented by linear springs. Furthermore, the deflections caused by the application of the tonometer were assumed to be small, allowing nonlinearities to be neglected. The elastic artery was also represented by a spring model [269]. This model was further developed in which the artery was represented by a cylindrical tube instead of a spring model. The overlying tissue and skin layer was neglected and the artery was assumed to be thin walled and with isotropic and homogeneous wall properties. By assuming a uniform deformation along the length of the arterial segment, the contact stress, as a function of distance from the centre of the circular artery, was calculated using curved beam mechanics [270]. This elaborate analysis

indicates the importance of correct placement of the sensor to obtain zero contact stress so that only the internal blood pressure is detected with sufficient hold-down force, but no additional deformational stress.

The application of the applanation tonometry principle to arteries followed directly from the ocular application, given the propensity for circular arterial segment to be flattened by an external force. However, this requires a rigid support for the artery. The most accessible and suitable anatomic location is the wrist, where the radial pulse can be readily palpated. The main sensor types are the single sensor at the end of a hand-held pencil-type holder where the element consists of a piezo-resistive transducer with dimensions much smaller than the arterial diameter, or an array of sensors strapped over the radial artery where the optimum signal is selected using computer-based algorithms [271].

As in the ocular application, the original aim was to obtain actual calibrated measurements of arterial pressure using the principles of applanation tonometry. In theory, this was deemed to be possible by eliminating the contact stress [272]. However, the practical application of this has not proved entirely successful [273]. As yet, there is no reliable and reproducible tonometric technique able to quantify the intra-arterial pressure in a way to match the sphygmomanometer with respect to the ease of use [271]. Notwithstanding the inability to obtain calibrated pressure values, the applanation principle is highly effective in recording the time-related change in intra-arterial pressure [269]. Thus, for an uncalibrated signal, the non-invasive tonometric pulse is similar to the intra-arterial pressure pulse. There is strong correspondence between the non-invasive tonometric radial artery pulse and the pressure measured with an intra-arterial 1 F micro-tip sensor located directly beneath the tonometer [266]. This property of arterial applanation tonometry has enabled the accurate registration of the

peripheral pulse. This has resulted in the development of devices that combine pulse wave analysis and the cuff sphygmomanometer [208]

Although the tonometric sensor is in solid state and with a high-frequency response in the kHz range, the overall response is determined by the transducer and the coupling to the artery through the surrounding tissue [270]. For a multi-sensor array device, the frequency response of the sensor is given as being flat for 0–50 Hz for a pressure applied directly to the sensor, although a much lower flat frequency response between was found for the complete system [268]. However, a good agreement was found with intra-arterial pressure waves with largest discrepancies occurring in early systole [266].

Difficulties are encountered with methods that are prone to the tonometer being displaced when the subject moves, leading to loss of the pulse signal. The Bpro and PulseTraxer devices (HealthSTATS International Pty Ltd, Singapore) use a hemispherical tonometer, which, according to manufacturer specifications, provides less sensitivity to exact positioning over the artery, facilitating measurements over longer periods and 24 h monitoring of the pulse [274]. Recent studies have shown the utility of adaptive filtering techniques for noise cancellation in the tonometric signal due to motion artefact [275]. Other devices employ computerized control systems to place an array of tonometric sensors on the strongest pulse detection point upon the wrist, subsequently choosing the optimal transducer element in the array to monitor the strongest waveform amplitude [208].

2.12.2 Vascular unloading methods

The volume clamp, or vascular unloading method introduced by Penaz (1973) [276], measures continuous peripheral arterial blood pressure. The method is incorporated in

a range of devices [208]. A photoplethysmography approach is used at the distal part of the phalanges to monitor vascular volume. Changes in vascular volume are compensated through a servo-nulling principle by adjustment of a pressure cuff around the phalanges. The finger cuff pressure required to maintain the constant blood volume is the instantaneous arterial pressure in the finger and describes a calibrated finger pressure pulse [277]. The device can be uncomfortable when used for long periods as the finger cuff pressure is greater than venous pressure and occludes venous return. The Portapres and other similar devices like BMV (CnSystems) attempt to alleviate this problem with alternate measurement on two fingers. Recent developments in the technology have shown an improvement in the association between finger pressure and brachial cuff pressure values [278].

The finger-cuff device gives a calibrated continuous recording of arterial pressure. It has been used extensively for applications requiring beat-to-beat information such as quantification of baroreceptor function and obstructive sleep apnoea [279, 280] as well as in microgravity simulations for space missions [281].

2.12.3 Fluid-filled pulse detectors

A fluid-filled sensing apparatus placed over the radial artery is the basis of the Vasotrac system (Medwave Inc, Danvers, USA) and operates by a method similar to oscillometry [282]. A pressure ramp is applied from a low pressure to systolic or supra-systolic level. The mean pressure is determined as the pressure corresponding to the maximum pulse amplitude. Systolic and diastolic pressures are estimated from waveform features using patented algorithms [283]. The continuously varying force means the device does not continuously measure blood pressure but provides a pulse

waveform approximately every 15 seconds that corresponds well with those measured using applanation tonometry [208] and invasive pressures [284]. However, there are some limitations in obese subjects [285]. The varying force also makes it suitable for measurements over extended time periods as it does not permanently occlude blood flow. It is also less sensitive to positioning errors.

2.12.4 Cuff devices for the detection of the arterial pulse

The arterial pulse can also be measured using a volume displacement technique in a statically inflated limb pressure cuff. Small oscillations in the cuff caused by arterial pulsations within the area covered by the cuff are detected by a piezo-electric pressure sensor. The Vicorder device (SkidmoreMedical) inflates pressure cuffs to sub-diastolic levels for the detection of the arterial pulse at the brachial, femoral and carotid regions [286]. The Arteriograph (Tensiomed) measures the terminal arterial pulse against a brachial pressure cuff inflated to super-systolic pressure, associating features of the terminal pulse with arterial stiffness parameters such as augmentation index and pulse wave velocity [287]. Similar techniques of supra-systolic cuff inflation are employed in the Pulsecor device using a wideband external pulse measurement with a broad bandwidth piezoelectric sensor located over the brachial artery under the distal edge of a sphygmomanometer cuff. The WEP signal is similar to the first derivative of the intra-arterial pressure signal and tracks changes following use of vasoactive agents [288].

2.12.5 Photoplethysmography

A common method for pulse wave recording is monitoring of the finger blood volume through photoplethysmography (PPG). Allen (2007) [229] has provided an extensive review on the broad field of PPG and its applications in physiological measurement and clinical use. Although the origins of the PPG are not fully established, the availability of low cost components and microprocessor technology has seen resurgence in the technique, employed in various commercial devices encompassing blood oxygen levels, transit time, cardiac output and beat-to-beat changes in arterial pressure, thus enabling studies of autonomic function. The reader is referred to the in-depth review by Allen (2007) on the use of PPG as an extension of the features of the non-invasive arterial pulse as discussed in this present topical review. Recent studies have highlighted the use of the PPG signal in relation to characterizing changes in blood volume [289]. Other studies extended the use of the PPG waveform to the detection of ventricular ejection times and pulse transit times during tilt manoeuvres [290]. A number of studies have made extensive use of the PPG signals for pulse waveform analysis and quantification of vascular function[290-292].

2.14 Cardio-ankle Vascular Index as a measure of Arterial Stiffness

Cardio-ankle vascular index (CAVI) is a measure of arterial stiffness from the aortic root through thoracic, abdominal, common iliac, femoral, and tibial arteries independent of arterial blood pressure[293, 294]. Basically, CAVI is obtained by recording the distance from the level of the aortic valve to the ankle and the time delay between the closing of the aortic valve to the detected change in arterial pressure wave at the ankle. Information require for computation of CAVI, including PWV, systolic and diastolic blood pressure as well as arterial pulse waveforms, can then be acquired

through the electrocardiogram, cardiac phonogram, and the pressure cuffs on the testing subject at the reference points [206].

In comparison to PWV, CAVI has two distinctive features. First, CAVI changes over a short period of time in response to alterations in circulatory condition. Second, CAVI reflects the state of smooth muscle contraction rather than changes in blood pressure [295]. CAVI has also been reported to represent both "functional" and "organic" stiffness [293]. The former represents the state of smooth muscle contraction that is subjected to changes in sympathetic tone and also pharmacological influence, whereas the latter signifies the physical properties such as sclerotic change of the arterial wall. While the administration of a selective beta 1-blocker, which decreases the contraction of cardiac muscle and blood pressure without affecting the vascular tone of arterial wall, led to a reduction in PWV in human subjects, the CAVI stayed unchanged. On the other hand, infusion of an alpha-1 adrenergic receptor blocker, which decreases blood pressure through a relaxation of the smooth muscle of peripheral arteries, caused a diminished CAVI score [294]. These findings, therefore, highlight the influence of vascular tone on the magnitude of CAVI in spite of its blood pressure independence.

2.14.1 CAVI: theories and computation

The propagating velocity of the arterial pressure pulse, ΔP, can be expressed in terms of "characteristic pulse wave velocity (PWVc)" as

$$PWVc = \sqrt{(K/\rho)}, \qquad (1)$$

where K is the elastic modulus of luminal volume change per unit length of artery and ρ is the density of blood [296].

However, arterial stiffness can be describe by its distensibility, or preferably "compressibility" [197], which is the relative change in volume in response to a change in blood pressure. That is, Compressibility = $(\Delta V/V)/\Delta P$, where ΔV = change in volume; V = original volume; ΔP = change in pressure.

Since the elastic modulus, K, is defined as the ratio of stress (the pulse pressure) to strain (the fractional volume change) per unit length of artery, it is the inverse of compressibility. This implies that

$$K = \Delta P/(\Delta V/V) \qquad (2)$$

Since $V = \pi R^2$ (where R is luminal radius), then $dV = 2\pi R dR$, and if ΔV (luminal volume change per unit length of artery) is small, $dV/V = \Delta V/V = 2\Delta R/R = 2\Delta D/D$ (where D is luminal diameter), then equation (2) may be written as:

$$K = \Delta P/(2\Delta D/D) \qquad (3)$$

Bergel [297] proposed that the loop tension, T, in the artery wall of thickness, h, was related to the luminal pressure, P, by the equation,

$$P = Th/R, \text{ that is, } T = PR/h \qquad (4)$$

Suppose that the changes in luminal radius, R, and arterial wall thickness, h, are negligible, then the relation between a small change in luminal pressure, ΔP, and the corresponding change in stress, ΔT, can be given by $\Delta T = \Delta PR/h$. The circumferential strain caused by ΔT will be $[2\pi(R + \Delta R) - 2\pi R]/2\pi R$, giving $\Delta R/R$.

Therefore, E_{inc}, the static incremental Young's modulus, which is stress/strain for the material of the arterial wall, can be defined as, $E_{inc} = (\Delta P \cdot R/h)/(\Delta R/R)$.

Hence, E_{inc}, the static incremental Young's modulus, can be rewritten as,

$$E_{inc} = \Delta P \cdot D^2/2h\Delta D \quad (5)$$

The equation was based on the assumption of blood flow inside an infinitely long, thin-walled elastic tube with an incompressible fluid and with the elasticity of the tube wall considered to be isotropic in the absence of any pressure reflections from the periphery.

Combining (1), (3), and (5) gives:

$$PWVc = \sqrt{(E_{inc} \cdot h/D \cdot \rho)} \quad (6)$$

The equation was commonly referred to as the Moens–Korteweg equation [298].

Alternatively, substituting (2) into (5) gives: $PWVc = \sqrt{\Delta P \cdot D/(2\rho \cdot \Delta D)}$, which can be rewritten as:

$$D/\Delta D = (2\rho/\Delta P) \cdot PWV^2 \quad (7)$$

Where PWV: pulse wave velocity; ΔP: pulse pressure; ρ: density of blood; D: luminal diameter; ΔD: change in luminal diameter.

Equation (7) thus derived provides a theoretical model, that was first proposed by Bramwell and Hill [299], that links the PWV, compressibility, pulse pressure, and blood density. Although the Bramwell and Hill equation was based on the Moens–Korteweg model, the former is believed to be more general because it does not assume thin-walled and homogeneous elastic arteries, as in the Moens–Korteweg model [300].

On the other hand, CAVI is calculated based on a stiffness parameter, β, which was first proposed by Hayashi et al [205] to assess the local stiffness of a blood vessel according to the change in vascular diameter in response to arterial pressure variance [205]. To fit into clinical practice, this blood pressure-independent parameter was later defined by Kawasaki *et al* (1987) [301] as:

$$\beta = \text{In}\,(Ps/Pd) \cdot (D/\Delta D) \qquad (8)$$

Using an echo phase tracing system, the proportional change in luminal diameter (ΔD/D) can be obtained. Provided that an exponential relation exists between intravascular pressure and arterial diameter under stable physiological condition theoretically because of the transfer of stress from the stretchable elastin to the stiff collagen fibres in the arterial wall as blood pressure increases (Figure 8A) [302], plotting the natural logarithm of systolic–diastolic pressure ratio (ln Ps/Pd) against the arterial wall extensibility (ΔD/D) would give a linear relation (Figure 8B). The mathematical expression of the β stiffness index may better account for non-linearity in the relationship between pressure change and diameter change and as a result of this mathematical property [191], it appears to demonstrate much less within-subject variations in response to blood pressure changes induced by nitroprusside infusions [294]. Therefore, the stiffness parameter β, which is the slope of the plot (Figure 8B), is theoretically independent of blood pressure at the time of measurement [293]. Hence, the higher the value of β, the lower the compressibility (or the higher the stiffness) of the arterial wall, and the steeper the plot (Figure 8B). Clinically, since accurate assessment of arterial stiffness according to the change in vascular luminal diameter is hampered by the measurement on merely a local segment of artery and also the requirement for specific sonographic equipment [206]. The proportional change in luminal diameter in Equation 8 can be replaced by substituting (7) into (8):

$$\beta = \text{In } (Ps/Pd) \cdot (2\rho/\Delta P) \cdot PWV^2. \quad (9)$$

For scale conversion from PWV, the following formula can be applied:

$$CAVI = a\beta + b \quad (10)$$

where a and b are scale conversion constants [293].

Hence, substituting (9) into (10) gives:

$$CAVI = a\,[(2\rho/\Delta P) \cdot \ln (Ps/Pd) \cdot PWV2] + b \quad (11)$$

PWV: pulse wave velocity from valve orifice to ankle; Ps: systolic blood pressure; Pd: diastolic blood pressure; ΔP: change in blood pressure; ρ: blood density.

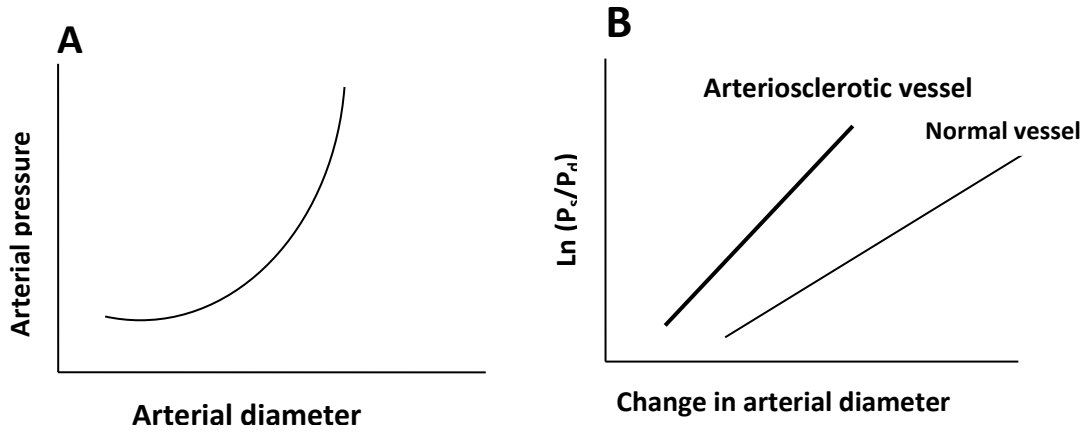


Figure 8. (A) An exponential relation between blood pressure and arterial diameter under stable physiological condition. (B) Linear relation between natural logarithm of systolic (P_s) − diastolic (P_d) pressure ratio (In P_s/P_d) and arterial wall distensibility.

2.15 Principle of operation of Arteriograph

The Arteriograph (TensioMed Kft., Budapest, Hungary) is a novel device that uses a simple upper arm cuff as a sensor to simultaneously measure all parameters, under strict stop-flow condition. The cuff is inflated to suprasystolic pressure, at least 35 mmHg over the actual systolic pressure, to totally occlude the brachial artery. This creates a stop-flow condition, generating a small diaphragm in the brachial artery at

the level of the upper edge of the over-pressurized cuff. Changes in the central pressure, early (direct) systolic wave, late (reflected) systolic wave and diastolic wave(s) reaches the diaphragm and cause a beat on the membrane like a drumstick.

Because the upper arm tissues are practically incompressible, the energy propagates and reaches the skin/over-pressurized cuff edge, where it causes a very small volume/pressure change in the cuff. These very small suprasystolic pressure changes are recorded by a high fidelity pressure sensor in the device. In this situation the conduit arteries of the upper arm (subclavian, axillary and brachial arteries) act like a cannula to transfer the central pressure changes to the edge-position sensor (similar to the central pressure measurement during cardiac catheterization). Because the arterial wall does not move beneath the cuff in stop-flow condition (occluded artery), the local influence of the characteristics of the wall of the brachial artery is practically eliminated and so the received oscillations are as results of pure pressure waves.

Validation of the working principle of the Arteriograph against an invasive counterpart was reported by Horváth et al [287]. The study measured PWV invasively during cardiac catheterization and compared the results with those produced by the Arteriograph. The mean PWV values for the Arteriograph and the invasive measurements were 9.46 ± 1.8 and 9.41 ± 1.8 ms^{-1}, respectively ($r = 0.91$, $P < 0.001$). The study did not have enough power due to its small size; however, the authors justified this on the basis that they used an invasive counterpart with a very strong evidence basis.

Trachet *et al*., (2010)[303] tested the validity of this working principle of Arteriograph, using a numerical model of the arterial tree to simulate pressures and flows in the normal configuration and in a configuration with an occluded brachial artery. They

found a pronounced secondary peak in the brachial pressure signal of the occluded model, but its timing was only related to brachial stiffness and not to aortic stiffness. They concluded that the Arteriograph picks up wave reflection phenomena confined to the brachial artery, and derived values of PWV rather reflect the stiffness of the brachial arteries.

Rajzer *et al*., (2008) [304] compared the measurement of aortic PWV by three noninvasive devices: Complior, SphygmoCor and Arteriograph. They observed that Aortic pulse wave velocity measured using Complior was significantly higher than that obtained using SphygmoCor or Arteriograph. They concluded that the differences in pulse wave velocity obtained by these noninvasive devices resulted primarily from using various methods for measuring travelled distance.

The above reports indicate that, the Ateriograph, despite its novelty, has not been accepted without criticism. This can be said of all the other noninvasive devices of measuring central haemodynamics.

2.13 Body composition and arterial stiffness

Body composition assessments are crucial for investigating physiology and pathophysiology of diabetes and CVDs. Recent researches increasingly use body composition assessment for better describing clinical phenotypes and for understanding the pathogenesis of many CVDs. The contribution of excess global (such as BMI) and regional adiposity (such as waist circumference [WC]) to the prediction of cardiovascular risk has been the subject of long-standing debate [305]. In the past, many studies suggested that the incidence of certain types of CVDs, particularly coronary heart disease and stroke, was greater in heavier individuals, but

only a few proposed that any obesity index makes an additional contribution to risk once the levels of coexisting risk factors such as dyslipidaemia, hypertension, insulin resistance, glucose intolerance, and T2DM had been taken into account [306]. The consensus had been that the increased risk among obese individuals was due primarily to the influence of the associated risk factor profile and not to the degree of obesity per se. Long-term follow-up studies, in both genders, have indicated clearly that obesity is a significant independent predictor of CV risk [307] and/or mortality [308].

Toto-Moukouo et al. [309] were the first to evaluate the mechanical properties of large arteries in obese individuals. PWV of the upper limbs was measured in 27 obese and 25 nonobese patients with sustained essential hypertension and was increased significantly in obese patients in comparison with nonobese patients. The result was independent of age, gender, and level of BP. In the overall population, a significant positive correlation (r =0.85, $P < 0.001$) was observed between the degree of obesity and PWV. A study of partial correlation coefficients indicated that fasting blood level, cholesterolemia, and triglyceridemia did not influence the relationship. After body weight reduction, BP decreased and systemic arterial compliance increased, indicating that body weight reduction is associated with an enhanced arterial elasticity, as a result of BP reduction or not [309].

In recent years, it has been shown in adults [310] and children [27] but not constantly [311] that individuals with obesity have an increase in aortic stiffness, independent of BP level, ethnicity, and age. However, increased aortic stiffness has been shown to be more related to body fat repartition (assessed by WC and visceral adiposity) than to increased BMI [312]. More recently, total trunk fat has been found to be associated adversely with PW [313]. Because visceral obesity is linked more to arterial stiffness than to BMI, aortic stiffness and/or local measurements of arterial elasticity have been

studied extensively in individuals with T2DM and individuals with metabolic syndrome [305]. Reduced elasticity has been observed in both central and peripheral arteries, in contrast with hypertension, in which peripheral arteries but not central arteries have normal values of elasticity indices [312].

2.16 Peripheral Neuropathy in T2DM

Peripheral neuropathy is the most common and intractable complication of diabetes [314]. It involves somatic sensory and motor nerves, as well as autonomic nerves. The prevalence of diabetic neuropathy ranges from 7% within 1 year of diagnosis to 50% for those with diabetes for >25 years [315]. If patients with subclinical levels of neuropathic disturbances are included, the prevalence might exceed 90% [316]. The presence of cardiovascular autonomic neuropathy dramatically shortens the patients' longevity and increases the mortality [317]. Loss of feeling in the lower limbs is a high risk for limb amputation, which occurs in 1–2% of diabetic patients and necessitates extreme cost [316].

Despite efforts to make an early diagnosis and to halt the progression of diabetic neuropathy, currently there is no effective treatment available at a global level, except for tight control of blood glucose. This might be as a result, at least in part, of insufficient clarification of the pathogenesis of diabetic neuropathy, complicated clinical pictures that do not necessarily reflect proper progression of the disease, or inadequate design of clinical trials. There might also be a possibility that the development of a candidate drug might not be based on genuine inciting factors [314, 318].

2.16.1 Risk Factors for Progression of Neuropathy

The duration of diabetes and glycated haemoglobin levels have been well associated with a high incidence of neuropathy [319]. Classically, the Diabetes Control and Complications Trials (DCCT) confirmed the beneficial effects of meticulous control of blood glucose on the incidence of chronic complications in 1441 T1DM patients[32]. In that study, intensive insulin treatment for 6.5 years lowered HbA1c levels (average 7%) by 2% compared with a conventionally treated group (average 9%) and successfully decreased the incidence of neuropathy by 60% (13 vs 5%) [32]. More striking are the so-called 'legacy effects' (glucose memory) of tight blood glucose control for the suppression of new development of neuropathy during a post-trial observation period for 8 years [320]. In T2DM patients, the Kumamoto study showed that intensive insulin treatment for 7 years improved nerve conduction velocity and the vibration perception threshold (VPT) compared with those conventionally treated [321]. In contrast, the UK prospective diabetes study (UKPDS) on 3867 T2DM patients did not find the effects of glucose control (to the extent of a 0.9% decrease in HbA1c) on the prevalence of neuropathy, whereas there was a significant reduction in the risk for retinopathy and nephropathy [322].

Tesfaye *et al*., (2005) [323] in the EURO-Diab group reported that blood glucose control, duration of diabetes, hypertension, hyperlipidaemia and smoking were all significant risk factors for the development of neuropathy in T1DM patients. The impact of hyperlipidaemia has also been emphasized by a follow-up study of the DCCT trial [324]. However, this trend is different in cohorts of other countries, because Japanese studies could not find a significant influence of the blood concentrations of triglyceride or cholesterol on the prevalence of neuropathy [325].

2.16.2 Anatomy and Vascular Supply of Peripheral Nervous System

Anatomical characteristics of the peripheral nervous system might explain why the pathogenesis of neuropathy is distinct from other microvascular complications [326]. Peripheral nerves are covered by perineurium, where only a few transperineurial arterioles penetrate into the endoneurium (Figure 9). The vascular supply in peripheral nerves is sparse and blood flow is likely to be compromised and lacks autoregulation [34]. This system makes peripheral nerves vulnerable to ischemia. Endoneurial microvessels are tightly connected with endothelial cells on their inner surface, but when destroyed they are leaky and affect the endoneurial tissue components[327]. Leaky vessels are mainly located in the ganglion with fenestrated vessels, and nerve terminals on the distal side are directly exposed to environments not covered by perineurium and are susceptible to traumatic injury.

Innervation of epineurial microvessels is involved in diabetes, resulting in impaired blood supply in diabetic nerves [328]. Endoneurial microvessels show thickened and multilayered basement membranes, cell debris of pericytes, as well as disrupted endothelial cells, and thus constitute salient structural changes in diabetic nerves [329].

Independent of vascular supply, three dimensions of neuronal architecture specific to the peripheral nervous system might account for the reason why the most distal side is susceptible in diabetes. Ganglion cells have extensively long axons covered by Schwann cells. The neuronal cell body is relatively small compared with the extremely long distance of axonal neurites, and thereby distal axons are innately too weak to support themselves for the long transport of nutrients, nerve trophic factors, as well as other signals [325, 329].

2.16.3 Pathological Background of Neuropathy

Most characteristic findings of the peripheral nervous system in diabetes patients are distal and sensory predominant nerve fibre degeneration, axonal loss and endoneurial microangiopathy [330, 331]. Both large and small caliber sizes of nerve fibres are affected. Based on this anatomical condition, Dyck et al. (1984; 1986) proposed that microvascular injury is the most probable factor for focal fibre loss and its summation appears to be the cause of diffuse fibre loss of distal predominant axonal neuropathy in diabetes [332, 333].

However, this explanation is too simplistic and does not explain why hyperglycaemia and duration of diabetes are crucial for occurrence of diabetes neuropathy. There also emerges a controversy as to whether there is any predominance for the involvement of small fibres in early diabetic neuropathy. Questions on this issue were further raised by the report that the focality of nerve fibre loss was not universally demonstrated, indicating that microangiopathy does not always account for the fibre loss [33].

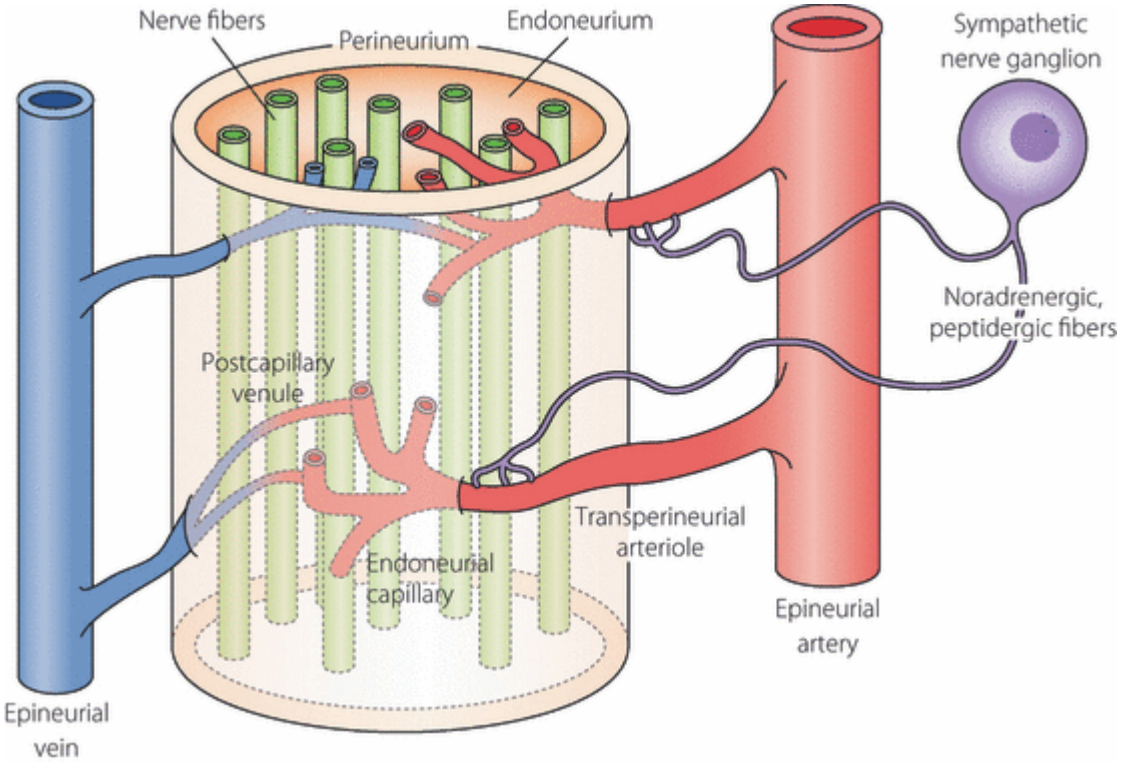


Figure 9. Vascular supply of the peripheral nervous system. Adapted from [329]

Nevertheless, vascular influence on the development of neuropathy was further supported by subsequent studies on humans. Malik *et al*., (2005) showed that patients who did not have clinically evident neuropathy at the time of nerve biopsy, but who showed high-grade microangiopathic changes of endoneurial microvessels later, developed overt neuropathy, whereas the patients without microvessel changes did not develop neuropathy [334]. The extent of microangiopathic changes correlated well with subsequent nerve fibre loss in diabetic nerves [335]. There is correlation between the thickness of the basement membrane of endoneurial microvessels and reduced myelinated fibre density [336].

2.17 Vascular Growth Factors

Angiogenesis, the formation of new blood vessels from pre-existing blood vessels, is a prominent feature in CVDs. The regulation of angiogenesis is not yet fully understood, however angiopoietins appear to play an important role and as a result have begun to receive considerable interest [35, 38]. Angiopoietins are ligands which bind to the transmembrane receptor Tie-2 and possibly Tie-1. These receptors are essential for the correct formation and development of blood vessels [337]. The Tie receptors are part of the receptor tyrosine kinase family that is separated into two main sub-groups according to their amino acid composition: The VEGF family includes Flt-4 and Flk-1/KDR, and a second family that includes Tek (Tie-2) and Tie (Tie-1) receptors. Angiogenesis is thought to depend on a balance of activators and inhibitors that regulate the 'angiogenic switch' [338].

