CAROTID FEMORAL PULSE WAVE VELOCITY AND CENTRAL BLOOD PRESSURE IN NORMOTENSIVE AND HYPERTENSIVE PARTICIPANTS AT THE UNIVERSITY TEACHING HOSPITAL IN LUSAKA, ZAMBIA.

BY

FESTUS MUSHABATI

BACHELOR OF VETERINARY MEDICINE (B.V.M), UNIVERSITY OF ZAMBIA.

A dissertation submitted to the University of Zambia in partial fulfillment of the requirements of the Masters of Science degree in Human Physiology.

THE UNIVERSITY OF ZAMBIA
SCHOOL OF MEDICINE
DEPARTMENT OF PHYSIOLOGICAL SCIENCES
LUSAKA

DECLARATION

I, FESTUS MUSHABATI, do hereby declare that this dissertation is a product of my own original work. I further attest that it has not been accepted in any previous application for the award of any qualification in any other institution (University of Zambia inclusive) and that all sources of information have been specifically acknowledged by means of acceptable referencing in accordance with the University of Zambia regulations.

Festus Mushabati (Candidate).

Signed ……..... Date.....

NOTICE OF COPYRIGHT

CERTIFICATE OF COMPLETION OF DISSERTATION

I, **FESTUS MUSHABATI**, do hereby declare that this dissertation is a product of my own original work. In submitting it for my MSc Human Physiology programme, I further attest that it has not been accepted in any previous application for the award of any qualification in any other institution (University of Zambia inclusive).

Signature..... Date.....

I, **FASTONE MATHEW GOMA (DR)**, having supervised and read this dissertation is satisfied that this is the original work of the author under whose name it is being presented. I confirm that the work has been completed satisfactorily and is ready for presentation to the examiners.

Supervisor's signature……Date……

Head of Department

Signature…… Date……..

Department of Physiological Sciences, School of Medicine, University of Zambia.

CERTIFICATE OF APPROVAL

This dissertation by **FESTUS MUSHABATI** has been approved as partially fulfilling the requirements for the award of the degree of Masters of Science in Human Physiology by the University of Zambia.

---------- ----------

Supervisor (DR F.M. GOMA) Signature

Examiner I ---------- Sign ---------- Date ----------

Examiner II ---------- Sign ---------- Date ----------

Examiner III ---------- Sign ---------- Date ----------

Head of Department

Signature..... Date.....

Department of Physiological sciences, School of Medicine, University of Zambia.

ABSTRACT

Carotid femoral pulse wave velocity and central blood pressure in normotensive and hypertensive participants at the UTH in Lusaka, Zambia.

Background and aim: Carotid femoral pulse wave velocity (cfPWV) is considered as the gold standard for directly estimating central arterial stiffness. Furthermore, the simultaneous measurement of central blood pressure (CBP) with cfPWV is augmentative. This is because the two are strong and independent predictors of cardiovascular events and mortality including hypertension in any given population. The present study sought to measure cfPWV and CBP in a population of Zambian normotensive (NT) and hypertensive participants (both treated hypertensives (HTC) and untreated hypertensives (HTN)) between 30 – 65 years of age.

Method and results: Carotid femoral PWV and CBP were simultaneously measured in 146 participants. A Complior® Analyse device (Version 1.9 Beta 2013; ALAM-Medical, France) was used by noninvasively accessing superficial pulses over the carotid– femoral segment. Major findings were that cfPWV values in HTN participants (N=23) were significantly higher than in NT (N=64) participants (11.4±4.2 vs 9.1±3.2, p=0.009). As for HTC participants (N=59), their cfPWV values (10.4±5.6) tended to approach those in HTN participants, with no statistical differences between the two. Furthermore, the mean cfPWV found in NT participants was regrettably higher than any found in previous studies. Carotid femoral PWV did not have a significantly age – related increase in all three blood pressure categories ($p > 0.100$, $R^2 < 0.08$). The same trend of results was observed for CBP values.

Conclusion: These findings show that central arteries (aorta, carotid, etc) of hypertensive participants were stiffer and thus less compliant than those in normotensive participants. This meant a poor BP control in the patients on treatment implying a further substantial risk of developing a major cardiovascular disorder like stroke considering their high cfPWV and central blood pressures.

Key words: carotid femoral pulse wave velocity, arterial stiffness, hypertension, central blood pressure, Zambian.

DEDICATION

This Dissertation is dedicated to:

My parents: Mr. Muselu Mushabati, Mrs. Alice Mushabati and Beauty Kaseba

For bringing me up a tough young man

My lady Mizinga and our daughter Tabiso as well as Cousins (Mr. and Mrs. Mayamba Jnr)

For believing in me

My dear friends

Whose hardwork laid a basis for part of my motivation to complete this work.

My Strength and Provider

Our Lord Jehovah

ACKNOWLEDGEMENTS

I thank our Almighty God for allowing me to complete this work in good health.

I sincerely thank my employers, The Copperbelt University, and MEPI for their financial support, without whom this research would not be possible.

Iam most grateful to my family and fiancée for their support throughout this work.

In addition I render my gratitude to my supervisor and mentor, Dr Fastone Mathew Goma, (Dean, School of Medicine), for his productive guidance and critiquing throughout this work.

Furthermore, I also thank the Nurses in clinic 5 of the UTH and technical staff of the Department of Physiological Sciences for their hardwork in being research assistants.

Lastly but not the list, I thank my colleagues especially Dr. Lukubi Lwiindi for being a consistent and good friend at work and socially. I also thank Prof Seter Siziya and Mr. Chester Kalinda, colleagues and Biostatisticians' at Copperbelt University, for their technical help with data analysis.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AC..... Arterial compliance

Aix..... Augmentation index

ANOVA…….....….....…. Analysis of variance

AP..... Amplification pressure

aPWV..... Aortic or carotid femoral pulse wave velocity

BP categories..... Blood pressure categories (NT, HTC and HTN).

BP..... Blood pressure

C..... Compliance; A measure of volume change (ΔV) in response to a change in blood pressure (ΔP); C = ΔV/ΔP)

CBP..... Central or aortic blood pressure

cfPWV..... Carotid femoral or aortic pulse wave velocity

CVD..... Cardiovascular disease

DBP..... Diastolic blood pressure

ERES Converge..... Ethical review board IRB, Zambia.

ESH/ESC..... European Society for Hypertension/European Society for Cardiologists for the management of arterial hypertension

HT..... Hypertension or hypertensive participant(s)

HTC..... Treated hypertensive participants

HTN..... Untreated hypertensive participants

MAP..... Mean arterial pressure or mean blood pressure

mmHg.....	Millimeters of mercury
NT	Normotensive control participants
P value or α.....	Level of statistical significance which is 0.05 or less.
PP	Pulse pressure
PWV.....	Pulse wave velocity
r.....	Pearson's coefficient of correlation
R^2	Coefficient of determination for the best fit line
SBP.....	Systolic blood pressure
SD…….....	Standard deviation
SOM…….	School of Medicine
SPSS.....	Statistical Package for Social Sciences
BMI.....	Body mass index
UNZA……..	University of Zambia
UNZABREC……...	University of Zambia Biomedical research ethics committee
UTH.....	University Teaching Hospital
WHO……..	World Health Organisation

DEFINITION OF KEY OPERATIONAL TERMS

Arterial compliance -	The reduced capability of an artery to expand or distend (during systole) and contract/recoil (during diastole) in response to arterial blood pressure changes.
Compliance -	A measure of volume change (ΔV) in response to a change in blood pressure (ΔP); $C = \Delta V/\Delta P$)
Carotid femoral or aortic pulse wave velocity -	The speed at which pulse wave travels on an arterial segment that is from the aorta or carotid artery to the femoral artery when the heart contracts in systole; it is a direct index of central arterial stiffness.
Zambian –	A citizen of Zambia (predominantly black in skin colour).
Central or aortic blood pressure -	The pressure in the main large elastic arteries (aorta, carotids, etc)
Normotensive participants -	Control participants whose brachial systolic and diastolic pressures were ≤ 139/89 mmHg), between 30 – 65 years and those who have never suffered any overt CVD disorder.
Hypertensive participants -	Participants whose brachial systolic and diastolic pressures were ≥ 140/90 mmHg or were taking anti-hypertensive drugs.

Chapter One
INTRODUCTION

1.1 Background

Pulse wave velocity (PWV) is the speed at which pulse wave travels on an arterial segment when the heart contracts in systole (Mackenzie et al., 2002a). In elastic arteries such as the aorta, common carotid and other large arteries, the pulse wave travels slowly, whilst in stiffer arteries, the pulse wave travels faster partly due to the increase in the number of reflection sites as it is in the muscular peripheral arteries (Mancia et al., 2007b, Laurent et al., 2006). Carotid-femoral PWV (cfPWV) is the speed at which pulse wave travels on an arterial segment that is from the carotid artery to the femoral artery when the heart contracts in systole. Cf PWV also called aortic PWV (aPWV) is used as a direct index of central arterial stiffness due to its' ability to independently predict cardiovascular morbidity and mortality in the general population including normotensive subjects and hypertensive patients (Mancia et al., 2007b, Laurent et al., 2006).

Arterial stiffness is described as the hardening of the arterial walls due to endothelial wall dysfunction as well as loss of elastin, an elastic fibre that keeps large elastic arteries such as the aorta and the carotids compliant. Compliance (C) is a measure of volume change (ΔV) in response to a change in blood pressure (ΔP); $C = \Delta V/\Delta P$). In a stiff vessel the volume change, and therefore compliance, is reduced for any given pressure change. Thus the loss of elastin in the large elastic fibres (increased central arterial stiffness) results into decreased arterial compliance which is also described as the reduced capability of an artery to expand or distend (during systole) and contract/recoil (during diastole) in response to arterial blood pressure changes (Mackenzie et al., 2002a, Cecelja and Chowienczyk, 2012).

The stiffening of arterial walls has been shown to increase physiologically with age as well as with cardiovascular related disorders such as hypertension, cardiac failure, diabetes mellitus and stroke (Nichols and O'Rourke, 2005). Of the various methods for the non invasive measurement of PWV, only carotid-femoral pulse wave velocity is

recognised as the gold standard for measuring aortic or central stiffness (Mancia et al., 2007b, Laurent et al., 2006). The joint European Society for Hypertension/European Society for Cardiologists for the Management of Arterial Hypertension (Reference Values for Arterial Stiffness, 2010) states that a cf PWV above 12 m/s predicts a high cardiovascular risk including hypertension. Furthermore, elevated cfPWV (and thus elevated central arterial stiffness) is a major cardiovascular risk independent of other usual risk factors. It has been shown that if PWV is improved, cardiovascular risk decreases (Guerin et al., 2001). For these reasons, aortic stiffness assessment with PWV is now a recommended procedure by several researchers and clinicians in developed countries in managing hypertension (Reference Values for Arterial Stiffness, 2010, Mancia et al., 2007b).

Central blood pressure (CBP) is the pressure in the main large arteries (aorta, carotids, etc) and peripheral blood pressure is pressure in the peripheral arteries (brachial, femoral, radial, etc). Central blood pressure is also described as the pressure the heart has to pump against to get blood to flow to the rest of the body (Roman et al., 2007). Higher central blood pressures mean that the heart must work harder to do its job (by overcoming the high afterload). This can eventually lead to left ventricular hypertrophy and heart failure. Central blood pressure also determines the pressure in the blood vessels feeding the brain. If central pressure is too high, it may cause aneurysms and strokes. Central BP represents the pressure load of target organs such as the heart, the brain and the kidneys (Wang et al., 2009). However, central blood pressure (central systolic pressure, central pulse pressure and central mean arterial pressure) is not equal to peripheral blood pressure (peripheral systolic pressure, peripheral pulse pressure and peripheral mean arterial pressure) measured with a standard cuff (sphygmomanometre) in the clinic (Roman et al., 2007). This is due to the peripheral site being closer to locations from which pulse waves reflect. Infact, it has been shown that central pressure has a superior cardiovascular prognostic value compared to brachial pressure in that, like cf PWV, it is an independent predictor of cardiovascular outcomes including hypertension and end organ damage (Roman et al., 2009, Roman et al., 2007, Agabiti-Rosei et al., 2007)

Hypertension is defined as sustained systolic blood pressure (SBP) of 140 mm Hg or more, or diastolic blood pressure (DBP) of 90 mm Hg or more, or taking antihypertensive medication (Mancia et al., 2013, Mancia et al., 2007b). It is classified as either primary (essential) hypertension or secondary hypertension; about 90–95% of cases are categorised as primary hypertension which means high blood pressure with no obvious underlying medical cause. The remaining 5–10% of cases categorised as secondary hypertension is caused by other conditions that affect the kidneys, arteries, heart or the endocrine system. Based on recommendations of the 2013 ESH/ ESC guidelines for the Management of hypertension (Mancia et al., 2013), the classification of BP (office measured blood pressure) for adults aged 18 years or older has been as follows:

Optimal: Systolic lower than 120 mm Hg, diastolic lower than 80 mm Hg

Normal: Systolic 120 – 129 mm Hg, diastolic 80 - 84 mm Hg

High Normal: Systolic 130-139 mm Hg, diastolic 85-89 mm Hg

Stage 1 (mild): Systolic 140-159 mm Hg, diastolic 90-99 mm Hg

Stage 2 (moderate): Systolic 160 - 179 mm Hg, diastolic 100 - 109 mm Hg

Stage 3 (severe): Systolic over 180 mm Hg, diastolic 110 mm Hg or greater

Isolated systolic hypertension: Systolic equal or greater than 140 mmHg and diastolic less than 90 mmHg.

