LOWER-LIMB ARTERIAL STIFFNESS: ASSESSMENT, NOVEL PHYSIOLOGICAL INSIGHT AND CLINICAL POTENTIAL

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Thesis Summary

There is an urgent need to identify novel cardiovascular biomarkers which can improve cardiovascular disease (CVD) risk prediction and permit timely-efficacious treatment; necessary to combat the unabated rise in global CVD. Arterial stiffness, representing the ability of an artery to accommodate changes in blood pressure by corresponding changes in dimension, has emerged as an important biomarker of CVD risk. Central (aortic) arterial stiffness, assessed using carotid-femoral pulse wave velocity (cfPWV), is the reference standard, improving the prediction of cardiovascular events beyond conventional risk factors. Whilst cfPWV is a powerful discriminator of CVD risk, its dependence on blood pressure limits its clinical utility. In contrast to cfPWV, the clinical value of lower-limb arterial stiffness has received little attention and its role in CVD risk is not well understood; its measurement may well improve CVD risk prediction by providing unique CVD risk information. The purpose of this thesis is to identify the clinical utility of lower-limb arterial stiffness, assessed using femoral-ankle PWV (faPWV). Using experimental and epidemiological research approaches, this thesis demonstrated that faPWV can be assessed simply with accuracy and precision and provides additional CVD risk information beyond conventional risk factors and existing lower-limb arterial health measures. The assessment of faPWV also permits determination of the aortic-femoral arterial stiffness gradient (af-SG), a novel biomarker of promising clinical utility. This thesis demonstrated that the af-SG has stronger associations with CVD than cfPWV alone, can be determined with acceptable precision, and is blood pressure independent. These findings indicate that the assessment of lower-limb arterial stiffness could be of clinical utility and may permit better identification of CVD risk.

Declaration

I declare that the work in this thesis was carried out in accordance with the regulations of the University of Gloucestershire and is original except where indicated by specific reference in the text.

No part of the thesis has been submitted as part of any other academic award. The thesis has not been presented to any other education institution in the United Kingdom or overseas.

Any views expressed in the thesis are those of the author and in no way represent those of the University.

Signed: **Date:** 08.12.21

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To my Rose.

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Thesis Publications

1. **Stone, K**., Fryer, S., Faulkner, J., Meyer, M.L., Heffernan, K., Kucharska-Newton, A., Paterson, C., Zeiff, G., Matsushita, K., Hughes, T.M., Tanaka, H., & Stoner, L. Associations of lower-limb atherosclerosis and arteriosclerosis with cardiovascular disease risk factors and disease in older adults: An Atherosclerosis Risk in Communities (ARIC) Study. *Atherosclerosis* (in press). https://doi.org/10.1016/j.atherosclerosis.2021.10.014.

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5. Fryer, S., **Stone, K.,** Paterson, C., Brown, M., MartinezAguirre-Betolaza, A., Creduer, D., Faulkner, J., Zieff, G., Kelsch, E., Burnet, K. & Stoner, L. (2019). Impact of a high fat meal combined with prolonged sitting on central and peripherial arterial stiffness: A pilot study. *American College of Sports Medicine (ACSM).* Orlando, FL, USA. May 28 - June 1, 2019.
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Abbreviations

ABI	ankle brachial index
aSEE	absolute standard error of estimate
ab-SG	aortic-brachial arterial stiffness gradient
af-SG	aortic-femoral arterial stiffness gradient
AGE	advanced glycation end-products
AIx	augmentation index
AIx@75	augmentation index normalized to a heart rate of 75 bpm
ARTERY	association for research into arterial structure and physiology
aSEE	absolute standard error of estimate
β	beta stiffness index
BMI	body mass index
BP	blood pressure
baPWV	brachial-ankle pulse-wave velocity
cfPWV	carotid-femoral pulse-wave velocity
CHD	coronary heart disease
CI	confidence interval
CKD	chronic kidney disease
CO	cardiac output
crPWV	carotid-radial pulse-wave velocity
cSBP	central systolic blood pressure
CVD	cardiovascular disease
d	Cohens d
DALY	disability-adjusted life year
DBP	diastolic blood pressure
ECG	electrocardiogram
ESC	European society of cardiology
faPWV	femoral-ankle pulse-wave velocity
FMD	flow mediated dilation
haPWV	heart-ankle pulse-wave velocity
hfPWV	heart-femoral pulse-wave velocity
HF	heart failure
HR	heart rate

IHD	ischemic heart disease
ICC	intra-class correlation coefficient
LV	left ventricular
MAP	mean arterial pressure
NO	nitric oxide
OR	odds ratio
P	pressure
PP	pulse pressure
PAD	peripheral arterial disease
Pb	backward aortic pressure
Pf	forward aortic pressure
PTA	posterior tibial artery
PTT	pulse transit time
PWV	pulse wave velocity
RAAS	renin angiotensin-aldosterone system
RC	reliability coefficient
RF	renal failure
RM	reflection magnitude
RSE	relative standard of error
SEM	standard error or measurement
SBP	systolic blood pressure
SFA	superficial femoral artery
sSEE	standardized standard error of estimate
SV	stroke volume
SVR	systemic vascular resistance
VSMC	vascular smooth muscle cells
WC	waist circumference
WHO	world health organisation
XCEL	sphygmoCor XCEL device
YLD	years lost to disability
YLL	years of life lost

1

Introduction

Background

Cardiovascular diseases (CVDs), compromising pathologies of the heart and blood vessels, are the leading cause of premature morbidity and mortality globally [1]. Contemporary prevention strategies typically employ population screening to identify an individual's risk of CVD progression or incident events [2] by determining the presence of conventional CVD risk factors, such as hypertension and diabetes [3]. But conventional risk factors do not fully explain cardiovascular risk [4,5], and accurate CVD risk prediction is essential for efficacious clinical-decision making and optimising prevention/treatment strategies [6,7]. Thus, there is a pressing need to identify novel tools or 'biomarkers' that reflect early pathological changes and improve CVD risk prediction [6]. One promising biomarker, representing the ability of an artery to accommodate changes in blood pressure (BP) by corresponding changes in dimension, is arterial stiffness. Research interest in arterial stiffness has chiefly centred on the clinical utility of central arterial stiffness [8,9], demonstrating it to be a powerful biomarker of CVD risk [10]. In contrast, the prognostic significance of arterial stiffness in the lower-limbs has received little attention; its measurement may well improve risk prediction accuracy by providing unique and additive CVD risk information [11]. Through a series of inter-related studies, the purpose of this thesis is to identify the clinical potential of lower-limb arterial stiffness.

Arterial stiffness can be assessed using pulse wave velocity (PWV) - the speed to which an arterial pulse propagates along the arterial wall [12]. Although PWV can be measured in any arterial segment, carotid-femoral PWV (cfPWV) has emerged as the reference standard [8,9]. This is because cfPWV is a measure of central (aortic) arterial stiffness, which increases significantly with age and pathologies, and is therefore a strong marker of vascular aging [13-16]. The cfPWV is now well established in epidemiological research [6] because it robustly predicts incident CVD events and mortality [17-19], providing better discrimination and reclassification of CVD risk than conventional risk factors alone, including BP and diabetes [10]. However, wider implementation of cfPWV into clinical practice is hampered by its dependence on mean arterial pressure (MAP) [20], which is known to be affected by a range of physiological, mechanical, and psychological factors [21-23]. Whilst arterial stiffness measures can be adjusted for MAP, it makes comparing stiffness-related outcomes between individuals, tracking changes over time, and determining optimal treatment strategies challenging [24]. More broadly, a persistent focus on cfPWV has ignored the integrated role that medium-sized peripheral conduit arteries play in the cardiovascular system. In particular, there can be important

pathophysiological changes within muscular arteries of the lower-limbs that contribute to CVD risk [25].