Angiopoietin 1 (ang-1), the first angiopoietin to be described, is secreted by pericytes, mesenchyme and VSMCs of the developing vasculature and is thought to stabilize the formation of newly formed blood vessels [339]. Angiopoietins utilise the same binding domain for the Tie receptors and were initially thought to be exclusive for these receptors, however, angiopoietins 1 and 2 have been shown to interact with other receptors, including integrins [340]. This suggests that the angiopoietins could be involved in mediating cell adhesion or migration through integrins, independent of the Tie-2 receptor. Angiopoietin 2 (ang-2) is secreted by endothelial cells (ECs) and VSMCs. Both ang-1 and -2 have been shown to be secreted by human cord blood $CD34^+$ cells and are necessary for EC differentiation and proliferation, respectively [37].

The role of ang-2 in the vasculature appears to be influenced by VEGF: in the absence of VEGF, ang-2 destabilizes the interaction between ECs and their support cells causing plasma leakage and promotes vascular regression. In the presence of VEGF,

ang-2 promotes angiogenesis by allowing ECs to be more responsive to VEGF mediated cell proliferation which is necessary for ECs repair and turn over [341]. Ang-2 and VEGF are thought to have a complementary effect on blood vessel growth which could be due to both ligands being able to activate the receptor Tie-2 [38].

Dysfunction in angiogenesis also has been suggested as a common origin for vascular complications in diabetes[342]. Also, previous studies reported elevation of plasma levels of VEGF, Ang-2 and soluble Tie-2 (sTie-2) in subjects with T2DM[35, 36, 343]. A selective increase of plasma levels of Ang-2 and sTie-2, but not Ang-1, is accompanied by neovascularization and endothelial abnormalities. Endothelial abnormalities are closely linked to the pathophysiology of microvascular and atherosclerotic vascular complications in T2DM[35, 39]. Although a previous study showed that raised levels of plasma Ang-2 and VEGF in diabetes are independent of concomitant vascular disease[36], the underlying mechanisms for the association of Ang-2 and sTie-2 with T2DM are not well understood.

Nadar *et al*., (2004) [344] studied patients with hypertension and reported changes in plasma angiogenic growth factors that correlate with angiogenesis seen in hypertension. They found that hypertensive patients had higher levels of plasma VEGF, Ang-1, Ang-2 and Tie-2 (all $P < 0.01$), but not platelet Ang-1, when compared with normotensive controls. Patients with target organ damage had higher levels of platelet VEGF and Ang-1 (both $P < 0.001$), and plasma Ang-1 ($P < 0.001$). Amongst the hypertensive subjects, plasma levels of VEGF correlated significantly with Ang-1, Ang-2, Tie-2 and platelet VEGF, whilst platelet VEGF correlated strongly with plasma levels of VEGF and Ang-1 (all $P < 0.05$) [344]. From their results, it is obvious that altered angiogenesis leads to target organ damage in hypertensive patients; however, what results in altered angiogenesis was not explored.

To explore the relationship between circulating vascular growth factors and central haemodynamics, thus implicate arterial stiffness as an active victim of altered angiogenesis, Zachariah *et al*., (2012) studied 3496 participants from the Framingham Heart Study third generation cohort and compared carotid femoral pulse wave velocity and forward pressure wave, mean arterial pressure, and the global reflection coefficient to circulating concentrations of ang-2, its soluble receptor; VEGF, its soluble receptor; hepatocyte growth factor; insulin-like growth factor 1; and its binding protein 3. They reported that, after adjusting for standard cardiovascular risk factors, serum insulin-like growth factor 1 concentrations were negatively associated with carotid femoral pulse wave velocity, mean arterial pressure, and reflection coefficient ($P < 0.01$ for all), whereas serum VEGF levels were positively associated with carotid femoral pulse wave velocity and mean arterial pressure ($P < 0.04$). Serum insulin-like growth factor binding protein 3 and soluble angiopoietin 2 receptor levels were positively related to mean arterial pressure and to forward pressure wave, respectively ($P < 0.05$)[40]. This study definitely showed that vascular growth factors, especially VEGF, are associated with central haemodynamics.

CHAPTER THREE

METHODOLOGY

3.1 Study Design

The study was a case control design. The study subjects were male and female patients with type 2 diabetes (T2DM) and age and gender matched nondiabetes individuals. The T2DM and nondiabetes subjects were further divided into hypertensives and nonhypertensive subjects. The design and protocol of the study was considered and granted approval by the University of Ghana Medical School Ethics and Protocol Review committee (Protocol ID number: MS-Et/M.2 – P.4.10/2012-2013).

3.2 Setting

The study was performed at the National Diabetes Management and Research Centre (NDMRC), Korle-Bu Teaching Hospital in Accra. The NDMRC was established in 1995 as a Centre of excellence for diabetes care, training, research and education. It provides ambulatory medical care and consultation, diabetes education, dietetics and eye services for diabetes care, management and prevention. The NDMRC works from 6:00 am to 16:00 pm on the weekdays. As part of its mandate, the NDMRC performs research activities in addition to patient care. Assessment of body composition and vascular function were performed at the Cardiovascular Research Laboratory, NDMRC.

3.3 Study subjects

The cases composed of already diagnosed T2DM patients attending the NDMRC for clinical care. T2DM status was essentially clinical and based on non-requirement of insulin at the time of diagnosis of diabetes and an older age of presentation (> 35 years). The controls were gender and age matched individuals with normal glucose tolerant status.

3.3.1 Definition of cases

1. Type 2 diabetes subjects: Individuals diagnosed of diabetes in adulthood (later than age 35), initially treated with lifestyle modification and/or oral hypoglycaemic medication.
    i. Hypertensive subjects (T2DM+HtN): Individuals with systolic BP ≥ 140 mmHg and/or diastolic BP ≥ 90 mmHg taken and/or being treated with antihypertensive medication.
    ii. Nonhypertensive diabetes subject (T2DM-HtN): Diabetes subjects without hypertension
2. Nondiabetes subjects: Individuals with no history of diabetes, FPG < 7.0 and plasma glucose after 2 hours post load plasma glucose < 11.2 mmol/L
    i. Hypertensive nondiabetes subjects (NDM+HtN): Nondiabetes individuals with systolic BP ≥ 140 mmHg and/or diastolic BP ≥ 90 mmHg or being treated with antihypertensive drugs.
    ii. Control subjects (CTRL): Nondiabetes individuals with no history of hypertension treatment and systolic BP < 140 mmHg and diastolic BP < 90 mmHg.

3.4 Eligibility criteria

3.4.1 Inclusion criteria

1. T2DM patients and nondiabetes individuals who were within the age range of 35 – 75 years were recruited as cases and controls respectively.

   Rationale: The lower border of the age range was set to be 35 years due to the adult presentation of T2DM and the upper cut-off of 75 years was because older age can affect vascular properties independent of T2DM.

2. For controls, the subject had FBG not greater than 6.9 mmol/l and blood glucose not more than 11.1 mmol/l after 2 hour of 75g glucose ingestion.
3. The subjects had to be of sound mind to clearly understand what the study was about and to willingly agree and sign or thumb-printed on the informed consent form.

3.4.2 Exclusion criteria

1. T2DM patients who were outside the required age range
2. Type 1 diabetes (T1DM) and latent autoimmune diabetes in adults (LADA) patients. These individuals were identified based on their requirement of insulin at diagnosis of diabetes.
3. Subjects with diabetic foot ulcers, amputation not through accident and arterial reconstruction surgery. These individuals were identified with their response to some items in the questionnaire administered.

   Rationale: These conditions are already indications of severe vascular damage.

3.5 Sample Size and Power Determination

Sample size and power were computed based on the expected standardized effect size [ratio of the planned mean difference (δ) to combine standard deviations (σ) between T2DM subjects and controls] of the various parameters being measured in the study. With the level of significance, α=5%, and power of 80% (β = 20%), the results are shown in the Table 3.1 below.

Table 3.0.1 Postulation for sample calculation

Parameter	Hypothesized effect	α	Power	N
Pulse wave velocity (PWV)	0.82	0.05	80%	52
Augmentation index (AIx)	0.75	0.05	80%	60
Cardio-ankle vascular index (CAVI)	0.70	0.05	80%	68

3.5 Subject Recruitment and Data collection

The T2DM patients arrive at the NDMRC early in the morning and register at the sample collection point with their Diabetes record book, popularly known as ‘diabetes traffic light book’. It was based on the traffic light book that systematic sampling was performed. IBM SPSS version 20 software was used to select the participants for the study based on the age, gender and hypertensive status. Other factors such as BMI, duration of diabetes and forms of diabetes treatment were not considered in the selection of subjects.

The selected patients were assembled in a separate room and the researcher explained to them as a group the nature, rationale, procedure, risks and benefits of the study. They were allowed to ask questions and the researcher provided answers to their concerns. Afterwards, the researcher met privately with the invited individuals and

addressed any personal concerns of the invitee, clarifying any doubts before recruiting him/her into the study by registration and signing or thumb-printing the informed consent form. The study subject was given a special identification number. Those who were unwilling to join the study or had any reservations were thanked for their time and allowed to go for their normal clinical routine care.

The controls were recruited from the general public who responded to the invitation to participate in the research. An invitation was sent out for research participants. Those willing to participate were contacted, mainly on the phone. A brief explanation of the nature of the research, detailed instruction of the fasting procedure was given to them. Afterwards, they were scheduled to come to the NDMRC to participate in the research. The evening prior to their appointment date, the researcher contacted them on phone to remind the volunteers of the fasting procedure. The study volunteers arrived at the NDMRC in the morning, between 6:30 – 8:30 am. Upon their arrival at the NDMRC, the nondiabetes invitees were taken through a process similar to the diabetes invitees, as stated in the preceding paragraph.

3.5.1 Fasting instructions

Prior to the participation in the research, subjects were asked to fast overnight between 8 – 12 hours. To prevent over fasting, subjects were asked to take light food like fruits, biscuits or slice of bread around 9 pm, before going to bed, and report to the NDMRC at 7:00 am. At the NDMRC, subjects were asked the time of their last food intake to estimate the duration of fasting. Subjects who did not meet the fasting requirement had their appointment rescheduled.

3.5.2 Questionnaire administration

A structured questionnaire was used to collect the following data from the study participants: sociodemographic characteristics [age, gender, highest education level, lifestyle (level of tobacco use, alcohol use, and medical history (personal and family history diabetes mellitus, hypertension, heart failure, renal failure, amputation and stroke)].

3.5.3 Blood pressure measurement

Systolic and diastolic blood pressures were measured using a semi-automated digital blood pressure monitor (Omron 991XL, Healthcare, Inc., Vernon Hills, IL) according to the NDMRC protocol.[44, 45] Before blood pressure measured, the subjects were asked the last time they passed out urine and were instructed to empty their bladder if they have not passed out urine within the last 4 hours. The blood pressure cuff was placed on the right arm of the subject lying in a supine position; with the lower edge of the cuff about 2-3 cm above the elbow crease and the bladder is centred over the brachial artery. The arm was rested on a table and raised so that the cuff was at level with the heart. The subject was allowed to rest for at least for 5 minutes to acclimatise with this condition. The blood pressure was measured three times; each measurement was spaced with at least 60 seconds interval with the succeeding measurement. The first measurement was discarded and the last two measurement were averaged the give the true blood pressure.

3.5.4 Anthropometry

Weight was measured in kilograms (kg) and reported in 0.1 kg, using a heavy-duty floor scale (Secca, Hamburg, Germany) with the subject standing upright on the platform, in light clothing with footwear and any heavy jewellery removed from the body; both feet directed forward and arm by the side of the body. Body height was measured in metres (m) and reported in 0.01 m, with a clinical measuring rule in a similar faction. Body mass index (BMI) was calculated by the ratio of the weight to square of the height. BMI was categorised as underweight (BMI < 18.50 kg/m^2), normal weight (BMI: 18.50 – 24.99 kg/m^2), overweight (BMI: 25.00 – 29.99 kg/m^2) and obese (BMI ≥ 30 kg/m^2) [345].

The waist and hip circumferences were measured in centimetres (cm) and reported in 0.1 cm using stretch-resistant tape measure. For waist circumference, the measurement was made at the approximate midpoint between the lower margin of the last palpable rib and the top of the iliac crest. The hip circumference measurement was taken around the widest portion of the buttocks. Waist and hip measurements were made with the tape held snugly, but not constricting, and at a level parallel to the floor [346]. Waist-to-hip ratio (WHR) was computed as waist girth/hip girth. Also, waist-to-stature ratio (WSR) was computed as waist girth/body height. The cut-off point for abnormal waist girth was set at 90 cm for males and 88 cm for females [347].

The body composition was measured using the Omron Body Composition Monitor (BF- 506, Omron Healthcare, Inc., Vernon Hills, IL, USA). The subject's data such as age, gender and height were entered into the equipment and the subject was asked to stand upright (straight torso) on the platform in the same condition as the weight measurement. The subject then grabbed the grip electrodes of the monitor by placing the palm of his or her hand on the top and the bottom of the electrodes while placing

his or her thumbs up, resting on the top of the unit, and stretches the arms forward to approximately 90° to the axis of the body. The device sends a non-detectable low electrical current of 50 kHz and 500mA through the body to determine the amount of fat tissue. Muscle, blood vessels and bone are body tissues with large water content, thus they conduct the electrical current with less resistance. Body fat has a lower electrical conductivity. The proportion of fat in the body is calculated using five variables: electric resistance, height, weight, age and sex.

3.5.5 Neurothesiometry

Neurothesiometry was performed in the subjects in a supine position using Horwell Neurothesiometer (Horwell Scientific, London, UK). The device produces ranges of vibrations corresponding to voltages of 0 – 50 V. Neurothesiometry was performed on right and left legs of each participant. The procedure was explained to the subjects and demonstrated on the subject's arm the vibration to expect before the actual test began. The plastic tip of the neurothesiometer was then placed lightly against the plantar pulp of the distal phalanx of the great toe. The principles of limit were applied; the knob of the device was then turned clockwise; increasing the voltage at a rate of 0.5 V/s, and thus the strength of vibration, until the subject first perceives the stimulus. This gives the ascending vibration perception threshold (VPT) of the subject. The procedure was repeated, this time beginning from a high voltage, corresponding to greater strength of stimulus, and decreasing the vibration by turning the device knob anticlockwise to reduce the voltage. The VPT in this case, is the voltage at which the subject indicates the disappearance of the stimulus. After the initial test to estimate the VPT, two other repetitions were performed to obtained VPTs of ascending and descending methods that do not differ by 5 V. A null stimulus

trial was added randomly to ensure the participant's comprehension and adherence. The average of the ascending and descending VPTs were considered as the VPT for that particular leg. Testing generally required less than 10 minutes depending on the comprehension and response of the research volunteer.

The VPT of subjects were categorised into three levels: normal (<15 V), elevated (15.0 – 24.5 V) and high (≥ 25 V). These groups were chosen on the basis that a VPT <15 V was unlikely to be associated with significant neuropathy, a VPT of ≥25 V has been shown to be associated with foot ulceration in cross-sectional and prospective studies[348, 349].

3.5.6 Tensiomed Arteriograph

The Arteriograph was used to measure brachial BPs, aortic systolic BP, aortic PWV, as well as brachial and aortic augmentation indices. The subject was thoroughly informed about the procedure involved in the assessment and thereafter, asked to lie calmly on the examination couch in a supine position in a quiet, temperature-controlled room (20 ± 2ºC) for at least 10 minutes. The subject's arm circumference was measured, using a nonelastic tape, at the midpoint between the shoulder girdle and tip of the elbow. The length of the subject's descending aorta, from the aortic root to the aortic bifurcation, was estimated as the jugulum-symphysis distance which was measured, using a specialized calliper, as the distance between the suprasternal notch to the tip of the pubic bone. The subject's data was entered into software (Tensiowin 2.4.10) and through infrared connection, the arteriograph was programmed. The appropriate BP cuff size, as recommended by the software based on the arm

circumference, was applied to the subject's right arm. The subject was told to remain calm before and during the cuff inflation.

The Arteriograph first measures the actual systolic and diastolic BPs oscillometrically, and then the device deflates the cuff. In a few seconds, the device starts inflating the cuff again, first to the actually measure diastolic pressure, then to the suprasystolic pressure, and records the signals for 8 s (optionally up to 10) at both cuff pressure levels [287]. All of the signals received by the device are transmitted wirelessly to a notebook.

The data analysis is performed by the Tensiowin software (version 2.4.10) designed for this purpose. The software of the device determines the augmentation index (AIx) by using the formula:

$$\text{AIx} = \frac{P_2 - P_1}{PP} \times 100$$

where P_1 is the amplitude of the first (direct) wave, P_2 is the amplitude of the late (reflected) systolic wave and PP is the pulse pressure.

To determine aortic PWV, the Arteriograph uses the physiological behaviour of the wave reflection, namely that the ejected direct (first systolic) pulse wave is reflected back mostly from the aortic bifurcation. The device measures the time interval between the peaks of the direct (first) and reflected (late) systolic wave (return time – RT). For both the invasive and noninvasive aortic PWV calculation, the distance from sternal notch to the upper edge of the pubic bone is used because this provides the nearest value of the true aortic length [350, 351]. Care was taken to avoid overestimation of the distance by measuring on the body surface. Instead, parallel, straight-line distance between these anatomical points was measured with a calliper.

The aortic PWV was calculated by using the formula:

$$\text{Aortic PWV (m/s)} = 2 \cdot (\text{juglum-symphysis distance})/\text{return time}$$

The calculation of the central SBP in the Arteriograph was based on the relationship between the brachial and central SBP on the basis of the late systolic wave amplitude. The BP measuring algorithm in the device has been validated [352].

3.5.7 Vasera

The VaSera VS-1500 (Fukuda-Denshi Company, Ltd, Tokyo, Japan) was used to measure cardio-ankle vascular index (CAVI) and heart-ankle PWV. After explaining to the subject the procedure involved in the assessment, he/she was allowed to rest in supine position for at least 10 minutes. Colour coded BP cuffs were wrapped around both arms and ankles of the subject; lead I ECG electrodes were placed on both wrists and a phonocardiogram was placed on the sternum to detect heart sound. The subject's data such as unique identifier, initials, date of birth, height and weight were entered into the equipment.

The measurement started with the four cuffs inflating simultaneously to 50 – 60 mmHg to detect brachial and ankle pulse waves. After a waiting time of 8 seconds, the cuffs are pressurized to measure the BPs of the right arm and ankle, followed by BP measurement of left arm and ankle 5 seconds afterwards.

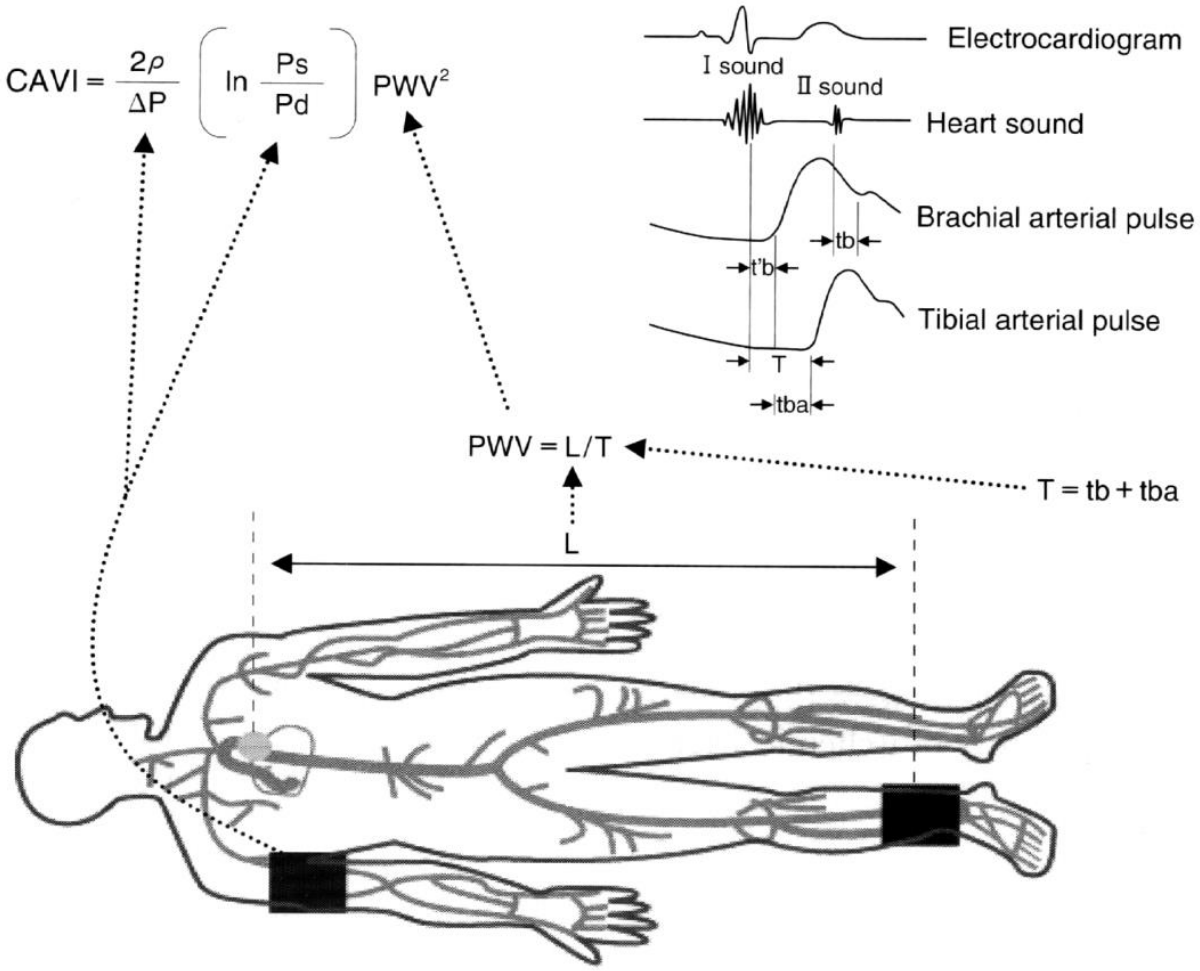


Figure 10. Measurement of Cardio-ankle vascular index (CAVI) in supine patient. Adapted from Shirai et al, 2011.

PWV was obtained by dividing the distance between the aortic valve and the ankle (L) by the time during which pulse waves travel that distance (T). T is difficult to obtain, because the exact time of the beginning of blood expulsion through the aortic valve is difficult to identify from the valve's opening sound. T was obtained by summing the time between the aortic valve's closing sound and the notch of the brachial pulse wave (tb) and the time between the rise of the brachial pulse wave and rise of the ankle pulse wave (tba), in place of the time between the aortic valve's opening sound and the rise of the brachial pulse wave (t'b) and tba. Theoretically, t′b and the time between the closing of aortic valve and the notch of the brachial pulse

wave, tb, are equal (Figure 10) [293, 353]. The scale conversion constants, 'a' and 'b', in Equation 11 on page 101 were computed automatically in the equipment.

After the measurements, the data obtained were analysed using VaSera® Data Management Software, VSS-10 software (Fukuda Denshi, Tokyo, Japan), and the CAVI, PWV and ABI values of right and left sides were calculated.

3.5.8 Blood sample collection, processing and storage

An amount of 10 ml of venous blood sample was collected from the antecubital area into vacutainer tubes, using single-use disposable sterile syringe under aseptic technique. The blood samples were collected into three vacutainer collection tubes: 4 ml into plain tube with clotting activator (red-top), 4 ml into sodium ethylenediaminetetraacetic acid (Na^+-EDTA) tubes (purple-top) and 2 ml into fluoride oxalate (ash-top) tubes. The collection tubes, containing the blood sample, were immediately chilled on ice prior to centrifugation. Within 15 minutes of sample collection, the collection tubes were centrifuged at 4000g: 5 minutes for fluoride oxalate collection tubes and 15 minutes for plain and Na^+-EDTA collection tubes. Plasma and serum samples were collected into Eppendorf tubes. Blood lipids and glucose were analysed immediately before storage. The plasma and serum samples were than stored at -80ºC.

3.5.9 Preparation and administration of glucose drink

An amount of 75g of anhydrous glucose was dissolved in 400 mL of water, sterilized by boiling, and refrigerated overnight at 8ºC in a well presented bottle. Dye and flavour was added to make it easier for the subjects to ingest. The subjects drank all the glucose solution within 5 minutes and sat on a bench in a waiting room for 2

hours, after which 2 mL of blood was taken from the antecubital area into fluoridated tubes for post glucose-load plasma glucose assay.

3.7 Biochemical analysis

3.7.1 Plasma glucose analysis

The level of glucose in the fasting plasma and 2-hour post glucose-load plasma glucose were measured with BS 120 chemical autoanalyser (Mindray, China), using Randox Gluc-Pap reagent (Randox Laboratory Reagents, UK), following the manufacturer's instructions. The analysis involves enzymatic oxidation of glucose to form equimolar amount of gluconic acid and hydrogen peroxide.

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} \text{gluconic acid} + H_2O_2$$

The hydrogen peroxide formed reacts, under the catalysis of peroxidase, with phenol and 4-aminophenazone to form a red-violet quinoneimine dye as indicator.

$$2H_2O_2 + \text{4-aminophenazone} + \text{phenol} \xrightarrow{\text{POD}} \text{quinoneimine} + 4\ H_2O$$

The concentration was determined by the equipment after reading the absorbance of the indicator at a wavelength of 500 nm.

3.7.2 Plasma Lipid Profile assay

Lipid profile of plasma was analysed using BS 120 chemical autoanalyser with Randox reagents, following the manufacturer's instructions.

The total amount of cholesterol (TChol) in the plasma was assayed after enzymatic hydrolysis and oxidation. Briefly, cholesterol ester in the plasma was hydrolysed by cholesterol esterase to form cholesterol and fatty acids.

Cholesterol ester + H_2O →cholesterol ester→ cholesterol + fatty acids

The cholesterol was oxidised afterward, by cholesterol oxidase to form cholestene-3-one and hydrogen peroxide.

Cholesterol + O_2 →cholesterol oxidase→cholestene-3-one + H_2O_2

Plasma triglycerides (TG) were assayed after enzymatic hydrolysis with lipases. Triglycerides are hydrolysed by lipases to form glycerol and fatty acids.

Triglycerides + H_2O →lipases→ glycerol + fatty acids

Phosphate is transferred from adenosine triphosphate (ATP) to glycerol, under catalysis of glycerolkinase (GK), to form glycerol-3-phosphate, which is oxidised by glycerol-3-phosphate oxidase (GPO) to form dihydroacetonephosphate and hydrogen peroxide.

Glycerol + ATP →GK→ glycerol-3-phosphate +ADP

Glycerol-3-phosphate + O_2 →GPO→ dihydroacetonephosphate + H_2O_2

The hydrogen peroxide formed reacts, under the catalysis of peroxidase, with phenol and 4-aminophenazone to form a red-violet quinoneimine dye as indicator.

$2H_2O_2$ +4-aminophenazone +phenol →POD→ quinoneimine + 4 H_2O

The concentration was determined by the equipment after reading the absorbance of the indicator at a wavelength of 500 nm.

HDL cholesterol was assayed by the precipitation method. 500 µL of diluted precipitant solution, containing phosphotungstic acid in the presence of magnesium, was added to 200 µL of the plasma sample. The sample was allowed to sit for 10 minutes at room temperature and centrifuged afterwards at 4000g for 10 minutes to precipitate low density lipoproteins and chylomicrons. The HDL cholesterol was assayed from the supernatant solution at an absorbance of 500nm.

The levels of LDL cholesterol were calculated from Frieldwald's equation [354], LDL = TChol – (HDL+TG/2.2).

The study subjects were further classified based on their lipid profile into various CVD risk groups according to the National Cholesterol Education Programme and Adult Treatment Panel III (NCEP/ATPIII).

3.7.3 Vascular Growth Factors Enzyme Immunoassay

The serum levels of vascular growth factors, VEGF, ang-1 and ang-2 were assayed using enzyme linked immunoassay (ELISA) method.

Sample Dilution

An amount of 50 µL of the serum samples was added to 450 µL of regent diluent to achieve 1:10 dilution. The samples were then stored in a refrigerator at a temperature of 2ºC and used for the assay within one week.

Plate Preparation

a. Plate Coating with Capture Antibody: Before the assay of each vascular growth factor, capture antibody was used to coat the wells in ELISA microplate overnight. For ang-1, mouse anti-human Ang-1 was reconstituted with 1.0 mL of PBS to obtain a concentration of 720 µg/mL, which was further diluted in PBS to achieve a working concentration of 4 µg/mL. For ang-2, mouse anti-human Ang-2 was reconstituted with 1.0 mL of PBS to achieve a concentration of 180 µg/mL, which was diluted further in PBS to a working concentration of 1.0 µg/mL. For VEGF, mouse anti-human $VEGF_{165b}$ was reconstituted with 1.0 mL of PBS to obtain a concentration of 360 µg/mL, and this was further diluted to in PBS a working concentration of 2.0 µg/mL. All these dilutions were performed without a carrier antibody. 100 µL of the diluted capture antibody solution was used to coat a 96-well microplate overnight at room temperature.
b. Washing: After overnight incubation, each well was aspirated and washing was done by filing each well with 400 µL of wash buffer twice, for a total of three times, using an autowasher. Any remaining wash buffer was removed by aspiration and blotting the microplate against paper towel.
c. The microplates were blocked by adding 300 µL of reagent diluent to each well and incubated for 1 hour.
d. Washing: The washing process was repeated as in step ‘b’.

Assay procedure

e. Sample and standard addition: In each well, 100 µL of sample or standard in reagent diluent was added, covered with adhesive strip and incubated for 2 hours at room temperature.
f. Washing: Washing process repeated as in step 'b'.
g. Detection antibody addition: a volume of 100 µL of detection antibody, diluted in reagent diluent with normal goat serum was added to each well, covered with a new adhesive strip and incubated for 2 hours at room temperature.
h. Washing: The washing process was repeated as in step 'b'.
i. A volume of 100 µL of diluted Streptavidin-HRP was added to each well, covered and incubated for, at room temperature, for 20 minutes.
j. The washing process was repeated as in step 'b'.
k. A volume of 100 µL of substrate solution was added to each well, incubated for 20 minutes at room temperature in darkness.
l. A volume of 50 µL of stop solution was added to each well.
m. The optical density was read at 450 nm using a microplate reader.

The optical densities were converted into concentrations by using four-parameter logistic curve fit programme, Auditable Data Analysis and. Management System for ELISA (ADAMSEL v1.1).

3.8 Statistical analysis

The data were analysed using IBM statistical package for social sciences software. The data with continuous variables were tested for normality using Shapiro-Wilk test

and variables with normal distribution were presented as mean±standard deviation, whereas non-normal distribution variables were presented as median (interquartile range). Marginal means of normally distributed variables were analysed across the various categories of study subjects using analysis of variance (ANOVA) test, with means adjusted for covariance when necessary using analysis of covariance (ANCOVA) tests. Independent student's T-test was used to analyse means of data with two predictors. Data with non-normal distribution were analysed across various categories of study subjects using Kruskal-Wallis test.

Categorical data were presented as frequency (percentage) and analysed by χ^2 test, with Yate's or Fisher's correction when necessary.