In addition, the severity of isolated systolic hypertension is also classified based on the systolic BP shown above as grade 1, 2 and 3. The current routine methods for evaluating hypertension (use of sphygmomanometre to measure patient BP, electrocardiography, laboratory diagnosis, medical history, physical examination and CVS risk factor analysis) in Zambia, Africa and most of the world at large still fall short of detecting both arterial stiffness or central blood pressure.

While the presence of collagen and elastin determine the intrinsic stiffness of the arterial wall and its ability to expand and recoil respectively, PWV (and thus arterial stiffness) also depends on the pressure exerted by blood on the wall and vice versa. Arterial stiffness increases at higher loading pressures and so does blood pressure with increasing arterial stiffness. Theoretically, this is because stress is transferred from

elastin to collagen fibres. In vivo arterial stiffness is increased by an acute rise in central blood pressure. Furthermore, stiffness is greater in hypertensive individuals when compared with age-matched controls or normotensive subjects. Sustained systolic blood pressure or hypertension may also accelerate structural changes to the arterial wall, particularly in hypertensive individuals in whom blood pressure therapy does not normalize arterial stiffness. In the presence of structural change (stiff arteries), peripheral blood pressure reduction might be expected to have less impact on arterial stiffness (Cecelja and Chowienczyk, 2012, Benetos, 2002).

In Zambia, less is known about cfPWV (central arterial stiffness) nor has central BP been investigated despite the high prevalence of hypertension being at 34.8% in urban areas (Goma et al., 2011). Therefore, the present study sought to investigate cfPWV and central BP in a population of Zambian normotensive and hypertensive participants. The results of the study would further become the basis for further research and reason to implement both cfPWV and CBP measurements alongside the use of sphygmomanometre in the management of hypertension. Apart from predicting future CVS events in normotensive subjects, this would ensure the optimum selection of anti-hypertensive therapy that would decrease both arterial stiffness (elevated cfPWV) and central BP as opposed to the current traditional approach that mainly aims to lower peripheral BP only in the hypertensive subjects.

1.2 Research questions

1. What are the cfPWV values in a population of Zambian normotensive and hypertensive participants at the UTH?
2. What are the CBP values in a population of Zambian normotensive and hypertensive participants at the UTH?
2. Are there any differences in the cfPWV and central BP values respectively of normotensive and hypertensive individuals seeking medical care at the UTH?
4. How do central blood pressure values of an individual compare with his/her corresponding peripheral blood pressure values?

1.3 Justification/Rationale

The current study sought to investigate aortic PWV and central BP in a population of Zambian normotensive subjects and hypertensive individuals. Information from this study would provide valuable insights into the pathophysiology and management of hypertension and other cardiovascular conditions. Further, the measurement of central arterial stiffness as well as central pressure may help predict the development of future cardiovascular outcomes especially hypertension in the 30 to 65 year olds. In addition, the results from this study may provide an informed basis for Zambian Cardiologists, Pharmacologists and Physiologists to recommend for the side by side use of cfPWV/central pressure with cuff pressure measurements in the clinical management of hypertension and stroke as included in the 2007 ESH/ ESC guidelines for the Management of hypertension. In the case of hypertensive and/or geriatric patients the results shall help to develop more effective treatment options to reduce both central arterial stiffness and central pressure thereby improving their prognosis as opposed to the traditional current approach which mainly aims to lower peripheral blood pressure only.

1.4 Objectives

1.4.1 Main objective

The main objective of this study was to determine carotid – femoral PWV and central BP respectively in a population of Zambian normotensive subjects (NT; brachial BP $\leq$ 139/89 mm Hg) and hypertensives individuals (untreated hypertensives (HTN); brachial BP $\geq$ 140/90 mm Hg or treated hypertensives (HTC); those on anti-hypertensive drugs) between 30 – 65 years of age.

1.4.2 Specific objectives

(i) To determine carotid – femoral PWV in Zambian normotensive subjects and hypertensive patients between 30– 65 years of age.

(ii) To determine CBP in Zambian normotensive subjects and hypertensive patients between 30– 65 years of age.

(iii) To compare cf PWV and central blood pressure in normotensive control subjects and hypertensive patients in the age categories.

(iv) To determine the relationship between central BP indices and their corresponding peripheral BP values in normotensive subjects and hypertensive individuals respectively.

Chapter Two

LITERATURE REVIEW

2.1 Pulse Wave Velocity and Central Blood Pressure

2.1.1 Pulse wave propagation, reflection and central blood pressure indices

During systole, blood is pushed into the aorta under considerable pressure generating a pulse wave. The most accepted model of the arterial tree is a propagative model (Windkessels model). This consists of a visco-elastic tube whose distributed elastic properties permit generation of a forward pressure wave from the aorta (Koelwyn et al., 2012). This wave then travels along the tube or arteries and whose numerous branch points and high level of resistance of tube's end (peripheral arteries and arterioles have highest arterial resistance) generate retrograde waves (Nichols and O'Rourke, 2005, Laurent et al., 2006). Note that the higher the arterial stiffness, the higher the speed of travel of forward and retrograde waves (higher PWV). Thus the shape of the pulse waveform is the result of the summation of a direct wave and reflected wave (retrograde wave) which both propagate along the arterial tree. The reflected wave is created by bifurcations, diameters changes, thus by impedance changes. To a first approximation, it can be considered that the reflected wave comes mainly from the lower body. Direct and reflected waves change shape during their travel along the arteries depending on the vessels characteristics.

In the aorta and carotid, both elastic arteries, direct and reflected wave are subject to Windkessel effect (energy is stored during systole for later restitution during the diastolic phase of the cardiac cycle). On the other hand, subclavian and brachial arteries are muscular arteries where only the propagation in tubes of decreasing diameter is to be taken into account. As a result, shapes of aortic and brachial pressure waveforms are very different leading to a difference in central and peripheral systolic pressure values. In addition, there is an amplification of pressures from the aorta or carotid toward the periphery (brachial) depending on vascular characteristics as discussed above (Koelwyn et al., 2012). In general, the amplification is higher in young healthy adults while it tends to decrease with age

and cardiovascular diseases because central pressures begin to approach those in the periphery. Furthermore, with increased arterial stiffness, as observed in older, black and hypertensive participants, the reflected wave travels more rapidly along the arterial tree. Thus, both small and large arteries contribute to early reflected waves (mainly due to increased total peripheral resistance) which arrive in early systole, superimpose on the forward wave, and boost the systolic blood pressure further, whereas diastolic blood pressure remains constant with only minor fluctuations (Cecelja and Chowienczyk, 2012). This in turn increases the afterload the left ventricle has to overcome in the aorta. Thus, because of pulse pressure amplification between central and peripheral arteries, it is inaccurate to use either brachial pulse pressure or brachial systolic pressure as surrogates for aortic or carotid pulse or systolic pressure to predict adverse CVD events including hypertension (Agabiti-Rosei et al., 2007).

2.2 Carotid femoral PWV (cfPWV)

The ideal methodology to obtain both PWV and CBP is with an invasive line. However this is not feasible in clinical routine and non invasive methods are often used instead (Pini et al., 2008, Palatini et al., 2011). Indeed, with similar geometry and structure and their close proximity, carotid and aortic arteries have virtually identical pressure waveforms and thus they are used interchangeably in this document as either carotid femoral PWV or aortic PWV (Koelwyn et al., 2012). Arterial stiffening and its hemodynamic consequences can be easily and reliably measured using a range of noninvasive techniques (Palatini et al., 2011). Of these methods, carotid-femoral pulse wave velocity (cf PWV) is considered the gold standard for measuring arterial stiffness because it is a well-recognized predictor of adverse cardiovascular outcomes including increased systolic blood pressure and pulse pressure in ageing and disease (Mancia et al., 2007a, Palatini et al., 2011).

Carotid-femoral PWV is a direct measurement of central arterial stiffness, and it corresponds to the widely accepted propagative model of the arterial system described above. Measured along the aortic and aorto-iliac pathway, it is the most clinically relevant, since the aorta and its first branches are what the left ventricle

(LV) ‘sees’ and are thus responsible for most of the pathophysiological effects of arterial stiffness. In addition, carotid-femoral PWV has been used in the epidemiological and clinical physiological studies demonstrating the predictive value of aortic stiffness for CV events. In contrast, PWV measured outside the aortic track, at the upper (brachial PWV) or lower limb (femoro-tibial PWV), has been said to have less predictive value in patients with end-stage renal disease (ESRD) and hypertension (Mancia et al., 2007a, O'Rourke and Nichols, 2005, Palatini et al., 2011, Mackenzie et al., 2002b).

The Complior Analyse System (Colson, Les Lilas, France) is often used and this employs dedicated mechanotransducers directly applied on the skin. This device is able to measure both cf PWV and Central BP simultaneously. The transit time is determined by means of a correlation algorithm between each simultaneous recorded wave. The operator is able to visualize the shape of the recorded arterial waves and to validate them. This system has been used in most of the epidemiological studies demonstrating the predictive value of PWV for CV events.

The assessment of PWV involves recording pulse waves at two different arterial sites that is carotid and femoral arterial sites, for a minimum of 10-15 seconds, to ensure measurement across at least one respiratory cycle (Van Bortel et al., 2002). PWV is usually measured using the foot-to-foot velocity method from various waveforms. These are usually obtained, transcutaneously at the right common carotid artery and the right femoral artery (i.e. ‘carotid-femoral’ PWV), and the time delay (Dt or Δt or transit time) measured between the feet of the two waveforms (Figure 1).

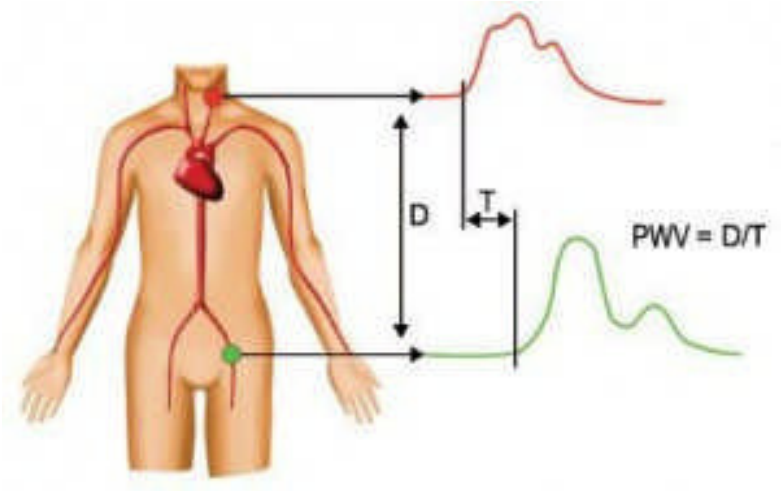


Figure 1. Measurement of carotid-femoral PWV with the foot to foot velocity method. Modified from (Laurent et al., 2006). D represents the transcutaneous distance from the carotid artery to the femoral artery on same side; T represents transit time from the foot of the carotid artery pulse to the foot of the femoral artery pulse.

Carotid femoral PWV is calculated using the following equation (Reference Values for Arterial Stiffness, 2010):

$$\text{CF PWV (m/s)} = \frac{\text{Carotid-Femoral Distance (D)}}{\text{Transit time (T or } \Delta t)} * 0.8$$

Where 0.8 is the correction factor for overestimation of cf PWV, D is the distance between measurement sites (C = carotid and F = femoral arteries sites) in meters and Δt is the pulse transit time in seconds.

2.2.1 PWV measure of distance

Several methodologies have been used to assess the length of the carotid-femoral pathway. From studies carried out with MRI, the best measure of CF distance is by multiplying the direct carotid-femoral distance by a correction factor of 0.8 (Reference Values for Arterial Stiffness, 2010). This is the methodology recommended in the latest Arterial stiffness consensus and the one selected by default at software installation of Complior analyse device. Thus the CF distance can be measured in a straight line between the carotid site in the neck region and the femoral site in the mid-

inguinal region with the participant in supine position, both on the right side, with a tape measure after which it is multiplied by the correction factor of 0.8. This method was preferred to others because it is the one with published reference cf PWV values in most studies (Reference Values for Arterial Stiffness, 2010).