The femoral-tibial arterial pathways of the lower-limbs are prone to developing both arteriosclerosis, the stiffening and thickening of the arterial wall, and atherosclerosis, a narrowing of the artery by the deposition of plaque - principal drivers for CVD [26]. Poor lower-limb arterial health indicates systemic pathophysiology [27]. Assessments of lower-limb arterial health therefore allow clinicians to detect local disease pathology and provide opportunity to estimate CVD risk. In clinical practice, atherosclerosis in the lower-limbs is assessed using the ankle brachial index (ABI) [28], and the presence of occlusive stenosis serves as an independent marker for heightened global atherosclerotic CVD risk [29-32]. Whilst lower-limb arterial stiffness, an arteriosclerotic process, can be assessed using femoral-ankle PWV (faPWV), unlike cfPWV and ABI, faPWV is not routinely determined in epidemiological research or clinical practice. Increased lower-limb arterial stiffness has been shown to impair local arterial blood flow [33] leading to hypoxia of peripheral tissues [34], and is associated with increased ventricular mass, myocardial stress and damage [35,36]. And although age related increases in faPWV are less marked than cfPWV, it is associated with other CVD risk factors, including hypertension and diabetes [36-38]. But the few studies which have examined the association of faPWV with global CVD risk have reported inconsistent findings [39-42]. Studies have demonstrated associations between increased faPWV and ischaemic heart disease (IHD) [39], and stroke, but not all CVDs [40]. Others have failed to show any prognostic value [41,42]. However, all of these studies were relatively small, and most were conducted in explicit, patient populations only, limiting inference to the general population. Further research is warranted in order to identify if faPWV is a robust biomarker for CVD, particularly in general community-dwelling populations where arguably CVD risk screening has the greatest impact [2].

In the healthy young, the arterial vasculature progressively stiffens from the elastic aorta to the muscular arteries of the periphery [26,43]. This stiffness gradient dampens the transmission of pulsatile forces, permitting smooth consistent blood flow to the micro-circulation [43]. However, the differential rate of age-related stiffening between the aorta and peripheral arteries means that cfPWV may exceed peripheral PWV in older adults [11,16]. This stiffness gradient reversal increases pressure wave transmission to the micro-circulation, contributing to end-organ damage [11,44]. To date, the available literature has only employed upper-limb PWV to derive the stiffness gradient, which can predict incident CVD and all-cause mortality, better than cfPWV alone [44-49], but only in high risk

patient populations. The aortic-to-femoral stiffness gradient (af-SG), a ratio of faPWV and cfPWV, may provide a more comprehensive picture of cardiovascular risk, given that the lower-limbs make a up a greater portion of the arterial tree are more prone to arteriosclerosis and atherosclerosis than the upper limbs [26]. One potential, but unconfirmed, advantage of the af-SG is a purported independence to MAP. Should the af-SG demonstrate MAP independence, it may be of significant clinical value, allowing clinicians to optimise treatment strategies and making the tracking of changes over-time more straightforward. However, no studies have investigated the association of the af-SG with CVD, and, like faPWV, its clinical utility is unknown.

In order to address these gaps in the literature, and identify whether faPWV and the af-SG have clinical potential, it must be identified whether they meet accepted criteria, such as those listed by the European Society of Cardiology [6,50] (**Figure 1.1**). Confirming all is a challenging prospect, as such this thesis will focus on identifying if measures of faPWV and af-SG can meet two fundamental criteria. *Firstly*, in order to facilitate adoption into epidemiological or clinical practice, determination of a biomarker must involve the use of techniques which are ***simple, accurate and reliable for widespread application*** **~ European Society of Cardiology Criteria 1.** To be of any clinical use in the screening of CVD risk, a measurement technique must be valid, reflecting underlying (patho)physiology with reasonable accuracy, and do so reliably, in order to permit objective monitoring over-time. *Secondly,* a biomarker must ***add cardiovascular risk information over and above established, standard markers*** **~ European Society of Cardiology Criteria 2**. In order to justify its measurement, a biomarker must add independent additional risk information beyond biomarkers that are already routinely used, such as conventional Framingham risk factors [51,52], ABI [28] and cfPWV [10].

1. **Measurement should be simple, accurate and precise for widespread application.**
2. **Incremental value** – Does it add cardiovascular risk information over and above established, standard risk markers?
3. **Prospective validation** – Does it predict development of future outcomes?
4. **Clinical utility** – Does it change predicted risk sufficiently to change recommended therapy?
5. **Clinical outcomes** - Does the use of the biomarker improve clinical outcomes, especially when tested in a randomized clinical trial?
6. **Cost effectiveness** - Does the use of the biomarker improve clinical outcomes sufficiently to justify any additional costs?
7. **Methodological consensus.**
8. **Reference values or cut-off values established.**

Figure 1.1 Cardiovascular disease biomarkers must meet a number of accepted criteria, as agreed by the European Society of Cardiology and endorsed by Association for Research into Arterial Structure and Physiology Society before they can be recommended and incorporated into practice [50].

Objective and Approach

The principal aim of this thesis is to identify the clinical utility of lower-limb arterial stiffness. To achieve this, the following objectives have been identified:

1) **To identify whether the standalone biomarker, faPWV:**
    1.1) can be determined with acceptable accuracy (validity) and precision (reliability) - European Society of Cardiology Criteria 1.
    1.2) can provide additional cardiovascular disease risk information beyond ABI, an existing marker of lower-limb arterial health - European Society of Cardiology Criteria 2.

2) **To identify whether the composite biomarker, af-SG:**
    2.1) can provide additional cardiovascular disease risk information beyond the criterion measure of arterial stiffness, cfPWV - European Society of Cardiology Criteria 2.
    2.2) can be determined with acceptable precision (reliability) - European Society of Cardiology Criteria 1.
    2.3) can demonstrate an independence to blood pressure.

Thesis Overview

According to the two overarching objectives described above, following a review of literature (**Chapter 2**), this thesis is divided into two main parts. **Part one** of the thesis is focussed on the standalone marker faPWV, including its measurement and its association with CVD. **Part two** of this thesis is focussed on the composite marker af-SG, including its association with CVD, its measurement and its dependence on BP. **Chapters 3 – 7** represent standalone studies, presented in a typical publication format. **Chapters 3 and 6** are laboratory-based experimental research studies investigating biomarker measurement accuracy and reliability, whereas **Chapters 4, 5 and 7** are epidemiological research studies involving secondary analysis of existing population demographic and CVD data. Finally, in **Chapter 8**, a summary of the main results is given and these results are discussed with respect to their implications for practice and future research.