Association between variables were analysed using Pearson's correlation for normally distributed data and Spearman's correlation for non-normally distributed data. Multiple regression analyses were performed to analyse the relationship between the various variables.

The level of significance was set at 95% and $p<0.05$ was considered statistically significant.

4. CHAPTER FOUR

RESULTS

4.1 Description of the Study Population

4.1.1 General Description

The number of volunteers that participated in the studies, their gender and age decade distribution, alcohol and smoking status had been presented in Table 4.1. There was no significant association of the distribution of study subjects among various categories with respect to their gender, age decade, alcohol intake or previous smoking status. None of the study subjects was a current smoker.

4.1.2 Anthropometric characteristics of study subjects

The mean levels of anthropometric features of study subjects were compared among different CVD risk categories and presented as in Table 4.2. A common trend seems to run through all the anthropometric features measured, except for visceral fat and waist-to-hip ratio which were statistically non-significant: subjects with hypertension, either diabetes or nondiabetes, had higher mean anthropometric levels than nonhypertensive subjects. Pairwise comparison indicates that hypertensive T2DM subjects have significant higher waist girth ($p=0.018$) and WHR ($p=0.015$) than nonhypertensive T2DM subjects. Only BMI ($p=0.043$) was higher in hypertensive nondiabetes subjects than the control subjects.

Table 4.1 General description of study subjects

Parameter	Subject's category n (%)					*p*
	T2DM+HtN	T2DM-HtN	NDM+HtN	CTRL	Total	
N	68 (35.1)	46 (23.7)	48 (24.7)	35 (16.5)	197 (100)	
Gender						
Male	31 (16.1)	31 (16.1)	17 (8.8)	16 (8.3)	95 (49.2)	0.574
female	37 (19.2)	15 (7.8)	31 (16.1)	15 (7.8)	98 (50.8)	
Age decade in years						
<50	24 (12.4)	20 (10.4)	10 (5.2)	18 (9.3)	72 (37.3)	
50 – 59	16 (8.3)	13 (6.7)	18 (9.3)	10 (3.1)	57 (27.5)	0.243
60+	28 (14.5)	13 (6.7)	20 (10.4)	7 (3.6)	68 (35.2)	
Alcohol intake	32 (16.6)	7 (3.6)	14 (7.3)	27 (14.0)	80 (41.5)	0.148
Previous smokers	13 (6.7)	11 (5.7)	7 (3.6)	6 (3.1)	37 (19.2)	0.742

Data presented in frequency (percentages). T2DM+HtN: hypertensive type 2 diabetes subjects; T2DM-HtN: nonhypertensive type 2 diabetes subjects; NDM+HtN: hypertensive nondiabetes subjects; CTRL: control subjects (normotensive nonhytensive subjects)

Table 4.2 Mean levels Anthropometric Indices of Study Subjects

Anthropometric parameter	**Subject's category ($\bar{x}$±SD)**				***p***
	T2DM+HtN	T2DM-HtN	NDM+HtN	CTRL	
BMI (kg/m^2)	30.61±6.03[§]	26.46±4.42	29.89±5.55[§]	28.53±5.32	0.001
Body fat (%)	36.26±12.72[§]	30.56±10.12	38.39±11.91[§]	33.34±13.71	0.009
Visceral fat	12.15±4.66	11.17±1.08	10.78±3.26	9.87±3.05	0.082
Waist girth (cm)	102.79±12.10[§*]	93.93±10.32	97.48±11.36[§]	93.51±13.11	0.001
WHR	0.93±0.07[*]	0.91±0.07	0.89±0.07	0.91±0.10	0.351
WSR	0.62±0.09[§]	0.56±0.07	0.60±0.08[§]	0.56±0.11	0.001

Data presented as mean±standard deviations. BMI: body mass index; WHR: waist-to-hip ratio; WSR: waist-to-stature ratio. §: $p<0.05$ when compared to nonhypertensive subjects; *: $p<0.05$ when compared to nonhypertensive diabetes subjects.

As shown in Table 4.3, there was no significant association between BMI levels distribution among various categories of study subjects, although overweight and obesity tended to be more represented in hypertensive T2DM subjects. With respect to waist girth levels, hypertensive T2DM subjects also tended to have greater proportion of high level of waist girth, but this was not significant. Most of the study subjects have significantly high WHR, with hypertensive and nonhypertensive T2DM subjects having the greater proportion.

4.1.3 Mean BP levels among study subjects

As shown in Table 4.4, the mean brachial BP indices of hypertensive subjects, either T2DM or nondiabetes, was significantly higher than the corresponding mean BP of nonhypertensive subjects with and without T2DM. Post hoc analysis (Bonferrroni) showed that there was no significant difference between the mean brachial BP indices of hypertensive and nonhypertensive T2DM subjects. Similarly, there was no significant difference between the mean brachial BP indices of nonhypertensive T2DM subjects and control subjects.

Table 4.3 Distribution of Classes of Anthropometric Indices among Study Subjects

Anthropometric index	Study subjects, n (%)				Total	*p*
	T2DM+HtN	T2DM-HtN	NDM+HtN	CTRL		
BMI levels						
Normal	10 (5.1)	17 (8.6)	11 (5.6)	10 (5.1)	39 (24.4)	
Overweight	26 (13.2)	22 (11.2)	14 (7.1)	14 (7.1)	76 (38.6)	0.308
Obese	32 (16.2)	8 (4.1)	24 (12.2)	9 (4.6)	73 (37.0)	
Waist girth levels						
Normal	22 (11.6)	29 (15.3)	14 (7.4)	15 (7.9)	80 (42.3)	0.361
High	46 (24.3)	17 (9.0)	32 (16.9)	14 (7.4)	109 (57.7)	
WHR levels						
Normal	13 (7.1)	13 (7.1)	14 (7.7)	13 (7.1)	53 (29.1)	0.010
High	52 (28.6)	32 (17.6)	31 (17.0)	14 (7.7)	129 (70.1)	

Data presented as frequency (percentage).

Table 4.4 Brachial BP of study subjects

Blood pressure indices (mm Hg)	**Diabetes status ($\bar{x}$±SD)**				***p***
	T2DM+HtN	T2DM-HtN	NDM+HtN	CTRL	
Systolic BP	152.98±27.29§	124.35±9.42	148±18.50§	123.61±10.38	<0.001
Diastolic BP	89.16±12.02§	73.91±7.54	81.10±13.08§	73.97±8.20	<0.001
PP	66.10±13.95§	50.43±6.90	61.10±12.00§	49.65±7.69	<0.001
MAP	140.92±17.43§	115.36±9.97	136.51±18.22§	115.17±11.49	<0.001
HR	76.66±11.48§	73.74±10.46	66.91±12.96§	65.45±11.16	<0.001

Data presented as mean±standard deviation. §: $p<0.05$ when compared with nonhypertensive subjects. PP: pulse pressure; MAP: mean arterial pressure; HR: heart rate

4.1.4. Biochemical Parameters among Study subjects

As shown in Table 4.5, there was significant difference between the mean levels of fasting plasma glucose, 2-hour post glucose-load plasma glucose, total plasma cholesterol, LDL cholesterol and nonHDL cholesterol across all the categories of the study subjects. Pairwise contrast analysis, performed to compare hypertensive and nonhypertensive subjects, showed that there was no significant difference between the mean levels of fasting plasma glucose between hypertensive T2DM subjects and nonhypertensive T2DM subjects (p=0.792). There were no significant difference in the mean levels of fasting plasma glucose (p=0.867) and 2-hour post glucose-load plasma glucose (p=0.786) between hypertensive nondiabetes subjects and controls subjects.

Pairwise contrast analysis also indicated that there was no significant difference between the mean levels of total plasma cholesterol between hypertensive T2DM and nonhypertensive T2DM subjects (p=0.055), but control subjects had significantly higher mean level of total plasma cholesterol than nondiabetes hypertensive subjects (p=0.030). Hypertensive T2DM subjects had significantly higher mean level of LDL cholesterol than nonhypertensive T2DM subjects (p=0.025) and control subjects also had higher mean level of LDL cholesterol than hypertensive nondiabetes subjects. The mean level of nonHDL cholesterol was significantly higher in hypertensive T2DM subjects than nonhypertensive T2DM subjects (p=0.024) and also, higher in controls than hypertensive nondiabetes subjects.

Table 4.5 Mean levels of Biochemical Parameters among Study Subjects

Biochemical parameter (mmol/L)	Study subjects ($\bar{x}$±SD)				*p*
	T2DM+HtN	T2DM-HtN	NDM+HtN	CTRL	
FPG	8.59±3.77[§]	8.41±3.03[§]	4.37±1.68	4.97±0.56[*]	<0.001
2h-PPG	NA	NA	6.05±2.65	6.17±2.16	<0.001
TChol	4.53±1.37[ψ]	4.07±1.15	4.49±1.98	5.33±1.36[#]	0.006
Trig	1.05±0.43	1.00±0.52	1.05±0.73	1.12±0.46	0.830
HDL	0.70±0.19	0.82±0.67	0.74±0.33	0.85±0.52	0.301
LDL	3.36±1.22[ψ]	2.80±1.31	3.27±1.61	3.96±1.31[#]	0.005
TChol/HDL	6.70±1.91	6.20±3.24	6.51±2.49	7.23±2.46	0.358
LDL/HDL	5.00±1.71	4.44±2.89	4.80±2.21	5.51±2.20	0.239
Trig/HDL	1.63±0.85	1.67±1.51	1.53±0.91	1.58±0.91	0.943
nonHDL	3.84±1.29[ψ]	3.26±1.34	3.75±1.84	4.48±1.35[#]	0.006

Data presented as marginal mean±standard deviations are provided. §: $p < 0.05$ when compared to nondiabetes subjects (NDM+HtN and CTRL). *: $p < 0.05$ when compared to controls. ψ: $p < 0.05$ when compared to nonhypertensive diabetes subjects. #: $p < 0.05$ when compared to other subject's categories.
FPG-fasting plasma glucose, 2h-PPG-2hour postglucose load plasma glucose, TChol-plasma total cholesterol, Trig-fasting plasma triglycerides, HDL-high density lipoprotein cholesterol, LDL-low density lipoprotein cholesterol, NA: not applicable.

The plasma lipids levels were categorised according to NCEP/ATPIII lipid risk categories [355] and the distribution was presented in Table 4.6. Most of the study subjects were within the desirable (low risk) levels of total plasma cholesterol distribution, with hypertensive T2DM subjects having the greater proportion of individuals in the low risk category, followed by nonhypertensive T2DM subjects, hypertensive non diabetes subjects and control subjects in that order. Also a greater proportion of the study subjects in all categories are within the normal (low risk) levels of triglyceride distribution, though the association of the proportions across the various categories of study subjects was non-significant. For HDL cholesterol distribution, most of the study subjects across the various categories were within the low (high risk) levels. For LDL cholesterol distribution, nearly half of the study subjects were with the borderline risk levels with nearly quarter proportion of the study subjects in the optimal (low risk) and high risk levels of distribution. The number of nondiabetes subjects in the high risk level of LDL cholesterol distribution was relatively more than that of T2DM subjects.

Table 4.6 Plasma lipid risk distribution according to NCEP/ATPIII criteria.

Plasma cholesterol level of risk		Study subjects n (%)				Total	p
		T2DM+HtN	T2DM-HtN	NDM+HtN	CTRL		
TChol	Desirable	48 (25)	38 (19.8)	31(16.1)	13 (6.8)	130 (67.7)	0.031
	Borderline	13 (6.8)	4 (8.7)	10 (5.2)	11 (5.7)	38 (19.8)	
	High	7 (3.6)	4 (2.1)	7 (3.6)	6 (3.1)	24 (12.5)	
Trig	Normal	61 (31.8)	40 (20.8)	43 (22.4)	26 (13.5)	170 (88.5)	0.593
	Borderline	5 (2.6)	4 (2.1)	3 (1.6)	2 (1.0)	14 (7.3)	
	High	2 (1)	2 (1)	2 (1)	2 (1)	8 (4.2)	
HDL	Normal	6 (3.1)	5 (2.6)	9 (4.7)	7 (3.6)	27 (14.1)	0.031
	Low	62 (32.3)	41 (21.4)	39 (20.3)	23 (12.0)	165 (85.9)	
LDL	Optimal	20 (10.5)	20 (10.5)	11 (5.8)	3 (1.6)	54 (28.3)	0.041
	Borderline	33 (17.3)	20 (10.5)	23 (12.0)	15 (7.9)	91 (47.6)	
	High	15 (7.9)	6 (3.1)	14 (7.3)	11 (5.8)	46 (24.1)	

Data presented as frequency (percentage). TChol-plasma total cholesterol, Trig-fasting plasma triglycerides, HDL-high density lipoprotein cholesterol, LDL-low density lipoprotein cholesterol.

4.2. Levels of arterial stiffness indices

The mean levels of arterial stiffness indices have been shown in Table 4.7. In all the indices, there was significant difference across the various categories of study subjects. Pairwise contrast comparison indicated that hypertensive T2DM had significantly higher aortic PWV than nonhypertensive T2DM ($p<0.001$), hypertensive nondiabetes ($p=0.046$) and controls ($p<0.001$). Hypertensive nondiabetes subjects had higher aortic PWV than nonhypertensive T2DM ($p=0.001$) and controls ($p<0.001$), whereas nonhypertensive T2DM subjects has higher aortic PWV than controls ($p=0.042$). With respect to aortic SBP and aortic PP, there were no significant difference between the mean levels for hypertensive T2DM and hypertensive nondiabetes subjects ($p=0.892$; $p=0.901$), as well as no significant difference between nonhypertensive T2DM and control subjects ($p=0.843$; $p=0.745$) respectively. Nondiabetes hypertensive subjects had significantly higher level of aortic AIx than hypertensive T2DM ($p=0.001$), nonhypertensive T2DM ($p<0.001$) and control subjects ($p=0.017$). There was no significant difference in AIx between hypertensive T2DM subjects and controls ($p=0.985$). Nonhypertensive T2DM subjects had the lowest levels of AIx compared to subjects in other categories.

The results of right and left CAVIs follow a common pattern: hypertensive T2DM had higher mean levels than nonhypertensive T2DM ($p=0.038$; $p=0.006$), and the nonhypertensive T2DM also had higher mean levels compared to nondiabetes hypertensive subjects ($p=0.021$; $p=0.037$), and the control subjects had the least mean levels ($p=0.001$; $p=0.007$) respectively. The results of right and left ha-PWV followed a similar pattern as aortic PWV. Hypertensive T2DM had significantly higher right and left ha-PWV than nonhypertensive T2DM ($p<0.048$; $p=0.006$), hypertensive nondiabetes ($p=0.038$; $p<0.001$) and controls ($p=0.008$; $p<0.001$) respectively.

Nondiabetes hypertensive subjects had higher right and left ha-PWV than nonhypertensive T2DM (p=0.049; p=0.043) and controls (p=0.047; p=0.038), whereas nonhypertensive T2DM subjects has higher aortic PWV than controls (p=0.042; 0.047) respectively.

Table 4.7 Adjusted mean levels of arterial stiffness indices among study subjects

Arterial stiffness indices	Study subjects ($\bar{x}$±SD)				*p*
	T2DM+HtN	T2DM-HtN	NDM+HtN	Controls	
Aortic PWV (m/s)	9.28±1.42$^{\#}$	8.24±1.25*	8.92±1.14$^{\psi}$	7.14±1.29	<0.001
Aortic SBP (mm Hg)	151.83±24.11$^{\varphi}$	116.21±11.82	147.44±23.55$^{\varphi}$	119.45±11.50	<0.001
Aortic PP (mm Hg)	58.78±22.26$^{\varphi}$	39.52±12.89	58.21±20.51$^{\varphi}$	42.84±13.90	<0.001
Aortic AIx	28.28±13.74	18.07±12.25$^{\#}$	37.86±11.58$^{\#}$	28.67±13.10	<0.001
Right CAVI	8.32±1.32$^{\#}$	7.70±1.09$^{\Omega}$	7.33±1.14*	7.09±0.97	<0.001
Left CAVI	8.13±1.1$^{\#}$	7.73±0.99$^{\Omega}$	7.31±1.09*	7.04±1.01	<0.001
Right ha-PWV (m/s)	8.47±1.12$^{\#}$	7.40±0.78*	7.91±1.06 $^{\psi}$	7.04±1.12	<0.001
Left ha-PWV (m/s)	8.41±1.10$^{\#}$	7.46±0.78*	7.97±1.30 $^{\psi}$	6.95±0.86	<0.001

Data presented in means±standard deviation after adjustment of gender, age, duration of diabetes, height, body mass index, heart rate and mean arterial pressure.
*: $p < 0.05$ when compared to controls. ψ: $p < 0.05$ when compared to nonhypertensive diabetes subjects. #: $p < 0.05$ when compared to other subject's categories.
Ω: $p < 0.05$ when compared to nondiabetes hypertensive subjects. φ: $p < 0.05$ when compared to nonhypertensive (T2DM-HtN and CTRL) subjects.
SBP: systolic blood pressure, PP: pulse pressure, AIx: aortic augmentation index, CAVI: cardio-ankle vascular index, ha-PWV: heart-ankle pulse wave velocity,

The gender differences in mean levels of arterial stiffness indices are shown in the Table 4.8 below. Male subjects had significantly higher levels of aortic PWV, aortic systolic BP, aortic PP and aortic Aix than female subjects after adjusting for diabetes and hypertension status, age, BMI, heart rate and mean blood pressure. However, there is no significant difference in the mean levels of right and left CAVI as well as ha-PWV between males and females.

Table 4.8 Gender differences in arterial stiffness indices

Arterial stiffness indices	**Study subjects ($\bar{x}$±SD)**		***p***
	Males	Females	
Aortic PWV (m/s)	8.23±1.48	9.02±1.34	<0.001
Aortic SBP (mm Hg)	131.54±26.17	142.69±27	0.004
Aortic PP (mm Hg)	48.09±19.49	59.05±19.05	<0.001
Aortic AIx	22.66±12.86	34.98±12.66	<0.001
Right CAVI	7.62±1.54	7.53±1.28	0.678
Left CAVI	7.63±1.18	7.63±1.20	0.089
Right ha-PWV (m/s)	7.82±1.13	7.80±1.16	0.871
Left ha-PWV (m/s)	7.84±1.44	7.73±1.18	0.663

Data presented in means±standard deviation after adjustment of gender, age, duration of diabetes, height, body mass index, heart rate and mean arterial pressure.
SBP: systolic blood pressure, PP: pulse pressure, AIx: aortic augmentation index, CAVI: cardio-ankle vascular index, ha-PWV: heart-ankle pulse wave velocity.

To assess the impact of diabetes on arterial stiffness indices, the mean levels of the of arterial stiffness indices for T2DM and nondiabetes subjects were compared, with adjustment for age, gender, BMI, height, heart rate and MAP. The results are as presented in Table 4.9 for all the parameters of arterial stiffness, with the exception of aortic SBP and aortic PP where no significant difference were found, T2DM subjects had significant higher mean levels compared to nondiabetes subjects. However, for aortic AIx, T2DM had lower mean level compared to nondiabetes subjects.

Table 4.9 Adjusted mean levels of arterial stiffness indices among T2DM and nondiabetes subjects.

Arterial stiffness indices	**Study subjects ($\bar{x}$±SD)**		***p***
	T2DM	NDM	
Aortic PWV (m/s)	8.75±1.50	8.38±1.31	0.046
Aortic SBP (mm Hg)	137.78±26.64	136.61±24.10	0.337
Aortic PP (mm Hg)	51.46±20.75	52.28±19.72	0.227
Aortic AIx	24.37±13.91	34.35±12.10	0.001
Right CAVI	7.86±1.35	7.26±1.07	0.001
Left CAVI	7.76±1.16	7.13±1.09	<0.001
Right ha-PWV (m/s)	7.94±1.14	7.59±1.08	0.041
Left ha-PWV (m/s)	7.95±1.09	7.56±1.27	<0.001

Data presented in means±standard deviation after adjustment of gender, age, duration of diabetes, height, body mass index, heart rate and mean arterial pressure.
SBP: systolic blood pressure, PP: pulse pressure, AIx: aortic augmentation index, CAVI: cardio-ankle vascular index, ha-PWV: heart-ankle pulse wave velocity.

To assess the impact of hypertension on indices of arterial stiffness, mean levels of arterial stiffness indices for hypertensive subjects were compared, after adjustment for gender, age, height, BMI and MAP to nonhypertensive subjects. The means are presented in Table 4.10. Hypertensive subjects had significantly higher levels of aortic PWV, aortic SBP, aortic PP, right and left CAVI, right and left ha-PWV, except for aortic AIx where there is no significant difference, compared to the nonhypertensive subjects.

Table 4.10 Adjust mean levels of arterial stiffness indices among hypertensive and nonhypertensive subjects.

Arterial stiffness indices	**Study subjects ($\bar{x}$±SD)**		***p***
	HTN	NHT	
Aortic PWV (m/s)	9.13±1.32	7.78±1.26	0.032
Aortic SBP (mm Hg)	150.05±23.87	117.39±11.76	0.049
Aortic PP (mm Hg)	59.02±20.84	40.82±13.40	0.046
Aortic AIx	32.38±13.42	22.06±13.61	0.510
Right CAVI	7.89±1.37	7.28±1.05	0.023
Left CAVI	7.72±1.24	7.23±1.03	0.029
Right ha-PWV (m/s)	8.19±1.12	7.21±0.82	0.044
Left ha-PWV (m/s)	8.17±1.21	7.20±0.84	0.011

Data presented in means±standard deviation after adjustment of gender, age, duration of diabetes, height, body mass index, heart rate and mean arterial pressure.
SBP: systolic blood pressure, PP: pulse pressure, AIx: aortic augmentation index, CAVI: cardio-ankle vascular index, ha-PWV: heart-ankle pulse wave velocity.

4.2.1 Association between indices of arterial stiffness, age and duration of diabetes

The association of arterial stiffness indices with chronological age and duration of diabetes had been presented in Table 4.11. All the indices of arterial stiffness had positive significant correlation with age; right and left CAVIs showed the highest correlation, followed by the PWVs, then the central haemodynamic indices. The duration of diabetes correlated positively with right and left CAVIs as well as the right and left head-to-ankle PWVs. In multiple regression analysis, after adjusting for subjects' category, duration of diabetes, BMI, waist circumference and WHR, age showed a significant association with aortic PWV (β=0.033, p=0.024), aortic PP (β=0.473, p=0.006), aortic AIx (β=0.276, p=0.031), right CAVI (β=0.037, p=0.008) and left CAVI (0.039, p<0.001) but not aortic SBP, right and left ha-PWVs. The perceived association of duration of diabetes and some of the indices of arterial stiffness were lost after similar adjustments.

Figure 11 shows the mean levels of arterial stiffness indices by age decade. In all indices, the high age decade (60+ years) had higher mean levels than the 50-59 year group, which also had higher level than lowest age decade (< 50 years).

Figure 12 shows the mean levels of central haemodynamic pressures by the various decades. In all parameters, the lowest age decade (< 50 years) subjects had lower mean levels of central pressures than the '50-59 years' and '60+ years' subjects, though there is no significant difference between the last two age decades.

Table 4.11 Correlation of Arterial Stiffness with Age and diabetes duration.

Arterial Stiffness Index	Age		Duration of diabetes	
	r	***p***	**r**	***p***
Aortic PWV (m/s)	**0.346**	<0.001	0.103	0.312
Aortic SBP (mm Hg)	**0.215**	0.003	-0.034	0.740
Aortic PP (mm Hg)	**0.314**	<0.001	0.027	0.785
Aortic AIx	**0.273**	<0.001	-0.067	0.501
Right CAVI	**0.504**	<0.001	**0.348**	<0.001
Left CAVI	**0.498**	<0.001	**0.278**	0.005
Right ha-PWV (m/s)	**0.414**	<0.001	**0.283**	0.004
Left ha-PWV (m/s)	**0.381**	<0.001	**0.253**	0.011

Data presented as Pearson's correlation coefficient, r.
SBP: systolic blood pressure, PP: pulse pressure, AIx: aortic augmentation index, CAVI: cardio-ankle vascular index, ha-PWV: heart-ankle pulse wave velocity.

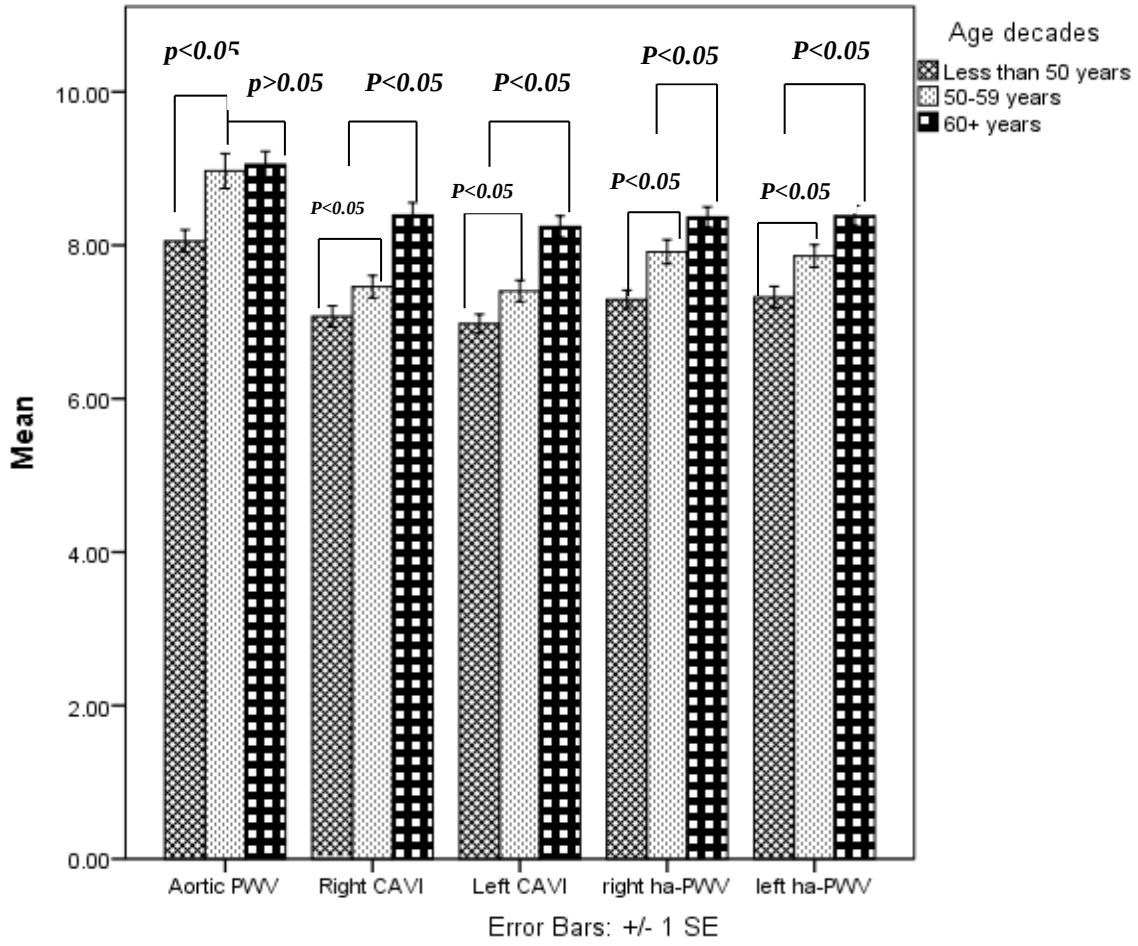


Figure 11. Mean levels of arterial stiffness indices by age decade.

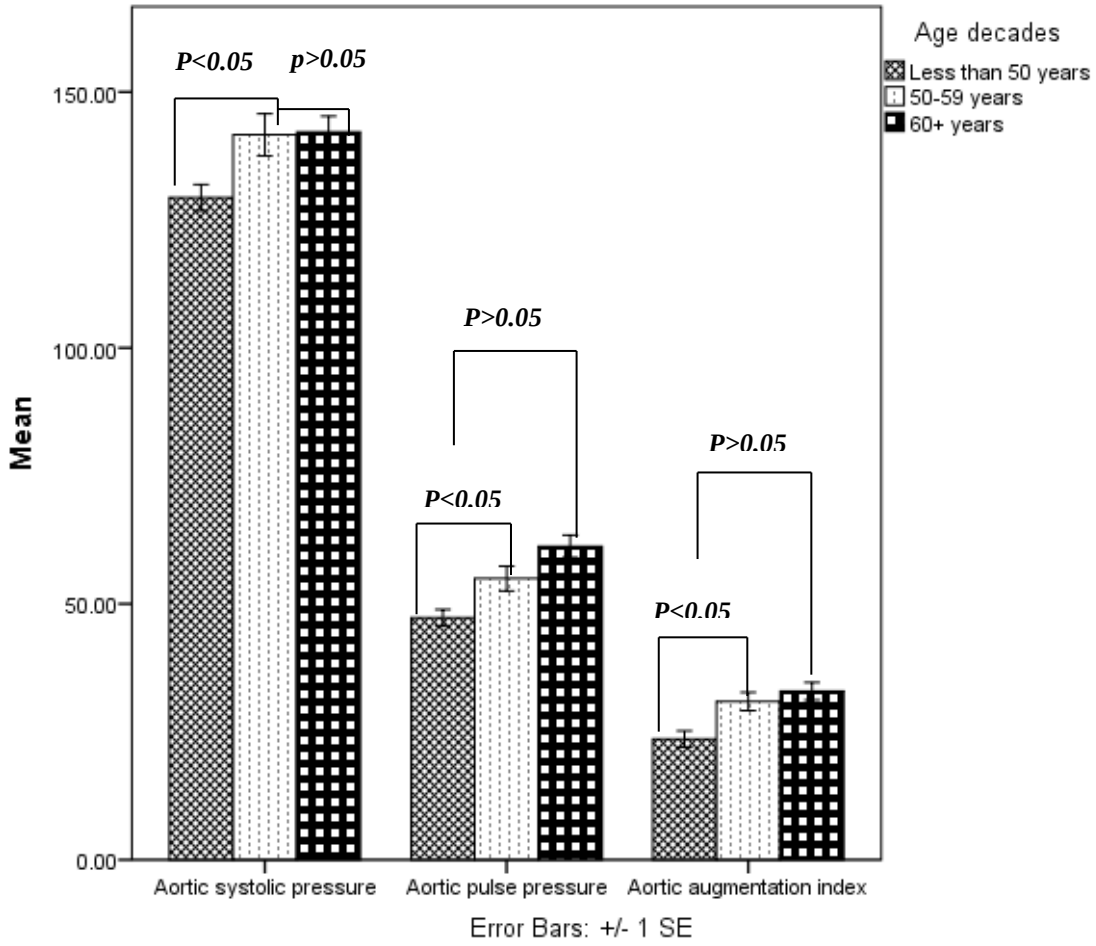


Figure 12. Central haemodynamic pressures by age decades

4.3. Arterial stiffness indices and anthropometric indices

The mean levels of arterial stiffness indices across various BMI groups were presented as shown in Table 4.12 Aortic PWV and aortic SBP differed significantly across various BMI groups. Pairwise analysis indicated that obese subjects had higher aortic PWV (p=0.032) and aortic SBP (p=0.041) than both overweight and normal weight groups, which do not differ significantly from each other.