2.2.2 Transit time and PWV algorithm

Several methodologies for the detection of the foot of the pulse can been used to assess the transit time and PWV. The intersecting tangent algorithm is the one now recommended by the European Heart Journal (Reference Values for Arterial Stiffness, 2010). It has been shown to be the methodology with the least 'beat' to 'beat' variability (Nichols and O'Rourke, 2005). This is the PWV algorithm selected by default at software installation. The pulse transit time is determined as the time delay between the arrival of the pulse wave at the two arterial sites, and is calculated using the following equation:

TT (transit time or Δt in seconds) **= T2 – T1**

Where **T2** is the pulse arrival time at the distal (femoral or radial) site and **T1** is the pulse arrival time at the proximal (carotid) site. The most commonly used method for estimating transit time is the foot-to-foot method (figure 1). The foot of the wave is defined at the end of diastole, when the steep rise of the waveform begins. The transit time is the time of travel of the foot of the wave over a known distance. Time at each site can be determined online or offline (Reference Values for Arterial Stiffness, 2010).

2.3 Challenges and limitations

Challenges and limitations with the femoral pressure waveform may include difficult to record accurately in participants with metabolic syndrome, obesity, diabetes, and peripheral artery disease. In the presence of aortic, iliac, or proximal femoral stenosis, the pressure wave may be attenuated and delayed. Abdominal obesity, particularly in men, and large breast size in women can make distance measurements inaccurate. The measurement of distance along the surface of the body is not a true anatomical representative of the arterial segment pathway and

therefore can introduce error into the PWV calculation. Pulse wave measurements at the two arterial sites should be collected simultaneously. (Koelwyn et al., 2012).

While arterial stiffening is primarily attributed to modifications to the intrinsic structure of the vessel especially with ageing and CVS disorders (O'Rourke and Hashimoto, 2007), several lifestyle factors and diseases can transiently decrease or increase pulse wave velocity and hence arterial stiffness. Caffeine consumption, smoking and resistance or strength exercises have been shown to temporarily increase arterial stiffness. On the contrary, alcohol consumption, food consumption, and aerobic exercise transiently decrease arterial stiffness. Chronic exposure to these factors, however, can lead to more permanent changes in arterial stiffness. Elevated resting arterial stiffness is observed in habitual smokers and individuals who consume excess caffeine and alcohol. Conversely, individuals who are habitually active, or who undergo an exercise training program are capable of attenuating or reversing age associated increases in arterial stiffness. Resting arterial stiffness is also affected by time of day, with larger arterial diameters (high arterial compliance and low arterial stiffness) and lower blood pressures reported at night (Koelwyn et al., 2012).

Traditional risk factors for cardiovascular disease are associated with increased arterial stiffening as observed in obesity, type 2 diabetes, hypertension, and hypercholesterolemia. Not surprisingly, elevated arterial stiffness is present in individuals with cardiovascular diseases such as coronary artery disease, heart failure and stroke (Weber et al., 2004, Koelwyn et al., 2012).

Chapter Three

MATERIALS AND METHODS

3.1 Study setting, study design, study population and inclusion/exclusion criteria.

This was a cross-sectional study involving 178 participants aged 30 to 65 years. Data was collected over a period of four weeks. Hypertensive participants (untreated hypertensive patients (HTN); brachial BP ≥ 140/90 mm Hg or treated hypertensive individuals (HTC); those on anti-hypertensive drugs) were volunteers visiting clinic 5 of the University Teaching Hospital. Normotensive subjects (NT; brachial BP ≤ 139/89 mm Hg) included people who escorted their relatives and friends to the hospital. Those with any known major cardiovascular disease including diabetes other than hypertension were excluded from the study.

3.2 Sample size determination

The sample size was calculated as shown below using equations of equal proportions with the following assumptions: Based on expected mean carotid pulse wave velocity (cfPWV) of 12m/s and 8m/s in hypertensive and normotensive participants respectively from previous studies (Reference Values for Arterial Stiffness, 2010), we needed to enroll 89 participants from each group in order to have 80% power to detect a 4m/s difference in cfPWV between the two using α=0.05. Simple random sampling was used in which every second participant was picked in either group.

The sample size was calculated as follows:

Proportions in two groups

- $N = \dfrac{[u\sqrt{\pi_1(1-\pi_1)+\pi_0(1-\pi_0)} + v\sqrt{2\pi(1-\pi)}]^2}{(\pi_0-\pi_1)^2}$

Where;

- N = sample size of **each** group
- π_0, π_1 Proportions (mean aortic PWV in hypertensives and normotensives respectively as obtained from other studies),
- π= Average of the proportions
- u = **0.84** for **80%** power
- v = Z statistic = **1.96** at α =0.05 (for a two tailed test)

Proportions in two groups

- $N = \dfrac{[u\sqrt{\pi_1(1-\pi_1)+\pi_0(1-\pi_0)} + v\sqrt{2\pi(1-\pi)}]^2}{(\pi_0-\pi_1)^2}$

$= \dfrac{[0.84\sqrt{8(7)+12(11)} + 1.96\sqrt{20*(9)}]^2}{(4)^2}$

= 89 (each group)

Total sample size = 178

3.3 Ethical clearance

Written ethical clearance was given by the ERES Converge (appendix 1.5) and confidentiality of participants' information was preserved as required for human research.

3.4 The Complior Analyse device (V1.9 Beta Version 2013; ALAM-Medical, France). This device comprised of software installed on a computer, an acquisition unit connected to the PC with a USB cable and specific colour coded arterial sensors for the carotid, femoral, brachial and radial arteries. This software was password protected and thus only the Principal Investigator could access participants' data. On the desktop, there were mandatory fields to be filled in manually such as participant's date of birth, sex, brachial cuff SBP and DBP pressures, BMI and the carotid – femoral distance measured by the measuring tape. Once the carotid and femoral sensors were placed on the neck and above the femoral artery in the groin area respectively, the screen would display and record (after selecting for cfPWV and CBP) carotid – femoral PWV and Central pressure analysis automatically. When the above were valid, the screen will display valid which can be stopped after 10 valid pulses for every participant provided the Principal Investigator was satisfied with the observed wave. The data was automatically recorded and stored on the PC for further analysis as well as on a laboratory data entry tool for back up (appendix 1.4).

3.5 Procedure for collecting data

The protocol used was as described in the Complior Analyse Operator's Manual V1.9 Beta Version 25/02/2013 which conforms to the 2007 ESC/ESH hypertension guidelines for the non-invasive measurement of pulse wave velocity in humans.

Each participant was invited for a nonrecurring study to clinic 5 of the UTH, Lusaka. This was done between 7am and 11am during the normal working days. The participant was introduced to the set-up and after the organizational procedures had been explained to him/her, he/she was asked to sign informed consent forms if agreeable to participate (appendix 1.1 and 1.2 respectively) and then interviewed as outlined in the interview schedule (see appendix 1.3).

Body height and weight of each participant were taken according to standard procedures. Maximum height was measured to the nearest 0.1 cm and Weight to the nearest 0.1 kg using the Invicta Stadiometer (IP 1465, Leicester, UK). After a 10-minute rest in the sitting position, brachial systolic blood pressure (SBP) and

diastolic blood pressure (DBP) and heart rate were measured using a digital BP machine, the OMRON HEM-757 (Omron, Kyoto, Japan) apparatus, with the BP cuff on the left upper arm. Appropriate cuff sizes were used for overweight participants. Two measurements were taken, with a 5-minute rest interval and the mean of the two recorded. The BP measurements were repeated in lying position (supine) after 2 minutes rest interval. Thereafter, carotid-femoral pulse wave velocity and central blood pressure were measured using noninvasively accessible superficial pulses using the Complior Analyse device (V1.9 Beta Version 2013; ALAM-Medical, France) over the carotid - femoral segment. The path length for the determination of aortic PWV was measured as the direct surface distance between the carotid and femoral artery sites using a measuring tape measure. The device was set to correct the CF distance entered manually by multiplying it with 0.8. PWV measurements were made on the right side of each participant while the participant was in supine position on the examination bed.

All measurements were taken by the Principal Investigator and a trained Research Assistant for all participants. Each participant received a report concerning their basic health information including PWV and central blood pressure results immediately. The entire procedure took about 15 – 20 minutes per participant. In the event of a participant being identified with any abnormalities (such as severe hypertension), the participant was referred to the Physician on-call or indeed the respective clinic in the UTH.

3.6 Data analysis

All statistical analyses including major graphs were done using SPSS software versions 16 with the aid of Microsoft excel for some graphs. Results were reported as means (standard deviation) using a statistical significance of $P \leq 0.05$ or a confidence interval of 95% unless stated otherwise. Pulse wave velocity and central blood pressure values per age category in each BP category were represented as means (standard deviation) and as well as with graphs. One way ANOVA (Analysis of Variance) was used to compare statistical differences in cf PWV and CBP respectively in the age group strata. Furthermore, a partial correlation with pvalue of 0.05 or less was used to check for associations between cfPWV and CBP

respectively with other variables after controlling for confounders. Linear regression was computed for certain variables to determine their relationship. In all analyses, equal variances were assumed. To ensure validity and reliability, results were compared with previous studies done elsewhere in similar populations that used this method.

Chapter Four

RESULTS

4.1 Description of study participants

Both the description of basic parametres (using independent student t test) and their comparison between two BP categories (using one way ANOVA) are summarised in tables 1 – 3. Participants were subdivided into one of the three BP categories such as Normotensive (control) participants (NT; brachial BP ≤ 139/89 mm Hg), Treated hypertensive participants (HTC; those on anti-hypertensive drugs) and Untreated hypertensive participants (HTN; brachial BP ≥ 140/90 mmHg). The total study population was 146 participants (after further excluding those who did not satisfy the inclusion criteria described above) of which 69 were normotensives, 59 were treated hypertensives and the remaining 23 were untreated hypertensive participants. The number of females was 42, 36 and 17 in NT (n=69), HTC (n=59) and HTN (n=23) participants respectively. Their mean age was 42±8.7, 47±8.8 and 47±8.9 years respectively. Compared with normotensive subjects, both categories of hypertensive subjects were older, had a higher body mass index, had the highest carotid femoral PWV and thus stiffer and less compliant arteries. In addition, the latter had higher central and corresponding brachial BP values and were as equally likely to have a history of hypertension and diabetes as NT subjects.

4.2 Carotid femoral pulse wave velocity and Central blood pressure in BP categories.

Tables 1 - 3 show comparisons of cfPWV and CBP values respectively between two BP categories. Compared to NT participants, HTC participants were significantly obese (table 1) with no significant difference in their cfPWV although the latter's was higher (9.1±3.2 versus 10.4±5.6, p= 0.104). The cf PWV of HTN participants was significantly higher than of NT (table 2), which depicts that central arteries of HTN were stiffer and thus less compliant (9.1±3.2 versus 11.4±4.2, p=0.009). This finding alone could partly account for the sustained elevated peripheral and central blood pressures in this group. On the contrary, there was no significant difference between

the cfPWV of HTC and HTN (table 3). Regrettably, this means that central arteries in the HTC were as stiff as those in the HTN.

Table 1. Comparison of parameters between normotensive and treated hypertensive participants.

Blood Pressure Category /Parametre	NT (n=64)	HTC (n=59)	Pvalue
Age (years)	42±8.7	47±8.8	0.005
Weight (kg)	70.5±13.9	79.1±14.3	0.001
Body Mass Index (kg/m^2)[a]	27.5±8.0	30.0±5.9	0.048
# (%) of alcohol drinkers	9(14.1%)	18(30.5%)	0.031
Walking/bicycle to work (days/week)	6±1.9	5±2.9	0.004
Brachial Systolic Blood Pressure	123±9.7	153±30.5	0.000
Brachial Diastolic Blood Pressure	79±7.1	98±19.4	0.000
Brachial Pulse Pressure	44±7.9	55±16.6	0.000
Brachial Mean Arterial Pressure*	94±7.2	116±22.3	0.000
Carotid Femoral PWV (m/s)	9.1±3.2	10.4±5.6	0.104
Central Systolic BP	114±10.2	145±33.7	0.000
Central Diastolic BP	79±7.1	98±19.4	0.000
Central Pulse Pressure(cPP)	35±8.0	47±25	0.000
Central Mean Arterial Pressure*	94±7.2	116±22.3	0.000

Data is expressed as mean±standard deviation. Blood pressure is expressed in mmHg; Carotid femoral PWV (cf PWV) is measured in metres per second (m/s). * Mean arterial pressure (MAP) was calculated as MAP = PP x 33% + DBP (Diastolic blood pressure). Pvalue represents statistical differences of the parametre between the BP categories (with α significant at ≤ 0.05). **Abbreviations;** N – Number of participants, NT - Normotensive participants, HTC - Hypertensive participants on treatment, HTN - Untreated hypertensive participants. [a]Body Mass Index (BMI) was calculated as BMI = Weight (Kg)/Height^2 (m^2).