PART ONE: Femoral-Ankle Pulse Wave Velocity.

In **Chapter 3** the accuracy of a simple oscillometric cuff-based device for determining faPWV in supine and seated postures is determined by comparing to a criterion test device, Doppler ultrasound *(objective 1.1)*. Measurement precision is determined by assessing faPWV on three separate days using the oscillometric device. European Society of Cardiology Criteria 1 is satisfied if the absolute standard error of estimate (SEE) between devices is $\leq$ 1.0 m/s, and the intra-class correlation coefficient (ICC) estimates of repeated measures is greater than 0.75. In **Chapter 4** the association of faPWV and ABI with conventional Framingham CVD risk factors and a composite measure of CVD (coronary heart disease, heart failure and stroke) in a population of community-dwelling older adults is described *(objective 1.2)*. European Society of Cardiology Criteria 2 is satisfied if faPWV measures independently explain a significantly greater proportion of the variance in prevalent CVD beyond Framingham risk factors and ABI.

PART TWO: Aortic-Femoral Arterial Stiffness Gradient.

In **Chapter 5** the associations of the af-SG and the criterion measure of arterial stiffness, cfPWV, with conventional CVD factors and CVD (hypertension, diabetes, coronary heart disease, heart failure and stroke) in a population of community-dwelling older adults is described *(objective 2.1)*. European

Society of Cardiology Criteria 2 is satisfied if af-SG measures explain a significantly greater proportion of the variance in CVD prevalence than Framingham risk factors and cfPWV. In **Chapter 6** ***(objective 2.2)*** the measurement precision of the af-SG is determined by assessing faPWV and cfPWV on three separate days using a simple oscillometric device ***(objective 2.2)***. European Society of Cardiology Criteria 1 is satisfied if ICC estimates of repeated measures is greater than 0.75. In **Chapter 7** the association of the af-SG with MAP, in healthy, hypertensive and diabetic populations is described ***(objective 2.3)***. The af-SG is considered MAP independent if associations with MAP are non-significant.

2

Review of Literature

OUTLINE

Cardiovascular Disease and Its Prevention

The Cardiovascular Disease Burden

Cardiovascular diseases (CVDs), compromising pathologies of the heart and blood vessels, are the leading cause of premature morbidity and mortality globally [1]. In 2019 the World Health Organization (WHO) estimated that globally the total years of life lost (YLL) prematurely to CVDs was more than 354 million, with the primary manifestation being cardiac and cerebrovascular events [53]. This represents a 20% increase in YLL to CVDs since 1990 [53], driven chiefly by the prevalence of CVD doubling to 523 million in this period [53,54]. But CVDs also have a significant intermediary impact upon people's lives, chiefly through mass disability. Concomitant with the rise in CVD prevalence, productive years of life lost to disability (YLD) due to CVDs also doubled from 17 million in 1990 to 35 million in 2019, and now represents the leading somatic cause of loss of productivity [53,54]. This decades-long rise in CVD burden is expected to continue, with the total 'healthy YLL' annually, the sum of YLD and YLL termed disability adjusted life-years (DALYs), forecast to rise more than 12% by 2030 [55,56]. The unabated rise in CVDs represents a significant economic challenge to health states [57,58], whilst having a tragic impact upon an individual's quality of life, particularly in low-income developing countries. Effective prevention strategies are essential if we are to limit the growing burden of CVDs.

Cardiovascular Disease Prevention

Two broad strategies are employed to prevent and reduce CVD burden, and typically operate in tandem. The first is the application of population-wide public health measures, creating a physical environment that promotes the adoption and maintenance of lifelong healthy lifestyles [59]. Often at policy level, initiatives target known behavioural (physical activity, tobacco use), psychosocial (chronic life stress, depression), biological (BP, diabetes), environmental (air pollution) and health system (access to care) risk factors for all non-communicable diseases, including cancer, respiratory illness, as well as CVDs [60]. Examples include the taxation of tobacco products or the enablement of active transport. These initiatives should make it easier for healthy people to stay healthy, and those at greater CVD risk or with established CVD, to change behaviours. However, population-wide strategies have consistent, but only modest impact on CVD morbidity and mortality [61-63], and can neglect individuals at an elevated risk of CVD, or those with an established CVD [59].

The second strategy, one at the individual level, is the screening and stratification of CVD risk throughout adulthood, but typically from middle age (> 40 years) [2]. Screening for the presence of known CVD risk factors permits the early detection and identification of adults at elevated risk of CVD, who may be asymptomatic [59], and, when followed with behavioural counselling or pharmaceutical treatment as appropriate, can prevent the progression of disease or recurrence of CVD events [64,65]. This 'high-risk' approach has become the focus of contemporary primary CVD prevention [2,66]. Successful CVD screening strategies are multifactorial [61], but are fundamentally contingent on the accurate estimation of an individual's CVD risk in order to inform efficacious clinical-decision making, including, the determination of need and intensity of pharmacological intervention, objective monitoring and control of risk, and, the systematic evaluation of the benefits, risk and costs of management strategies [6,7]. Inaccurate risk assessment may overlook high-risk individuals or result in overdiagnosis, leading to inappropriate or hazardous treatment that wastes precious financial resources [67]. To accurately assess CVD risk, clinicians and epidemiological researchers need to determine the presence of pertinent markers for CVD, and, do so using tools which are accurate and precise.

Primary Cause of Cardiovascular Disease

Although the aetiology of CVD is complex, the cause of most CVDs is the structural and functional changes of central and large conduit arteries, induced by the pathogenic processes of arteriosclerosis and atherosclerosis [68]. These phenomena impact two principle, and interrelated, arterial functions: i) to act as pipes permitting adequate blood supply from the heart to distal tissues and organs - the 'conduit' function; and, ii) to dampen pressure oscillations caused by the intermittent nature of left ventricular (LV) ejection, permitting continuous blood flow to the microcirculation – the 'cushioning function' [26,43,69]. Arteriosclerosis refers to the stiffening and thickening of the arterial wall and attenuates the cushioning function of arteries; typically associated with heart failure (HF) [70,71], LV hypertrophy [72,73], and target organ damage, including stroke and chronic kidney disease (CKD) [74]. Atherosclerosis refers to the narrowing of the artery by the deposition of atheromatous plaque, leading to the restriction of blood flow and ischaemia of distal tissues, a frequent cause of strokes [75], ischaemic heart disease (IHD) [76] and peripheral arterial disease (PAD) [77]. Both arteriosclerosis and atherosclerosis begin early in life [78,79], contribute to normal vascular aging and progress

gradually throughout adulthood [80]. However, the rate of pathogenesis is strongly influenced by the interplay between biological and lifestyle factors [81].

Identifying Cardiovascular Disease Risk

Epidemiological and clinical studies over the past 50 years have led to many of the primary risk factors for the acceleration of arteriosclerosis and atherosclerosis, and thus CVD, being well established, identifiable and controllable [81]. First identified through seminal work from the Framingham Heart [51,52] and Seven Countries studies [82], these primary risk factors include; age, sex, hypertension, dyslipidaemia, diabetes, smoking and, lack of physical activity - which combined with a poor diet leads to obesity [81]. These 'conventional' risk factors have been shown to account for up to 75% of CVD risk [3]. Incorporating these conventional global risk factors, which commonly coexist and act multiplicatively, into simple multivariable risk prediction models (e.g. Framingham risk score, QRISK2) enables practitioners to estimate an individual's total risk of developing specific CVDs, including CHD [83], HF [84] and stroke [85], or general all arterio- and atherosclerotic CVD risk [86,87], with reasonable accuracy. Importantly there is strong evidence that controlling levels of modifiable risk factors (e.g. BP, physical activity) has a beneficial effect on CVD progression and mortality [88-90].