Table 4.12 Mean levels of arterial stiffness indices across various BMI groups.

Arterial stiffness indices	BMI groups ($\bar{x}$±SD)			*p*
	Obese	Overweight	Normal	
Aortic PWV (m/s)	9.20±1.24#	8.38±1.40	8.07±1.54	<0.001
Aortic SBP (mm Hg)	145.84±25.81#	133.33±22.03	131.69±28.07	0.003
Aortic PP (mm Hg)	55.27±25.27	50.16±17.97	47.33±22.75	0.222
Aortic AIx	30.35±14.00	28.25±13.65	24.33±16.39	0.206
Right CAVI	7.62±1.33	7.44±1.59	7.67±1.08	0.483
Left CAVI	7.46±1.23	7.46±1.12	7.58±1.21	0.460
Right ha-PWV (m/s)	7.77±1.11	7.61±1.43	7.97±1.19	0.430
Left ha-PWV (m/s)	7.69±1.13	7.60±1.39	8.10±1.44	0.204

Data presented as means±standard deviation after adjustment of gender, age, duration of diabetes, height, heart rate and mean arterial pressure. #: $p<0.05$ when compared with either overweight or normal BMI.
SBP: systolic blood pressure, PP: pulse pressure, AIx: aortic augmentation index, CAVI: cardio-ankle vascular index, ha-PWV: heart-ankle pulse wave velocity.

4.3.1 Effect of age and body composition variation on arterial stiffness indices

Analysis of covariance was performed to assess the effect of age decade, gender and waist circumference, WHR and BMI group on the variation of various arterial stiffness indices across the different categories of study subjects.

It was found that subject's category ($p<0.001$, $\eta^2=0.125$), waist circumference ($p=0.003$, partial $\eta^2=0.052$) and age decade ($p<0.001$, partial $\eta^2=0.081$) were

significantly associated with aortic PWV and exclusively explains the variation of 12.5%, 5.2% and 8.1% of aortic PWV respectively. Only subject's category was significantly associated with aortic systolic pressure ($p<0.001$, partial $\eta^2=0.331$) and explains 33.1% of the variation in aortic SBP independent of other factors. Aortic PP was significantly associated with subject's category ($p<0.001$, partial $\eta^2=0.140$) and age decade ($p=0.007$, partial $\eta^2=0.041$) and independently explain 14% and 4.1% of the variation in aortic PP respectively. With respect to aortic AIx, it was significantly associated with gender ($p<0.001$, partial $\eta^2=0.110$), age decade ($p=0.005$, partial $\eta^2=0.046$) and subject's category ($p<0.001$, partial $\eta^2=0.196$). Gender, age decade and subject's category independently explain the variation of aortic AIx by 11%, 4.6% and 19.6% respectively.

In the model also, right side CAVI was significantly associated with waist circumference ($p=0.001$, partial $\eta^2=0.063$), WHR ($p=0.028$, partial $\eta^2=0.028$), gender ($p=0.017$, partial $\eta^2=0.034$), age decade ($p<0.001$, partial $\eta^2=0.090$) and subjects category ($p=0.006$, partial $\eta^2=0.071$). Waist circumference, WHR, gender, age decade and subject's category independently explains the variation in the right side CAVI by 6.3%, 2.8%, 3.4%, 9.0% and 7.1% respectively. The left side CAVI was also associated with WHR ($p=0.048$, partial $\eta^2=0.23$), age decade ($p<0.001$, partial $\eta^2=0.144$) and subject's category ($p<0.001$, partial $\eta^2=0.136$). The variation in the left CAVI can be accounted for independently, 4.8% by WHR, 14.4% by age decade and 13.6% by subject's category.

The right side ha-PWV was associated significantly with WHR ($p=0.021$, partial $\eta^2=0.031$), age decade ($p=0.011$, partial $\eta^2=0.038$) and subject's category ($p<0.001$, partial $\eta^2=0.127$). The variation in right side ha-PWV can be accounted for independently, 3.1% by WHR, 3.8% by age decade and 12.7% by subject's category.

Likewise, left side ha-PWV was associated significantly with WHR (p=0.041, partial η^2=0.025), age decade (p=0.004, partial η^2=0.049) and subject's category (p<0.001, partial η^2=0.126). The variation in right side ha-PWV can be accounted for independently, 2.5% by WHR, 4.9% by age decade and 12.6% by subject's category.

4.4. Association between arterial stiffness indices and plasma lipid levels.

From Table 4.13, only plasma triglyceride was significantly associated with aortic PWV. Regression analysis yielded a significant positive relationship (beta=0.391, p=0.045) between plasma triglycerides and aortic PWV, after adjusting for other types of plasma lipids and categories of study subjects. Also, there was a significant positive association between aortic pulse pressure and total cholesterol, LDL cholesterol, nonHDL cholesterol, LDL/HDL as well as total cholesterol/HDL.

Table 4.13 Association between plasma lipids and arterial stiffness.

Arterial stiffness index		**Plasma Lipids**							
		TChol	TG	HDL	LDL	nonHDL	LDL/HDL	TChol/HDL	TG/HDL
Aortic PWV	r	0.105	**0.147**	-0.016	0.095	0.105	0.098	0.097	0.126
	p	0.158	0.048	0.834	0.200	0.159	0.194	0.199	0.094
Aortic SBP	r	0.113	0.108	0.025	0.100	0.103	0.048	0.034	0.049
	p	0.118	0.137	0.736	0.167	0.155	0.515	0.641	0.505
Aortic PP	r	**0.215**	0.104	0.051	**0.182**	**0.200**	**0.159**	**0.147**	0.055
	p	0.003	0.156	0.492	0.013	0.006	0.032	0.048	0.460
Aortic AIx	r	0.039	**0.167**	0.017	0.022	0.044	-0.019	0.002	0.147
	p	0.597	0.024	0.817	0.763	0.556	0.802	0.983	0.051

Arterial stiffness index		**Plasma Lipids**							
		TChol	TG	HDL	LDL	nonHDL	LDL/HDL	TChol/HDL	TG/HDL
Right CAVI	r	0.041	0.038	0.008	0.032	0.035	-0.019	-0.024	0.013
	p	0.577	0.609	0.908	0.662	0.629	0.801	0.750	0.862
Left CAVI	r	0.009	0.007	-0.037	0.011	0.007	-0.003	-0.014	0.006
	p	0.899	0.925	0.618	.882	0.919	0.973	0.854	0.935
Right ha-PWV	r	0.058	0.125	0.057	0.031	0.050	-0.042	-0.035	0.063
	p	0.430	0.087	0.438	0.671	0.498	0.574	0.633	0.392
Left ha-PWV	r	0.001	0.095	-0.021	-0.023	-0.005	-0.036	-0.027	0.080
	p	0.988	0.193	0.770	0.754	0.950	0.624	0.713	0.280

Data presented as Pearson's correlation coefficient, r.
TChol: plasma total cholesterol, Trig: fasting plasma triglycerides, HDL: high density lipoprotein cholesterol, LDL: low density lipoprotein cholesterol.
SBP: systolic blood pressure, PP: pulse pressure, AIx: aortic augmentation index, CAVI: cardio-ankle vascular index, ha-PWV: heart-ankle pulse wave velocity.

4.5 Regression analysis of brachial BPs and indices of arterial stiffness

Multiple regression analysis was used to model indices of arterial stiffness with brachial BPs. As shown in the Table 4.14, aortic PWV was positively related to systolic brachial pressure and heart rate, but not diastolic brachial pressure. Also, aortic pulse pressure was positively related to systolic brachial pressure but negatively related to diastolic brachial pressure and heart rate. Aortic AIx was mathematically related to both brachial diastolic pressure and heart rate but not systolic pressure. Both right and left side CAVIs were related to systolic brachial pressure and only left CAVI showed a negative significant relation with diastolic pressure.

Table 4.14 Multiple regression analysis of arterial stiffness indices with brachial BPs.

Arterial stiffness index	Brachial BPs					
	SBP		DBP		HR	
	β	*p*	β	*p*	β	*p*
Aortic PWV (m/s)	0.024±0.007	<0.001	0.010±0.012	0.414	0.032±0.008	<0.001
Aortic PP (mm Hg)	0.858±0.090	<0.001	-0.539±0.155	0.001	-0.434±0.091	<0.001
Aortic AIx	0.055±0.071	0.438	0.443±0.116	<0.001	0.032±0.008	<0.001
Right CAVI	0.017±0.008	0.028	-0.016±0.012	0.186	0.006±0.008	0.443
Left CAVI	0.022±0.007	0.001	-0.023±0.011	0.044	0.002±0.007	0.829

4.6 Peripheral sensory neuropathy

4.6.1 Mean VPT levels of study subjects

As shown in Table 4.15, the mean VPT of the right leg is not significantly different from that of the left leg. The mean VPTs of the right and left leg of diabetes subjects with or without hypertension is significantly higher than the mean VPTs of nondiabetes subjects with or without hypertension respectively.

Table 4.15 Adjusted mean levels of right and left legs VPT of study subjects

VPT (V)	**Study subjects ($\bar{x}$±SD)**				**p**
	T2DM+HtN	T2DM-HtN	NDM+HtN	CTRL	
Right leg	11.43±9.21#	11.27±14.62#	7.43±3.36	7.78±4.87	0.013
Left leg	12.43±7.94#	11.31±6.08#	8.65±5.89	8.47±7.53	0.010

Data presented as means±standard deviation after adjustment of gender, age, duration of diabetes, height, BMI and mean arterial pressure. #: $p<0.05$ when compared with nondiabetes subjects.

As shown in table 4.16, the prevalence of neuropathy was higher in subjects with hypertensive T2DM subjects in both right and left legs. However, both hypertensive and nonhypertensive T2DM subjects had equivalent proportion of elevated VPT.

Table 4.16 Distribution of VPT levels among the study subjects.

Leg	VPT level	Study subjects n (%)				Total	p
		T2DM+HtN	T2DM-HtN	NDM+HtN	CTRL		
Right	Normal	49 (25.9)	33 (17.5)	43 (22.8)	24 (12.7)	149 (78.8)	0.055
	Elevated	11 (5.8)	10 (5.3)	4 (2.1)	5 (2.5)	30 (15.9)	
	High	7 (3.7)	1 (0.5)	1 (0.5)	1 (0.5)	10 (5.3)	
Left	Normal	51 (28.5)	36 (20.1)	41 (22.9)	21 (11.7)	149 (83.2)	0.011
	Elevated	9 (5.0)	7 (3.9)	2 (1.1)	4 (2.2)	22 (12.3)	
	High	7 (3.9)	1 (0.5)	0	0	8 (4.5)	

Data presented as frequency (percentage).

4.6.2 Association between VPT, age and duration of diabetes

As shown in Table 4.17, age was positively associated with right and left leg VPT, but diabetes duration was only associated positively with left leg VPT. Multiple regression of age and duration of diabetes with right and left leg VPTs by force entry indicated that, only diabetes duration was associated positively with left leg VPT (β=0.279, p=0.030) after adjustment of age.

Table 4.17 Correlation between VPT, age and duration of diabetes.

VPT	**Age**		**Diabetes duration**	
	r	*p*	r	*p*
Right leg	**0.171**	0.018	0.112	0.263
Left leg	**0.229**	0.002	**0.284**	0.004

Data presented as Pearson's correlation coefficient, r.

4.6.3 Association between body composition indices and VPT.

As shown in Table 4.18, VPT correlated negatively with percentage body fat and positively with WHR.

Table 4.18 Correlation between VPT and body composition indices.

Body composition index	Right leg VPT		Left leg VPT	
	r	*p*	r	*p*
Body mass index (kg/m^2)	-0.089	0.224	-0.120	0.110
Body fat (%)	-0.100	0.171	**-0.181**	0.015
Visceral fat	0.040	0.583	0.065	0.391
Waist girth (cm)	0.011	0.878	0.068	0.366
WHR	0.105	0.154	**0.172**	0.021
WSR	-0.063	0.393	-0.046	0.542

Data presented as Pearson's correlation coefficient, r. WHR: waist-to-hip ratio; WSR: waist-to stature ratio

4.6.4. Association between biochemical parameters and VPT

As shown in Table 4.19, only FBG correlated positively with VPT.

Table 4.19 Correlation between VPT and biochemical parameters

Biochemical parameters	Right leg VPT		Left leg VPT	
	r	*p*	r	*p*
FBG	**0.181**	0.013	**0.170**	0.018
2h-PPG	0.009	0.935	0.066	0.574
TC	0.036	0.625	0.050	0.510
TG	0.018	0.807	-0.009	0.902
HDL	0.014	0.847	.0079	0.293
LDL	0.026	0.725	-0.013	0.865
TChol/HDL	-0.003	0.966	-0.082	0.283
LDL/HDL	-0.026	0.724	-0.101	0.183
TG/HDL	0.029	0.694	0.011	0.886

Data presented as Pearson's correlation coefficient, r.
TChol: plasma total cholesterol, Trig: fasting plasma triglycerides, HDL: high density lipoprotein cholesterol, LDL: low density lipoprotein cholesterol. VPT: vibration perception threshold

4.7 Relationship between arterial stiffness indices and VPT

The mean levels were compared across various categories of VPT as shown in Table 4.20, after adjustment of gender, age, duration of diabetes and MAP. Aortic PWV, right and left ha-PWV were found to be significant across the VPT categories. Pairwise analysis indicated that in all the parameters that were significant, the high

VPT group had higher mean levels than elevated and normal VPT groups; the latter two had no significant difference among them.

Table 4.20 Mean levels of indices of arterial stiffness in various VPT groups

Arterial stiffness indices	**VPT groups**			***p***
	Normal	Elevated	High	
Aortic PWV (m/s)	8.58±1.44	8.63±1.31	9.77±1.47#	0.048
Aortic SBP (mm Hg)	136.43±24.61	140.03±28.62	149.25±27.79	0.326
Aortic PP (mm Hg)	51.52±20.04	56.77±17.09	47.10±26.94	0.306
Aortic AIx	29.13±14.01	28.53±14.53	19.85±15.64	0.166
Right CAVI	7.52±1.306	7.95±1.13	7.89±1.27	0.196
Left CAVI	7.39±1.18	7.82±1.04	7.78±1.25	0.152
Right ha-PWV (m/s)	7.71±1.09	8.04±1.02*	8.58±1.55#	0.017
Left ha-PWV (m/s)	7.71±1.16	8.01±1.07*	8.43±1.41#	0.049

Data presented as means±standard deviation after adjustment of gender, age, duration of diabetes, height and mean arterial pressure. #: $p<0.05$ when compared with either elevated or normal VPT levels. *: $p<0.05$ when compared with the normal VPT level.
SBP: systolic blood pressure, PP: pulse pressure, AIx: aortic augmentation index, CAVI: cardio-ankle vascular index, ha-PWV: heart-ankle pulse wave velocity.

The correlation between the indices of arterial stiffness and right and left VPTs were performed and presented in Table 4.21. Right and left CAVIs as well as right and left ha-PWVs were positively associated with right and left VPTs.

Table 4.21 Correlation between arterial stiffness indices and VPT

Arterial stiffness indices	**VPT**			
	Right leg		Left leg	
	r	*p*	r	*p*
Aortic PWV	0.094	0.207	0.119	0.120
Aortic SBP	0.136	0.067	0.060	0.438
Aortic PP	0.081	0.268	0.037	0.618
Aortic AIx	-0.013	0.857	-0.111	0.144
Right CAVI	**0.172**	0.020	**0.216**	0.004
Left CAVI	**0.210**	0.004	**0.236**	0.002
Right ha-PWV	**0.269**	<0.001	**0.260**	0.001
Left ha-PWV	**0.264**	<0.001	**0.237**	0.002

Data presented as Pearson's correlation coefficient, r.
SBP: systolic blood pressure, PP: pulse pressure, AIx: aortic augmentation index, CAVI: cardio-ankle vascular index, ha-PWV: heart-ankle pulse wave velocity.

4.8 Levels of Vascular growth factors

The distribution of the vascular growth factors were tested for normality with Shapiro-Wilk test; ang-1, but not ang-2 and VEGF, was normally distributed across the various categories of study subjects. Hence, the distributions of vascular growth factors had been presented in Table 4.22 as means±SD for ang-1 and median (interquartile range) for ang-2 and VEGF. The mean level of ang-1 in hypertensive T2DM subjects is significantly higher than that in nonhypertensive T2DM, hypertensive nondiabetes subjects and the controls. There was no significant difference among the latter three groups on pairwise analysis. Kruskal–Wallis test was used to test the distribution of mean ranks (medians) of ang-2 and VEGF across the various categories of study subjects. Ang-2 median distribution, but not VEGF, was significantly different across the various categories of study subjects.

Table 4.22 Distribution of vascular growth factors among study subjects

Vascular growth factors	Study subjects, ($\bar{x}$±SD) or median (interquartile range)				*p*
	T2DM+HtN	T2DM-HtN	NDM+HtN	CTRL	
Ang-1 (nmol/L)	44.44±21.05	35.15±21.82	35.08±10.47	37.71±13.07	0.019
Ang-2 (pmol/L)	858.8 (515.20-1448.60)	710.35 (396.20-1063.90)	736.5 (390.80-1168.70)	449.65 (180.00-891.20)	0.016
VEGF (pmol/L)	86.5 (30.90-187.20)	56.5 (16.50-246.40)	51.75 (20.20-117.25)	35.25 (20.30-104.50)	0.258

Data presented as means±standard deviation or median (interquartile range). #: $p<0.05$ when compared with other subject categories.
Ang-1: angiopoietin-1, ang-2: angiopoietin-2, VEGF: vascular endothelial growth factor.

Association between vascular growth factors and arterial stiffness indices were assessed in binary correlational analysis and presented in Table 4.23. Ang-2 was the only vascular growth factor that significantly correlated positively with central haemodyanmics; aortic SBP, aortic PP and aortic AIx.

Table 4.23 Correlation of vascular growth factors with arterial stiffness indices.

Arterial stiffness indices	**Vascular growth factors**					
	Ang-1		Ang-2		VEGF	
	r	*p*	r	*p*	r	*p*
Aortic PWV	0.087	0.248	0.114	0.142	0.039	0.607
Aortic SBP	0.103	0.174	**0.279**	<0.001	0.064	0.402
Aortic PP	0.113	0.123	**0.209**	0.005	0.052	0.482
Aortic AIx	0.047	0.533	**0.186**	0.015	0.053	0.482
Right CAVI	0.041	0.582	0.070	0.360	-0.052	0.487
Left CAVI	0.028	0.711	0.072	0.347	-0.063	0.404
Right ha-PWV	0.064	0.391	0.097	0.202	0.010	0.895
Left ha-PWV	0.009	0.907	0.071	0.355	0.029	0.698

Data presented as Pearson's correlation coefficient, r.
SBP: systolic blood pressure, PP: pulse pressure, AIx: aortic augmentation index, CAVI: cardio-ankle vascular index, ha-PWV: heart-ankle pulse wave velocity.

As shown in Table 4.24, ang-1, but not ang-2 or VEGF, significantly correlated negatively with right and left leg VPT.

Table 4.24 Correlation between vascular growth factors and VPT

VPT		**Vascular growth factors**		
		Ang-1	Ang-2	VEGF
Right	r	**-0.121**	0.022	0.099
	p	0.010	0.773	0.183
Left	r	**-0.215**	-0.034	0.089
	p	0.004	0.663	0.242

Data presented as Pearson's correlation coefficient, r.

CHAPTER FIVE

DISCUSSION, RECOMMENDATION AND CONCLUSION

5.1 Indices of arterial stiffness and the methodology of measurement

In this study, arterial stiffness was measured using aortic pulse wave velocity (PWV), cardio-ankle vascular index (CAVI), heart-ankle (ha) PWV and indices of central haemodyanamics. Few studies in Africa had reported PWV in apparently healthy subjects [59, 356-359] or hypertensive subjects [62, 360]. In all these studies, aortic PWV was measured as carotid-femoral (cf) PWV using Sphymocor (AtCor Medical Pty. Ltd., West Ryde, New South Wales, Australia) and study subjects rarely included diabetes patients. In the present study, a novel equipment, Arteriograph (TensioMed Kft., Budapest, Hungary), which has been validated with invasive techniques [287] and found to accurately measure aortic PWV, aortic SBP, aortic AIx and aortic PP was used. Based on the principle of measurement and type of study subjects recruited, this study might be unique and the findings may differ from those reported by other investigators in sub-Saharan Africa.

No study was found in literature that has reported the levels of arterial stiffness using CAVI and ha-PWV in Africa. This might be the first study to report the utility of CAVI and ha-PWV in sub-Saharan Africans. CAVI is a new index of arterial stiffness and it is unique by its theoretical independence on BP fluctuations at the time of measurement [206, 294]. Schutte *et al.*, (2011) [358] reported that arterial stiffness in Africans, unlike as found in Caucasians, is not age-related but BP related; this finding lends support to the preposition that the use of CAVI as index of arterial stiffness might be more suitable for indigenous Africans, in order to minimise the covariance

of BP [353]. However, in contrast to the report of Schutte *et al*., (2011)[358], the findings of this study indicated that indices of arterial stiffness are related to both age and BP.

The ha-PWV measures the degree of arterial stiffness from the aortic root to the posterior tibial artery; a longer length of artery. The disadvantage of this technique is that the level of stiffness in the central arteries cannot be distinguished from that in the peripheral arteries. However, ha-PWV can provide a comparative index for stiffness the whole arterial system[361, 362]. This index is occasionally reported in literature, but its equivalent form, the brachial-ankle (ba) PWV, had been widely reported to predict CVD mortality [363]. Also, a recent study had reported a high association between ba-PWV and cf-PWV [364], though the ba-PWV overestimated cf-PWV by 20%. The findings of this study, in which ha-PWV varies among subject's groups with different CVD risk levels (Table 4.7), suggest that ha-PWV can be used to discriminate subjects with various CVD risks.

5.2 Arterial stiffness indices and nonmodifiable CVD risk factors

5.2.1 Gender

In this study, three major nonmodifiable CVD risk factors were measured: gender, age and duration of diabetes. Because the objective of the study did not include the effects of these nonmodifiable CVD risk factors, study subjects were strategically recruited to ensure minimised effects of gender and age on the arterial stiffness indices. However, the findings of this study, as presented in Table 4.8, showed that female subjects had higher aortic PWV, aortic SBP, aortic PP and aortic AIx than male subjects, even after adjusting for gender, age, duration of diabetes, height, BMI, body fat and MAP. This result was spectacular because other factors such as mean age (54.03 vs 53.63,

p=0.800), age decade (p=0.314), study subject's category (p=0.574) and duration of diabetes (7.76 vs 8.95, p=0.383) did not differ significantly between male and female subjects. Also, comparison of mean levels of brachial BP indices between males and female yielded no difference.

It was reported by Ahinastos *et al*., (2003)[365] that before puberty, females had higher arterial stiffness than males, but males develop stiffer arteries while females develop distensible artery after menopause. A study in Belgium, in which the subjects were stratified by gender and age decade, no difference in arterial stiffness was observed among male and female subjects [366]. A recent study that focused on the gender differences in arterial stiffness reported that Algerian women have no difference in carotid-femoral PWV in comparison to men, after adjustment for covariates [357]. The findings of these studies are similar to the mean values of CAVI and ha-PWV observed in this study, which showed no gender difference among the study subjects (Table 4.8). However, studies conducted in T2DM subjects in London found greater level of arterial stiffness in females than in males [367].

A clue to the explanation for the gender differences in the aortic stiffness was given in a study reported by Coutinho *et al*., (2013) [368], in which greater proximal aortic stiffness was found in women than men. The authors rationalised that this might be the reason why women might be more susceptible to the deleterious effects of greater pulsatile and early arterial load on diastolic function and ventricular-arterial interaction. They inferred from the findings that higher aortic stiffness in women might contribute to the greater risk of heart failure with preserved ejection fraction in women [368]. This explanation by Coutinho *et al*., (2013) has been criticized by O'Rourke and Safar (2013) [369], who severely criticised the findings of Coutinho *et al*., (2013). O'Rourke and Safar (2013) suggested that because women had smaller body

size in comparison to men, the aortic size is inferable small as well, and this would result in high level of resistance and therefore arterial stiffness. However, Weng *et al*., (2013) [370] measured brachial-ankle PWV and found women had higher levels than men, and attributed this difference to the higher levels of metabolic syndrome in women than in men. The present study was not powered enough to test the hypotheses propounded by O'Rourke and Safar (2013) as well as Weng *et al*., (2013) as the plausible explanation to the high levels of mean aortic PWV, aortic SBP, aortic PP and AIx, but not in CAVI or ha-PWV values, in women than in men. Whether the observed gender difference in central haemodynamic parameters is due to lifestyle, body size, CVD risk factors or inherent genetic difference between men and women may be addressed by future studies with better design to test these hypotheses.

5.2.2 Age and duration of diabetes

The actual contribution of age to arterial stiffness in relatively known. However, arteries stiffen as one ages [189, 244, 350, 371]. This study age correlated with arterial stiffness (Table 4.11). The effect of aging on arterial stiffness as shown in the study, might be similar to what had been reported in literature[59, 62, 371]. However, Schutte *et al*., (2011) [358] reported that arterial stiffness in Africans is not age-related but BP related.

Both aortic PWV [367, 372] and CAVI [206, 293, 353] had been found to increase with age. As regards the mechanism involved, most studies have reported an association of age with the thickening of proximal 'non-load bearing' intima and media of the arterial system, principally due to cellular hyperplasia [244]. The media had been found to not thicken appreciably with age though, individual elastin lamellae shrink and become

separated by increasing amounts of non-load-bearing material; whereas in healthy young subjects, the intima and the media are indistinguishable. These events culminate in the aorta becoming dilated, stiffening and tortuous [371]. Dilation and stiffening of the central arteries reduce their buffering and cushioning function of the pulsatile blood flow generated by the rhythmic cardiac ejection.

Stiffening of the aorta increases PWV, leading to devastating effect on the heart, brain and the kidney [373]. As the walls of large arteries become stiffer, central systolic arterial pressure increases, diastolic arterial pressure decreases, and the pulse pressure increases for a given pattern of left ventricular ejection [374]. However, it is very difficult to demonstrate *in vivo* the effect of aging alone on arterial stiffness, because aging itself is associated with various CVD risk factors that contribute to the dramatic change in arterial stiffness [374].

Duration of diabetes has been found to be associated with macro- and micro-vascular complications [375, 376]. In this study, indices of arterial stiffness measured with Vasera (CAVI and ha-PWV) were found to be significantly associated with duration of diabetes, whereas aortic stiffness and central pressure indices were not associated with duration of diabetes. Most studies involving T2DM subjects did not report an association of diabetes duration with arterial stiffness, but those who reported one confirmed the positive correlation between diabetes duration and arterial stiffness [367, 377]. Ahlgren *et al*., (1999) [378] reported of an association between diabetes duration and abdominal aorta stiffening in type 1 diabetes (T1DM) subjects and suggested that prolonged diabetes duration might be responsible for the increased susceptibility of diabetes patients to the development of CVD.

The non-significant association between diabetes duration and aortic stiffness in this study can be explain by two main reasons. First, it would be erroneous, as was done in this study, to estimate the duration of diabetes in T2DM subjects in Ghana from the time of diagnosis, because most of Ghanaians with diabetes are unaware of their condition [106] until quite late in the course of the disease. Although the subjects in this study provided their duration of diabetes from the time of diagnosis, macrovascular damage might have begun many years before the development and diagnosis of overt T2DM [6]. Secondly, the measurement of aortic PWV and stiffness indices are restricted to only central elastic arteries which are influenced greatly by pulsatile ejections of the heart. However, CAVI and ha-PWV measure the stiffness in a wider range of arterial segment including central elastic arteries and peripheral muscular arteries, making the indices more sensitive to variation in organic stiffness [377]. This might have accounted for the demonstration of association of duration of diabetes with CAVI and ha-PWV, but not aortic PWV and central pressure indices.

5.3 Arterial stiffness and modifiable risk factors: body composition, blood pressure and biochemical parameters

5.3.1 Body composition

Body composition had been identified to independently predict CVD morbidity and mortality [307, 308]. In this study, as shown in Tables 4.12, most of the indices of arterial stiffness significantly increased with body composition indices. This is similar to what has been found in other studies that investigated the association between body composition and arterial stiffness[27, 312]. This study showed that the contribution of body composition to arterial stiffness might be moderate. However, this might be

remarkable, considering the fact that the contribution of body composition was independent of other classical CVD risk factors such as age and BP.

From this study, BMI and waist girth had higher association with central haemodynamics and aortic PWV when compared to other indices of body composition. This means that general obesity and specifically central obesity [379] is associated with increased arterial stiffness. It has been documented that obesity increases CVD risk by raising BP of healthy and diseased individuals [380]. Arterial stiffness might be the mechanism linking obesity to elevated BP. Only WHR was associated with right and left CAVIs as well as ha-PWV. Since CAVI reflects organic stiffness [206], WHR could be inferred to reflect organic stiffness. Aortic AIx was associated with percentage body fat and WSR but not the other indices of body composition. Since aortic AIx reflects endothelial function [381] of the central and peripheral arteries, changes in percentage body fat and WSR might have a relationship with the functioning of the endothelium.

This study also showed the level of arterial stiffness indices was higher in obese subjects, but no significant difference between over overweight and normal weight subjects was found. The classification of indices of body composition has been criticised recently as not related to mortality [382, 383] as it was previously thought to predict [384]. Waist girth has been reported to be a more powerful predictor of CVD mortality than BMI or WHR. However, the body stature in Ghanaian population is quite different from the Caucasian population from which these indices were derived [385]. In Ghana and other indigenous populations of sub-Saharan Africa, the utility of BMI category, waist girth and other indices of body composition as CVD risk indices are yet to be determined [386, 387].

The exact pathophysiologic mechanism linking global and regional adiposity to increased arterial stiffness is not clear. Adipose tissue, known to be a major energy storage organ, is now well recognised as a complex and highly active metabolic and endocrine organ. Visceral adipocytes have elevated lipolytic activity that results in increased free fatty acids release into the portal vein, leading to accumulation of fatty acids in liver, pancreas, and muscles [305]. This phenomenon might contribute to insulin resistance without appreciable increase in fasting blood glucose, but impair the regulation of postprandial plasma glucose. Insulin resistance is associated with an increased level of arterial stiffness [263]. Furthermore, other mechanisms could be involved, such as increases in circulating proinflammatory cytokines or leptin [388]. Indeed, high levels of leptin have been documented in individuals with obesity [389] and have been found to be correlated with an increase in arterial stiffness [390]. In addition to hypothalamic receptors, receptors for leptin have been observed on the vascular endothelium and on smooth muscle cells [391]. Accordingly, leptin can exert receptor-mediated influence on vessel tone and growth and, in cell culture, stimulate vascular smooth muscle proliferation and migration [392]. In addition, leptin induces oxidative stress in endothelial cells, and this action triggers the transcription of oxidant-sensitive genes that participate in loss of arterial elasticity [393].