Compared to NT, both HTC and HTN respectively had significantly higher central blood pressures (tables 2 and 3). For example, the mean central systolic and central pulse pressures for HTC were 31mmHg and 12mmHg ($p < 0.001$ respectively) higher

than for NT. The mean values for the same parametres in HTN were even much higher (42mmHg and 23mmHg, $p < 0.001$ respectively) than their corresponding values in NT. Regrettably, there were no significant differences between CBP values of HTC and HTN participants (table 3) implying a substantially greater risk of developing worse CVD events as compared to HTN participants.

Table 2. Comparison of parameters between normotensive and untreated hypertensive participants.

Blood Pressure Category /Parametre	NT (n=64)	HTN (n=23)	Pvalue
Age (years)	42±8.7	47±8.9	0.022
Weight (kg)	71±13.9	75±14.4	0.191
Body Mass Index (kg/m^2)	27.5±8.0	28.3±5.1	0.670
# (%) Moderate work	60(93.8%)	18(78.3%)	0.051
Walking/bicycle to work (days/week)	6±1.9	6±2.4	0.283
Brachial Systolic Blood Pressure	123±9.7	156±17.7	0.000
Brachial Diastolic Blood Pressure	79±7.1	98±11.3	0.000
Brachial Pulse Pressure	44±7.9	58±12.4	0.000
Brachial Mean Arterial Pressure*	94±7.2	117±12.4	0.000
Carotid Femoral PWV (m/s)	9.1±3.2	11.4±4.2	0.009
Central Systolic BP	114±10.2	156±56.2	0.000
Central Diastolic BP	79±7.1	98±11.3	0.000
Central Pulse Pressure (cPP)	35±8.0	58±55.7	0.002
Central Mean Arterial Pressure*	94±7.2	117±12.4	0.000

NB. Data is expressed as mean±standard deviation. Blood pressure is expressed in mmHg; Carotid femoral PWV (cfPWV) is measured in metres per second (m/s).

Table 3. Comparison of parameters between treated hypertensive and untreated hypertensive participants.

Blood Pressure Category /Parametre	HTC (n=59)	HTN (n=23)	Pvalue
Age (years)	47±8.8	47±8.9	0.979
Weight (kg)	79.1±14.3	75±14.4	0.466
Body Mass Index (kg/m^2)	30.0±5.9	28.3±5.1	0.535
# (%) Moderate work	47(79.7%)	18(78.3%)	0.985
Walking/bicycle to work (days/week)	5±2.9	6±2.4	0.412
Brachial Systolic Blood Pressure	153±30.5	156±17.7	0.832
Brachial Diastolic Blood Pressure	98±19.4	98±11.3	0.997
Brachial Pulse Pressure	55±16.6	58±12.4	0.541
Brachial Mean Arterial Pressure*	116±22.3	117±12.4	0.977
Carotid Femoral PWV (m/s)	10.4±5.6	11.4±4.2	0.678
Central Systolic BP	145±33.7	156±56.2	0.865
Central Diastolic BP	98±19.4	98±11.3	0.997
Central Pulse Pressure (cPP)	47±25	58±55.7	0.737
Central Mean Arterial Pressure*	116±22.3	117±12.4	0.778

NB. Data is expressed as mean±standard deviation. Blood pressure (BP) is expressed in mmHg; Carotid femoral PWV (cf PWV) is measured in metres per second (m/s).

4.3 Carotid femoral PWV and Central BP in age categories.

The summary of cfPWV and CBP values according to age category is shown in figure 2 - 4. Even though neither cfPWV nor Central BP values reached significant differences (figure 2 - 4, $p > 0.100$) across the three age categories for each of the BP category, the observed cfPWV and/or CBP values showed an increase with increasing age category (figures 2 - 4). Hence the 30 – 39 age category had the least mean values for both cfPWV and CBP, followed by the 40 – 49 category and highest measurements being observed in the 50 – 65 age category in all BP categories.

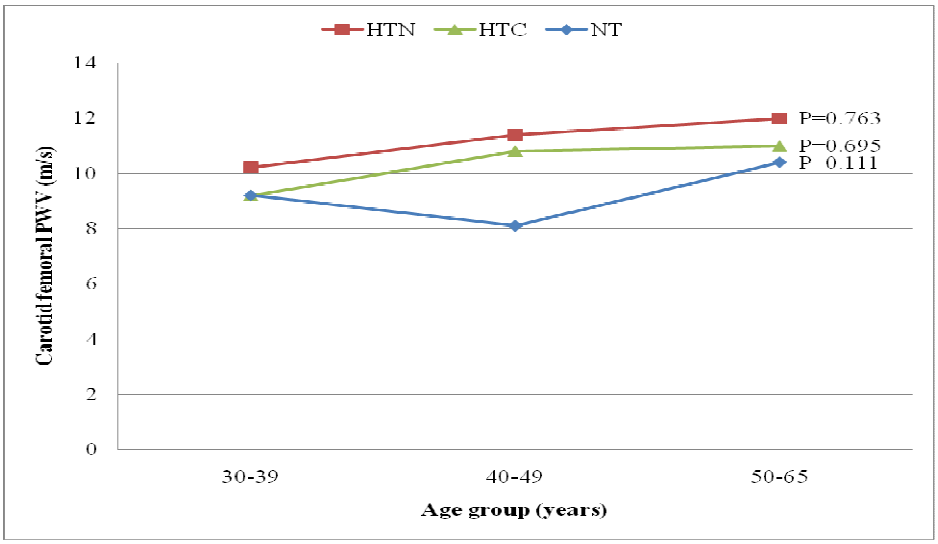


Figure 2. Carotid femoral pulse wave velocity (cfPWV) according to age category in all three BP categories. A pvalue ≤ 0.05 depicts significant differences in cfPWV among the age categories in each blood pressure (BP) category. Points signify mean values.

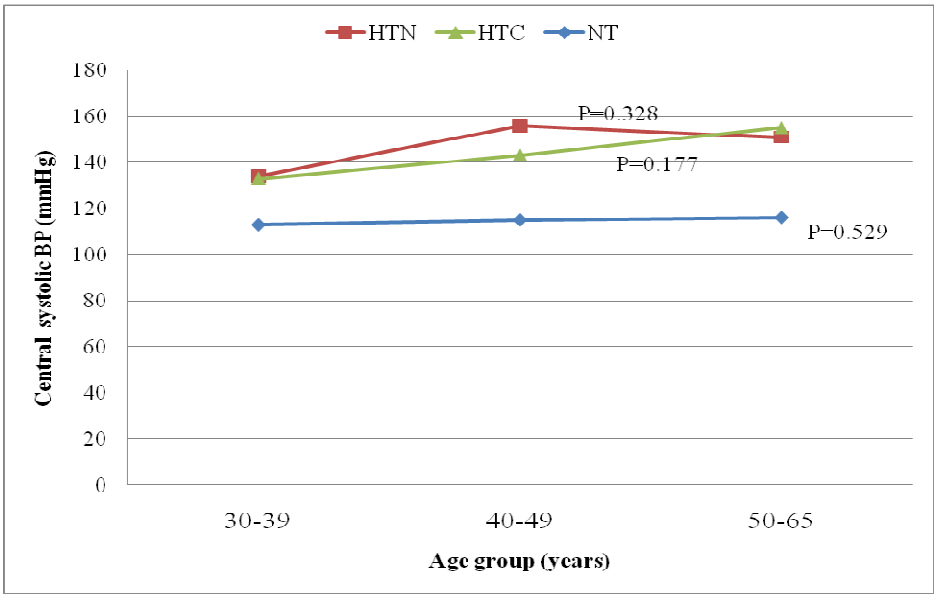


Figure 3. Central systolic blood pressure (cSBP) according to age category in all three BP categories. A pvalue ≤ 0.05 depicts significant differences in cSBP among the age categories in each blood pressure (BP) category. Points signify mean values.

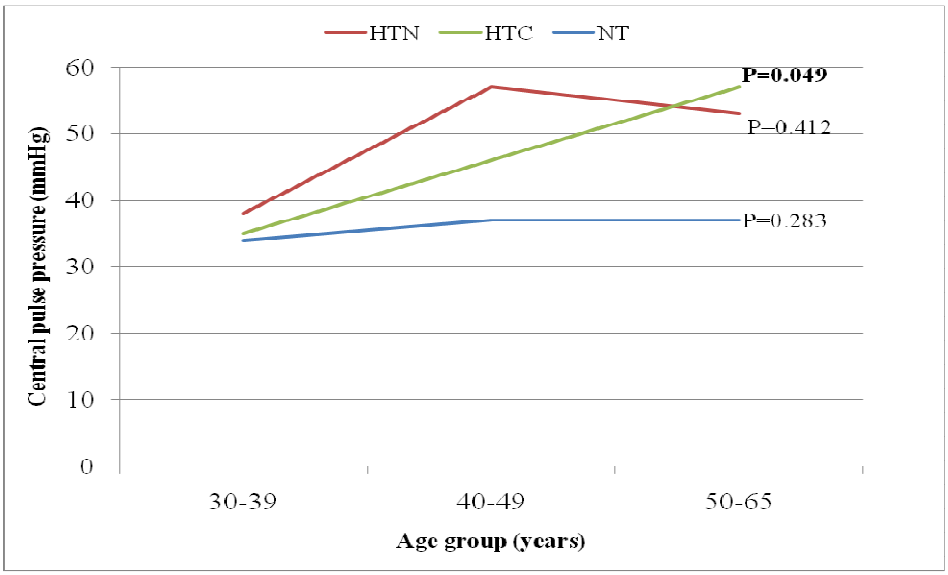


Figure 4. Central pulse pressure (cPP) according to age category in all three BP categories. A pvalue ≤ 0.05 depicts significant differences in cPP among the age categories in each blood pressure (BP) category. Points signify mean values.

Furthermore, HTN subjects showed the highest cfPWV and CBP values in all age categories than any of the two BP categories. On the contrary, NT subjects showed the lowest cfPWV and CBP values with increasing age, with HTC values being only slightly lower than those in HTN subjects (with the exception of central pulse pressure in the HTC subjects which showed significant results, p = 0.049; figure 4).

4.4 Central versus brachial blood pressures.

A summary of comparisons between central and corresponding peripheral (brachial) blood pressure is shown in figures 5 and 6. Both NT and HTC subjects had significantly lower central systolic and central pulse pressures when compared with their corresponding brachial values ($p < 0.01$ respectively). On the contrary, central pressures were not significantly different from their corresponding brachial values in the HTN subjects ($p > 0.800$). In addition, both central and brachial pressures of HTC subjects (although significantly different from each other, $p < 0.01$ with central values being lower) were observed to be closer to values of HTN subjects than to those of NT participants.

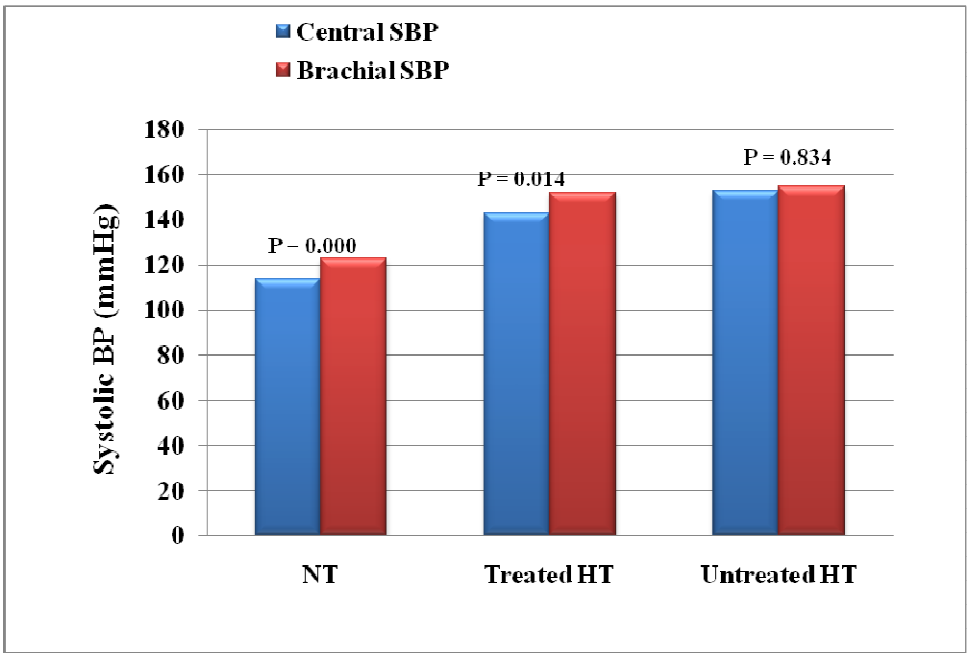


Figure 5. Comparison of central systolic blood pressure (central SBP) vs brachial systolic blood pressure (brachial SBP) in each BP category. $P \leq 0.05$ (from paired student t-tests) indicates significant differences between central and brachial systolic pressure in each BP category.