Despite this, many aspects of CVD pathophysiology remain poorly understood, and conventional risk factors cannot fully explain excess CVD risk. For example, 50% of individuals with CVD do not have any risk factors [91,92], and many adults suffering fatal CVD events do not have any prior symptoms [93,94]. The detection of sub-clinical arteriosclerosis and atherosclerosis is therefore essential, and may open a window of opportunity to prevent the occurrence of CVD through timely treatment. This prognostic gap has led to the search for new tools, or 'biomarkers', to aid CVD risk screening and stratification [6]. The National Institutes for Health defines a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" [95]. With regard to CVD prevention, cardiovascular biomarkers reflect early functional or morphological changes, before the overt manifestation of CVD. One promising biomarker whose role in the development of CVD has received significant attention in the last decade, representing the ability of an artery to accommodate changes in BP by corresponding changes in diameter, is arterial stiffness.

Arterial Stiffness

Arterial stiffness is a general term that describes the rigidity of the arterial wall, which in turn has functional consequences for the artery, since it affects the manner in which pressure, blood flow and arterial diameter change in response stroke volume (SV) [50].

Structural Basis of Arterial Stiffness

In order to understand the impact of how changes in artery mechanical behaviour can lead to CVD and heighten risk, it is necessary to know the composition of an artery and how the biomaterial is organised.

The stiffness of the arterial wall is determined by the intrinsic properties of its constituents, their relative proportion, functional relationships, as well as geometrical factors such as the size of the vessel and its thickness [96]. The arterial wall is comprised of endothelial cells, vascular smooth muscle cells (VSMCs), and proteins, principally collagen and elastin, arranged in a highly organised matrix [26], depicted and described in **Figure 2.1**. The endothelium, a mono-cellular layer, lines the inside of the entire vascular system, forming part of intima. Endothelial cells contribute little passive elasticity, but their structural and functional integrity are important in the maintenance of vessel wall circulatory function. The endothelium acts as semi-permeable barrier regulating molecule transfer and contributes to the regulation of vascular tone via the production of vasoactive molecules in response to endogenous factors, such as ischemia and mechanical stimuli like shear stress [97]. VSMCs in the media layer are a major constituent of the artery wall, but whilst contributing to structure and tension, are not regarded as elastic material. But VSMCs facilitate dynamic regulation of vascular tone and arterial diameter, being acted upon by vasoactive molecules such as nitric oxide and endothelin, triggering smooth muscle dilation or contraction respectively [98].

Elastin and collagen are the predominant elastic materials of the arterial wall, and play a crucial role in artery mechanics. They are precisely orientated and interlocked with VSMCs in the media, with this collective acting mechanically as a homogenous material [26]. Elastin bands or sheets act as

concentric plates, taking on the primary load bearing function and providing reversible extensibility during cyclic loading of the cardiac cycle [99]. In an unloaded artery, the elastic lamellae appear undulated and wavy. As the pressure increases, they straighten as they begin to bear load, being mostly straight at physiologic pressures [100]. Collagen fibres, located in the media, are inherently stiffer, provide strength and are arranged circumferentially, dispersed equally, and to a greater degree than elastin, are initially un-stretched. As the pressure increases above physiologic values, more collagen fibres become load bearing and their stiffness limits arterial distension – a safety net preventing failure at high pressure [26].

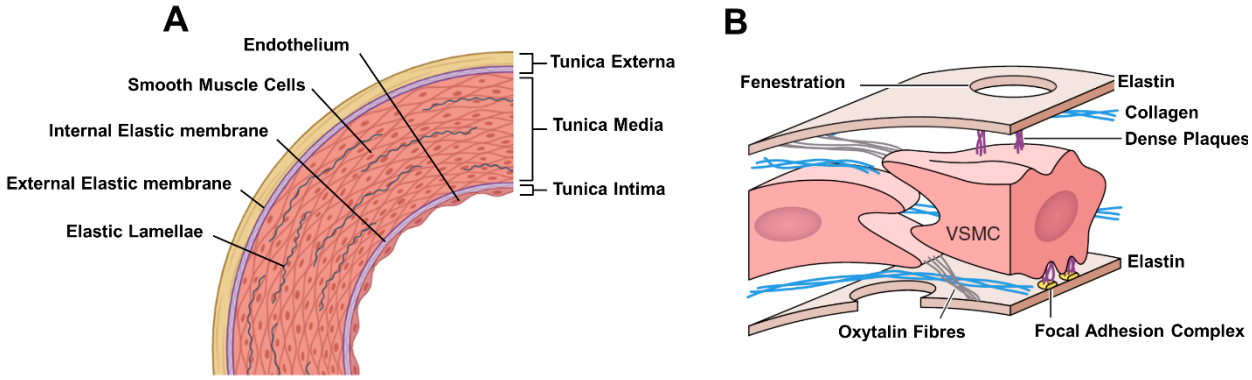

Figure 2.1 Structure of the arterial wall. **A:** Organisation of endothelium, vascular smooth muscle cells (VSMC) and elastic components, with intima-media-externa layering. The tunica intima consists of endothelium and a thin layer of elastin and collagen fibres. The tunica externa is a region of collagen and some elastin tissues that merges with the surrounding connective tissue consisting of nerves and small blood vessels. The tunica media forms the major part of the wall properties, consisting of concentrically organized musculo-elastic complexes. **B:** Organisation of VSMCs, elastin and collagen within a musculo-elastic complex of a large artery. VSMCs are embedded between two layers of elastic lamellae. Collagen fibres run along the lamellae. The stiffness of the arterial wall material of large arteries is dependent the stiffness of each component (VSMC, elastin, collagen) and their functional relationships (image B adapted from Lacolley *et al.* [98]).

The distribution of arterial wall components and their organisation changes markedly between central arteries and that of peripheral arteries, described and depicted in **Figure 2.2**. Elastin predominates in the proximal aorta, whereas collagen fibres and smooth muscle cells predominate beyond the femoral arteries [26]. Muscular arteries also rely to a greater extent on the elasticity of internal and external lamina to accommodate distension. As the composition of the arterial wall changes, so does its stiffness, leading to a gradual central to peripheral arterial stiffness gradient. This difference in vessel composition and stiffness reflects the differential roles that elastic central, medium

and muscular peripheral (resistance) arteries play in the cardiovascular system. Central arteries (carotid, aorta, femoral) perform both conduit and cushioning functions. Their large diameters and high elastic potential mean they exert low resistance on blood flow, essential for efficient conduit function. But central arteries are also compliant because they need to cushion blood flow and pressure oscillations caused by intermittent LV ejection and ensure peripheral organ perfusion occurs at a steady flow and pressure [93]. In contrast, whilst medium and small muscular peripheral arteries have lower elastic potential than central arteries, and therefore have less of a cushioning role, their greater propensity for alterations in vascular tone mean they are proficient conduits [98]. The contraction of VSMCs allows for large variation in the vessel lumen and permits the effective short-term distribution of flow to vascular beds according to metabolic demand.