5.3.2 Brachial blood pressure

Brachial blood pressure indices were found to be associated with indices of arterial stiffness as shown in Table 4.14. From this study, systolic BP and pulse rate were independently associated with aortic PWV. It is commonly believed that arterial stiffening is accelerated by higher systolic BP because of the structural and functional

alterations in the walls of the central elastic arteries in response to the chronically elevated distending pressures [221, 394]. In the Framingham Heart Study, the longitudinal increase in pulse pressure, a surrogate measure of arterial stiffness, was greater in subjects with higher baseline SBP [395]. However, the LIFE [235] and REASON [24] studies indicated that aortic PWV provides addition beneficial information about the state of the arteries independent of the systolic brachial BP.

HR had been reported to increase with aortic PWV but drastically reduce aortic PP. HR is a haemodynamic property that had been suspected to link aortic stiffness to pulsatile ejection of the left ventricle (PP). Albaladejo *et al*., (2001)[396] stimulated the HR of patients with pace maker monitoring system and reported significant increase in brachial BP indices but reduction in carotid PP. They also reported decrease in timing of wave reflection leading to increase in aortic PWV without any significant change in arterial stiffness [396]. In CAFÉ study, HR was found to have no impact on the brachial BP indices, but showed significant inverse relationship with aortic SBP and PP [397]. The possible explanation for the findings of the current study (Table 4.14) and the reported findings above might be what O'Rourke (1970) reported that increased HR decreases left ventricular ejection time and increase aortic to brachial amplification [227]. This finding implies that PP amplification is likely to increase with HR and, thus, to be responsible for significant differences between aortic and brachial SBP and DBP levels. This also provides explanation to the 'white coat' hypertension phenomena observed in clinical setting [398].

Contrary to the theoretical postulation of CAVI independence of BP at the time of measurement[206, 294, 295, 353], the findings of this study showed that brachial SBP was significantly related to CAVI (Table 4.14). The study by Shirai *et al*., (2011) [294] which confirmed CAVI independence of BP utilised an intervention that altered drastically

the BP of the subjects without changing CAVI appreciably. This study was an observational one in which BP was measure simultaneously with CAVI. Further studies would be required to investigate the relation between CAVI and BP in Ghanaian subjects.

5.3.3 Biochemical parameters

In this study, aortic PP showed a significant relationship with lipid panel markers. It was observed in this study that the plasma lipids levels in various categories of study subjects with different levels of CVD risk factors were not in agreement with NCEP/ATP III report [355]. The reason for the controls (nonhypertensive nondiabetes subjects) had higher levels of total cholesterol and LDL cholesterol compared to the other high CVD risk groups require further investigation [386, 387]. One explanation might be that the other study subjects with disease condition (hypertension and/or T2DM) were undergoing treatment and this might have contributed to controlling their cholesterol levels to the optimum. All the same, some researchers have disputed the utility of plasma lipids as CVD risk predictor in sub-Saharan African populations[386, 387, 399]. In this study, the HDL cholesterol levels were low irrespective of the degree of CVD risk category of the study subjects, [355]. Low levels of HDL cholesterol and high levels of total and LDL cholesterol, suspected to increase atherogenicity index, contrast the reported low incidence of atherosclerotic macrovascular diseases such as coronary heart disease [46, 49] and stroke [46, 48] in sub-Saharan Africa.

5.4 Arterial stiffness across the categories of study subjects

5.4.1 Impact of diabetes on arterial stiffness

The impact of diabetes on arterial stiffness was assessed as shown in Table 4.9. The findings showed that diabetes increases aortic PWV, CAVIs and ha-PWVs without any appreciable increase in aortic pressure indices. This finding is similar to what other studies had reported. Lacy *et al*., (2004) [400] reported an increased cf-PWV in diabetes subjects compared to nondiabetes subjects, but no significant difference in AIx. In the Tanno and Sobetsu Study [401], diabetes subjects had higher levels of ankle-brachial PWV than subjects with impaired fasting glucose, who also had a higher levels compared to nondiabetes controls. Kimoto *et al*., (2003) [372] demonstrated that in subjects with diabetes, the stiffening of the arteries preferentially occurs in the central arteries than in the peripheral arteries.

Hyperglycaemia and insulin resistance have been implicated to be the likely cause of arterial stiffness in diabetes subjects[402, 403]. In animal models of insulin-resistant diabetes, chronic hyperglycaemia and hyperinsulinemia increase local angiotensin II production and expression of vascular angiotensin II type I receptors via stimulation of TGF-β_1 [404], upregulate plasminogen activator inhibitor-1 [405], and downregulate matrix metalloprotease activity [406]; all these events play critical roles in central artery remodelling through vessel wall hypertrophy and fibrosis. The proliferative effects of insulin occur because insulin resistance impairs PI 3-kinase dependent signalling, while having little effect on the growth-promoting mitogen-activated kinase pathways[407-409]. In addition, insulin resistance enhances nonenzymatic glycation of proteins with covalent cross-linking of collagen, making modified collagen deposited into the intima-media of the arterial wall resistant to degradation of collagenases [408].

Other elements occurring early in the insulin-resistant state, such as elevated levels of LDL cholesterol, free fatty acids, and endothelin-1[252, 410], decreased levels of adiponectin and natriuretic peptides, may impair endothelial function and increase arterial stiffness as well [153].

Hyperglycaemia in T2DM causes increased oxidative stress by generating reactive oxygen species, leading to increased glycosylation of functional proteins and glucose autoxidation with activation of the polyol pathway and generation of reactive oxygen species, including superoxide, hydrogen peroxide, and hydroxyl radicals [153, 408]. Elevated glucose levels may also reduce NO synthase activity by enhancing oxidation of tetrahydrobiopterin, an essential cofactor for NO synthase. As a result, NO production from arginine and molecular oxygen is reduced and electrons are transferred to molecular oxygen to further increase superoxide and peroxynitrite levels [171, 411]. These mechanisms decrease arterial compliance and increase the level of stiffness in the arterial wall.

5.4.2 Impact of hypertension on arterial stiffness

The results of this study, as presented in Table 4.10, showed that hypertension increases the level of arterial stiffness in both T2DM and nondiabetes subjects, after adjustment for gender, age, height, BMI and MAP. Few studies in Africa have reported on arterial stiffness in hypertensive subjects. In a multicentre trial of the effectiveness of antihypertensive medication, Ezeala-Adikaibe et al (2012) [62] reported of the levels of arterial stiffness in hypertensive subjects, there was no comparable nonhypertensive controls in the study. The mean levels of aortic PWV, aortic SBP, aortic PP and aortic AIx reported in that study [62] were lesser than the findings of this

study. This could be as a result of the different methodology of measuring arterial stiffness indices in this study and the one reported by Ezeala-Adikaibe *et al*., (2012) [62]. Also in the Ezeala-Adikaibe *et al*., (2012) [62] study, the subjects were mild-to-moderate untreated hypertensives and the results were not adjusted for age, gender and BMI. In South Africa, Shiburi *et al*., (2006) [356] reported on the levels of aortic PWV, aortic PP and aortic AIx in apparently healthy subjects of African descent and suggested reference intervals, subjected to validation. Their reported reference range was lower than the mean value of the controls in this study. Again, this can be explained by the difference in methodology and also, environmental influences. For example, ambient temperature is known to affect haemodynamic measurements [412]. In the current study, arterial stiffness was assessed in a temperature controlled room (22°C). Other studies failed to report the temperature under which arterial stiffness was measured.

In African population, the mechanistic process underlining arterial stiffening has not been thoroughly investigated. Schutte *et al*., (2010) [413] found that asymmetrical dimethylarginine, an inhibitor of nitric oxide, was correlated positively to PWV, implying the involvement of reduced nitric oxide levels in the levels of arterial stiffening. Redelinghuys *et al*., (2011) [414] reported an attenuated association between indices of central haemodynamics and PWV after adjustment for covariates; casting doubts on the independent involvement of subclinical inflammation in the stiffening of the arteries as had been observed in African-Americans [415]. Clues about arterial stiffness impact in people of African descent can be drawn from studies involving Africa-Americans. Barring the differences resulting from genetic admixture [416, 417] and the environment [417, 418], the Africa-American population have health dynamics closer to indigenous Africans than the Caucasian population. Hence, the association between

arterial stiffness in Africa-American population can be used to explain the levels of arterial stiffness indices and central haemodynamics observed in Africans. The prevalence of high blood pressure in Africa-Americans, early onset of hypertension and target organ damage has been attributed to arterial stiffness and greater central haemodynamic load on the heart of Africa-Americans [419]. Arterial stiffness in African-American populations had been attributed to reduced activity of endothelial nitric oxide activity in the blood vessel [420].

The mechanism underlining arterial stiffening in hypertension is similar to that in diabetes. High blood pressure is associated with degeneration of compliant elastin fibres and deposition of stiffer collagen; the two mechanisms considered as key causes of age-related arterial stiffening. Moreover, blood pressure plays a significant role in determining vessel wall structure, with remodelling occurring to compensate for changes in wall stress. One potential mechanism is through matrix metalloproteinases, which modulate extracellular matrix proteins. When angiotensin II was given to normotensive mice, matrix metalloproteinase 9 activity was induced, resulting in enhanced collagen degradation. This improves the intrinsic distensibility of elastic arteries and, thus, blunts any blood pressure rise [421]. Impairment of this compensatory mechanism may, therefore, contribute to increased stiffness. The organization of elastic fibres is also important. Inhibition of the vascular adhesion protein semicarbazide-sensitive amine oxidase in a rat model results in reduced elastin fibre cross-linking, leading to morphological changes. This, in turn, increases vascular fragility and arterial stiffness [422]. In humans, aortic calcification has also been shown to be positively associated with both aortic stiffness and isolated systolic hypertension [423].

5.5 Peripheral sensory neuropathy and arterial stiffness

The prevalence of peripheral sensory neuropathy was assessed as perception to vibration using neurothesiometer. This method had been found to predict foot ulceration [348] in high risk subjects. The findings of this study, as presented in Table 4.16, showed low prevalence of neuropathy (VPT ≥ 25V): 5.3% of the right leg and 4.5% of the right leg. The difference in the leg-specific prevalence might be attributed to the difference in leg-specific alteration in microvasculature. The prevalence of peripheral sensory neuropathy in this study population is very low compared to other reported studies. The prevalence of neuropathy in T2DM subjects in Sweden was 34% [424]. In Singapore, the prevalence of PSN was found to be 25.5% in diabetes subjects [425].

The low prevalence of PNS in this study might be attributed to good management of diabetes and the associated microvascular complications at the NDMRC. It might also be that PSN is presented differently in Ghanaian with T2DM, other than vibration perception. Also, there might be a possibility that the subjects did not understand the protocol well and/or gave a false feedback about perceived vibration sensation [314]. The utility and validity of neurothesiometer to detect PSN in sub-Saharan Africans need to be determined. The major limitation of neurothesiometry in screening for PSN is the subject's dependency and this can be overcome by using an objective form of assessment such as nerve conduction testing [426], might be able to estimate the actual burden of neuropathy in T2DM patients in Ghana.

Most study reports from Africa utilized a questionnaire method[427, 428] or tuning fork and/or monofilament [428-431] to screen for sensory neuropathy. In all these studies, the reported prevalence of neuropathy, screen using questionnaire, far exceeded what was

found in this study: Jarso *et al*., (2011)[427] reported 53%, Roaeid and Kadiki (2011) [430] reported 47.1%, Mwendwa *et al*., (2005) [428] reported 28%, Ndip *et al*., (2006) [431] reported 27.3% and El-Nahas *et al*., (2008) [429] reported 10.2%. These reports indicate large variation in PSN, which could be attributed to the mode of sampling and/or clinical management of diabetes.

The findings of this study, as presented in Tables 4.17-19, showed that VPT correlated with age, duration of diabetes, body fat, WHR and FPG. Bergenheim *et al*., (1992) [432] reported an association of diabetes with age, height and duration of diabetes. Tesfaye *et al*., (2005) [323] in the EURO-Diab group reported that blood glucose control, duration of diabetes and hyperlipidaemia were all significant risk factors for the development of neuropathy in T1DM patients. However, the findings of this study indicated that plasma lipids were not associated significantly with VPT, lending support to the report of Yagihashi *et al*., (2011) [325], that plasma concentration of triglycerides or cholesterol were not associated with diabetic neuropathy.

The findings of this study, as presented in Table 4.21, showed that subjects with PSN had higher levels of aortic PWV, ha-PWV than subjects without PSN. Also, Table 4.22 showed that VPT increases significantly with CAVIs and ha-PWV. These findings are similar to that of reported studies investigating the association of neuropathy and arterial stiffness; though these studies utilized subjective neurological examination for diagnosis of neuropathy[433, 434]. Yokoyama *et al*., (2007) [433] reported that ba-PWV increases together with levels of neuropathy in T2DM subjects. Also, Kim *et al*., (2011) [434] reported CAVI as a significant predictor of neuropathy in T2DM subjects.

The link between arterial stiffness and neuropathy can be explained by the unique anatomy of vascular supply to peripheral nerves. Peripheral nerves, in contrast to central nervous system, are supplied by the upper branches of arteries supplying musculature of the limbs. Hence, it could be expected that stiffness in the peripheral arteries can affect the peripheral nerves as well. Also, as reported by Smith *et al*., (1977) [34], vascular supply to peripheral nerves are sparse and lacks autoregulation, making the nerves vulnerable to ischemia as a result of stiffness in elastic arteries. In addition, Edmonds *et al*., (1982) [435] and Young *et al*., (1993) [436] observed higher levels of arterial medial calcification in diabetes patients with neuropathic symptoms when compared to diabetes patients with neuropathy. This means that diabetes causes arterial medial calcification, resulting in raised arterial stiffness, which might also lead to neuropathy [437].

5.6 Vascular growth factors and arterial stiffness

As shown in Table 4.22, the levels of Ang-1 and Ang-2, but not VEGF, were significantly high in hypertensive T2DM compared to other categories of study subjects. Lim *et al*., (2004) [35] found that in T2DM subjects, plasma Ang-2 and VEGF, but not Ang-1, were selectively elevated and associated with indices of endothelial damage/dysfunction, regardless of the type of vascular disease. In Asian subjects, Anuradha *et al*., (2010) [438] reported higher levels of Ang-2 in subjects with impaired glucose metabolism, diabetes and hypertension. The predictive abilities of vascular growth factors were reported by Iribarren *et al*., (2011) [439] that Ang-2 can independently predict acute myocardial infarction.

Ang-1 binds to angiopoietin receptor, Tie-2, to prolonged endothelial cell survival, maintains the endothelium in a quiescent state [440], and supports the maturation of new vessels [441]. It had been established that ang-2, which is a competitive inhibitor of ang-1 for Tie-2 binding, modulates endothelial cell biology and destabilizes blood vessels to facilitate angiogenesis [35]. Ang-2 is a key angiogenic hypoxia-induced growth factor [341]. Moreover, in diabetes mellitus, chronic hyperglycaemia causes an accelerated formation of advanced glycated end-products (AGE) and mitochondrial overproduction of reactive oxygen species (ROS). The resulting toxic and oxidative stress in vascular endothelium promotes micro-and macro-vascular complications [141]. Amongst multiple pathological changes in gene expression, AGE and ROS lead to the up-regulation of ang-2 mRNA expression [141], which promotes vascular permeability, destabilization and sprouting [442].

As shown in Table 4.23, ang-2, but not ang-1 and VEGF, significantly increases with central BP indices. This explains the ability of serum ang-2 levels to predict the occurrence of acute myocardial syndrome [439]. VEGF has been found in a community study to be associated with endothelial dysfunction[443]. In another community study, vascular growth factors were found to correlate with cf-PWV and central haemodynamic indices[40]. Unlike the design of the current study, the two community studies were designed with a large sample size and hence, highly powered to detect any little form of association as significant.

5.7 Vascular growth factors and VPT

Table 4.24 showed that ang-1 is correlated with VPT. Because ang-1 is a competitive inhibitor of ang-2 and prevents vascular turn over, it is likely that neuropathy might

be related to low angiogenesis of blood vessels or abnormalities in the vasa nervorum. Ultrastructural morphometric analysis of T2DM subjects showed nerve capillary pathology in the skin and muscle with significant reduction in the endoneurial capillary density, increased capillary basement membrane and total diffusion barrier thickening, resulting in reduced oxygen diffusing capacity in T2DM subjects with neuropathy compared to controls [334, 444]. Electron microscopy showed thickening of vessel walls resulting from endothelial proliferation in the vasa nervorum in patients with peripheral neuropathy[445]. Ang-1 has been implicated in the alteration in microcirculation, at least in the renal glomerular endothelial cell barrier [446]. These study reports might explain the association of ang-1 with VPT.

It might probably be incorrect to conclude that microvascular abnormality is the primary trigger of neuropathic complications, an assumption that ignores direct neuronal damage by other factors such as hyperglycaemia and oxidative damage. Instead, there is significant evidence that a unique neuroscience of diabetic neuropathy, independent of microangiopathy, exists [329, 447]. Hence, vascular growth factor might not show independent association with diabetic neuropathy.

5.8 Limitation of the study

5.8.1 Study design

1. Cross sectional data was collected in this study. Hence, causality cannot be inferred from the findings of this study. A longitudinal study can be used to assess the causes of arterial stiffening in Ghanaian population.

2. Older subjects were recruited for this study. This confounds the results with natural aging process on arterial stiffness. Also, the older population had been exposed to many vasculotoxic stimuli such as second-hand smoking, acute inflammatory disease and even some diets that may have an effect on the stiffness of the arteries. Especially in female subjects and to some extent the males subjects as well, the levels of hormones that might influence the level of arterial stiffness were not assessed.

3. Diabetes and hypertensive subjects recruited into this study were mostly on some form of treatment for their condition. This likely confounds the real stiffness caused by these conditions. Also, due to the management of diabetes and hypertension, the biochemical parameters, especially fasting plasma triglycerides and plasma cholesterols, were possibly not reflecting the extent of vascular damage assessed.

4. The sample size used in the study was not powered enough to detect the association of vascular growth factors with arterial stiffness and VPT. However, based on the findings of this study, future studies can compute sample size with adequate power to test the association between CVD risk factors, diabetes and vascular growth factors.

5.8.2 Methodology

1. Arterial path length was estimated from specific anatomical points on the body surface with a tape measure or ‘anthropometric calliper’. This method erroneously assumes that the arterial segment is straight (not tortuous) and little inter-subject variability exist. All the same, this form of estimation of

arterial length been shown to be closer to the real arterial path length measured with magnetic resonance imaging[350, 351]. Whether the assumptions of Sugawara *et al*., (2010) [351] are true for estimating aortic path length in an African population is unknown.

2. The estimation of aortic PWV and central haemodynamic indices were based on the generalised transfer function [64] which has been shown to vary less in various experimental conditions such as induced vasodilation [64], exercise [225] and Valsalva manoeuvre [214]. The use of the generalised transfer function for estimation of central haemodynamic indices has been questioned [208]. Whether the generalised transfer function is independent of gender, age, disease state or geographical barriers need to be determined [232]. The physiological differences in arterial haemodynamics [358] and variation in disease presentation [48] between indigenous Africans and Caucasians give the impression that the transfer function linking the brachial artery pulse waveform to the aortic pulse waveform might be different among the two populations.

3. CAVI was computed based on the β-stiffness of the carotid and femoral arteries, which are elastic in nature [205]. Whether the relationship between the arterial stiffness of the logarithm of pulse pressure of fibrous arteries, brachial and posterior tibial artery [293], used in CAVI computation is similar to that of elastic artery has not been examined. Also, the constants ‘a’ and ‘b’ used to transform β-stiffness to CAVI [353] needs to be determine as to its variation within gender, disease state and geographical barriers.

4. The use of neurothesiometer is dependent on the ability of the study subject to accurately sense and indicate the exact level of voltage he/she perceives

vibration. This limits the sensitivity and specificity of the technique to detect neuropathy.

5. The level of microangiopathy was not assessed in this study. Hence, the relative contribution of microvessel versus macrovessel abnormalities to end-organ damage cannot be inferred.

5.10 Conclusions

This study has shown that coexistence of diabetes and hypertension increases arterial stiffness than diabetes or hypertension alone in Ghanaians after adjustment for possible confounders. CAVI and ha-PWV were found to more effective in discriminating subjects with various CV risk than aortic indices. The association between arterial stiffness indices and lipid profile markers were inconclusive. Female subjects were found to have higher levels of arterial stiffness than their male counterparts. Arterial stiffness was found to increase with age, duration of diabetes and body composition. WHR was associated with most indices of arterial stiffness better than the other indices of body composition.

The study showed a low prevalence of PSN. It was mostly found in hypertensive T2DM subjects. Arterial stiffness was related to VPT levels suggesting a possible role in PSN. The possible association between VPT and some markers in lipid panel would require further investigation.

Also, impaired angiogenesis was found to be associated with arterial stiffness, especially ang-2, which was associated with central pressure indices. In this study, hypertensive T2DM had high level of impaired angiogenesis compared to the other

patient groups; suggesting a possible association of imbalanced vascular growth factors in hypertensive T2DM. The study also showed that subjects with high VPT had impaired angiogenesis, suggesting a possible role of impaired microvascular turn over in neuropathy in Ghanaian subjects. Of the indices of arterial stiffness, CAVI and ha-PWV were found to be associated with VPT better than aortic indices.

The used of arterial stiffness as a biomarker for arterial age in sub-Saharan African population is still in the infancy stage and more research in a population based setting or with longitudinal design will be required to establish the utility and benefits of arterial stiffness assessment.

5.9 Recommendations

1. A study involving treatment naïve diabetes and hypertensive subjects might be able to determine the actual burden of arterial stiffness attributable to hypertension and diabetes. Also, this design could be used to test the association between arterial stiffness and biochemical parameters.

2. Longitudinal studies could be design to assess the impact of diabetes and hypertension with time. The study could be designed to assess the ability of various indices of arterial stiffness to predict end-organ damage. The study could involve younger subjects who might be free from most vasculotoxic agents.

3. The validity of the various tools used to assess aortic stiffness and central pressure indices need to be determined in Ghanaian population, taking into account age, gender, disease state and various risk factors. The validity of the

generalised transfer function can be difficult to assess in low resource setting like sub-Saharan Africa, but noninvasive ultrasound technique can be used to verify the findings of the tools used to assess arterial stiffness.

4. As performed in other studies, a community-based study could be design to assess the levels of vascular growth factors in Ghanaian population.

REFERENCES

1. Morrish, N., Wang, S.L., Stevens, L., Fuller, J. & Keen, H. Mortality and causes of death in the WHO Multinational Study of Vascular Disease in Diabetes. *Diabetologia* **44**, 14-21 (2001).

2. Feldman, C.L. *et al.* Vulnerable Anatomy; The Role of Coronary Anatomy and Endothelial Shear Stress in the Progression and Vulnerability of Coronary Artery Lesions: Is Anatomy Destiny?, in *Asymptomatic Atherosclerosis*. (ed. M. Naghavi) 495-506 (Humana Press, New York; 2010).

3. Moffatt, R.J. & Stamford, B. (CRC Press, Florida; 2006).

4. Rydén, L. *et al.* Guidelines on diabetes, pre-diabetes, and cardiovascular diseases: executive summary: The Task Force on Diabetes and Cardiovascular Diseases of the European Society of Cardiology (ESC) and of the European Association for the Study of Diabetes (EASD). *European heart journal* **28**, 88-136 (2007).

5. Haffner, S.M., Stern, M.P., Hazuda, H.P., Mitchell, B.D. & Patterson, J.K. Cardiovascular risk factors in confirmed prediabetic individuals: Does the clock for coronary heart disease start ticking before the onset of clinical diabetes? *JAMA : the journal of the American Medical Association* **263**, 2893-2898 (1990).

6. Haffner, S.M., Mykkänen, L., Festa, A., Burke, J.P. & Stern, M.P. Insulin-Resistant Prediabetic Subjects Have More Atherogenic Risk Factors Than Insulin-Sensitive Prediabetic Subjects: Implications for Preventing Coronary Heart Disease During the Prediabetic State. *Circulation* **101**, 975-980 (2000).

7. Santiago, J.V. Lessons from the diabetes control and complications trial. *Diabetes* **42**, 1549-1554 (1993).

8. Turner, R. *et al.* Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet* **352**, 837-853 (1998).

9. Advance Collaborative Group *et al.* Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. *The New England journal of medicine* **358**, 2560-2572 (2008).

10. Duckworth, W.C. *et al.* The duration of diabetes affects the response to intensive glucose control in type 2 subjects: the VA Diabetes Trial. *Journal of diabetes and its complications* **25**, 355-361 (2011).

11. Action to Control Cardiovascular Risk in Diabetes Study Group *et al.* Effects of intensive glucose lowering in type 2 diabetes. *The New England journal of medicine* **358**, 2545-2559 (2008).

12. Selvin, E. *et al.* Meta-analysis: glycosylated hemoglobin and cardiovascular disease in diabetes mellitus. *Annals of internal medicine* **141**, 421-431 (2004).

13. Stettler, C. *et al.* Glycemic control and macrovascular disease in types 1 and 2 diabetes mellitus: meta-analysis of randomized trials. *American heart journal* **152**, 27-38 (2006).

14. Holman, R.R., Paul, S.K., Bethel, M.A., Matthews, D.R. & Neil, H.A.W. 10-year follow-up of intensive glucose control in type 2 diabetes. *New England Journal of Medicine* **359**, 1577-1589 (2008).

15. U. K. Prospective Diabetes Study Group Tight Blood Pressure Control and Risk of Macrovascular and Microvascular Complications in Type 2 Diabetes: UKPDS 38. *BMJ: British Medical Journal* **317**, 703-713 (1998).

16. Cooper-DeHoff, R.M. *et al.* Tight blood pressure control and cardiovascular outcomes among hypertensive patients with diabetes and coronary artery disease. *JAMA: The Journal of the American Medical Association* **304**, 61-68 (2010).

17. ACCORD Study Group *et al.* Effects of intensive blood-pressure control in type 2 diabetes mellitus. *The New England journal of medicine* **362**, 1575-1585 (2010).

18. Anderson, R.J. *et al.* Blood pressure and cardiovascular disease risk in the Veterans Affairs Diabetes Trial. *Diabetes care* **34**, 34-38 (2011).

19. de Galan, B.E. *et al.* Lowering blood pressure reduces renal events in type 2 diabetes. *Journal of the American Society of Nephrology : JASN* **20**, 883-892 (2009).

20. Zoungas, S. *et al.* Combined effects of routine blood pressure lowering and intensive glucose control on macrovascular and microvascular outcomes in patients with type 2 diabetes: New results from the ADVANCE trial. *Diabetes care* **32**, 2068-2074 (2009).

21. Kannel, W.B., Gordon, T. & Schwartz, M.J. Systolic versus diastolic blood pressure and risk of coronary heart disease. The Framingham study. *The American journal of cardiology* **27**, 335-346 (1971).

22. Madhavan, S., Ooi, W.L., Cohen, H. & Alderman, M.H. Relation of pulse pressure and blood pressure reduction to the incidence of myocardial infarction. *Hypertension* **23**, 395-401 (1994).

23. Fang, J., Madhavan, S., Cohen, H. & Alderman, M.H. Measures of blood pressure and myocardial infarction in treated hypertensive patients. *Journal of hypertension* **13**, 413-419 (1995).

24. de Luca, N. *et al.* Regression of left ventricular mass in hypertensive patients treated with perindopril/indapamide as a first-line combination: the REASON echocardiography study. *American journal of hypertension* **17**, 660-667 (2004).

25. Yusuf, S. *et al.* Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. The Heart Outcomes Prevention Evaluation Study Investigators. *The New England journal of medicine* **342**, 145-153 (2000).

26. Laurent, S. *et al.* Abridged version of the expert consensus document on arterial stiffness. *Artery Research* **1**, 2-12 (2007).

27. Cruickshank, J.K., Rezailashkajani, M. & Goudot, G. Arterial Stiffness, Fatness, and Physical Fitness: Ready for Intervention in Childhood and Across the Life Course? *Hypertension* **53**, 602-604 (2009).

28. Luft, F.C. Molecular mechanisms of arterial stiffness: new insights. *Journal of the American Society of Hypertension* **6**, 436-438 (2012).

29. Cruickshank, K. *et al.* Aortic pulse-wave velocity and its relationship to mortality in diabetes and glucose intolerance. *Circulation* **106**, 2085-2090 (2002).

30. Westerhof, N., Lankhaar, J.W. & Westerhof, B.E. The arterial windkessel. *Medical and Biological Engineering and Computing* **47**, 131-141 (2009).

31. Mbanya, J.C. & Sobngwi, E. Diabetes in Africa. Diabetes microvascular and macrovascular disease in Africa. *Journal of cardiovascular risk* **10**, 97-102 (2003).

32. Diabetes Control and Complications Trial Research Group The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group. *The New England journal of medicine* **329**, 977-986 (1993).

33. Llewelyn, J.G., Thomas, P.K., Gilbey, S.G., Watkins, P.J. & Muddle, J.R. Pattern of myelinated fibre loss in the sural nerve in neuropathy related to Type 1 (insulin-dependent) diabetes. *Diabetologia* **31**, 162-167 (1988).

34. Smith, D.R., Kobrine, A.I. & Rizzoli, H.V. Absence of autoregulation in peripheral nerve blood flow. *Journal of the Neurological Sciences* **33**, 347-352 (1977).

35. Lim, H.S., Blann, A.D., Chong, A.Y., Freestone, B. & Lip, G.Y.H. Plasma Vascular Endothelial Growth Factor, Angiopoietin-1, and Angiopoietin-2 in Diabetes: Implications for cardiovascular risk and effects of multifactorial intervention. *Diabetes care* **27**, 2918-2924 (2004).

36. Lim, H.S., Lip, G.Y. & Blann, A.D. Angiopoietin-1 and angiopoietin-2 in diabetes mellitus: relationship to VEGF, glycaemic control, endothelial damage/dysfunction and atherosclerosis. *Atherosclerosis* **180**, 113-118 (2005).

37. Thomas, M. & Augustin, H.G. The role of the Angiopoietins in vascular morphogenesis. *Angiogenesis* **12**, 125-137 (2009).

38. Trollope, A.F. & Golledge, J. Angiopoietins, abdominal aortic aneurysm and atherosclerosis. *Atherosclerosis* **214**, 237-243 (2011).

39. Lieb, W. *et al.* Clinical and genetic correlates of circulating angiopoietin-2 and soluble Tie-2 in the community. *Circulation. Cardiovascular genetics* **3**, 300-306 (2010).

40. Zachariah, J.P. *et al.* Circulating vascular growth factors and central hemodynamic load in the community. *Hypertension* **59**, 773-779 (2012).