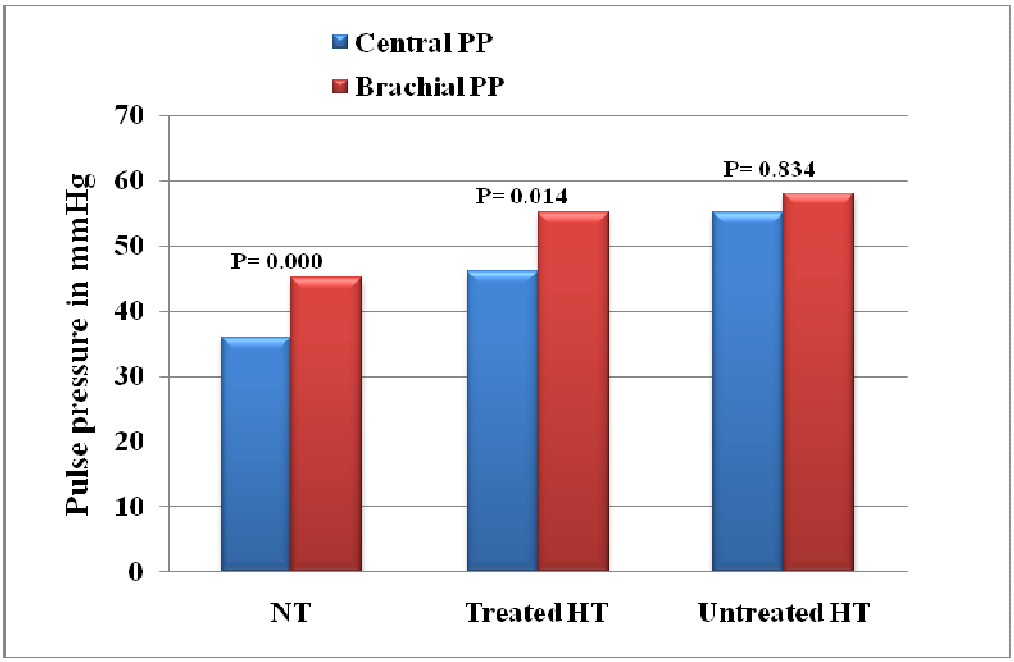


Figure 6. Comparison of central pulse pressure (central PP) vs brachial pulse pressure (brachial PP) in each BP category. $P \leq 0.05$ (from paired student t-tests) indicates

significant differences between central and brachial pulse pressure in each BP category.

4.5 Correlations and regression of cfPWV or central BP with age or corresponding brachial BP.

Partial Pearson's correlation and linear regression analyses' were conducted to examine the relationship between cfPWV or Central BP with age as well as central BP with corresponding peripheral values (table 4 and figures 7 and 8). As can be seen in table 4, cfPWV of both NT and HTC subjects were weakly though positively and insignificantly correlated with age after adjusting for confounders (r = 0.075, p = 0.588 and r = 0.057, p= 0.699 respectively). No correlations of any parametres (cfPWV or CBP) were found for HTN subjects after adjusting for confounders.

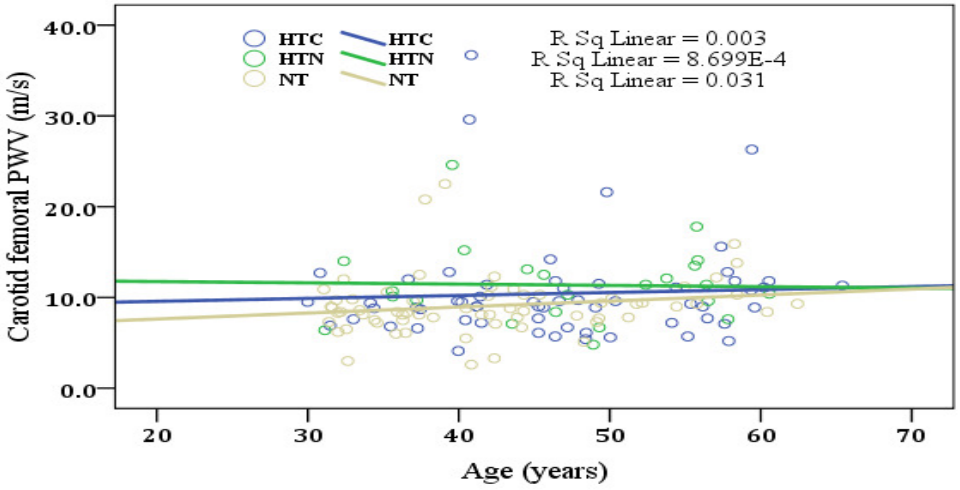


Figure 7. Carotid femoral pulse wave velocity (cf PWV) vs age. Regression lines denote the results of linear regression cfPWV on age for blood pressure (BP) categories. R sq. linear – R^2 (coefficient of determination).

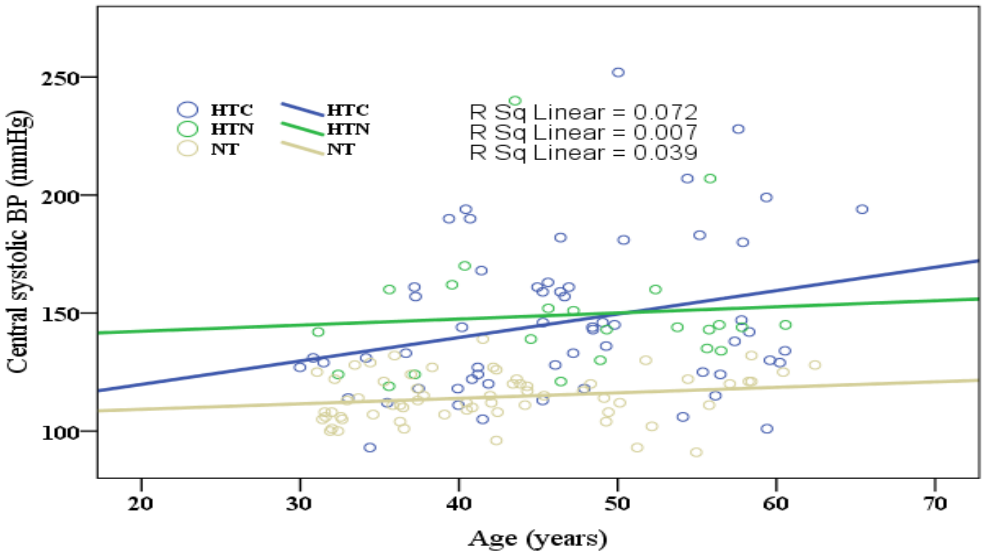


Figure 8**.** Central systolic blood pressure (cSBP) vs age. Regression lines denote the results of linear regression of cSBP on age for blood pressure (BP) categories. R sq. linear – R^2 (coefficient of determination).

Compared to NT subjects, HTC subjects showed stronger and significant correlations of central MAP and central PP with age after adjusting for confounding variables respectively. The strongest and most significant correlations were found between central pulse pressure, central mean arterial pressure, central systolic pressure and their corresponding brachial pressures ($r > 0.75$, $p = 0.000$ respectively, table 4). Infact, it was very easy to predict brachial blood pressures from their corresponding central pressures in both NT and HTC subjects ($R^2 > 0.60$ respectively) and even to a lesser extent in the HTN subjects ($R^2 > 0.20$) (figures 9 – 11).

Table 4. Partial correlations between age or central blood pressure and the carotid femoral PWV or corresponding brachial blood pressure respectively in all BP categories.

	Age (years) [a]					
	NT (n=64)		**HTC (n=59)**		**HTN (n=23)***	
	R	Pvalue	R	Pvalue	R	Pvalue
Carotid Femoral PWV (m/s)	.075	.588	.057	.699		
Central systolic BP	.012	.934	.018	.902		
Central MAP	.135	.287	.349	.015		
Central PP	.100	.470	.356	.013		
	Central blood pressure indices					
Peripheral blood pressures indices	Central systolic BP [b]					
	NT (n=64)		**HTC (n=59)**		**HTN (n=23)***	
	R	Pvalue	R	Pvalue	R	Pvalue
Brachial systolic BP	.793	.000	.788	.000		
	Central pulse pressure [c]					
	NT (n=64)		**HTC (n=59)**		**HTN (n=23)***	
	R	Pvalue	R	Pvalue	R	Pvalue
Brachial PP	1.000	.000	1.000	.000		
	Central mean arterial pressure [d]					
	NT (n=64)		**HTC (n=59)**		**HTN (n=23)***	
	R	Pvalue	R	Pvalue	R	Pvalue
Brachial MAP	1.000	.000	1.000	.000		

NB. r – Pearson's linear correlation coefficient, all correlations (r) with $\alpha \leq 0.05$ were significant (strongly related). *Partial correlations (linear) were insignificant for all variables under untreated hypertensive (HTN) patients after adjusting for confounders.

[a] Adjusted for gender, smoking, alcohol consumption, history of diabetes & hypertension, days/week one does vigorous &/or moderate exercise, body mass index, brachial systolic BP & central systolic BP.
[b] Adjusted for age, cf PWV, gender, smoking, alcohol consumption, history of diabetes & hypertension, days/week one does vigorous &/or moderate exercise & body mass index.
[c] Adjusted for age, cf PWV, gender, smoking, alcohol consumption, history of diabetes & hypertension, days/week one does vigorous &/or moderate exercise, body mass index, brachial systolic BP & central systolic BP.
[d] Adjusted for age, cf PWV, gender, smoking, alcohol consumption, history of diabetes & hypertension, days/week one does vigorous &/or moderate exercise, body mass index, brachial systolic BP & central systolic BP.

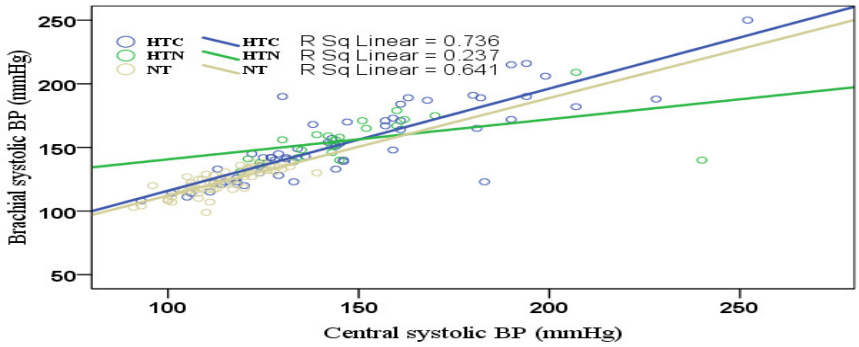


Figure 9. Brachial systolic BP vs central systolic BP. Regression lines denote the results of linear regression of brachial systolic BP on central systolic blood pressure BP for all blood pressure categories.

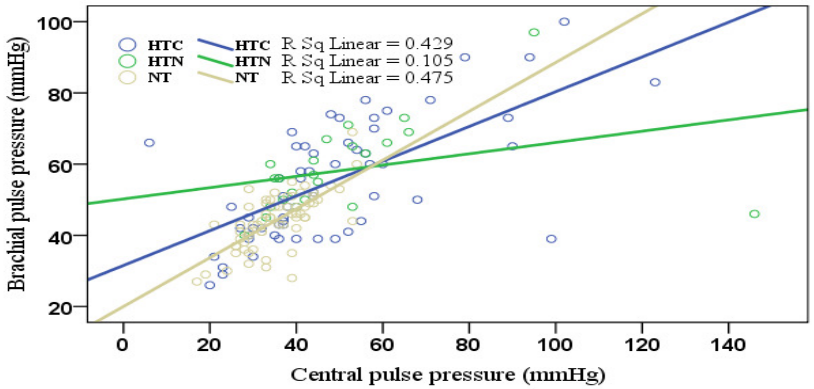


Figure 10. Brachial pulse pressure (brachial PP) vs central pulse pressure (central PP). Regression lines denote the results of linear regression of brachial PP on central PP for all blood pressure categories.

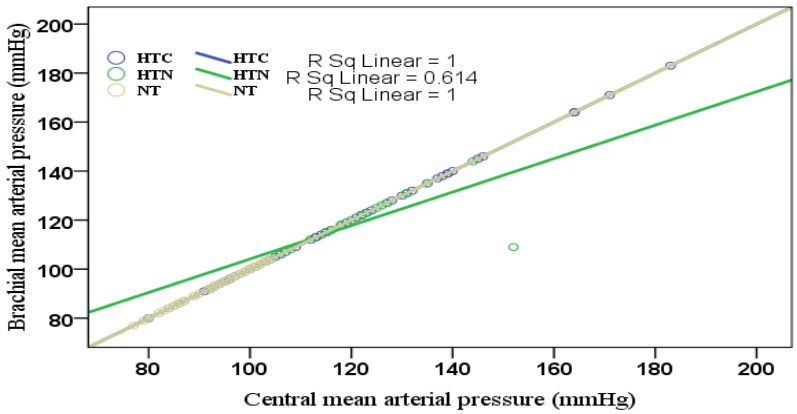


Figure 11**.** Brachial mean blood pressure (brachial MAP) vs central mean blood pressure. Regression lines denote the results of linear regression brachial MAP on central MAP for all blood pressure categories.