The intrinsic elastic properties of the biomaterials in the arterial wall can be characterised directly, independent of vessel geometry. But the transfer of load bearing predominance from elastin to collagen fibres highlights the non-linear behaviour of an artery when subjected to stretching/pressurisation over a large range (pressure-area relationship), with a functional stiffening of an artery, and greater resistance to distension, at higher volumes/pressures [12,26]. Arterial stiffness then can only be defined in terms of a given pressure, since stiffness increases with increases in BP. Also, a thick-walled artery of a given material stiffness will distend less in response to a given pressure than a thin-walled one with the same material properties [96]. Thus, in practice, arterial stiffness is typically quantified as a 'functional stiffness', incorporating both intrinsic material properties of the arterial wall, BP, as well as geometrical factors such as the size of the vessel and its thickness [12].

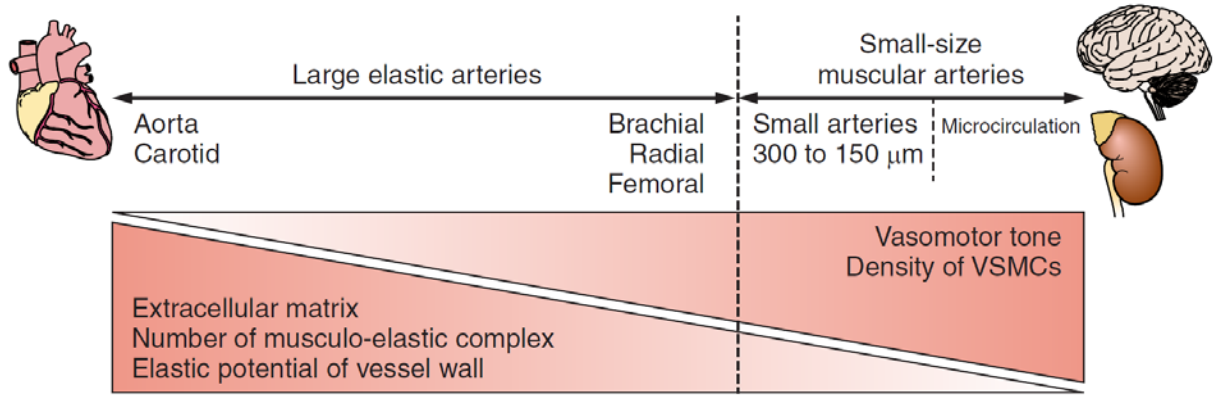

Figure 2.2 The structure of the arterial tree is heterogenous. The left red triangle illustrates the high elastic potential of proximal central arteries, but a progressive reduction in muscular-elastic complexes

reduces elastic potential moving distally. The enlarging of the right triangle indicates the potential for changes in vasomotor tone of peripheral arteries increases moving distally, due to the increasing density of vascular smooth muscle cells (adapted from Lacolley *et al.* [98]).

Measurement of Arterial Stiffness

Arterial stiffness can be assessed at a systemic, regional and local level (**Table 2.1**) [8]. Systemic arterial stiffness reflects global buffering properties of the arterial system [101], but can only be estimated from simple models of the circulation (e.g. *Windkessel* model) following pulse waveform analysis at a peripheral site [8]. Whilst providing useful data for various haemodynamic analyses, it is not recommended as a valid surrogate for direct arterial stiffness measurements [102]. By contrast, regional and local arterial stiffness can be measured directly, and non-invasively, at various sites along the arterial tree. A major advantage of the regional and local evaluations of arterial stiffness is that they are based on direct measurements of parameters strongly linked to wall stiffness [8].

Measure	Description	Calculation / Unit
Systemic		
Augmentation Pressure	Difference between early and late systolic peak of pulse wave contour, divided by PP height.	(mm Hg or as % of PP)
Regional		
Pulse Wave Velocity	Speed of travel of the pulse along an arterial segment	Distance/ Δt (m/s)
Local		
Distensibility	Relative diameter (or area) change for a pressure increment; the inverse of elastic modulus.	$\Delta D/\Delta P \cdot D$) (mm Hg^{-1})
Compliance	Absolute diameter (or area) change for a given pressure step at fixed vessel length.	$\Delta D/\Delta P$ (cm/mm Hg) or cm^2/mm Hg)
Elastic Modulus	The pressure step required for (theoretical) 100% stretch from resting diameter at fixed vessel length.	$\Delta P \cdot D/\Delta D$) (mm Hg)
Stiffness Index	Ratio of logarithm (systolic/diastolic pressures) to (relative change in diameter).	β = In (Ps/Pd) / [(Ds-Dd)/Dd] (nondimensional)

Table 2.1 Definition and units of various indices of arterial stiffness.

Abbreviations: P, pressure; PP, pulse pressure D, diameter; t, time; s, systolic; d, diastolic.

Local arterial stiffness measures are calculated as the ratio of pulse pressure (PP) to the relative change in diameter, with diameter typically obtained using ultrasound [50]. Several parameters of local arterial stiffness have been proposed, which are all related to the mechanical stiffness of the artery, including compliance, distensibility and elastic modulus [26]. The main limitation of these parameters is that their estimation requires arterial pressure measurement at the site of diameter assessment, which can only be determined via catheterisation in deep-seated vessels (e.g. the aorta). Non-invasive assessment of pressure at substitute (peripheral) arteries leads to increased measurement inaccuracy [103]. Moreover, their assessment necessitates expensive equipment and a high degree of expertise. Therefore, although of utility for mechanistic analyses in pathophysiology and therapeutic studies, local measurement of arterial stiffness is unsuitable for large scale epidemiological research or routine clinical practice [104]. At present, the only method recommended for large scale epidemiological or clinical use [105], which is simple and accurate enough to be considered as a diagnostic procedure, is pulse wave velocity (PWV), a measure of regional arterial stiffness.

Assessing PWV, the speed to which an arterial pulse propagates along the arterial wall, has become synonymous with the evaluation of arterial stiffness [58]. Indeed, the measurement of PWV is generally accepted as the most simple, non-invasive, robust, and reproducible method to determine arterial stiffness [12]. The association between stiffness and PWV can be proved theoretically, based on the propagative circulatory model. It is recognised from the Bramell-Hill equation (eq.1) that PWV is inversely related to the square root of the distensibility (D) [106]; the higher the PWV, the stiffer the blood vessel. And PWV is a functional assessment of stiffness of an artery as a hollow structure, and depends the geometry of the artery (thickness, h; and radius, R), the intrinsic elastic properties of the arterial wall (E) and blood density (ρ), according to the Moens-Kortweg equation (eq.2):

(1) Bramwell Hill equation: $\mathrm{PWV} = \frac{1}{\sqrt{\rho D}}$

(2) Moens-Korteweg equation: $\mathrm{PWV} = \sqrt{\frac{Eh}{pR}}$

Note that geometrical and mechanical properties of the arterial tree are heterogenous, as such PWV is not constant [26]. Therefore, regional measures of PWV will only be an integrated measure of the stiffness properties of arterial segment under study.