41. Yach, D., Hawkes, C., Gould, C.L. & Hofman, K.J. The global burden of chronic diseases: overcoming impediments to prevention and control. *JAMA : the journal of the American Medical Association* **291**, 2616-2622 (2004).

42. Stamler, J., Vaccaro, O., Neaton, J.D. & Wentworth, D. Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial. *Diabetes care* **16**, 434-444 (1993).

43. Cederholm, J. *et al.* Blood pressure and risk of cardiovascular diseases in type 2 diabetes: further findings from the Swedish National Diabetes Register (NDR-BP II). *Journal of hypertension* **30**, 2020-2030 (2012).

44. Amoah, A.G. Hypertension in Ghana: a cross-sectional community prevalence study in greater Accra. *Ethnicity & disease* **13**, 310-315 (2003).

45. Amoah, A.G.B., Owusu, S.K. & Adjei, S. Diabetes in Ghana: a community based prevalence study in Greater Accra. *Diabetes research and clinical practice* **56**, 197-205 (2002).

46. Kengne, A.P., Amoah, A.G. & Mbanya, J.C. Cardiovascular complications of diabetes mellitus in sub-Saharan Africa. *Circulation* **112**, 3592-3601 (2005).

47. Kengne, A.P. & Patel, A. How Important Is Diabetes as a Risk Factor for Cardiovascular and Other Diseases in Older Adults? *PLoS Med* **3**, e424 (2006).

48. Connor, M.D., Modi, G. & Warlow, C.P. Differences in the nature of stroke in a multiethnic urban South African population: the Johannesburg hospital stroke register. *Stroke; a journal of cerebral circulation* **40**, 355-362 (2009).

49. Amoah, A. Spectrum of cardiovascular disorders in a national referral centre, Ghana. *East African medical journal* **77** (2009).

50. Melikian, N. *et al.* Asymmetric Dimethylarginine and Reduced Nitric Oxide Bioavailability in Young Black African Men. *Hypertension* **49**, 873-877 (2007).

51. Patel, P.D., Velazquez, J.L. & Arora, R.R. Endothelial dysfunction in African-Americans. *International journal of cardiology* **132**, 157-172 (2009).

52. U.K. Prospective Diabetes Study Group Ethnicity and cardiovascular disease. The incidence of myocardial infarction in white, South Asian, and Afro-Caribbean patients with type 2 diabetes (U.K. Prospective Diabetes Study 32). *Diabetes care* **21**, 1271-1277 (1998).

53. Chaturvedi, N., Jarrett, J., Morrish, N., Keen, H. & Fuller, J.H. Differences in mortality and morbidity in African Caribbean and European people with non-insulin dependent diabetes mellitus: results of 20 year follow up of a London cohort of a multinational study. *Bmj* **313**, 848-852 (1996).

54. Folsom, A.R. *et al.* A prospective study of coronary heart disease in relation to fasting insulin, glucose, and diabetes. The Atherosclerosis Risk in Communities (ARIC) Study. *Diabetes care* **20**, 935-942 (1997).

55. Westerhof, N., Stergiopulos, N. & Noble, M.I.M. *Snapshots of hemodynamics: An Aid for Clinical Research and Graduate Education*, Edn. 2nd. (Springer, New York; 2010).

56. Koenig, W., Löwel, H., Baumert, J. & Meisinger, C. C-Reactive Protein Modulates Risk Prediction Based on the Framingham Score. *Circulation* **109**, 1349-1353 (2004).

57. Adler, A.I. *et al.* UKPDS 59: Hyperglycemia and Other Potentially Modifiable Risk Factors for Peripheral Vascular Disease in Type 2 Diabetes. *Diabetes care* **25**, 894-899 (2002).

58. Criqui, M.H. *et al.* Ethnicity and Peripheral Arterial Disease The San Diego Population Study. *Circulation* **112**, 2703-2707 (2005).

59. Lemogoum, D. *et al.* Effects of Hunter-Gatherer Subsistence Mode on Arterial Distensibility in Cameroonian Pygmies. *Hypertension* **60**, 123-128 (2012).

60. Lemogoum, D. *et al.* Arterial Stiffness and Wave Reflections in Patients With Sickle Cell Disease. *Hypertension* **44**, 924-929 (2004).

61. Ngatchou, W. *et al.* Effects of antiretroviral therapy on arterial stiffness in Cameroonian HIV-infected patients. *Blood pressure monitoring* **Publish Ahead of Print**, 10.1097/MBP.1090b1013e328363ee328343 (2013).

62. Ezeala-Adikaibe, B. *et al.* Central haemodynamics reveal significant potential for prevention in Black hypertensive patients born and living in sub-Saharan Africa. *Artery Research* **6**, 41-48 (2012).

63. M'Buyamba-Kabangu, J.R. *et al.* Efficacy of newer versus older antihypertensive drugs in black patients living in sub-Saharan Africa. *J Hum Hypertens* (2013).

64. Karamanoglu, M., O'rourke, M.F., Avolio, A.P. & Kelly, R.P. An analysis of the relationship between central aortic and peripheral upper limb pressure waves in man. *European heart journal* **14**, 160-167 (1993).

65. Laurent, S. Defining vascular aging and cardiovascular risk. *Journal of hypertension* **30**, S3-S8 (2012).

66. Rezai, M.R., Goudot, G., Conchubhair Winters, J.D.F., Wu, F.C. & Cruickshank, J.K. Calibration mode influences central blood pressure differences between SphygmoCor and two newer devices, the Arteriograph and Omron HEM-9000. *Hypertension Research* **34**, 1046-1051 (2011).

67. International Diabetes Federation *Diabetes Atlas*, Edn. 4. (IDF, Brussels; 2009).

68. Knop, F., Vilsbøll, T., Madsbad, S., Holst, J. & Krarup, T. Inappropriate suppression of glucagon during OGTT but not during isoglycaemic iv glucose infusion contributes to the reduced incretin effect in type 2 diabetes mellitus. *Diabetologia* **50**, 797-805 (2007).

69. Rizza, R.A. Pathogenesis of fasting and postprandial hyperglycemia in type 2 diabetes: implications for therapy. *Diabetes* **59**, 2697-2707 (2010).

70. Mazzone, T., Chait, A. & Plutzky, J. Cardiovascular disease risk in type 2 diabetes mellitus: insights from mechanistic studies. *The Lancet* **371**, 1800-1809 (2008).

71. West, K.M. Substantial Differences in the Diagnostic Criteria Used by Diabetes Experts. *Diabetes* **24**, 641-644 (1975).

72. World Health Organization WHO Expert Committee on Diabetes Mellitus: second report. *World Health Organization technical report series* **646**, 1-80 (1980).

73. World Health Organization Diabetes mellitus. Report of a WHO Study Group. *World Health Organization technical report series* **727**, 1-113 (1985).

74. Alberti, K.G.M.M. & Zimmet, P.Z. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. Provisional report of a WHO Consultation. *Diabetic Medicine* **15**, 539-553 (1998).

75. Kuzuya, T. & Matsuda, A. Classification of diabetes on the basis of etiologies versus degree of insulin deficiency. *Diabetes care* **20**, 219-220 (1997).

76. Hoet, J., Reusens, B., Dahri, S., El-Hajjaji, H. & Remacle, C. Protein malnutrition during pregnancy in the rat has an intergenerational effect on the endocrine pancreas. *Proceedings of 16th Int Cong Nutrition, Montreal, Canada* **11**, 70 (1997).

77. Tripathy, B.B. & Samal, K.C. Overview and Consensus Statement on Diabetes in Tropical Areas. *Diabetes/Metabolism Reviews* **13**, 63-76 (1997).

78. The Committee of the Japan Diabetes Society on the Diagnostic Criteria of Diabetes, M. *et al.* Report of the Committee on the Classification and Diagnostic Criteria of Diabetes Mellitus. *Journal of Diabetes Investigation* **1**, 212-228 (2010).

79. Reece, E.A., Leguizamón, G. & Wiznitzer, A. Gestational diabetes: the need for a common ground. *Obstetric Anesthesia Digest* **30**, 84 (2010).

80. Xiang, A.H., Kjos, S.L., Takayanagi, M., Trigo, E. & Buchanan, T.A. Detailed physiological characterization of the development of type 2 diabetes in Hispanic women with prior gestational diabetes mellitus. *Diabetes* **59**, 2625-2630 (2010).

81. American Diabetes Association Standards of Medical Care in Diabetes—2012. *Diabetes care* **35**, S11-S63 (2012).

82. Pociot, F. *et al.* Genetics of type 1 diabetes: what's next? *Diabetes* **59**, 1561-1571 (2010).

83. Polychronakos, C. & Li, Q. Understanding type 1 diabetes through genetics: advances and prospects. *Nature Reviews Genetics* **12**, 781-792 (2011).

84. Giugliano, D., Ceriello, A. & Esposito, K. Glucose metabolism and hyperglycemia. *The American journal of clinical nutrition* **87**, 217S-222S (2008).

85. McFarlane, S.I. *et al.* Near-normoglycaemic remission in African-Americans with Type 2 diabetes mellitus is associated with recovery of beta cell function. *Diabetic Medicine* **18**, 10-16 (2001).

86. McLarty, D.G., Pollitt, C. & Swai, A.B.M. Diabetes in Africa. *Diabetic Medicine* **7**, 670-684 (1990).

87. Motala, A.A., Omar, M.A.K. & Pirie, F.J. Epidemiology of diabetes in Africa. *The epidemiology of diabetes mellitus (2nd edn). Chichester: Wiley*, 133-146 (2008).

88. Levitt, N.S. Diabetes in Africa: epidemiology, management and healthcare challenges. *Heart (British Cardiac Society)* **94**, 1376-1382 (2008).

89. National Diabetes Data Group Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. *Diabetes* **28**, 1039-1057 (1979).

90. King, H. & Rewers, M. Diabetes in adults is now a Third World problem. World Health Organization Ad Hoc Diabetes Reporting Group. *Ethnicity & disease* **3**, S67-74 (1993).

91. Mbanya, J.C.N., Motala, A.A., Sobngwi, E., Assah, F.K. & Enoru, S.T. Diabetes in sub-Saharan Africa. *The Lancet* **375**, 2254-2266 (2010).

92. Guariguata, L. By the numbers: New estimates from the IDF Diabetes Atlas Update for 2012. *Diabetes research and clinical practice* **98**, 524-525 (2012).

93. Gill, G., Mbanya, J.-C., Ramaiya, K. & Tesfaye, S. A sub-Saharan African perspective of diabetes. *Diabetologia* **52**, 8-16 (2009).

94. Perret, J.L., Bifane, E., Ngou-Milama, E., Moussavou-Kombila, J.B. & Nguemby-Mbina, C. [Types of sugar diabetes encountered in internal medicine in Gabon]. *Medecine tropicale : revue du Corps de sante colonial* **56**, 55-58 (1996).

95. Perret, J.L. & Nguemby-Mbina, C. [Characteristics of the development of insulin need in primary diabetes of adults in Gabon]. *Annales de la Societe belge de medecine tropicale* **71**, 243-249 (1991).

96. Wilkin, T.J. The accelerator hypothesis: weight gain as the missing link between Type I and Type II diabetes. *Diabetologia* **44**, 914-922 (2001).

97. McFarlane, S.I. *et al.* Near-normoglycaemic remission in African-Americans with Type 2 diabetes mellitus is associated with recovery of beta cell function.

98. Li, Y. *et al.* Induction of Long-term Glycemic Control in Newly Diagnosed Type 2 Diabetic Patients Is Associated With Improvement of β-Cell Function. *Diabetes care* **27**, 2597-2602 (2004).

99. Majaliwa, E.S. *et al.* Type 1 diabetes mellitus in the African population: epidemiology and management challenges. *Acta bio-medica : Atenei Parmensis* **79**, 255-259 (2008).

100. Motala, A.A. Diabetes trends in Africa. *Diabetes/metabolism research and reviews* **18**, S14-S20 (2002).

101. Motala, A.A., Omar, M.A.K. & Pirie, F.J. in The Epidemiology of Diabetes Mellitus Edn. 2nd (eds. J.-M. Ekoe, M. Rewers, R. Williams & P. Zimmet) 113- 146 (John Willey & Sons, Chichester, West Sussex, UK; 2008).

102. Christensen, D. *et al.* Prevalence of glucose intolerance and associated risk factors in rural and urban populations of different ethnic groups in Kenya. *Diabetes research and clinical practice* **84**, 303-310 (2009).

103. Dodu, S. The incidence of diabetes mellitus in Accra (Ghana); a study of 4,000 patients. *The West African medical journal* **7**, 129 (1958).

104. Dodu, S. & De Heer, N. A diabetes case-finding survey in Ho, Ghana. *Ghana Med J* **3**, 75-80 (1964).

105. Amoah, A. Comprehensive Care in a Low-Income Country: The Ghana Experience. *Diabetes Voice* **47**, 20-22 (2002).

106. Amoah, A. Undiagnosed diabetes and impaired glucose regulation in adult Ghanaians using the ADA and WHO diagnostic criteria. *Acta diabetologica* **39**, 7-13 (2002).

107. Preis, S.R. *et al.* Trends in Cardiovascular Disease Risk Factors in Individuals With and Without Diabetes Mellitus in the Framingham Heart Study. *Circulation* **120**, 212-220 (2009).

108. Ferrannini, E. & Cushman, W.C. Diabetes and hypertension: the bad companions. *The Lancet* **380**, 601-610 (2012).

109. Ferrannini, E. *et al.* Insulin Resistance, Insulin Response, and Obesity as Indicators of Metabolic Risk. *Journal of Clinical Endocrinology & Metabolism* **92**, 2885-2892 (2007).

110. Ibrahim, M.M. & Damasceno, A. Hypertension in developing countries. *Lancet* **380**, 611-619 (2012).

111. Krolewski, A.S. *et al.* Hypertension, orthostatic hypotension and the microvascular complications of diabetes. *Journal of chronic diseases* **38**, 319-326 (1985).

112. Solini, A. *et al.* Diverging association of reduced glomerular filtration rate and albuminuria with coronary and noncoronary events in patients with type 2 diabetes: the renal insufficiency and cardiovascular events (RIACE) Italian multicenter study. *Diabetes care* **35**, 143-149 (2012).

113. Barret-Connor, E., Criqui, M.H., Klauber, M.R. & Holdbrook, M. Diabetes and hypertension in a community of older adults. *Am J Epidemiol* **113**, 276-284 (1981).

114. Haffner, S.M. *et al.* Greater effect of glycemia on incidence of hypertension in women than in men. *Diabetes care* **15**, 1277-1284 (1992).

115. Simpson, N.E. Heritabilities of liability to diabetes when sex and age at onset are considered. *Annals of human genetics* **32**, 283-303 (1969).

116. Florey, C.V., Uppal, S. & Lowy, C. Relation between blood pressure, weight, and plasma sugar and serum insulin levels in schoolchildren aged 9-12 years in Westland, Holland. *British medical journal* **1**, 1368-1371 (1976).

117. Stamler, J. *et al.* Multivariate analysis of the relationship of six variables to blood pressure: findings from Chicago community surveys, 1965--1971. *Journal of chronic diseases* **28**, 499-525 (1975).

118. Salomaa, V.V. *et al.* Glucose tolerance and blood pressure: long term follow up in middle aged men. *Bmj* **302**, 493-496 (1991).

119. Medalie, J.H., Papier, C.M., Goldbourt, U. & Herman, J.B. Major factors in the development of diabetes mellitus in 10,000 men. *Arch Intern Med* **135**, 811-817 (1975).

120. Natali, A. *et al.* Metabolic characteristics of prehypertension: role of classification criteria and gender. *Journal of hypertension* **27**, 2394-2402 (2009).

121. Kannel, W.B. *et al.* Overall and coronary heart disease mortality rates in relation to major risk factors in 325,348 men screened for the MRFIT. Multiple Risk Factor Intervention Trial. *American heart journal* **112**, 825-836 (1986).

122. Fox, C.S. Cardiovascular disease risk factors, type 2 diabetes mellitus, and the Framingham Heart Study. *Trends in cardiovascular medicine* **20**, 90-95 (2010).

123. Fox, C.S. *et al.* Increasing Cardiovascular Disease Burden Due to Diabetes Mellitus: The Framingham Heart Study. *Circulation* **115**, 1544-1550 (2007).

124. Chen, G., McAlister, F.A., Walker, R.L., Hemmelgarn, B.R. & Campbell, N.R. Cardiovascular outcomes in framingham participants with diabetes: the importance of blood pressure. *Hypertension* **57**, 891-897 (2011).

125. Fox, C.S. *et al.* Trends in the Incidence of Type 2 Diabetes Mellitus From the 1970s to the 1990s: The Framingham Heart Study. *Circulation* **113**, 2914-2918 (2006).

126. Meigs, J.B. Epidemiology of Type 2 Diabetes and Cardiovascular Disease: Translation From Population to Prevention The Kelly West Award Lecture 2009. *Diabetes care* **33**, 1865-1871 (2010).

127. Fox, C.S., Coady, S., Sorlie, P.D. & et al. Trends in cardiovascular complications of diabetes. *JAMA : the journal of the American Medical Association* **292**, 2495-2499 (2004).

128. Levitzky, Y.S. *et al.* Impact of Impaired Fasting Glucose on Cardiovascular DiseaseThe Framingham Heart Study. *Journal of the American College of Cardiology* **51**, 264-270 (2008).

129. Meigs, J.B. *et al.* Impact of insulin resistance on risk of type 2 diabetes and cardiovascular disease in people with metabolic syndrome. *Diabetes care* **30**, 1219-1225 (2007).

130. Wilson, P.W., D'Agostino, R.B., Parise, H., Sullivan, L. & Meigs, J.B. Metabolic syndrome as a precursor of cardiovascular disease and type 2 diabetes mellitus. *Circulation* **112**, 3066-3072 (2005).

131. Banerji, M.A. & Chaiken, R.L. Insulin Resistance and the Metabolic Syndrome, in *Principles of Diabetes Mellitus* 531-555 (Springer, 2010).

132. Laakso, M. Cardiovascular Disease in Type 2 Diabetes From Population to Man to Mechanisms. *Diabetes care* **33**, 442-449 (2010).

133. Renard, C.B. *et al.* Diabetes and diabetes-associated lipid abnormalities have distinct effects on initiation and progression of atherosclerotic lesions. *Journal of Clinical Investigation* **114**, 659-668 (2004).

134. Gerrity, R.G., Natarajan, R., Nadler, J.L. & Kimsey, T. Diabetes-induced accelerated atherosclerosis in swine. *Diabetes* **50**, 1654-1665 (2001).

135. Piga, R., Naito, Y., Kokura, S., Handa, O. & Yoshikawa, T. Short-term high glucose exposure induces monocyte-endothelial cells adhesion and transmigration by increasing VCAM-1 and MCP-1 expression in human aortic endothelial cells. *Atherosclerosis* **193**, 328-334 (2007).

136. Yamagishi, S.-i. & Matsui, T. Advanced glycation end products, oxidative stress and diabetic nephropathy. *Oxidative Medicine and Cellular Longevity* **3**, 101-108 (2010).

137. Semba, R.D., Nicklett, E.J. & Ferrucci, L. Does accumulation of advanced glycation end products contribute to the aging phenotype? *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences* **65**, 963-975 (2010).

138. Lopes-Virella, M.F. *et al.* Levels of Oxidized LDL and Advanced Glycation End Products–Modified LDL in Circulating Immune Complexes Are Strongly Associated With Increased Levels of Carotid Intima-Media Thickness and Its Progression in Type 1 Diabetes. *Diabetes* **60**, 582-589 (2011).

139. Chao, P.-c., Huang, C.-n., Hsu, C.-c., Yin, M.-c. & Guo, Y.-r. Association of dietary AGEs with circulating AGEs, glycated LDL, IL-1α and MCP-1 levels in type 2 diabetic patients. *European Journal of Nutrition* **49**, 429-434 (2010).

140. Chen, K. & Keaney Jr, J.F. Evolving concepts of oxidative stress and reactive oxygen species in cardiovascular disease. *Current atherosclerosis reports* **14**, 476-483 (2012).

141. Selvaraju, V. *et al.* Diabetes, oxidative stress, molecular mechanism, and cardiovascular disease-an overview. *Toxicology Mechanisms and Methods* **22**, 330-335 (2012).

142. Santilli, F. *et al.* Postprandial hyperglycemia is a determinant of platelet activation in early type 2 diabetes mellitus. *Journal of Thrombosis and Haemostasis* **8**, 828-837 (2010).

143. Libby, P., Okamoto, Y., Rocha, V.Z. & Folco, E. Inflammation in atherosclerosis. *Circulation journal : official journal of the Japanese Circulation Society* **74**, 213-220 (2010).

144. Nomura, S. *et al.* Correlation between adiponectin and reduction of cell adhesion molecules after pitavastatin treatment in hyperlipidemic patients with type 2 diabetes mellitus. *Thrombosis research* **122**, 39-45 (2008).

145. Avogaro, A., Albiero, M., Menegazzo, L., de Kreutzenberg, S. & Fadini, G.P. Endothelial Dysfunction in Diabetes. *Diabetes care* **34**, S285-S290 (2011).

146. Bakker, W., Eringa, E.C., Sipkema, P. & van Hinsbergh, V.W.M. Endothelial dysfunction and diabetes: roles of hyperglycemia, impaired insulin signaling and obesity. *Cell and tissue research* **335**, 165-189 (2009).

147. Georgescu, A. Vascular dysfunction in diabetes: The endothelial progenitor cells as new therapeutic strategy. *World journal of diabetes* **2**, 92-97 (2011).

148. Dasu, M.R., Devaraj, S., Zhao, L., Hwang, D.H. & Jialal, I. High glucose induces toll-like receptor expression in human monocytes mechanism of activation. *Diabetes* **57**, 3090-3098 (2008).

149. Dasu, M.R., Ramirez, S. & Isseroff, R.R. Toll-like receptors and diabetes: a therapeutic perspective. *Clinical Science* **122**, 203-214 (2012).

150. Kuniyasu, A. *et al.* CD36-mediated endocytic uptake of advanced glycation end products (AGE) in mouse 3T3-L1 and human subcutaneous adipocytes. *FEBS letters* **537**, 85-90 (2003).

151. Okuda, L.S. *et al.* Advanced glycated albumin impairs HDL anti-inflammatory activity and primes macrophages for inflammatory response that reduces reverse cholesterol transport. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids* **1821**, 1485-1492 (2012).

152. Lee, Y.J., Kim, J.S., Kang, D.G. & Lee, H.S. Buddleja officinalis suppresses high glucose-induced vascular smooth muscle cell proliferation: role of mitogen-activated protein kinases, nuclear factor-κB and matrix metalloproteinases. *Experimental Biology and Medicine* **235**, 247-255 (2010).

153. Vicent, D. *et al.* The role of endothelial insulin signaling in the regulation of vascular tone and insulin resistance. *Journal of Clinical Investigation* **111**, 1373-1380 (2003).

154. Sainio, A., Jokela, T., Tammi, M.I. & Järveläinen, H. Hyperglycemic conditions modulate connective tissue reorganization by human vascular smooth muscle cells through stimulation of hyaluronan synthesis. *Glycobiology* **20**, 1117-1126 (2010).

155. Heickendorff, L., Ledet, T. & Rasmussen, L. Glycosaminoglycans in the human aorta in diabetes mellitus: a study of tunica media from areas with and without atherosclerotic plaque. *Diabetologia* **37**, 286-292 (1994).

156. McDonald, T.O. *et al.* Diabetes and arterial extracellular matrix changes in a porcine model of atherosclerosis. *Journal of Histochemistry & Cytochemistry* **55**, 1149-1157 (2007).

157. Sheng, X., Murphy, M.J., MacDonald, T.M. & Wei, L. Effect of statins on total cholesterol concentrations and cardiovascular outcomes in patients with diabetes mellitus: a population-based cohort study. *European journal of clinical pharmacology* **68**, 1201-1208 (2012).

158. Mooradian, A.D. Dyslipidemia in type 2 diabetes mellitus. *Nature clinical practice endocrinology & metabolism* **5**, 150-159 (2009).

159. Tie, G. *et al.* Oxidized low-density lipoprotein induces apoptosis in endothelial progenitor cells by inactivating the phosphoinositide 3-kinase/Akt pathway. *Journal of vascular research* **47**, 519-530 (2010).

160. Moore, K.J. & Tabas, I. Macrophages in the pathogenesis of atherosclerosis. *Cell* **145**, 341-355 (2011).

161. Benn, M. Apolipoprotein B levels, APOB alleles, and risk of ischemic cardiovascular disease in the general population, a review. *Atherosclerosis* **206**, 17-30 (2009).

162. Shamekh, R. *et al.* Endogenous and diet-induced hypercholesterolemia in nonhuman primates: effects of age, adiposity, and diabetes on lipoprotein profiles. *Metabolism: clinical and experimental* **60**, 1165-1177 (2011).

163. Wang, Y. *et al.* Prolonged caloric restriction in obese patients with type 2 diabetes mellitus decreases plasma CETP and increases apolipoprotein AI levels without improving the cholesterol efflux properties of HDL. *Diabetes care* **34**, 2576-2580 (2011).

164. Isoda, K., Folco, E.J., Shimizu, K. & Libby, P. AGE-BSA decreases ABCG1 expression and reduces macrophage cholesterol efflux to HDL. *Atherosclerosis* **192**, 298-304 (2007).

165. Zhou, H., Tan, K.C., Shiu, S.W. & Wong, Y. Increased serum advanced glycation end products are associated with impairment in HDL antioxidative capacity in diabetic nephropathy. *Nephrology dialysis transplantation* **23**, 927-933 (2008).

166. Navab, M., Reddy, S.T., Van Lenten, B.J. & Fogelman, A.M. HDL and cardiovascular disease: atherogenic and atheroprotective mechanisms. *Nature Reviews Cardiology* **8**, 222-232 (2011).

167. Tsompanidi, E.M., Brinkmeier, M.S., Fotiadou, E.H., Giakoumi, S.M. & Kypreos, K.E. HDL biogenesis and functions: role of HDL quality and quantity in atherosclerosis. *Atherosclerosis* **208**, 3-9 (2010).

168. Donath, M.Y. & Shoelson, S.E. Type 2 diabetes as an inflammatory disease. *Nature Reviews Immunology* **11**, 98-107 (2011).

169. Hogue, J.C. *et al.* Differential effect of atorvastatin and fenofibrate on plasma oxidized low-density lipoprotein, inflammation markers, and cell adhesion molecules in patients with type 2 diabetes mellitus. *Metabolism: clinical and experimental* **57**, 380-386 (2008).

170. Hotamisligil, G. Mechanisms of TNF-α-induced insulin resistance. *Experimental and clinical endocrinology & diabetes* **107**, 119-125 (2009).

171. Giacco, F. & Brownlee, M. Oxidative Stress and Diabetic Complications. *Circulation Research* **107**, 1058-1070 (2010).

172. Raff, H. & Levitzky, M.G. *Medical Physiology: A system approach*. (McGraw Hill New York; 2011).

173. Moore, K.L., Dalley, A.F. & Agur, A.M.R. *Clinically oriented anatomy*, Edn. 7th. (Lippincott Williams & Wilkins, Baltimore; 2013).

174. Schäberle, W. Extracranial Cerebral Arteries, in *Ultrasonography in Vascular Diagnosis* 291-375 (Springer, Heidelberg; 2011).

175. AbuRahma, A.F. Overview of cerebrovascular disease, in *Noninvasive Vascular Diagnosis*, Edn. 3rd. (eds. A.F. AbuRahma & J.J. Berga) (Springer, New York; 2010).

176. Schäberle, W. Peripheral arteries, in *Ultrasonography in Vascular Diagnosis* 45-163 (Springer, Heidelberg; 2011).

177. AbuRahma, A.F. *Noninvasive peripheral arterial diagnosis*. (Springer, 2010).

178. Ballal, N.H. Mathematical modeling of arterial blood flow using Windkessel model. *Sudan Medical Monitor (SMM)* **6** (2011).

179. Rhodin, J.A.G. Architecture of the Vessel Wall, in *Comprehensive Physiology* (John Wiley & Sons, Inc., 2011).

180. Gussenhoven, E.J. *et al.* Arterial wall characteristics determined by intravascular ultrasound imaging: an in vitro study. *Journal of the American College of Cardiology* **14**, 947-952 (1989).

181. Rey, F.E. & Pagano, P.J. The reactive adventitia fibroblast oxidase in vascular function. *Arteriosclerosis, thrombosis, and vascular biology* **22**, 1962-1971 (2002).

182. Nichols, W.W., O'Rourke, M.F. & Vlachopoulos, C. *McDonald's blood flow in arteries: theoretical, experimental and clinical principles*. (CRC Press, 2011).

183. Harkness, M.L., Harkness, R. & McDonald, D. The collagen and elastin content of the arterial wall in the dog. *Proceedings of the Royal Society of London. Series B-Biological Sciences* **146**, 541-551 (1957).

184. Wagenseil, J.E. & Mecham, R.P. Vascular Extracellular Matrix and Arterial Mechanics. *Physiological Reviews* **89**, 957-989 (2009).

185. Åstrand, H. *et al.* In vivo estimation of the contribution of elastin and collagen to the mechanical properties in the human abdominal aorta: effect of age and sex. *Journal of applied physiology* **110**, 176-187 (2011).

186. London, G.M. & Pannier, B. Arterial functions: how to interpret the complex physiology. *Nephrology dialysis transplantation* **25**, 3815-3823 (2010).

187. Chirinos, J.A. & Segers, P. Noninvasive Evaluation of Left Ventricular Afterload Part 1: Pressure and Flow Measurements and Basic Principles of Wave Conduction and Reflection. *Hypertension* **56**, 555-562 (2010).

188. Chirinos, J.A. & Segers, P. Noninvasive Evaluation of Left Ventricular Afterload Part 2: Arterial Pressure-Flow and Pressure-Volume Relations in Humans. *Hypertension* **56**, 563-570 (2010).

189. Lee, H.-Y. & Oh, B.-H. Aging and arterial stiffness. *Circulation Journal* **74**, 2257 (2010).

190. Quinn, U., Tomlinson, L.A. & Cockcroft, J.R. Arterial stiffness. *JRSM Cardiovascular Disease* **1** (2012).

191. Chirinos, J.A. Arterial stiffness: basic concepts and measurement techniques. *Journal of cardiovascular translational research* **5**, 243-255 (2012).

192. Mitchell, G.F. Clinical achievements of impedance analysis. *Medical and Biological Engineering and Computing* **47**, 153-163 (2009).

193. Mitchell, G.F. Arterial stiffness and wave reflection: biomarkers of cardiovascular risk. *Artery Research* **3**, 56-64 (2009).

194. Mitchell, G.F. *et al.* Changes in arterial stiffness and wave reflection with advancing age in healthy men and women the Framingham Heart Study. *Hypertension* **43**, 1239-1245 (2004).