Chapter Five

DISCUSSION

The main result of this study is the determination and establishment of carotid – femoral PWV and central BP values respectively in a population of Zambian normotensive and hypertensive participants. This is the first study in Black indigenous Zambians that attempts to understand the pathophysiology of hypertension by using an already widely accepted non invasive method to measure both cf PWV and CBP in an individual. These results represent a critical step in the simultaneous implementation of PWV and CBP as clinical tools for detecting subclinical organ damage in routine patient workup.

Carotid femoral pulse wave velocity and central arterial stiffness

In this cross sectional study, we demonstrate that being hypertensive (both treated and untreated subjects) is associated with elevated cfPWV values compared with being normotensive as supported in other studies (Laurent et al., 2001). In this context, a significant finding is the notable difference of cfPWV values between NT control participants and HTN participants (9.1±3.2 versus 11.4±4.2, p=0.009). In addition, HTN participants had consistently elevated cfPWV values for any given age or age category compared to NT participants. On the contrary, this increase in cfPWV with age was found not be significant for any of the three BP categories (table 5). Unfortunately, the mean cfPWV found in Zambian normotensive participants was a record highest; about 1.0 m/s higher than that found in previous studies involving similar populations of black and white normotensive control participants (9.1m/s versus 8.1m/s) (Schutte et al., 2011, Reference Values for Arterial Stiffness, 2010, Morris et al., 2013). Thus Zambian normotensive subjects were more likely to develop a CVD disease including hypertension than their Caucasian subjects.

These findings depicts that central arteries of HTN participants are stiffer and thus less compliant than in NT participants at any given age below 65 years. In addition, the finding that increase in cfPWV was not significantly associated with advancing age even after adjusting for confounders strongly supports already existing evidence that

arteries in black populations could already be stiff from birth or early years of life when compared to white populations (Schutte et al., 2011, Morris et al., 2013). Unfortunately, this situation increases the risk of developing worse CVD outcomes such as stroke and heart failure in the already burdened hypertensive individuals. Regrettably, even normotensive and treated hypertensive participants, although both had similar and lower cfPWV values, were still likely to develop a major cardiovascular disorder considering their high cfPWV and central pressures when compared to reference values in previous studies (Schutte et al., 2011, Reference Values for Arterial Stiffness, 2010, Morris et al., 2013). Physiologically, elevated cfPWV and (thus elevated central arterial stiffness) and elevated central pressure both increase the afterload the left ventricle must overcome during systole in order to supply adequate blood flow to peripheral organs and tissues; the heart, brain and kidneys inclusive (Laurent et al., 2006, Laurent et al., 2001). Eventually, if anti – hypertensive therapy that is able to lower both central arterial stiffness and central blood pressure (central systolic and central pulse pressure) is not started soon; irreversible arterial wall damage ensues including left or bilateral ventricular hypertrophy. The result is obviously poor CVD prognosis with possible death (Schutte et al., 2011, Morris et al., 2013).

Relationship of central blood pressure with age and corresponding brachial pressure

Compared to NT participants, both HTC and HTN participants showed significantly higher values of the central systolic pressure, central pulse pressure and central mean arterial pressure ($p < 0.001$ in all comparisons, tables 1 and 2). Central systolic, pulse and mean arterial pressures (with the exception of central PP for HTC participants; $r=0.356$, $p=0.013$), just like cfPWV, lacked significant age-related increases with advancing age in all the three BP categories even after controlling for confounding variables ($r < 0.15$, $p > 0.05$). This finding supports previous studies (McEniery et al., 2005, Cecelja and Chowienczyk, 2012) in which both cfPWV and CBP did not significantly increase with increasing age until over the age of 60 years in black

compared to white populations. This trend is as observed for cfPWV in that central pressures in black populations may already be elevated from birth or early years of life (Schutte et al., 2011, Morris et al., 2013).

On the contrary, there were strong positive and significant associations between central systolic, central pulse and central mean arterial pressure values and their corresponding brachial values in both NT and HTC participants ($r > 0.75$, $p = 0.000$ between all comparisons) after adjusting for confounding variables. In HTN participants, there were no associations and apparently no significant differences (after adjusting for confounders) observed between central BP values and their corresponding peripheral values (table 7 and figure 3) as seen in NT and HTC participants. This finding supports current evidence that central BP values in the HTN participants were already elevated and approaching their corresponding elevated brachial pressure values (Wang et al., 2009, Roman et al., 2007) and thus adding to the burden of hypertension in these individuals. Furthermore, these findings are consistent with other studies (Wang et al., 2009, Agabiti-Rosei et al., 2007) in which central systolic and central pulse pressures were found to be related to their corresponding brachial pressures respectively in the normotensives and treated hypertensives.

Stiffer aortic and carotid arteries as observed in HTN and HTC participants leads to reduced aortic compliance, so that central pressure generated for any given volume of stored blood becomes amplified or increased. Furthermore, arterial stiffening (and hence an increase in cfPWV) results in more reflected waves arriving back to the aorta and carotid in late systole, coinciding and augmenting systolic and pulse pressure of the forward pulse wave. Thus the resulting sustained elevation of central systolic and pulse pressure leads to target organ damage and cardiovascular events including hypertension more than corresponding brachial pressure would do since central pressure is directly related to the afterload imposed on the heart (Wang et al. 2011).

Chapter six

CONCLUSION AND RECOMMENDATIONS

The findings of this study show that aortic and carotid arteries, both elastic blood vessels, of hypertensive participants were stiffer and thus less compliant than those in normotensive participants. This was probably due to endothelial wall dysfunction as well as loss of elastin which could partly be explained by arterial stress resulting from the sustained elevated blood pressures. In addition, central systolic, diastolic and pulse pressures of both treated and untreated hypertensive participant were significantly elevated compared to those in normotensive control participants. This means a poor BP control in the patients on treatment as seen from their results; meaning that even treated hypertensive participants still had substantial risk of developing a major cardiovascular disorder like stroke considering their high cfPWV and central pressures.

To the knowledge of the author, this is the first study in Zambia and among the few in Africa to have simultaneously evaluated cfPWV and CBP in the same study. Furthermore, these findings challenge Physiologists, Cardiologists and Pharmacologists alike to improve and carryout more research in the overall pathophysiology and management of hypertension. In this regard, it is hoped that Cardiologists use this information in their choice of antihypertensive therapy to choose therapies which do not only lower peripheral (brachial) pressures but also reduce cfPWV and central pressures which are significant and independent predictors of cardiovascular outcomes and death.

REFERENCES

AGABITI-ROSEI, E., MANCIA, G., O'ROURKE, M. F., ROMAN, M. J., SAFAR, M. E., SMULYAN, H., WANG, J. G., WILKINSON, I. B., WILLIAMS, B. & VLACHOPOULOS, C. 2007. Central blood pressure measurements and antihypertensive therapy: a consensus document. *Hypertension,* 50**,** 154-60.

BENETOS, A. 2002. Determinants of Accelerated Progression of Arterial Stiffness in Normotensive Subjects and in Treated Hypertensive Subjects Over a 6-Year Period. *Circulation,* 105**,** 1202-1207.

CECELJA, M. & CHOWIENCZYK, P. 2012. Role of arterial stiffness in cardiovascular disease. *JRSM Cardiovasc Dis,* 1.

GOMA, F. M., NZALA, S. H., BABANIYI, O., SONGOLO, P., ZYAAMBO, C., RUDATSIKIRA, E., SIZIYA, S. & MUULA, A. S. 2011. Prevalence of hypertension and its correlates in Lusaka urban district of Zambia: a population based survey. *Int Arch Med,* 4**,** 34.

GUERIN, A. P., BLACHER, J., PANNIER, B., MARCHAIS, S. J., SAFAR, M. E. & LONDON, G. M. 2001. Impact of aortic stiffness attenuation on survival of patients in end-stage renal failure. *Circulation,* 103**,** 987-992.

KOELWYN, G. J., CURRIE, K. D., MACDONALD, M. J. & EVES, N. D. 2012. Ultrasonography and Tonometry for the Assessment of Human Arterial Stiffness. *InTech*

LAURENT, S., BOUTOUYRIE, P., ASMAR, R., GAUTIER, I., LALOUX, B., GUIZE, L., DUCIMETIERE, P. & BENETOS, A. 2001. Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. *Hypertension,* 37**,** 1236-1241.

LAURENT, S., COCKCROFT, J., VAN BORTEL, L., BOUTOUYRIE, P., GIANNATTASIO, C., HAYOZ, D., PANNIER, B., VLACHOPOULOS, C., WILKINSON, I. & STRUIJKER-BOUDIER, H. 2006. Expert consensus document on arterial stiffness: methodological issues and clinical applications. *European heart journal,* 27**,** 2588-2605.

MACKENZIE, I., WILKINSON, I. & COCKCROFT, J. 2002a. Assessment of arterial stiffness in clinical practice. *Qjm,* 95**,** 67-74.

MACKENZIE, I. S., WILKINSON, I. B. & COCKCROFT, J. R. 2002b. Assessment of arterial stiffness in clinical practice. *QJM,* 95**,** 67-74.

MANCIA, G., DE BACKER, G., DOMINICZAK, A., CIFKOVA, R., FAGARD, R., GERMANO, G., GRASSI, G., HEAGERTY, A. M., KJELDSEN, S. E., LAURENT, S., NARKIEWICZ, K., RUILOPE, L., RYNKIEWICZ, A., SCHMIEDER, R. E., BOUDIER, H. A. & ZANCHETTI, A. 2007a. 2007 ESH-ESC Practice Guidelines for the Management of Arterial Hypertension: ESH-ESC Task Force on the Management of Arterial Hypertension. *J Hypertens,* 25**,** 1751-62.

MANCIA, G., DE BACKER, G., DOMINICZAK, A., CIFKOVA, R., FAGARD, R., GERMANO, G., GRASSI, G., HEAGERTY, A. M., KJELDSEN, S. E., LAURENT, S., NARKIEWICZ, K., RUILOPE, L., RYNKIEWICZ, A., SCHMIEDER, R. E., STRUIJKER BOUDIER, H. A., ZANCHETTI, A., VAHANIAN, A., CAMM, J., DE CATERINA, R., DEAN, V., DICKSTEIN, K., FILIPPATOS, G., FUNCK-BRENTANO, C., HELLEMANS, I., KRISTENSEN, S. D., MCGREGOR, K., SECHTEM, U., SILBER, S., TENDERA, M., WIDIMSKY, P., ZAMORANO, J. L., KJELDSEN, S. E., ERDINE, S., NARKIEWICZ, K., KIOWSKI, W., AGABITI-ROSEI, E., AMBROSIONI, E., CIFKOVA, R., DOMINICZAK, A., FAGARD, R., HEAGERTY, A. M., LAURENT, S., LINDHOLM, L. H., MANCIA, G., MANOLIS, A., NILSSON, P. M., REDON, J., SCHMIEDER, R. E., STRUIJKER-BOUDIER, H. A., VIIGIMAA, M., FILIPPATOS, G., ADAMOPOULOS, S., AGABITI-ROSEI, E., AMBROSIONI, E., BERTOMEU, V., CLEMENT, D., ERDINE, S., FARSANG, C., GAITA, D., KIOWSKI, W., LIP, G., MALLION, J. M., MANOLIS, A. J., NILSSON, P. M., O'BRIEN, E., PONIKOWSKI, P., REDON, J., RUSCHITZKA, F., TAMARGO, J., VAN ZWIETEN, P., VIIGIMAA, M., WAEBER, B., WILLIAMS, B., ZAMORANO, J. L., THE TASK FORCE FOR THE MANAGEMENT OF ARTERIAL HYPERTENSION OF THE EUROPEAN

SOCIETY OF, H. & THE TASK FORCE FOR THE MANAGEMENT OF ARTERIAL HYPERTENSION OF THE EUROPEAN SOCIETY OF, C. 2007b. 2007 Guidelines for the management of arterial hypertension: The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *Eur Heart J,* 28**,** 1462-536.

MANCIA, G., FAGARD, R., NARKIEWICZ, K., REDÁN, J., ZANCHETTI, A., BÖHM, M., CHRISTIAENS, T., CIFKOVA, R., DE BACKER, G. & DOMINICZAK, A. 2013. 2013 Practice guidelines for the management of arterial hypertension of the European Society of Hypertension (ESH) and the European Society of Cardiology (ESC): ESH/ESC Task Force for the Management of Arterial Hypertension. *Journal of hypertension,* 31**,** 1925-1938.

MCENIERY, C. M., YASMIN, HALL, I. R., QASEM, A., WILKINSON, I. B., COCKCROFT, J. R. & INVESTIGATORS, A. 2005. Normal vascular aging: differential effects on wave reflection and aortic pulse wave velocity: the Anglo-Cardiff Collaborative Trial (ACCT). *J Am Coll Cardiol,* 46**,** 1753-60.