In practice, PWV is typically measured over an arterial segment by determining the transit time of an arterial pulse between proximal and distal measuring sites [8,9]. Combined with a measure of distance travelled, usually assimilated to the surface distance between the two recording sites, PWV is calculated as: distance (meters) divided by transit time (seconds). Carotid-femoral PWV (cfPWV) a measure of aortic arterial stiffness is the reference standard [50,105], as it is a strong predictor of CVD risk and mortality [10,19,107]. But PWV can be measured on any artery or between any arterial sites, including peripheral arteries, using femoral-ankle PWV (faPW: lower-limb) and brachial-ankle PWV (central-lower-limb composite), which can provide novel risk information and are being evaluated for their prognostic and diagnostic capabilities because of their ease of measurement (**Figure 2.3 A**) [11].

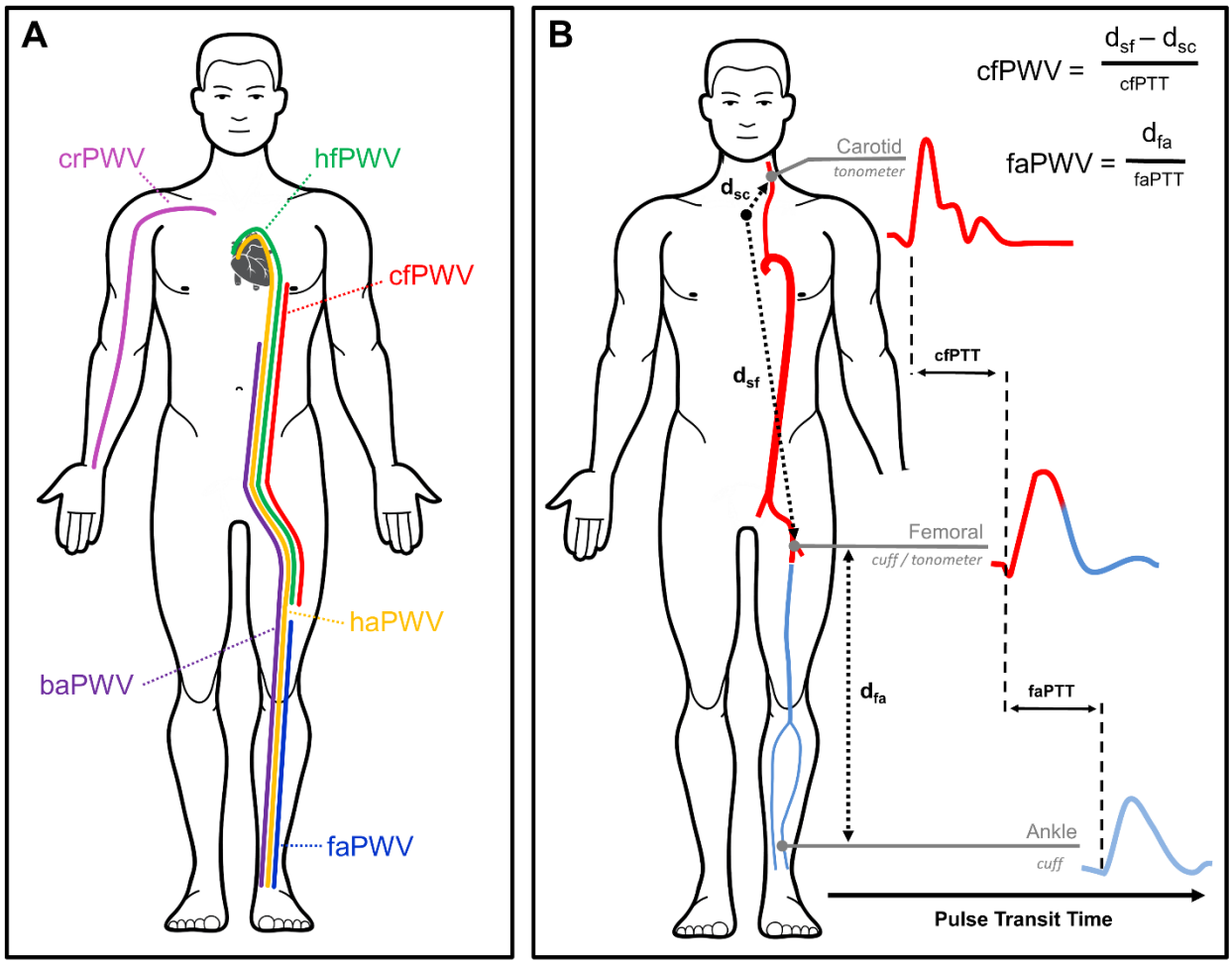

Figure 2.3. Indices of pulse wave velocity (PWV). **A**: Effective path lengths for carotid-femoral PWV, heart-femoral PWV, heart-ankle PWV, brachial-ankle PWV, carotid-radial PWV and femoral-ankle PWV. **B:** Schematic of the foot-to-foot method for determining cfPWV and faPWV measures.

cfPWV is estimated as the distance between the sternal notch and the femoral sampling site (dsf) minus the sternal notch to carotid sampling site (dsc), divided by the time delay (pulse transit time) between carotid and femoral waveforms (cfPTT). Note cfPTT is calculated using the subtraction method to correct for the fact pulse waves travel simultaneously towards the carotid and femoral arteries. faPWV is estimated as the distance between femoral and ankle sampling sites divided by the time delay between femoral and ankle waveforms (faPTT).

The most common method used to determine transit time is the foot-to-foot method, whereby the foot of the wave represents the end of diastole directly prior to the steep rise of the subsequent waveform and is used as the point of reference (**Figure 2.3 B**) [8,9]. The mechanical perturbation or signal that arises from the propagating pulse can be detected using a number of technologies with sufficiently high temporal resolution and with discernible fiducial points, including applanation tonometry or oscillometry (local pressure measurement, distension), Doppler ultrasound (flow velocity), or photoplethysmography (measuring local changes in volume) [50,108]. Ideally measurements are performed simultaneously; but ECG-gated sequential measurements are acceptable. Whilst all technologies have shown acceptable accuracy and precision [50], they can differ in their practicality. For example, although high-fidelity applanation tonometry and ultrasound is well accepted for PWV measurement, they require a trained operator, and detection/imaging of relevant arteries can be challenging in obese populations or those with advanced atherosclerosis [109]. By contrast emerging oscillometric devices, whereby a pressure cuff is placed around the neck or limbs to record pulse wave arrival, offer simple, automated, user-independent determination of PWV, and have demonstrated excellent reliability, and so are of high epidemiological and clinical utility [110,111].

Considerations for Arterial Stiffness Measurement

Regardless of the PWV technique employed, a number of physiological and methodological factors can influence the confound arterial stiffness measures. These factors require due consideration in order to minimise their impact, permit high quality data to be obtained, and allow for correct data interpretation [50].