195. Kaess, B.M. *et al.* Aortic stiffness, blood pressure progression, and incident hypertension. *JAMA: The Journal of the American Medical Association* **308**, 875 (2012).

196. Boutouyrie, P. *et al.* Pharmacological modulation of arterial stiffness. *Drugs* **71**, 1689-1701 (2011).

197. Gosling, R.G. & Budge, M.M. Terminology for describing the elastic behavior of arteries. *Hypertension* **41**, 1180-1182 (2003).

198. Hughes, S.M., Dixon, L.J. & McVeigh, G.E. Arterial stiffness and pulse wave velocity: problems with terminology. *Circulation* **109**, e3-e3 (2004).

199. Sciarra, G. & Vidoli, S. Generalized Hooke's law for isotropic second gradient materials. *arXiv preprint arXiv:1008.2879* (2010).

200. Giuliodori, M.J., Lujan, H.L., Briggs, W.S., Palani, G. & DiCarlo, S.E. Hooke's law: applications of a recurring principle. *Advances in Physiology Education* **33**, 293-296 (2009).

201. Costanzo, F. & Brasseur, J.G. The Invalidity of the Laplace Law for Biological Vessels and of Estimating Elastic Modulus from Total Stress vs. Strain: a New Practical Method. *arXiv preprint arXiv:1302.1067* (2013).

202. Chirinos, J.A. *et al.* Ethnic differences in arterial wave reflections and normative equations for augmentation index. *Hypertension* **57**, 1108-1116 (2011).

203. Peterson, L.H., Jensen, R.E. & Parnell, J. Mechanical properties of arteries in vivo. *Circulation Research* **8**, 622-639 (1960).

204. Gosling, R. Extraction of physiological information from spectrum-analysed Doppler-shifted continuous-wave ultrasound signals obtained non-invasively from the arterial system. *IEE Medical Electronic Monographs* **13**, 73-125 (1976).

205. Hayashi, K., Handa, H., Nagasawa, S., Okumura, A. & Moritake, K. Stiffness and elastic behavior of human intracranial and extracranial arteries. *Journal of biomechanics* **13**, 175-184 (1980).

206. Sun, C.-K. Cardio-ankle vascular index (CAVI) as an indicator of arterial stiffness. *Integrated blood pressure control* **6**, 27 (2013).

207. Womersley, J. Oscillatory flow in arteries: the constrained elastic tube as a model of arterial flow and pulse transmission. *Physics in medicine and biology* **2**, 178 (1957).

208. Avolio, A.P., Butlin, M. & Walsh, A. Arterial blood pressure measurement and pulse wave analysis—their role in enhancing cardiovascular assessment. *Physiological Measurement* **31**, R1 (2010).

209. Dick, D., Kendrick, J., Matson, G. & Rideout, V. Measurement of nonlinearity in the arterial system of the dog by a new method. *Circulation Research* **22**, 101-111 (1968).

210. Armentano, R.L., Barra, J.G., Levenson, J., Simon, A. & Pichel, R.H. Arterial Wall Mechanics in Conscious Dogs Assessment of Viscous, Inertial, and Elastic Moduli to Characterize Aortic Wall Behavior. *Circulation Research* **76**, 468-478 (1995).

211. Burattini, R., Gnudi, G., Westerhof, N. & Fioretti, S. Total systemic arterial compliance and aortic characteristic impedance in the dog as a function of pressure: A model based study. *Computers and Biomedical Research* **20**, 154-165 (1987).

212. Langewouters, G., Wesseling, K. & Goedhard, W. The pressure dependent dynamic elasticity of 35 thoracic and 16 abdominal human aortas *in vitro* described by a five component model. *Journal of biomechanics* **18**, 613-620 (1985).

213. Studinger, P., Lénárd, Z., Reneman, R. & Kollai, M. Measurement of aortic arch distension wave with the echo-track technique. *Ultrasound in medicine & biology* **26**, 1285 (2000).

214. De Melis, M. *et al.* Blood pressure waveform analysis by means of wavelet transform. *Medical & biological engineering & computing* **47**, 165-173 (2009).

215. Parker, K.H. An introduction to wave intensity analysis. *Medical & biological engineering & computing* **47**, 175-188 (2009).

216. Wang, J.-J., Shrive, N.G., Parker, K.H. & Tyberg, J.V. "Wave" as defined by wave intensity analysis. *Medical & biological engineering & computing* **47**, 189-195 (2009).

217. Tyberg, J.V. *et al.* Wave intensity analysis and the development of the reservoir–wave approach. *Medical & biological engineering & computing* **47**, 221-232 (2009).

218. Hughes, A. & Parker, K. Forward and backward waves in the arterial system: impedance or wave intensity analysis? *Medical & biological engineering & computing* **47**, 207-210 (2009).

219. Avolio, A., Butlin, M. & Xu, K. Reflections on determinants of augmentation index. *Journal of hypertension* **30**, 267-268 (2012).

220. Heffernan, K.S. *et al.* Elevated augmentation index derived from peripheral arterial tonometry is associated with abnormal ventricular–vascular coupling. *Clinical physiology and functional imaging* **30**, 313-317 (2010).

221. O'Rourke, M.F. & Adji, A. Noninvasive studies of central aortic pressure. *Current hypertension reports* **14**, 8-20 (2012).

222. Takazawa, K., Kobayashi, H., Shindo, N., Tanaka, N. & Yamashina, A. Relationship between radial and central arterial pulse wave and evaluation of central aortic pressure using the radial arterial pulse wave. *Hypertension Research* **30**, 219 (2007).

223. Butlin, M., Qasem, A. & Avolio, A.P. in Engineering in Medicine and Biology Society (EMBC), 2012 Annual International Conference of the IEEE 2591-2594 (IEEE, 2012).

224. Stok, W.J., Westerhof, B.E., Guelen, I. & Karemaker, J.M. Aortic pressure wave reconstruction during exercise is improved by adaptive filtering: a pilot study. *Medical & biological engineering & computing* **49**, 909-916 (2011).

225. Kroeker, E.J. & Wood, E.H. Comparison of simultaneously recorded central and peripheral arterial pressure pulses during rest, exercise and tilted position in man. *Circulation Research* **3**, 623-632 (1955).

226. Rowell, L.B., Brengelmann, G.L., Blackmon, J.R., Bruge, R.A. & Murray, J.A. Disparities Between Aortic and Peripheral Pulse Pressures Induced by Upright Exercise and Vasomotor Changes in Man. *Circulation* **37**, 954-964 (1968).

227. O'Rourke, M.F. Influence of ventricular ejection on the relationship between central aortic and brachial pressure pulse in man. *Cardiovascular research* **4**, 291-300 (1970).

228. Lasance, H., Wesseling, K. & Ascoop, C. Peripheral pulse contour analysis in determining stroke volume. *Progress report* **5**, 59-62 (1976).

229. Allen, J. Photoplethysmography and its application in clinical physiological measurement. *Physiological Measurement* **28**, R1 (2007).

230. Pauca, A.L., O'Rourke, M.F. & Kon, N.D. Prospective evaluation of a method for estimating ascending aortic pressure from the radial artery pressure waveform. *Hypertension* **38**, 932-937 (2001).

231. Westerhof, B.E. *et al.* Individualization of transfer function in estimation of central aortic pressure from the peripheral pulse is not required in patients at rest. *Journal of applied physiology* **105**, 1858-1863 (2008).

232. Shih, Y., Cheng, H., Sung, S., Hu, W. & Chen, C. Comparison of two generalized transfer functions for measuring central systolic blood pressure by an oscillometric blood pressure monitor. *Journal of Human Hypertension* (2012).

233. Hahn, J., Reisner, A.T., Jaffer, F.A. & Asada, H. Subject-specific estimation of central aortic blood pressure using an individualized transfer function: A preliminary feasibility study. *Information Technology in Biomedicine, IEEE Transactions on* **16**, 212-220 (2012).

234. Hope, S.A., Tay, D.B., Meredith, I.T. & Cameron, J.D. Use of Arterial Transfer Functions for the Derivation of Central Aortic Waveform Characteristics in Subjects With Type 2 Diabetes and Cardiovascular Disease. *Diabetes care* **27**, 746-751 (2004).

235. Dahlöf, B. *et al.* Cardiovascular morbidity and mortality in the Losartan Intervention For Endpoint reduction in hypertension study (LIFE): a randomised trial against atenolol. *The Lancet* **359**, 995-1003 (2002).

236. Williams, B. *et al.* Differential Impact of Blood Pressure–Lowering Drugs on Central Aortic Pressure and Clinical Outcomes Principal Results of the Conduit Artery Function Evaluation (CAFE) Study. *Circulation* **113**, 1213-1225 (2006).

237. Roman, M.J. *et al.* Central pressure more strongly relates to vascular disease and outcome than does brachial pressure the strong heart study. *Hypertension* **50**, 197-203 (2007).

238. Mallion, J.-M. *et al.* Twenty-four-hour ambulatory blood pressure monitoring efficacy of perindopril/indapamide first-line combination in hypertensive patients: the REASON study. *American journal of hypertension* **17**, 245-251 (2004).

239. Bjarnegård, N. & Länne, T. Arterial properties along the upper arm in humans: age-related effects and the consequence of anatomical location. *Journal of applied physiology* **108**, 34-38 (2010).

240. Wilkinson, I.B. *et al.* Heart rate dependency of pulse pressure amplification and arterial stiffness. *American journal of hypertension* **15**, 24-30 (2002).

241. Fyhrquist, F. *et al.* Pulse pressure and effects of losartan or atenolol in patients with hypertension and left ventricular hypertrophy. *Hypertension* **45**, 580-585 (2005).

242. Schriefl, A.J., Zeindlinger, G., Pierce, D.M., Regitnig, P. & Holzapfel, G.A. Determination of the layer-specific distributed collagen fibre orientations in human thoracic and abdominal aortas and common iliac arteries. *Journal of The Royal Society Interface* **9**, 1275-1286 (2012).

243. Zarins, C.K., Xu, C. & Glagov, S. Pathophysiology of Vascular Disease, in *Peripheral Endovascular Interventions* 11-28 (Springer, 2010).

244. O'Rourke, M.F. Arterial aging: pathophysiological principles. *Vascular Medicine* **12**, 329-341 (2007).

245. Watanabe, M., Sawai, T., Nagura, H. & Suyama, K. Age-related alteration of cross-linking amino acids of elastin in human aorta. *The Tohoku journal of experimental medicine* **180**, 115-130 (1996).

246. Mitchell, G.F. *et al.* Aortic diameter, wall stiffness, and wave reflection in systolic hypertension. *Hypertension* **51**, 105-111 (2008).

247. Zieman, S.J., Melenovsky, V. & Kass, D.A. Mechanisms, Pathophysiology, and Therapy of Arterial Stiffness. *Arteriosclerosis, thrombosis, and vascular biology* **25**, 932-943 (2005).

248. Fritze, O. *et al.* Age-related changes in the elastic tissue of the human aorta. *Journal of vascular research* **49**, 77-86 (2011).

249. Mannello, F. & Raffetto, J.D. Matrix metalloproteinase activity and glycosaminoglycans in chronic venous disease: the linkage among cell biology,

pathology and translational research. *American journal of translational research* **3**, 149 (2011).

250. Zieman, S. & Kass, D. Advanced Glycation Endproduct Crosslinking in the Cardiovascular System. *Drugs* **64**, 459-470 (2004).

251. Sell, D.R. & Monnier, V.M. Molecular basis of arterial stiffening: role of glycation–a mini-review. *Gerontology* **58**, 227-237 (2012).

252. Aggoun, Y. *et al.* Arterial mechanical changes in children with familial hypercholesterolemia. *Arteriosclerosis, thrombosis, and vascular biology* **20**, 2070-2075 (2000).

253. Urbina, E.M. *et al.* Triglyceride to HDL-C Ratio and Increased Arterial Stiffness in Children, Adolescents, and Young Adults. *Pediatrics* (2013).

254. Versari, D., Daghini, E., Virdis, A., Ghiadoni, L. & Taddei, S. The ageing endothelium, cardiovascular risk and disease in man. *Experimental physiology* **94**, 317-321 (2009).

255. Osherov, A.B., Gotha, L., Cheema, A.N., Qiang, B. & Strauss, B.H. Proteins mediating collagen biosynthesis and accumulation in arterial repair: novel targets for anti-restenosis therapy. *Cardiovascular research* **91**, 16-26 (2011).

256. Garrido, A.M. & Griendling, K.K. NADPH oxidases and angiotensin II receptor signaling. *Molecular and cellular endocrinology* **302**, 148-158 (2009).

257. Cat, A.N.D. & Touyz, R.M. Cell signaling of angiotensin II on vascular tone: novel mechanisms. *Current hypertension reports* **13**, 122-128 (2011).

258. Jablonski, K.L. *et al.* Dietary Sodium Restriction Reverses Vascular Endothelial Dysfunction in Middle-Aged/Older Adults With Moderately Elevated Systolic Blood Pressure. *Journal of the American College of Cardiology* **61**, 335-343 (2013).

259. Appel, L.J. *et al.* The importance of population-wide sodium reduction as a means to prevent cardiovascular disease and stroke. *Circulation* **123**, 1138-1143 (2011).

260. Shigemura, N. *et al.* Angiotensin II Modulates Salty and Sweet Taste Sensitivities. *The Journal of Neuroscience* **33**, 6267-6277 (2013).

261. Jin, Y. *et al.* Evaluation of Carotid Artery Stiffness in Obese Children Using Ultrasound Radiofrequency Data Technology. *Journal of Ultrasound in Medicine* **32**, 105-113 (2013).

262. Urbina, E., Gao, Z., Khoury, P., Martin, L. & Dolan, L. Insulin resistance and arterial stiffness in healthy adolescents and young adults. *Diabetologia* **55**, 625-631 (2012).

263. Ho, C. *et al.* Arterial stiffness is strongly associated with insulin resistance in Chinese--a population-based study (Taichung Community Health Study, TCHS). *Journal of atherosclerosis and thrombosis* **18**, 122 (2011).

264. Henry, R. *et al.* The metabolic syndrome in elderly individuals is associated with greater muscular, but not elastic arterial stiffness, independent of low-grade inflammation, endothelial dysfunction or insulin resistance—The Hoorn Study. *Journal of Human Hypertension* **23**, 718-727 (2009).

265. Cecelja, M. & Chowienczyk, P. Dissociation of aortic pulse wave velocity with risk factors for cardiovascular disease other than hypertension a systematic review. *Hypertension* **54**, 1328-1336 (2009).

266. O'Rourke, M.F. & Adji, A. An updated clinical primer on large artery mechanics: implications of pulse waveform analysis and arterial tonometry. *Current opinion in cardiology* **20**, 275-281 (2005).

267. Martin, X. Normal intraocular pressure in man. *Ophthalmologica* **205**, 57-63 (2010).

268. Kim, E.G., Nam, K.C., Heo, H. & Huh, Y. in Engineering in Medicine and Biology Society, 2009. EMBC 2009. Annual International Conference of the IEEE 3771-3774 (IEEE, 2009).

269. Eige, J., Newgard, P. & Pressman, G. Direct force-measuring transducer used in blood pressure research. (1965).

270. Eckerle, J.S. Tonometry, arterial. *Encyclopedia of medical devices and instrumentation* (2006).

271. Matthys, K. & Verdonck, P. Development and modelling of arterial applanation tonometry: a review. *Technology and Health Care* **10**, 65-76 (2002).

272. Salvi, P., Safar, M.E. & Parati, G. Arterial applanation tonometry: technical aspects relevant for its daily clinical use. *Journal of hypertension* **31**, 469-471 (2013).

273. Matthys, K.S. *et al.* Long-term pressure monitoring with arterial applanation tonometry: A non-invasive alternative during clinical intervention? *Technology and Health Care* **16**, 183-193 (2008).

274. Garcia-Ortiz, L. *et al.* Comparison of two measuring instruments, B-pro and SphygmoCor system as reference, to evaluate central systolic blood pressure and radial augmentation index. *Hypertension Research* **35**, 617-623 (2012).

275. Ciaccio, E.J. & Drzewiecki, G.M. Tonometric arterial pulse sensor with noise cancellation. *Biomedical Engineering, IEEE Transactions on* **55**, 2388-2396 (2008).

276. Penaz, J. in Digest of the 10th international conference on medical and biological engineering, Vol. 104 (Dresden, 1973).

277. Chung, E., Chen, G., Alexander, B. & Cannesson, M. Non-invasive continuous blood pressure monitoring: a review of current applications. *Frontiers of medicine*, 1-11 (2013).

278. Schattenkerk, D.W.E. *et al.* Nexfin noninvasive continuous blood pressure validated against Riva-Rocci/Korotkoff. *American journal of hypertension* **22**, 378-383 (2009).

279. Marrone, O. *et al.* Blood pressure changes after automatic and fixed CPAP in obstructive sleep apnea: relationship with nocturnal sympathetic activity. *Clinical and experimental hypertension* **33**, 373-380 (2011).

280. Cortelli, P., Lombardi, C., Montagna, P. & Parati, G. Baroreflex modulation during sleep and in obstructive sleep apnea syndrome. *Autonomic Neuroscience* (2012).

281. Mano, T., Nishimura, N. & Iwase, S. Sympathetic neural influence on bone metabolism in microgravity (Review). *Acta Physiologica Hungarica* **97**, 354-361 (2010).

282. Gump, B.B. *et al.* Low-level Pb and cardiovascular responses to acute stress in children: The role of cardiac autonomic regulation. *Neurotoxicology and teratology* **33**, 212-219 (2011).

283. Ng, K.-G., Peh, W.L. & Chua, N.K. (Google Patents, 2011).

284. Ahn, B. & Kim, J. Measurement and characterization of soft tissue behavior with surface deformation and force response under large deformations. *Medical image analysis* **14**, 138 (2010).

285. Hager, H. *et al.* A comparison of noninvasive blood pressure measurement on the wrist with invasive arterial blood pressure monitoring in patients undergoing bariatric surgery. *Obesity surgery* **19**, 717-724 (2009).

286. O'Brien, E. *et al.* European Society of Hypertension International Protocol revision 2010 for the validation of blood pressure measuring devices in adults. *Blood pressure monitoring* **15**, 23-38 (2010).

287. Horvath, I.G. *et al.* Invasive validation of a new oscillometric device (Arteriograph) for measuring augmentation index, central blood pressure and aortic pulse wave velocity. *Journal of hypertension* **28**, 2068-2075 (2010).

288. Payne, R.A., Wilkinson, I.B. & Webb, D.J. Arterial Stiffness and Hypertension Emerging Concepts. *Hypertension* **55**, 9-14 (2010).

289. Middleton, P.M. *et al.* Changes in left ventricular ejection time and pulse transit time derived from finger photoplethysmogram and electrocardiogram during moderate haemorrhage. *Clinical physiology and functional imaging* **29**, 163-169 (2009).

290. Javed, F., Savkin, A.V., Chan, G.S., Mackie, J.D. & Lovell, N.H. Recent advances in the monitoring and control of haemodynamic variables during haemodialysis: a review. *Physiological Measurement* **33**, R1 (2012).

291. Kim, S.W. *et al.* A new method of screening for diabetic neuropathy using laser Doppler and photoplethysmography. *Medical & biological engineering & computing* **46**, 61-67 (2008).

292. Chan, G.S. *et al.* Augmented photoplethysmographic low frequency waves at the onset of endotoxic shock in rabbits. *Physiological Measurement* **31**, 1605 (2010).

293. Shirai, K. *et al.* Cardio-ankle vascular index (CAVI) as a novel indicator of arterial stiffness: theory, evidence and perspectives. *Journal of atherosclerosis and thrombosis* **18**, 924 (2011).

294. Shirai, K. *et al.* Contradictory effects of β1-and α1-aderenergic receptor blockers on cardio-ankle vascular stiffness index (CAVI)--CAVI independent of blood pressure. *Journal of atherosclerosis and thrombosis* **18**, 49 (2011).

295. Kim, B., Takada, K., Oka, S. & Misaki, T. Influence of blood pressure on cardio-ankle vascular index (CAVI) examined based on percentage change during general anesthesia. *Hypertension Research* **34**, 779-783 (2011).

296. Frank, O. Die theorie der pulswellen. *Z Biol* **85**, 91-130 (1926).

297. Bergel, D. The dynamic elastic properties of the arterial wall. *The Journal of physiology* **156**, 458-469 (1961).

298. Bramwell, J.C. & Hill, A.V. The velocity of the pulse wave in man. *Proceedings of the Royal Society of London. Series B, Containing Papers of a Biological Character* **93**, 298-306 (1922).

299. Bramwell, J.C. & Hill, A. Velocity of transmission of the pulse-wave: and elasticity of arteries. *The Lancet* **199**, 891-892 (1922).

300. Dogui, A. *et al.* Consistency of aortic distensibility and pulse wave velocity estimates with respect to the Bramwell-Hill theoretical model: a cardiovascular magnetic resonance study. *J Cardiovasc Magn Reson* **13** (2011).

301. Kawasaki, T., Sasayama, S., Yagi, S.I., Asakawa, T. & Hirai, T. Non-invasive assessment of the age related changes in stiffness of major branches of the human arteries. *Cardiovascular research* **21**, 678-687 (1987).

302. Roach, M.R. & Burton, A.C. The reason for the shape of the distensibility curves of arteries. *Canadian journal of biochemistry and physiology* **35**, 681-690 (1957).

303. Trachet, B. *et al.* Numerical validation of a new method to assess aortic pulse wave velocity from a single recording of a brachial artery waveform with an occluding cuff. *Annals of Biomedical Engineering* **38**, 876-888 (2010).

304. Rajzer, M.W. *et al.* Comparison of aortic pulse wave velocity measured by three techniques: Complior, SphygmoCor and Arteriograph. *Journal of hypertension* **26**, 2001-2007 (2008).

305. Safar, M.E., Czernichow, S. & Blacher, J. Obesity, arterial stiffness, and cardiovascular risk. *Journal of the American Society of Nephrology* **17**, S109-S111 (2006).

306. Stamler, R., Ford, C.E. & Stamler, J. Why do lean hypertensives have higher mortality rates than other hypertensives? Findings of the Hypertension Detection and Follow-up Program. *Hypertension* **17**, 553-564 (1991).

307. Wang, T.J. *et al.* Obesity and the risk of new-onset atrial fibrillation. *JAMA: The Journal of the American Medical Association* **292**, 2471-2477 (2004).

308. McGee, D.L. Body mass index and mortality: a meta-analysis based on person-level data from twenty-six observational studies. *Annals of epidemiology* **15**, 87-97 (2005).

309. Toto-Moukouo, J., Achimastos, A., Asmar, R., Hugues, C. & Safar, M. Pulse wave velocity in patients with obesity and hypertension. *American heart journal* **112**, 136-140 (1986).

310. Dengo, A.L. *et al.* Arterial destiffening with weight loss in overweight and obese middle-aged and older adults. *Hypertension* **55**, 855-861 (2010).

311. Rodrigues, S.L. *et al.* Body Mass Index Is Not Independently Associated With Increased Aortic Stiffness in a Brazilian Population. *American journal of hypertension* (2012).

312. Nordstrand, N. *et al.* The relationship between various measures of obesity and arterial stiffness in morbidly obese patients. *BMC cardiovascular disorders* **11**, 7 (2011).

313. Lee, M. *et al.* Associations between trunk, leg and total body adiposity with arterial stiffness. *American journal of hypertension* (2012).

314. Boulton, A.J.M. *et al.* Diabetic Neuropathies: A statement by the American Diabetes Association. *Diabetes care* **28**, 956-962 (2005).

315. Pirart, J. Diabetes mellitus and its degenerative complications: a prospective study of 4,400 patients observed between 1947 and 1973. *Diabetes care* **1**, 168-188 (1978).

316. Vinik, A.I. *et al.* Diabetic Neuropathies. *Diabetes care* **15**, 1926-1975 (1992).

317. Vinik, A.I. & Ziegler, D. Diabetic Cardiovascular Autonomic Neuropathy. *Circulation* **115**, 387-397 (2007).

318. Tesfaye, S. *et al.* Diabetic Neuropathies: Update on Definitions, Diagnostic Criteria, Estimation of Severity, and Treatments. *Diabetes care* **33**, 2285-2293 (2010).

319. Pop-Busui, R., Lu, J., Lopes, N. & Jones, T.L.Z. Prevalence of diabetic peripheral neuropathy and relation to glycemic control therapies at baseline in the BARI 2D cohort. *Journal of the Peripheral Nervous System* **14**, 1-13 (2009).

320. Martin, C.L. *et al.* Neuropathy Among the Diabetes Control and Complications Trial Cohort 8 Years After Trial Completion. *Diabetes care* **29**, 340-344 (2006).

321. Ohkubo, Y. *et al.* Intensive insulin therapy prevents the progression of diabetic microvascular complications in Japanese patients with non-insulin-dependent diabetes mellitus: a randomized prospective 6-year study. *Diabetes research and clinical practice* **28**, 103-117 (1995).

322. UK Prospective Diabetes Study (UKPDS) Group Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *The Lancet* **352**, 837-853 (1998).

323. Tesfaye, S. *et al.* Vascular Risk Factors and Diabetic Neuropathy. *New England Journal of Medicine* **352**, 341-350 (2005).

324. Vincent, A.M., Hinder, L.M., Pop-Busui, R. & Feldman, E.L. Hyperlipidemia: a new therapeutic target for diabetic neuropathy. *Journal of the Peripheral Nervous System* **14**, 257-267 (2009).

325. Yagihashi, S. *et al.* in Journal of the Peripheral Nervous System, Vol. 16 S152-S153 (Wiley-Blackwell Commerce Place, 350 Main St, Malden 02148, MA USA; 2011).

326. Kihara, M., Weerasuriya, A. & Low, P.A. Endoneurial blood flow in rat sciatic nerve during development. *The Journal of physiology* **439**, 351-360 (1991).

327. Sima, A.A., Nathaniel, V., Prashar, A., Bril, V. & Greene, D.A. Endoneurial microvessels in human diabetic neuropathy. Endothelial cell dysjunction and lack of treatment effect by aldose reductase inhibitor. *Diabetes* **40**, 1090-1099 (1991).

328. Beggs, J., Johnson, P.C., Olafsen, A. & Watkins, C.J. Innervation of the Vasa Nervorum: Changes in Human Diabetics. *Journal of Neuropathology & Experimental Neurology* **51**, 612-629 (1992).

329. Yagihashi, S., Mizukami, H. & Sugimoto, K. Mechanism of diabetic neuropathy: Where are we now and where to go? *Journal of Diabetes Investigation* **2**, 18-32 (2011).

330. Yagihashi, S. & Matsunaga, M. Ultrastructural Pathology of Peripheral Nerves in Patients with Diabetic Neuropathy. *The Tohoku journal of experimental medicine* **129**, 357-366 (1979).

331. Dyck, P.J. & Giannini, C. Pathologic Alterations in the Diabetic Neuropathies of Humans: A Review. *Journal of Neuropathology & Experimental Neurology* **55**, 1181-1193 (1996).

332. Dyck, P.J. *et al.* Spatial pattern of nerve fiber abnormality indicative of pathologic mechanism. *The American journal of pathology* **117**, 225-238 (1984).

333. Dyck, P.J. *et al.* The spatial distribution of fiber loss in diabetic polyneuropathy suggests ischemia. *Annals of neurology* **19**, 440-449 (1986).

334. Thrainsdottir, S. *et al.* Endoneurial Capillary Abnormalities Presage Deterioration of Glucose Tolerance and Accompany Peripheral Neuropathy in Man. *Diabetes* **52**, 2615-2622 (2003).

335. Malik, R.A. *et al.* Sural nerve pathology in diabetic patients with minimal but progressive neuropathy. *Diabetologia* **48**, 578-585 (2005).

336. Yagihashi, S. Pathology and pathogenetic mechanisms of diabetic neuropathy. *Diabetes/Metabolism Reviews* **11**, 193-225 (1995).

337. Suri, C. *et al.* Requisite role of angiopoietin-1, a ligand for the TIE2 receptor, during embryonic angiogenesis. *Cell* **87**, 1171 (1996).

338. Hanahan, D. & Folkman, J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell* **86**, 353 (1996).

339. Patan, S. Vasculogenesis and angiogenesis, in *Angiogenesis in Brain Tumors* 3-32 (Springer, 2004).

340. Dallabrida, S.M. *et al.* Integrin binding angiopoietin-1 monomers reduce cardiac hypertrophy. *The FASEB Journal* **22**, 3010-3023 (2008).

341. Lobov, I.B., Brooks, P.C. & Lang, R.A. Angiopoietin-2 displays VEGF-dependent modulation of capillary structure and endothelial cell survival in vivo. *Proceedings of the National Academy of Sciences* **99**, 11205-11210 (2002).

342. Tremolada, G., Lattanzio, R., Mazzolari, G. & Zerbini, G. The therapeutic potential of VEGF inhibition in diabetic microvascular complications. *American journal of cardiovascular drugs : drugs, devices, and other interventions* **7**, 393-398 (2007).

343. Tuo, Q.H. *et al.* Critical role of angiopoietins/Tie-2 in hyperglycemic exacerbation of myocardial infarction and impaired angiogenesis. *American journal of physiology. Heart and circulatory physiology* **294**, H2547-2557 (2008).

344. Nadar, S.K., Blann, A.D., Kamath, S., Beevers, D.G. & Lip, G.Y. Platelet indexes in relation to target organ damage in high-risk hypertensive patients: a substudy of the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT). *Journal of the American College of Cardiology* **44**, 415-422 (2004).

345. World Health Organization *Waist circumference and waist–hip ratio: report of a WHO expert consultation*. (WHO Document Production Services, Geneva, Switzerland; 2011).

346. World Health Organization (World Health Organization, Geneva; 2008).

347. Levitt, N. *et al.* Type 2 diabetes clinical practice guidelines for sub-Saharan Africa. *Brussel: International Diabetes Federation Africa Region* (2006).

348. Young, M., Manes, C. & Boulton, A. Vibration perception threshold predicts foot ulceration: a prospective study. *Diabetic Med* **9**, 542 (1992).

349. Boulton, A.J.M. *et al.* Impaired Vibratory Perception and Diabetic Foot Ulceration. *Diabetic Medicine* **3**, 335-337 (1986).

350. Sugawara, J., Hayashi, K., Yokoi, T. & Tanaka, H. Age-associated elongation of the ascending aorta in adults. *JACC. Cardiovascular imaging* **1**, 739-748 (2008).

351. Sugawara, J., Hayashi, K., Yokoi, T. & Tanaka, H. Carotid-Femoral Pulse Wave Velocity: Impact of Different Arterial Path Length Measurements. *Artery Res* **4**, 27-31 (2010).

352. Nemeth, Z., Moczar, K. & Deak, G. Evaluation of the Tensioday ambulatory blood pressure monitor according to the protocols of the British Hypertension Society and the Association for the Advancement of Medical Instrumentation. *Blood Press Monit* **7**, 191-197 (2002).