MORRIS, A. A., PATEL, R. S., BINONGO, J. N., POOLE, J., AL MHEID, I., AHMED, Y., STOYANOVA, N., VACCARINO, V., DIN-DZIETHAM, R., GIBBONS, G. H. & QUYYUMI, A. 2013. Racial differences in arterial stiffness and microcirculatory function between Black and White Americans. *J Am Heart Assoc,* 2**,** e002154.

NICHOLS, W. W. & O'ROURKE, M. F. 2005. McDonald's blood flow in arteries: theoretical, experimental and clinical principles. . *London, UK*.

O'ROURKE, M. F. & HASHIMOTO, J. 2007. Mechanical factors in arterial aging: a clinical perspective. *J Am Coll Cardiol,* 50**,** 1-13.

O'ROURKE, M. F. & NICHOLS, W. W. 2005. Aortic diameter, aortic stiffness, and wave reflection increase with age and isolated systolic hypertension. *Hypertension,* 45**,** 652-8.

PALATINI, P., CASIGLIA, E., GASOWSKI, J., GLUSZEK, J., JANKOWSKI, P., NARKIEWICZ, K., SALADINI, F., STOLARZ-SKRZYPEK, K.,

TIKHONOFF, V., VAN BORTEL, L., WOJCIECHOWSKA, W. & KAWECKA-JASZCZ, K. 2011. Arterial stiffness, central hemodynamics, and cardiovascular risk in hypertension. *Vasc Health Risk Manag,* 7**,** 725-39.

PINI, R., CAVALLINI, M. C., PALMIERI, V., MARCHIONNI, N., DI BARI, M., DEVEREUX, R. B., MASOTTI, G. & ROMAN, M. J. 2008. Central but not brachial blood pressure predicts cardiovascular events in an unselected geriatric population: the ICARe Dicomano Study. *J Am Coll Cardiol,* 51**,** 2432-9.

REFERENCE VALUES FOR ARTERIAL STIFFNESS, C. 2010. Determinants of pulse wave velocity in healthy people and in the presence of cardiovascular risk factors: 'establishing normal and reference values'. *Eur Heart J,* 31**,** 2338-50.

ROMAN, M. J., DEVEREUX, R. B., KIZER, J. R., LEE, E. T., GALLOWAY, J. M., ALI, T., UMANS, J. G. & HOWARD, B. V. 2007. Central pressure more strongly relates to vascular disease and outcome than does brachial pressure: the Strong Heart Study. *Hypertension,* 50**,** 197-203.

ROMAN, M. J., DEVEREUX, R. B., KIZER, J. R., OKIN, P. M., LEE, E. T., WANG, W., UMANS, J. G., CALHOUN, D. & HOWARD, B. V. 2009. High central pulse pressure is independently associated with adverse cardiovascular outcome the strong heart study. *J Am Coll Cardiol,* 54**,** 1730-4.

SCHUTTE, A. E., HUISMAN, H. W., SCHUTTE, R., VAN ROOYEN, J. M., MALAN, L., MALAN, N. T. & REIMANN, M. 2011. Arterial stiffness profiles: investigating various sections of the arterial tree of African and Caucasian people. *Clin Exp Hypertens,* 33**,** 511-7.

VAN BORTEL, L. M., DUPREZ, D., STARMANS-KOOL, M. J., SAFAR, M. E., GIANNATTASIO, C., COCKCROFT, J., KAISER, D. R. & THUILLEZ, C. 2002. Clinical applications of arterial stiffness, Task Force III: recommendations for user procedures. *American journal of hypertension,* 15**,** 445-452.

WANG, K.-L., CHENG, H.-M., CHUANG, S.-Y., SPURGEON, H. A., TING, C.-T., LAKATTA, E. G., YIN, F. C., CHOU, P. & CHEN, C.-H. 2009. Central or

peripheral systolic or pulse pressure: which best relates to target-organs and future mortality? *Journal of hypertension,* 27**,** 461.

WEBER, T., AUER, J., O'ROURKE, M. F., KVAS, E., LASSNIG, E., BERENT, R. & EBER, B. 2004. Arterial stiffness, wave reflections, and the risk of coronary artery disease. *Circulation,* 109**,** 184-189.

APPENDICES

1.1 INFORMATION SHEET

Title of study; **Carotid-femoral pulse wave velocity and central blood pressure in normotensive subjects and hypertensive patients at the University Teaching Hospital in Lusaka, Zambia.**

Dear Participant,

This is to request you to take part in this study that is being carried out by Dr. Festus Mushabati., a student in Physiology at UNZA, School of Medicine. The study will focus on measuring the main predictor of high blood pressure in both those with normal and high blood pressure. Although majority of the hypertension cases are due to unknown causes, studies done elsewhere suggest that our arteries harden with age, hypertension and other cardiovascular diseases. Therefore, efforts should be made in predicting the hardening of these arteries so that doctors can help prevent and treat the problem better.

First, a physical examination will be done on you by doctors working at the UTH in clinic 5 and filter clinic to determine your health status. You will be asked to give information on your age, sex, past medical history, family history, and common symptoms. Thereafter, you will be asked to go to Clinic 5. There we will measure how hard your arteries are. Please be informed that the procedure is neither painful nor does it involve piercing through your body. This will take less than 10 minutes and you will be informed of your results immediately.

There will be no monetary gain to you and the results obtained will be used to gather information which will contribute to the management of high blood pressure. Your participation in this study is purely voluntary and therefore, you are allowed to withdraw any time during the study and your action will not affect your acquisition of health services. All the information you will provide will be kept confidential. For any questions and concerns, please contact the following Persons on the addresses below:

Dr Mushabati Festus, UNZA School of Medicine, Department of Physiological Sciences, Lusaka, Mobile Phone; **0977/65 – 819003,** Email: facemushabati@yahoo.com

Dr. Munalula Nkandu, ERES Converge, 33 Joseph Mwilwa Road, Rhodes Park, Lusaka, Tel: **+260 955 155 633, +260 955 155 634, +260 966 765 503**, Email: eresconverge@yahoo.com.

1.2 INFORMED CONSENT FORM

The purpose of the study has adequately been explained to me and I understand the aim, benefits, risks and confidentiality of the study. I further understand that; if I agree to take part in this study, I can withdraw at any time without having to give an explanation and that taking part in this study is purely voluntary.

I _____

(Names)

Consent to participate in this study

Signed; _____ date; _____

(Participant)

Participant's signature or thumb print

Signed; _____ date; _____

(Witness)

Name of the Researcher/Interviewer; _____

Signed; _____ date; _____

PERSONS TO CONTACT FOR ANYTHING:

Mushabati Festus. (Dr), University of Zambia, School of Medicine, Department of Physiological Sciences, P.O. Box 50110, Lusaka, Zambia. Mobile Phone; **0977/65 – 819003** Email: facemushabati@yahoo.com

Dr. Munalula Nkandu, ERES Converge, 33 Joseph Mwilwa Road, Rhodes Park, Lusaka, Tel: **+260 955 155 633, +260 955 155 634, +260 966 765 503**, Email: eresconverge@yahoo.com.

1.3 Budget

Item	**Detail**	**Cost estimate ZMW**
ERES CONVERGE submission fee	ERES CONVERGE submission fee	600
Stationery and printing (period of 8 months)	Secretarial and printing services	2,000
Personnel (research laboratory assistant)	Being payment of a research assistant for assistance in capturing individual data: K5OO/month x 1 × 3 months	1,500
Complior analyze device* and other materials and accessories	measuring tape, cuff spygmomanometre, examination gloves, colour printer and cartridges NB: Complior analyze device* will be provided by the department	6,000
Transport refund fee (1 × 178 participants)	Transport refund fee to participants K50/day / participant × 178 participants	8,900
Thesis Preparation costs	Professional printing and binding of 5 copies, poster design	4,000
Contingency @ 10% of total	Miscellaneous	2,300
Grand Total		**25, 300**

1.4 Timeline (Ghantt chart)

ACTIVITY/ DATE	Sept 2013	Nov/Dec, 2013	Jan/March 2014	April 2014	May 2014	June 2014	July 2014	August 2014	Sept/Feb 2014	March, 2015
Present Proposal to Supervisor and Department										
Present proposal at GPPF										
Submit proposal to ERES CONVERGE										
ERES review and approval										
Enroll participants and collect data										
Analyze data										
Write thesis, submit to supervisor for corrections and Defence at GPPF										
Submit final thesis for examination										
Submit final approved bound copies to DRGS and await graduation										

1.5 INTERVIEW SCHEDULE

Dear Participant

You have been chosen to participate in this study being conducted by Dr. Mushabati, a postgraduate student from the Department of Physiological sciences - University of Zambia, School of Medicine. The study will focus on measuring the main predictor of high blood pressure in both those with normal and high blood pressure. You are kindly requested to cooperate with persons administering this questionnaire. Please be assured that the study ethics of confidentiality will be strictly adhered to in this interview**.**

GENERAL INFORMATION

1. ID/Study No.: …….
2. Age (at last birth day)……. years
3. Sex: Male..... Female.....
4. Marital status single [ ] married [ ] divorced [ ] widowed [ ]
5. Occupation
   ……..
6. Nationality Zambian [ ] Non - Zambian [ ]
   a) If Non - Zambian please state Nationality ……

SMOKING

7. Do you currently smoke any tobacco products such as cigarettes, cigars or pipes?
   a) Yes *(IF YES GO TO QUESTION 8)*
   b) No *(IF NO GO TO QUESTION 9)*
8. Do you currently smoke products daily?
   a) Yes *(IF YES GO TO QUESTION 9)*
   b) No *(IF NO GO TO QUESTION 10)*
9. On average, how many of the following products do you smoke each day/week?

	DAILY	WEEKLY
MANUFACTURED CIGARETTES		
HAND-ROLLED CIGARETTES		
PIPES FULL OF TOBACCO		
CIGARS		
OTHER.....		

ALCOHOLIC CONSUMPTION

10. Do you drink alcoholic beverages?
    a) Yes [ ] IF YES GO TO QUESTION 11
    b) no [ ] IF NO GO TO QUESTION 12
11. How often do you consume alcohol?
    a) Everyday
    b) 3-5 times a week
    c) Once a week
    d) Only on weekends
    e) On special occasions

FAMILY/PERSONAL MEDICAL HISTORY

12. Is there history of diabetes mellitus in your family? Yes [ ] no [ ] I don't know [ ]
13. Is there history of hypertension in your family? Yes [ ] no [ ] I don't know [ ]
14. Have you ever been told by a doctor that you have diabetes? Yes [ ] no [ ]
15. Are you currently taking insulin or pills to control diabetes? Yes [ ] no [ ]
    a) If yes please state the type……
16. Are you currently taking medication prescribed by a doctor to lower your blood pressure? Yes [ ] no [ ]
    a) If yes please state the type……
17. Have you ever had or been treated for any cardiovascular disease other than hypertension such as heart attack, chest pain from heart disease (angina) including a stroke (cerebrovascular accident or incident), etc? Yes [ ] no [ ]
    a) If yes please state the condition.....

PHYSICAL ACTIVITY

18. Does your work involve vigorous- intensity activity that causes large increases in breathing or heart rate like carrying or lifting heavy loads, digging or construction work for at least 10 minutes continuously? Yes [ ] no [ ]
19. In a typical week, on how many days do you do vigorous intensity activities as part of your work? _____days
20. Does your work involve moderate- intensity activity that causes small increases in breathing or heart rate such as brisk walking or carrying or lifting light loads, for at least 10 minutes continuously? Yes [ ] no [ ]

21. In a typical week, on how many days do you walk, jog or bicycle for at least 10 minutes continuously to get to and from places?
    _____days

1.6 LABORATORY DATA ENTRY TOOL (AS ENTERED DIRECTLY ON PC)

A. PERSONAL INFORMATION

1. ID.....….....
2. SEX.....
3. DATE OF BIRTH.....
4. STUDY PARTICIPANT NO.....
5. VISIT DATE.....
6. AGE (calculated automatically from DOB)……..

B. <u>BODY MASS INDEX (BMI)</u>

Height (cm)..... Weight (kg).....

BMI (Weight/ Height^2) kg.cm^{-2} .....