The most significant physiological variable affecting arterial stiffness is the vessel distending pressure, or MAP [26]. Indeed, PWV is highly dependent on MAP [112,113], as increased MAP augments the arterial wall tension and adds functional arterial stiffness, but in a non-linear manner. In turn, MAP is known to be affected by a range of physiological, mechanical, and psychological

factors [21-23]. Arterial stiffness may also be impacted by heart rate (HR), although this relationship is less well defined. Studies have shown positive [114,115], inverse [116] or no association [117]. MAP, and likely HR, should be recorded at the time of an arterial stiffness measurement, and taken into consideration when interpreting stiffness data. To limit the acute impact of physiological confounding, as well as prevent possible errors due to temporary changes in participant conditions, a number of standard operating procedures have been recommended when determining PWV [50] (**Table 2.2**). For example, since BP and vascular function is influenced by circadian and diurnal variation, it is typical to control experimental time of day [118]. A fasted state is also a typical requirement, as macro- and micro-nutrients can greatly affect vascular function [119]. It is also important to control environmental conditions in including noise and ambient temperature as they have both been reported to impact BP and the vasculature [120,121].

Confounding Factor	In Practice
Room Temperature	Controlled environment kept at 22±1°C.
Rest	At least 10 min in recumbent position.
Time of Day	Similar time of the day for repeated measurements
Smoking/Eating	Subjects have to refrain, for at least 3 h before measurements, particularly caffeine.
Alcohol	Refrain from drinking alcohol 10 h before measurements.
Speaking/Sleeping	Subjects may neither speak nor sleep during measurements.
Position	Supine position is preferred.
Measurement Error	Calliper use for PWV distance measurement is preferred. A minimum of 10 cardiac cycles should be analysed Duplicate pulse wave velocity measures are recommended (within 0.5 m/s).

Table 2.2. Recommendations for standardisation of conditions (adapted from Townsend *et al.* [50]).

To account for any changes in MAP, statistical models including linear and logistic regression can/should be used to adjust for confounding and isolate the PWV relationship of interest [122,123]. However, adjustment for MAP may not completely control for confounding, as i) BP is typically a systemic rather than a local measure, ii) average MAP does not consider inter-individual differences in PWV-pressure relationship, and iii) does not consider the non-linearity of the PWV pressure relationship [20,24,123]. As a consequence, there have been many attempts to develop arterial stiffness indices which are MAP independent, including the β stiffness index [124] and the cardio-ankle vascular

index (CAVI) [125]. The β is a local stiffness measure determined using ultrasound and calculated from changes in diameter, whereas CAVI can be calculated from local changes in diameter or regional PWV measures such as haPWV. However, whilst β and CAVI theoretically account for MAP, Spronck *et al.* [126] recently demonstrated that both measures are not entirely pressure-independent. The use of a proposed fixed reference pressure value to correct this issue is also argued to be conceptually un-appealing, given that PWV measures typically encompass various heterogenous vascular territories [24].

The most important methodological confounder of PWV measurement is the calculation of wave distance travelled. Accurate transit time recording should be coupled with a precise, and reproducible, measure of true arterial lumen length [127]. But a limitation of most (except magnetic resonance imaging) PWV techniques is that the arterial length has to be approximated from body surface measurement, which may not reflect true arterial morphology. Accurate measurements, even with callipers, can be difficult in the presence of abdominal obesity, and does not consider potential arterial tortuosity of older arteries. Small inaccuracies may influence the absolute value of PWV [128]. As final consideration, many PWV measurement techniques, particularly applanation tonometry and ultrasound, are operator-dependent, and require training to ensure high quality recordings [50]. Elliot *et al.* demonstrated that ideally 30 'practice' assessments are required to demonstrate excellent reliability [129], but once trained, measurement variability between technicians using oscillometric based techniques is acceptable [130].

Arterial Stiffening with Age - "man is as old as his arteries"

Normal aging, in the absence of disease, is associated with several major arteriosclerotic changes in the structural and mechanical properties of the arterial wall – they dilate, thicken and stiffen.

Elastin fibres are predominantly deposited in the media of the arterial wall during fetal growth and infancy, and have an extremely low turnover rate *in vivo* due to low elastin expression in adults [99]. This longevity means they are subject to the accumulation of deleterious insults. Throughout the lifetime, cyclical wall stress can lead to elastic lamellae becoming fractured and discontinuous, attenuating their low-stretch bearing component, and shifting load bearing onto stiffer collagen fibrils, increasing arterial wall stiffness [131]. This phenomenon also accounts for the significant arterial dilation observed with age (**Figure 2.4**), with changes being most marked in the aorta (~0.017

mm/year) [132], but also occurring in the lower-limb with femoral diameter increasing by 0.5% per year [133]. At the same time, arteries can lose longitudinal elasticity, leading to elongation and increased tortuosity [134]. The arterial wall, again particularly in central arteries, may also stiffen due to the calcification of the elastic lamellae. Increased calcium deposits (medial elastocalcinosis) in the media with age facilitates the direct binding of calcium to elastin fibres, and there is a strong correlation between arterial wall calcium content and arterial wall stiffness [100,135].

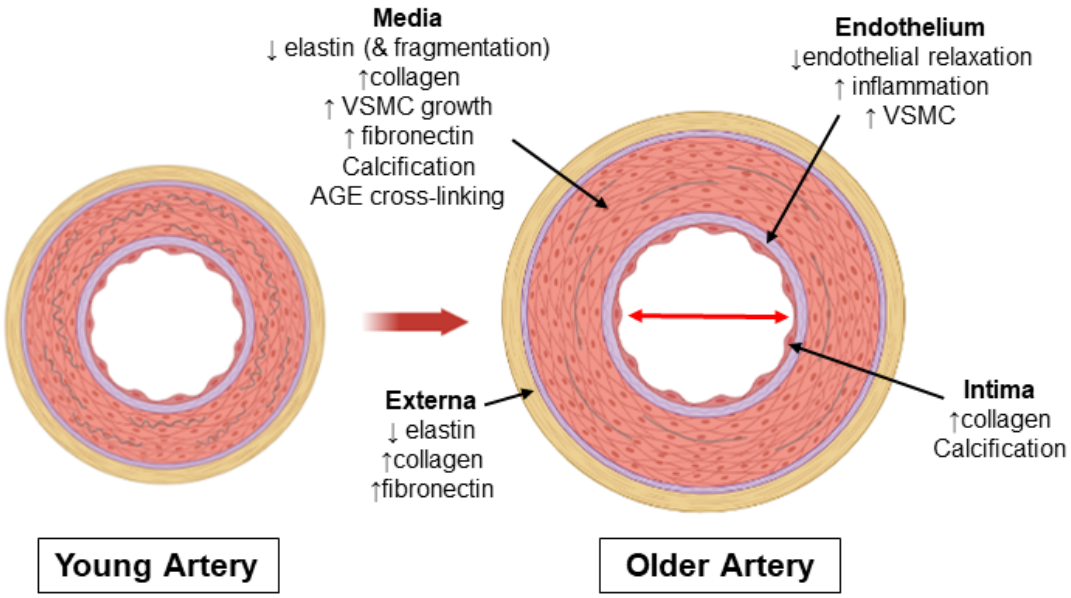

Figure 2.4 Mechanisms of arterial stiffening in the arterial wall. Abbreviations: AGE: advanced glycation end-products; VSMC, vascular smooth muscle cells.