353. Shirai, K. *et al.* Evaluation of Blood Pressure Control using a New Arterial Stiffness Parameter, Cardio-ankle Vascular Index (CAVI). *Current hypertension reviews* **9**, 66-75 (2013).

354. Friedewald, W.T., Levy, R.I. & Fredrickson, D.S. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical chemistry* **18**, 499-502 (1972).

355. National Cholesterol Education Program Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of

High Blood Cholesterol in Adults (Adult Treatment Panel III) Final Report. *Circulation* **106**, 3143 (2002).

356. Shiburi, C.P. *et al.* Reference Values for SphygmoCor Measurements in South Africans of African Ancestry&ast. *American journal of hypertension* **19**, 40-46 (2006).

357. Temmar, M. *et al.* Elderly Algerian women lose their sex-advantage in terms of arterial stiffness and cardiovascular profile. *Journal of hypertension* (2013).

358. Schutte, A.E. *et al.* Arterial Stiffness Profiles: Investigating Various Sections of the Arterial Tree of African and Caucasian People. *Clinical and experimental hypertension* **33**, 511-517 (2011).

359. Magalhães, P. *et al.* Age- and gender-specific reference values of pulse wave velocity for African adults: preliminary results. *AGE*, 1-11 (2013).

360. Norton, G.R. *et al.* Is prehypertension an independent predictor of target organ changes in young-to-middle-aged persons of African descent? *Journal of hypertension* **26**, 2279-2287 (2008).

361. Shang, Q., Tam, L., Li, E., Yip, G. & Yu, C. Increased arterial stiffness correlated with disease activity in systemic lupus erythematosus. *Lupus* **17**, 1096-1102 (2008).

362. Kim, W.J. *et al.* The association between regional arterial stiffness and diabetic retinopathy in type 2 diabetes. *Atherosclerosis* **225**, 237-241 (2012).

363. Turin, T.C. *et al.* Brachial–ankle pulse wave velocity predicts all-cause mortality in the general population: findings from the Takashima study, Japan. *Hypertension Research* **33**, 922-925 (2010).

364. Tsuchikura, S. *et al.* Brachial-ankle pulse wave velocity as an index of central arterial stiffness. *Journal of atherosclerosis and thrombosis* **17**, 658 (2010).

365. Ahimastos, A.A., Formosa, M., Dart, A.M. & Kingwell, B.A. Gender Differences in Large Artery Stiffness Pre- and Post Puberty. *Journal of Clinical Endocrinology & Metabolism* **88**, 5375-5380 (2003).

366. van der Heijden-Spek, J.J. *et al.* Effect of Age on Brachial Artery Wall Properties Differs From the Aorta and Is Gender Dependent: A Population Study. *Hypertension* **35**, 637-642 (2000).

367. De Angelis, L. *et al.* Sex Differences in Age-Related Stiffening of the Aorta in Subjects With Type 2 Diabetes. *Hypertension* **44**, 67-71 (2004).

368. Coutinho, T., Borlaug, B.A., Pellikka, P.A., Turner, S.T. & Kullo, I.J. Sex Differences in Arterial Stiffness and Ventricular-Arterial Interactions. *Journal of the American College of Cardiology* **61**, 96-103 (2013).

369. O'Rourke, M.F. & Safar, M.E. Sex Differences and Arterial StiffnessDie Methode ist Alles. *Journal of the American College of Cardiology* **62**, 258-258 (2013).

370. Weng, C. *et al.* Sex-Specific Association Between the Metabolic Syndrome and Arterial Stiffness in 8300 Subjects. *The American journal of the medical sciences* (2013).

371. Hickson, S.S. *et al.* The relationship of age with regional aortic stiffness and diameter. *JACC: Cardiovascular Imaging* **3**, 1247-1255 (2010).

372. Kimoto, E. *et al.* Preferential Stiffening of Central Over Peripheral Arteries in Type 2 Diabetes. *Diabetes* **52**, 448-452 (2003).

373. Nilsson, P.M. *et al.* Early vascular ageing in translation: from laboratory investigations to clinical applications in cardiovascular prevention. *Journal of hypertension* **31**, 1517-1526 (2013).

374. Lakatta, E.G. & Levy, D. Arterial and Cardiac Aging: Major Shareholders in Cardiovascular Disease Enterprises: Part I: Aging Arteries: A “Set Up” for Vascular Disease. *Circulation* **107**, 139-146 (2003).

375. Brun, E. *et al.* Diabetes duration and cause-specific mortality in the Verona Diabetes Study. *Diabetes care* **23**, 1119-1123 (2000).

376. Valensi, P., Paries, J. & Attali, J. Cardiac autonomic neuropathy in diabetic patients: Influence of diabetes duration, obesity, and microangiopathic complications—the French multicenter study. *Metabolism: clinical and experimental* **52**, 815-820 (2003).

377. Aso, K. *et al.* Brachial-Ankle Pulse Wave Velocity Is Useful for Evaluation of Complications in Type 2 Diabetic Patients. *Hypertension Research* **26**, 807-813 (2003).

378. Ahlgren, A.R., Sundkvist, G., Wollmer, P., Sonesson, B. & Länne, T. Increased aortic stiffness in women with type 1 diabetes mellitus is associated with diabetes duration and autonomic nerve function. *Diabetic medicine : a journal of the British Diabetic Association* **16**, 291-297 (1999).

379. Visscher, T.L. *et al.* A comparison of body mass index, waist-hip ratio and waist circumference as predictors of all-cause mortality among the elderly: the Rotterdam study. *International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity* **25**, 1730-1735 (2001).

380. van Dijk, S., van den Meiracker, A., van der Cammen, T., Mattace Raso, F. & van der Velde, N. Central but not peripheral fat mass percentage is associated with blood pressure components in the elderly. *Age and Ageing* **41**, 534-540 (2012).

381. Hayward, C.S., Kraidly, M., Webb, C.M. & Collins, P. Assessment of endothelial function using peripheral waveform analysis: A clinical application. *Journal of the American College of Cardiology* **40**, 521-528 (2002).

382. Chrusch, A.T. & Skolnik, N. A Review of "BMI-Mortality Paradox and Fitness in African American and Caucasian Men with Type 2 Diabetes". *Clinical Diabetes* **31**, 73-75 (2013).

383. Keith, S.W., Fontaine, K.R. & Allison, D.B. Mortality rate and overweight: Overblown or underestimated? A commentary on a recent meta-analysis of the associations of BMI and mortality. *Molecular Metabolism* **2**, 65-68 (2013).

384. Andres, R. Beautiful Hypotheses and Ugly Facts: The BMI-Mortality Association. *Obesity Research* **7**, 417-419 (1999).

385. Kengne, A.P., Njamnshi, A.K. & Mbanya, J.C. Cardiovascular Risk Reduction in Diabetes in Sub-Saharan Africa: What should the Priorities be in the Absence of Global Risk Evaluation Tools? *Clinical Medicine Insights: Cardiology* **2**, 25 (2008).

386. Gaillard, T., Schuster, D., Osei, K. & Reed, J. in Proceedings from the Global Summit on Cardio Renal Metabolic Syndrome in People of the African Diaspora, Columbus, Ohio, 2 December 2007., Vol. 19 (International Society on Hypertension in Blacks, 2009).

387. Osei, K. Metabolic syndrome in blacks: are the criteria right? *Current diabetes reports* **10**, 199-208 (2010).

388. Blaha, M.J. *et al.* Association Between Obesity, High-Sensitivity C-Reactive Protein≥ 2 mg/L, and Subclinical Atherosclerosis Implications of JUPITER from the Multi-Ethnic Study of Atherosclerosis. *Arteriosclerosis, thrombosis, and vascular biology* **31**, 1430-1438 (2011).

389. Myers Jr, M.G., Leibel, R.L., Seeley, R.J. & Schwartz, M.W. Obesity and leptin resistance: distinguishing cause from effect. *Trends in Endocrinology & Metabolism* **21**, 643-651 (2010).

390. Windham, B.G. *et al.* Influence of leptin, adiponectin, and resistin on the association between abdominal adiposity and arterial stiffness. *American journal of hypertension* **23**, 501-507 (2010).

391. Benkhoff, S. *et al.* Leptin potentiates endothelium-dependent relaxation by inducing endothelial expression of neuronal NO synthase. *Arteriosclerosis, thrombosis, and vascular biology* **32**, 1605-1612 (2012).

392. Álvarez, G. *et al.* The effects of adiponectin and leptin on human endothelial cell proliferation: a live-cell study. *Journal of vascular research* **49**, 111-122 (2012).

393. Fernández-Sánchez, A. *et al.* Inflammation, oxidative stress, and obesity. *International journal of molecular sciences* **12**, 3117-3132 (2011).

394. O'Rourke, M.F. & Nichols, W.W. Aortic Diameter, Aortic Stiffness, and Wave Reflection Increase With Age and Isolated Systolic Hypertension. *Hypertension* **45**, 652-658 (2005).

395. Franklin, S.S. *et al.* Hemodynamic Patterns of Age-Related Changes in Blood Pressure: The Framingham Heart Study. *Circulation* **96**, 308-315 (1997).

396. Albaladejo, P. *et al.* Heart Rate, Arterial Stiffness, and Wave Reflections in Paced Patients. *Hypertension* **38**, 949-952 (2001).

397. Williams, B., Lacy, P.S., Cafe & the, A.I. Impact of heart rate on central aortic pressures and hemodynamics: analysis from the CAFE (Conduit Artery Function Evaluation) study: CAFE-Heart Rate. *Journal of the American College of Cardiology* **54**, 705-713 (2009).

398. de la Sierra, A. Definition of White Coat Hypertension Ambulatory Blood Pressure, Self-Measured Blood Pressure, or Both? *Hypertension* **62**, 16-17 (2013).

399. Sumner, A.E. "Half the dsylipidemia of insulin resistance" is the dyslipidemia [corrected] of insulin-resistant Blacks. *Ethnicity & disease* **19**, 462-465 (2009).

400. Lacy, P.S. *et al.* Increased pulse wave velocity is not associated with elevated augmentation index in patients with diabetes. *Journal of hypertension* **22**, 1937-1944 (2004).

401. Ohnishi, H. *et al.* Pulse Wave Velocity as an Indicator of Atherosclerosis in Impaired Fasting Glucose: The Tanno and Sobetsu Study. *Diabetes care* **26**, 437-440 (2003).

402. Salomaa, V., Riley, W., Kark, J.D., Nardo, C. & Folsom, A.R. Non–Insulin-Dependent Diabetes Mellitus and Fasting Glucose and Insulin Concentrations Are Associated With Arterial Stiffness Indexes The ARIC Study. *Circulation* **91**, 1432-1443 (1995).

403. Emoto, M. *et al.* Stiffness indexes β of the common carotid and femoral arteries are associated with insulin resistance in NIDDM. *Diabetes care* **21**, 1178-1182 (1998).

404. Ogihara, T. *et al.* Angiotensin II-induced insulin resistance is associated with enhanced insulin signaling. *Hypertension* **40**, 872-879 (2002).

405. Samad, F., Pandey, M., Bell, P.A. & Loskutoff, D.J. Insulin continues to induce plasminogen activator inhibitor 1 gene expression in insulin-resistant mice and adipocytes. *Molecular Medicine* **6**, 680 (2000).

406. Nagareddy, P. *et al.* Inhibition of matrix metalloproteinase-2 improves endothelial function and prevents hypertension in insulin-resistant rats. *British journal of pharmacology* **165**, 705-715 (2012).

407. Boura-Halfon, S. & Zick, Y. Phosphorylation of IRS proteins, insulin action, and insulin resistance. *American Journal of Physiology - Endocrinology And Metabolism* **296**, E581-E591 (2009).

408. Saltiel, A.R. & Kahn, C.R. Insulin signalling and the regulation of glucose and lipid metabolism. (2001).

409. Zeng, G. *et al.* Roles for insulin receptor, PI3-kinase, and Akt in insulin-signaling pathways related to production of nitric oxide in human vascular endothelial cells. *Circulation* **101**, 1539-1545 (2000).

410. Safar, M.E. & Frohlich, E.D. (eds.) *Atherosclerosis, Large Arteries and Cardiovascular Risk*, Vol. 44. (S. Karger AG, Basel; 2007).

411. Ceradini, D.J. *et al.* Decreasing intracellular superoxide corrects defective ischemia-induced new vessel formation in diabetic mice. *The Journal of biological chemistry* **283**, 10930-10938 (2008).

412. Miersch, A. *et al.* Influence of seasonal variation on blood pressure measurements in children, adolescents and young adults. *Pediatric nephrology* **28**, 2343-2349 (2013).

413. Schutte, A.E. *et al.* Dimethylarginines: their vascular and metabolic roles in Africans and Caucasians. *European Journal of Endocrinology* **162**, 525-533 (2010).

414. Redelinghuys, M. *et al.* Lack of Independent Association Between C-Reactive Protein and Central Aortic Hemodynamics in Black Africans with High Risk of Cardiovascular Disease. *American journal of hypertension* **24**, 1094-1101 (2011).

415. Woodard, G.A. *et al.* C-reactive protein is associated with aortic stiffness in a cohort of African American and white women transitioning through menopause. *Menopause (New York, NY)* **18**, 1291 (2011).

416. Adeyemo, A. *et al.* A genome-wide association study of hypertension and blood pressure in African Americans. *PLoS genetics* **5**, e1000564 (2009).

417. Cappuccio, F.P. *et al.* Body size and blood pressure: an analysis of Africans and the African diaspora. *Epidemiology (Cambridge, Mass.)* **19**, 38 (2008).

418. Rotimi, C.N. *et al.* A genome-wide search for type 2 diabetes susceptibility genes in West Africans. *Diabetes* **53**, 838-841 (2004).

419. Heffernan, K.S., Jae, S.Y., Wilund, K.R., Woods, J.A. & Fernhall, B. Racial differences in central blood pressure and vascular function in young men. *American Journal of Physiology - Heart and Circulatory Physiology* **295**, H2380-H2387 (2008).

420. Li, R. *et al.* Relation of endothelial nitric oxide synthase gene to plasma nitric oxide level, endothelial function, and blood pressure in African Americans*. *American journal of hypertension* **17**, 560-567 (2004).

421. Flamant, M. *et al.* Role of Matrix Metalloproteinases in Early Hypertensive Vascular Remodeling. *Hypertension* **50**, 212-218 (2007).

422. Mercier, N. *et al.* Modifications of Arterial Phenotype in Response to Amine Oxidase Inhibition by Semicarbazide. *Hypertension* **50**, 234-241 (2007).

423. McEniery, C.M. *et al.* Aortic Calcification Is Associated With Aortic Stiffness and Isolated Systolic Hypertension in Healthy Individuals. *Hypertension* **53**, 524-531 (2009).

424. Karvestedt, L. *et al.* Peripheral sensory neuropathy associates with micro- or macroangiopathy: results from a population-based study of type 2 diabetic patients in Sweden. *Diabetes care* **32**, 317-322 (2009).

425. Ding, J. *et al.* Early retinal arteriolar changes and peripheral neuropathy in diabetes. *Diabetes care* **35**, 1098-1104 (2012).

426. Bril, V. & Perkins, B.A. Comparison of vibration perception thresholds obtained with the Neurothesiometer and the CASE IV and relationship to nerve conduction studies. *Diabetic Medicine* **19**, 661-666 (2002).

427. Jarso, G., Ahmed, A. & Feleke, Y. The prevalence, clinical features and management of periphral neuropathy among diabetic patients in Tikur Anbessa and St. Paul's

Specialized University Hospitals, Addis Ababa, Ethiopia. *Ethiopian medical journal* **49**, 299-311 (2011).

428. Mwendwa, F.M., Otieno, C.F., Kayima, J.K., Amayo, E.O. & Otieno, P.O. Risk factor profile and the occurrence of microvascular complications in short-term type 2 diabetes mellitus at Kenyatta National Hospital, Nairobi. *East Afr Med J* **82**, S163-172 (2005).

429. El-Nahas, M.R., Gawish, H.M.S., Tarshoby, M.M., State, O.I. & Boulton, A.J.M. The prevalence of risk factors for foot ulceration in Egyptian diabetic patients. *Practical Diabetes International* **25**, 362-366 (2008).

430. Roaeid, R. & Kadiki, O. Prevalence of long-term complications among Type 2 diabetic patients in Benghazi, Libya. *Journal of Diabetology* **3**, 5 (2011).

431. Ndip, E.A., Tchakonte, B. & Mbanya, J.C. A study of the prevalence and risk factors of foot problems in a population of diabetic patients in cameroon. *The international journal of lower extremity wounds* **5**, 83-88 (2006).

432. Bergenheim, T., Borssén, B. & Lithner, F. Sensory thresholds for vibration, perception and pain in diabetic patients aged 15–50 years. *Diabetes research and clinical practice* **16**, 47-52 (1992).

433. Yokoyama, H., Yokota, Y., Tada, J. & Kanno, S. Diabetic neuropathy is closely associated with arterial stiffening and thickness in type 2 diabetes. *Diabetic Medicine* **24**, 1329-1335 (2007).

434. Kim, E.S. *et al.* Diabetic Peripheral Neuropathy Is Associated With Increased Arterial Stiffness Without Changes in Carotid Intima–Media Thickness in Type 2 Diabetes. *Diabetes care* **34**, 1403-1405 (2011).

435. Edmonds, M., Morrison, N., Laws, J. & Watkins, P. Medial arterial calcification and diabetic neuropathy. *British medical journal (Clinical research ed.)* **284**, 928 (1982).

436. Young, M., Adams, J., Anderson, G., Boulton, A. & Cavanagh, P. Medial arterial calcification in the feet of diabetic patients and matched non-diabetic control subjects. *Diabetologia* **36**, 615-621 (1993).

437. Jeffcoate, W., Rasmussen, L.M., Hofbauer, L. & Game, F. Medial arterial calcification in diabetes and its relationship to neuropathy. *Diabetologia* **52**, 2478-2488 (2009).

438. Anuradha, S., Mohan, V., Gokulakrishnan, K. & Dixit, M. Angiopoietin-2 levels in glucose intolerance, hypertension, and metabolic syndrome in Asian Indians (Chennai Urban Rural Epidemiology Study-74). *Metabolism: clinical and experimental* **59**, 774-779 (2010).

439. Iribarren, C. *et al.* Circulating angiopoietins-1 and -2, angiopoietin receptor Tie-2 and vascular endothelial growth factor-A as biomarkers of acute myocardial infarction: a prospective nested case-control study. *BMC Cardiovasc Disord* **11**, 31 (2011).

440. Fiedler, U. & Augustin, H.G. Angiopoietins: a link between angiogenesis and inflammation. *Trends in immunology* **27**, 552-558 (2006).

441. Asahara, T. *et al.* Tie2 receptor ligands, angiopoietin-1 and angiopoietin-2, modulate VEGF-induced postnatal neovascularization. *Circ Res* **83**, 233-240 (1998).

442. Kim, K.A. *et al.* Dysfunction of endothelial progenitor cells under diabetic conditions and its underlying mechanisms. *Archives of pharmacal research* **35**, 223-234 (2012).

443. Lieb, W. *et al.* Vascular endothelial growth factor, its soluble receptor, and hepatocyte growth factor: clinical and genetic correlates and association with vascular function. *European heart journal* **30**, 1121-1127 (2009).

444. Malik, R.A. *et al.* Microangiopathy in human diabetic neuropathy: relationship between capillary abnormalities and the severity of neuropathy. *Diabetologia* **32**, 92-102 (1989).

445. Powell, H.C., Rodriguez, M. & Hughes, R.A.C. Microangiopathy of vasa nervorum in dysglobulinemic neuropathy. *Annals of neurology* **15**, 386-394 (1984).

446. Satchell, S.C., Anderson, K.L. & Mathieson, P.W. Angiopoietin 1 and vascular endothelial growth factor modulate human glomerular endothelial cell barrier properties. *Journal of the American Society of Nephrology* **15**, 566-574 (2004).

447. Yagihashi, S. *et al.* Neuropathy in diabetic mice overexpressing human aldose reductase and effects of aldose reductase inhibitor. *Brain : a journal of neurology* **124**, 2448-2458 (2001).

APPENDIX 1: Data capture tool

Section A: Demographic Data

Date……

A. Folder No…… Identification Number.....

NAME INITIALS			
Gender(Male=1;Female=2)		Date of birth/Age at last birthday(years)	

Marital Status (Married= 1; Not Married=2)	

Highest Educational Level Completed No formal Schooling=1; Less than Primary School=2; Primary School=3; Middle/JSS completed=4; SSS/A Level completed=5; Tertiary completed=6; Other=7, if so, specify…….	

Occupation Unskilled labourer/orderly/watchman=1; Technician/driver/Artisan/Tradesperson/Security=2; Clerical/Audit/Accounts/Messenger/Office worker=3; Nurse/Dietician/Technologist/Secretary/Transport or Estate officer/ Senior security officers=4; Lawyer/Doctor/Dentist/Architect/Senior Management/ Certified Accountant/Engineer=5; Business Person (Managerial Status) =6; Student=7; House wife=8; Other=9, if so, Specify……	
Employment Status: Employed=1 Unemployed=2	

Retirement Status: Normal Retirement and Not working=1; Normal Retirement but working =2; Not Retired=3; Early Retirement on Health grounds=4, if so, specify health reason……	

Residential Address……
Landmark to nearest to House……
Telephone……../……
Name of a relative/friend……**TEL(Mobile)**……

How long have you had diabetes? _____years_____months (if less than a year).	

Section B: Risk Factor Data

{For questions 8-11 Use 1 =Yes, and 2=No}

(1) Do you currently smoke cigarettes regularly? _____

a) if yes, how many sticks on average do you smoke a day? _____

b) How old were you when you first started to smoke? _____

(2) Have you ever smoked cigarettes? _____

a) if yes, how long did you smoke for?_____yrs _____ months

b) if yes, how many sticks did you smoke a day? _____

(3) Do you have a close relation who is a smoker? _____

a) if yes, who? _____ (eg. wife, sister, father, son, grandfather, etc)

(b) Did you live with that person(s)? _____ (Yes=1; No=2)

(4) Do you currently drink alcohol? _____

(a) if yes, how many drinks on average do you have in a week? _____

(b)if not that frequent, how many drinks in a month? _____

(Please note that 1 unit of alcohol = half a pint of beer, a tot of gin / whisky, a glass of wine)

Section C: Family History

{For questions 5 & 6 Use 1 =Yes, 2=No and 3=Don't know}

(5) Do you have anyone in your family (not a spouse) with diabetes? _____

if yes, who has _____(list all)

(6) Do you have anyone in your family with any of these conditions?

(a)Hypertension_____

(b)Stroke_____

(c)High blood, cholesterol or lipids_____

(d)Limb amputation that is not through an accident _____

(e)Coronary artery bypass surgery_____

(f)Angina_____

(g)Vascular surgery - Varicose veins? _____

(h) Heart attack_____

(i) Heart disease_____

(j) Gout_____

Section D: Past History

{For questions 7 & 8 Use 1=Yes, 2=No and 3=Don't know}

(7) Have you ever been told by a healthcare person that you have any of the following?

(a) Hypertension_____

(b) Heart attack_____

(c) Stroke_____

(d) Heart disease_____

(e) High blood cholesterol or lipids_____

(f) Limb amputation that is not through an accident_____

(g) Coronary artery bypass surgery_____

(h) Angina_____

(i) Gout_____

(j) Varicose veins_____

(k) Vascular surgery_____

(8) Have you ever had a toe/foot leg amputated?_____

(a) If yes, was it through an accident?_____

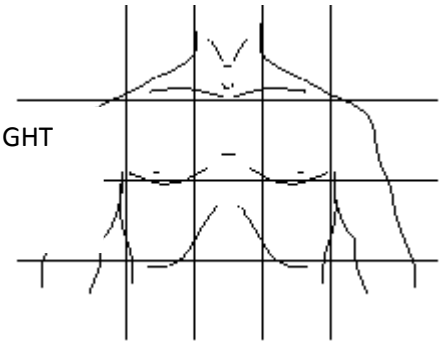
Section E: Chest Pain on Exertion

{For questions 9 a-h, Use 1 =Yes, 2=No and 3=Don't know}

(a) Do you ever have any pain or discomfort in your chest?___(If No, then continue from Q18)

(b) Where do you get this pain or discomfort? (please mark **x** on the appropriate place)

RIGHT



LEFT

(c) When you walk at an ordinary pace on the level does this produce the pain?_____

(d) When you walk uphill or hurry, does this produce the pain?_____

(e) Does the pain or discomfort in your chest go away if you stand still?___

(f) When you get any pain or discomfort in your chest on walking, what do you do? _____

(1=stop, 2= slow down, 3=continue at same pace, 4=not applicable)

(g) How long does the pain take to go away?____

(1=10 minutes or less, 2=more than 10 minutes)[1]

(h). Have you noticed anything that brings on the chest pain?_____

(i) If yes, what?_____

1=Exertion (rushing, climbing stairs, going uphill, running etc)

2=Emotion (excitement, anger, nightmares)

3=others if so specify _____

What relieves the pain? _____ (list all)

Section F : Peripheral vascular/neuropathy symptoms

{For questions 10(a-e) , Use 1 =Yes, 2=No and 3=Don't know}

(a) Do you have pain or discomfort in your leg when walking?_____

If yes, (NB: If No, then continue from Q28)

(b) Does this pain ever begin when you are standing still or sitting?___

(c)Do you get pain if you walk uphill or hurry?___

(d)Do you get pain if you walk at an ordinary pace on the level?____

(e)Does pain disappear within 10 minutes or less when you stand still?___

(f)What happens if you stand still?_____(1=pain goes away, 2=pain persist, 3=other, specify)

(g)Where do you get the pain or discomfort?_____(1=buttock, 2=thigh, 3=calf, 4=other, specify)

Section G: **Anthropometry/Physical Measurements**

(a) Height in (m) _____ Weight (kg) _____

(b) Body fat_____ Visceral fat_____ BMI_____ Resting Metabolism_____

(c) Waist girth 1 (cm) _____ Waist girth 2 (cm) _____

(d) Hip " 1 (cm)_____ Hip " 2(cm)_____

(e)SBP1_____ DBP1_____ Heart rate_____/min (rest for 10 minutes)

(f) SBP2_____ DBP2 _____ Heart rate_____/min

(g) SBP3_____ DBP3_____ Heart rate_____/min

What treatment do you currently take for your diabetes diet only=1; daonil/glibenclamide (other sulplonylurea)=2; metformin/Glucophage=3 daonil+metformin=4 insulin=5; thiazolidindione = 6 other=7 (combination?), specify_____	
Apart from your current treatment, have you had other treatment for diabetes? (yes=1; No=2)	
If yes, which treatment? diet only=1; daonil/glibenclamide (other sulplonylurea)=2; metformin/Glucophage=3 daonil+metformin=4 insulin=5; thiazolidindione = 6 other=7 (combination?), specify_____	
How long were you on that treatment? _____yrs____months (if less than a year)	

Section J: Medication History

Other Medications

Medication	**Yes/No**
Anti hypertensive	
ACEi/ARBs	
B-blocker	
Diuretic	
Calcium channel blockers	
Centrally acting	
Antiplatelet	
Statin	
Anti obesity drugs	
Herbal medication	
Hematenics/vitamins	
Other, specify	

APPENDIX 2: Consent form

Participant ID Number: _____ Participant Name: _____

Study Title: Endothelial Dysfunction in Type 2 Diabetes Subjects

Diabetes is an important cause of diseases of the heart and tubes that carry blood (blood vessels). Subjects with Diabetes also tend to develop problems with inner lining (endothelium) of special blood vessels called arteries, leading to the formation of fatty blockages (atherosclerosis) and stiffness in the arteries. How important problems with endothelium (endothelial dysfunction) and stiffness in the arteries are in Ghanaians with diabetes is not known. Also, the factors that cause these conditions in Ghanaians with diabetes need to be determined. Doctors and scientists from the National Diabetes Management and Research Centre, Korle Bu, Accra are conducting a research to find out how many persons with diabetes compared to those without diabetes have endothelial dysfunction and arterial stiffness.

You are to understand that taking part in this research is entirely voluntary. You are further to note that you may refuse to take part or withdraw from the study at any time without anyone objecting.

You are likely to spend the best part of the morning at the Diabetes Centre. For you to qualify to be part of this research, you should be between the ages of 35 and 65 years. If you want to take part in the research, you would be asked to come to the hospital after you have fasted overnight for 8-12 hours and report to the Diabetes Clinic at 7:00 in the morning. We will ask you to provide information about yourself, your health and that of your family. You may feel uncomfortable providing such information. In addition, your blood pressure, height, weight and amount of fat in your body will be measured. In addition, some special medical equipment that measure blood pressure and stiffness of the blood vessel will be applied to your arms and legs. These procedures are painless and might give slight tingling sensations for few seconds when the cuffs inflate. Also, amount of blood equivalent to 3 teaspoonfuls will be drawn to measure substances in the bloodstream that may be abnormal if you have endothelial dysfunction or stiffness of the arteries. This amount of blood is not very different from what you will normally be asked to provide when you first come to our centre. You are assured that this amount will not affect your health. You may be asked to take a glucose drink if you do not already have diabetes to find out if you have diabetes or not. All the tests we will do for you in connection with this research will be free of charge.

Information we collect on you in this study will be kept confidential and secure. The information will only be available to the doctors and scientists conducting this study. You are further assured that if a report of this study is prepared for the scientific and medical community you will not be identified by name.

You may experience a minor bruise and/or temporary discomfort at the site of the blood draw and this risk is no more than you will normally be exposed to for having a blood draw routinely at our hospital. We will reduce the discomfort by asking experienced staff to take the blood. All your test results will be explained to you. As you take part in this study, you will know you have atherosclerosis, stiff arteries or diabetes if you do not already know and you will be referred to a doctor for further testing and management. The study will, help us appreciate the importance of the

above conditions in Ghanaians with diabetes. The research may also help us put in place strategies to screen for these conditions to allow early treatment.

Is there something you do not understand or do you have any questions or concerns about this Research? Should you later wish to have any matter or question relating to this study clarified do not hesitate to contact Mr. Kwame Yeboah, Department of Physiology, University of Ghana Medical School, P.O. Box 4236, Accra. (Tel number, 024 17 855 61), the principal investigator for this study, or Prof Albert Amoah, the Director of the Diabetes Management and Research Centre, to answer any questions you may have.

CONSENT

I have fully explained to _____ the nature and purpose of the above described research, its procedures, risks and benefits. I have allowed the subject to ask questions and have answered and to the best of my ability, all questions relating to the study.

_____ _____ _____

Signature Full Name of Staff Member Date

I _____, have read (or have had read to me in a language that I fully understand) the proposed study and that I have understood what is going to be done. Also, any concerns I have, have fully been addressed. My signature or thumbprint below indicates that I have understood what is going to be done and that I agree to take part in the study.

_____ Date:_____

(Signature/thumbprint of Subject)

_____ Date:_____

(Signature: Witness)

APPENDIX 3: ETHICAL APPROVAL