C. <u>BRACHIAL BLOOD PRESSURES (measured using cuff sphygmomanometre)</u>

1. LYING (AFTER 3 MINUTES OF REST)

S/N	**SYSTOLIC (mmHg)**	**DIASTOLIC(mmHg)**	**PULSE**
1			
2			
AVERAGE			

2. STANDING (AFTER 1 MINUTE)

S/N	**SYSTOLIC (mmHg)**	**DIASTOLIC(mmHg)**	**PULSE**
1			
2			
AVERAGE			

D. <u>DISTANCES (measured using measuring tape)</u>

MEASUREMENT	DISTANCE (cm)	INITIALS
RIGHT CAROTID TO FEMORAL ARTERY SITE		C – F

E. COMPLIOR ANALYSE MEASUREMENTS (lying in supine position on bed AFTER 3 MINUTES)

1. CENTRAL BLOOD PRESSURE: SBP/DBP (mmHg) | | |

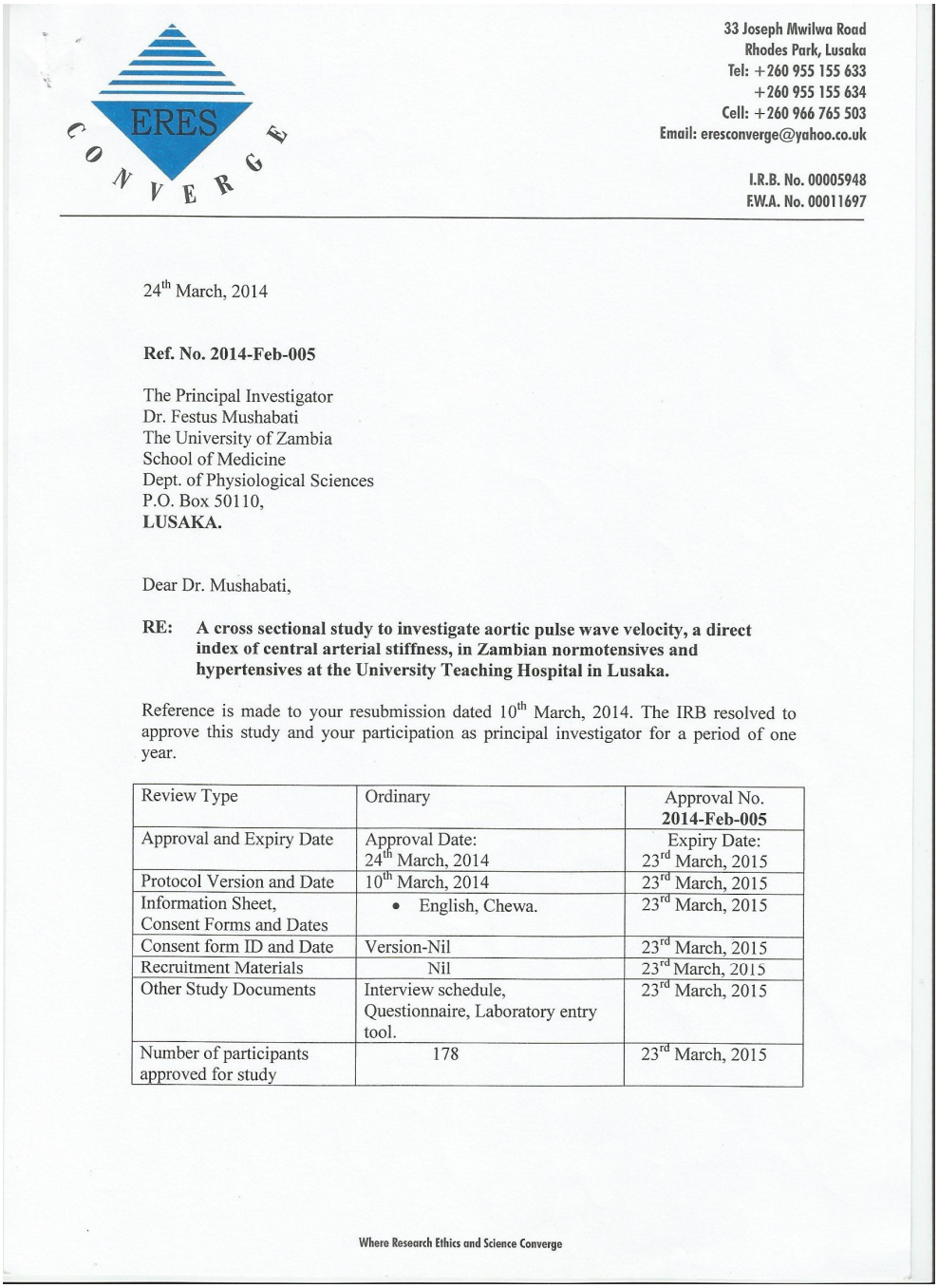
2. Carotid-femoral Aix | | | |

3. Carotid-femoral PWV

	PWV (metres/second)	HEART RATE	TRANSIT TIME	TRANSCRIBED
CAROTID FEMORAL				

4. DIAGRAM OF AORTIC PULSE WAVE VELOCITY (automatically recorded on the PC)

1.7 ERES Converge Clearance Letter.



33 Joseph Mwilwa Road
Rhodes Park, Lusaka
Tel: +260 955 155 633
+260 955 155 634
Cell: +260 966 765 503
Email: eresconverge@yahoo.co.uk

I.R.B. No. 00005948
F.W.A. No. 00011697

24th March, 2014

Ref. No. 2014-Feb-005

The Principal Investigator
Dr. Festus Mushabati
The University of Zambia
School of Medicine
Dept. of Physiological Sciences
P.O. Box 50110,
LUSAKA.

Dear Dr. Mushabati,

RE: A cross sectional study to investigate aortic pulse wave velocity, a direct index of central arterial stiffness, in Zambian normotensives and hypertensives at the University Teaching Hospital in Lusaka.

Reference is made to your resubmission dated 10th March, 2014. The IRB resolved to approve this study and your participation as principal investigator for a period of one year.

Review Type	Ordinary	Approval No. **2014-Feb-005**
Approval and Expiry Date	Approval Date: 24th March, 2014	Expiry Date: 23rd March, 2015
Protocol Version and Date	10th March, 2014	23rd March, 2015
Information Sheet, Consent Forms and Dates	• English, Chewa.	23rd March, 2015
Consent form ID and Date	Version-Nil	23rd March, 2015
Recruitment Materials	Nil	23rd March, 2015
Other Study Documents	Interview schedule, Questionnaire, Laboratory entry tool.	23rd March, 2015
Number of participants approved for study	178	23rd March, 2015

Where Research Ethics and Science Converge

Specific conditions will apply to this approval. As Principal Investigator it is your responsibility to ensure that the contents of this letter are adhered to. If these are not adhered to, the approval may be suspended. Should the study be suspended, study sponsors and other regulatory authorities will be informed.

Conditions of Approval

- No participant may be involved in any study procedure prior to the study approval or after the expiration date.
- All unanticipated or Serious Adverse Events (SAEs) must be reported to the IRB within 5 days.
- All protocol modifications must be IRB approved prior to implementation unless they are intended to reduce risk (but must still be reported for approval). Modifications will include any change of investigator/s or site address.
- All protocol deviations must be reported to the IRB within 5 working days.
- All recruitment materials must be approved by the IRB prior to being used.
- Principal investigators are responsible for initiating Continuing Review proceedings. Documents must be received by the IRB at least 30 days before the expiry date. This is for the purpose of facilitating the review process. Any documents received less than 30 days before expiry will be labelled "late submissions" and will incur a penalty.
- Every 6 (six) months a progress report form supplied by ERES IRB must be filled in and submitted to us.
- ERES Converge IRB does not "stamp" approval letters, consent forms or study documents unless requested for in writing. This is because the approval letter clearly indicates the documents approved by the IRB as well as other elements and conditions of approval.

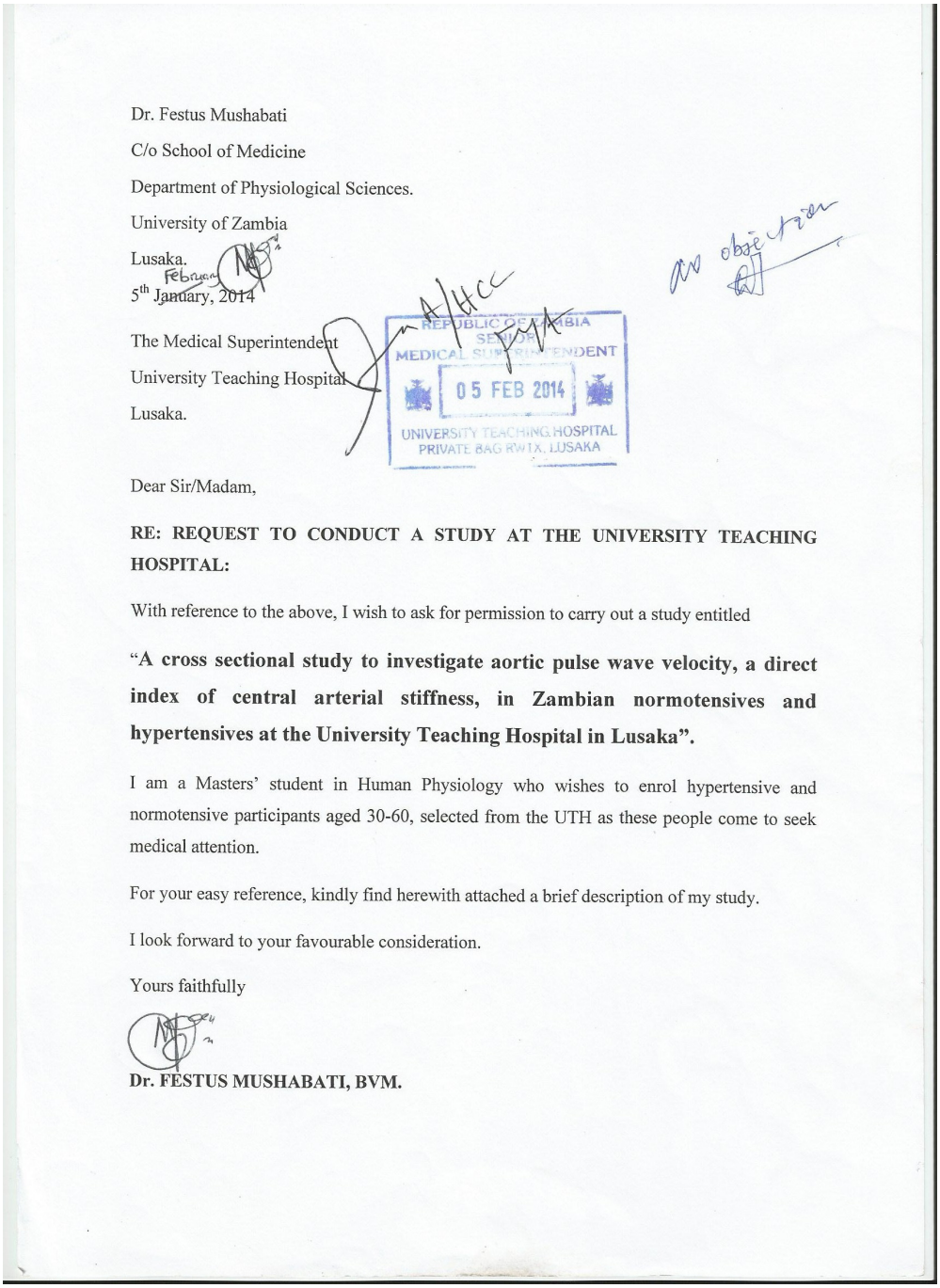
Should you have any questions regarding anything indicated in this letter, please do not hesitate to get in touch with us at the above indicated address.

On behalf of ERES Converge IRB, we would like to wish you all the success as you carry out your study.

Yours faithfully,
ERES CONVERGE IRB

Dr. E. Munalula-Nkandu
BSc (Hons), MSc, MA Bioethics, PgD R/Ethics, PhD
CHAIRPERSON

1.8 UTH authorisation letter to conduct research



Dr. Festus Mushabati

C/o School of Medicine

Department of Physiological Sciences.

University of Zambia

Lusaka.

5th January, 2014

The Medical Superintendent

University Teaching Hospital

Lusaka.

REPUBLIC OF ZAMBIA
MEDICAL SUPERINTENDENT
05 FEB 2014
UNIVERSITY TEACHING HOSPITAL
PRIVATE BAG RW1X, LUSAKA

Dear Sir/Madam,

RE: REQUEST TO CONDUCT A STUDY AT THE UNIVERSITY TEACHING HOSPITAL:

With reference to the above, I wish to ask for permission to carry out a study entitled

"A cross sectional study to investigate aortic pulse wave velocity, a direct index of central arterial stiffness, in Zambian normotensives and hypertensives at the University Teaching Hospital in Lusaka".

I am a Masters' student in Human Physiology who wishes to enrol hypertensive and normotensive participants aged 30-60, selected from the UTH as these people come to seek medical attention.

For your easy reference, kindly find herewith attached a brief description of my study.

I look forward to your favourable consideration.

Yours faithfully

Dr. FESTUS MUSHABATI, BVM.

1.9 Published article online from this work.

Mushabati F, Goma FM, Lwiindi L, Siulapwa NJ, Siziya S. (January 2015). Central arterial stiffness in Zambian normotensive and hypertensive participants. *Jour of Med Sc & Tech*; 4(1); Page No: 30 – 35.