In contrast to elastin, collagen is continuously degraded and deposited permitting blood vessels to grow and remodel in response to changes in the mechanical and biological environment [136]. In the young and healthy, this process is tightly regulated, but with age, collagen accumulates throughout the intima-media-externa complex, attenuating the elastin/collagen ratio [99]. Collagen fibres replace VSMCs and bundle near lamellae units leading to medial fibrosis [137,138]. This, along with the greater proliferation and migration of VSMCs in the intima and media with age, can lead to the greater wall thickness in both proximal elastic and distal medium sized arteries (**Figure 2.4**) [98]. The stiffness of elastin and collagen fibres is also increased by the accumulation of advanced glycation end-products (AGEs), leading to the formation of additional protein-protein cross-links along the length of both molecules [139].

As well as direct changes described above, the arterial wall may also be impacted by overarching pathways such as endothelial dysfunction and inflammation. Impaired endothelial function with age, due to a reduction in nitric oxide production [140], leads to increased resting vascular smooth muscle tone, which in the longer term may contribute to arterial remodelling through the confuscation of matrix protein synthesis [141]. There may be a cyclical relationship between these phenomena, as stiffening further worsens endothelial function by promoting a decline in nitric oxide, and this in turn, worsens stiffening. This may be more relevant for medium sized vessels given their inherent greater VSMC volume [96].

Finally, chronic low-grade inflammation, a complex non-specific protective response of vascular tissue to injury, is now well accepted as a major determinant of arterial remodelling. Aging disrupts the highly regulated homeostasis of arterial extracellular matrix through imbalances in regulatory pathways, such as the renin-angiotensin system (RAAS). Upregulation of renin-angiotensin activates pro-inflammatory pathways, altering the ratio of reactive oxygen species relative to antioxidant defences [142]. The subsequent release of inflammatory cytokines and oxidative stress impairs nitric oxide production (and thus endothelial function), and can trigger structural changes in the arterial wall through the breakdown of elastin, the proliferation of VSMCs and the calcification of the vessel wall [143].

Accelerated Arterial Stiffening

There are a number of independent biological and behavioural risk factors that may accelerate the progression of arterial stiffness beyond increases in chronological age. The most prominent of which will be briefly discussed here.

Blood Pressure elevation is undeniably the most important factor in the development of CVD and arterial stiffening in the short and long term [81,144]. As previously discussed, arterial stiffness and BP are interdependent, the arterial wall becomes stiffer with increasing distension due to the greater engagement of collagen fibres at higher distending pressures [145]. However, sustained increases in BP, and the concomitant greater cyclical loading, promote matrix synthesis and hypertrophy of the medial layer causing subsequent increases in vessel stiffness and structural stiffening [98]. Of course, increased stiffness then elevates BP, highlighting the insidious positive feedback loop between these parameters.

Diabetes is the strongest metabolic risk factor for CVD [81]. Several studies have shown arterial stiffness measures to be consistently higher in diabetic patients than healthy adults [146,147], with a preferential stiffening of central over peripheral arteries [148]. Positive correlations also exist between insulin resistance and fasting blood glucose concentrations [149]. Although there are multiple pathways, diabetes can accelerate arterial remodelling by augmenting production of AGEs that cross-link with collagen and elastin [150]. Chronic hyperglycaemia accentuates activity of RAAS, stimulating vascular hypertrophy [151].

Obesity was originally thought to be an in-direct causative factor, but has been shown to be an independent determinant of arterial stiffness [152,153]. Body mass index (BMI), waist circumference (WC), and body fat percentage are all robust predictors of accelerated aortic stiffening, as measured using PWV, after adjustment for age, sex, MAP and metabolic risk factors [154]. Obesity related reductions in elasticity have been observed in both central and peripheral arteries [155]. Sympathetic nervous activity (increased HR and BP), metabolic (hyperglycaemia, insulin resistance), endothelial dysfunction (reduced NO) and inflammatory (increased circulating pro-inflammatory cytokines & leptin) processes have all been presented as potential mediators [155].

Smoking has the strongest association with CVD of all the modifiable risk factors [3,81]. But its link to CVD is primarily thought to be via atherosclerotic mechanisms, including inflammation and elevated prothrombotic factors [156]. Its association with arterial stiffness is inconsistent [157,158]. Kool *et al.* reported that smoking a single cigarette can acutely reduce distensibility and compliance, likely a result of increased in BP and HR, but there was no difference in vessel stiffness between habitual and non-smokers [159]. But others have shown that aortic stiffening and augmentation index (AIx), a systemic measure of arterial stiffness, positively associate with smoking status [160].

Physical inactivity has consistently been associated with accelerated arterial stiffening [161,162], and is the second strongest behavioural determinant of CVD [3,81]. Moreover, in a longitudinal study of 5,196 adults Ahmad-Abhari *et al.* [163] demonstrated that higher levels of moderate-to-vigorous physical activity, as well as avoidance of sedentary behaviour (defined as very low intensity behaviours such as sitting), were each associated with slower age-related progression of aortic stiffness, independent of conventional vascular risk factors. Physical activity is associated with improved endothelial function, as a result of elevated shear stress and reduced production of vasoconstrictors (e.g. endothelin), as well as reduced low-grade inflammation due to decreased oxidative stress, which may explain its protective role [164].

Sex has a substantial impact on the rate of disease progression and prevalence of different CVDs [165]. These sex differences are also observed in the development of arterial stiffness with women experiencing greater increases in pulse pressure and arterial stiffness with age [15]. Moreover, stiffness and CVD risk increase linearly in men whereas women experience a curvilinear aging trend, with a flatter curve for young women and a steep increase in stiffness and CVD events post-menopause [15,166,167]. Although this suggests distinct sex-specific mechanisms, little work has examined potential sex differences, with most research using males only, limiting our understanding [166].

Racial disparities in arterial stiffness progression is now well supported by clinical and epidemiological research evidence. Most studies performing race-specific comparisons report that populations of African descent (and often Hispanic) have higher arterial stiffness than white populations throughout the life course [168,169]. Raised BP often accompanies elevated arterial stiffness, however, contributing factors to early vascular aging with certain racial backgrounds remain poorly understood [168].

In addition to those described above, various pathophysiological conditions and risk factors have been associated with arterial stiffening, including; low birth weight [170], menopause status [171], family history of hypertension, diabetes and myocardial infarction [172]; additional cardiovascular risk factors such as hypercholesterolaemia [173] and metabolic syndrome [174]; and the presence of CVD itself, including CHD, HF, and stroke [107,175]. However, when evaluating the degree of arterial stiffness, the major parameters to be considered are age, BP, sex, and race, and to a lesser degree the classical risk factors diabetes and smoking [74]. These factors have strong stiffness independent links with CVD, which can confound (distort or mask) the association between arterial stiffness and CVD. For clarity, a confounder is a variable that influences both the dependent variable and independent variable, causing a spurious association. Awareness and control of these factors, as covariate adjustments in regression models for example, may be necessary to discern the true association between arterial stiffness and CVD [74].

Arterial Stiffening and Arterial Function

The age and/or lifestyle induced stiffening, thickening and dilation of arteries has complex haemodynamic effects that have a major impact on cardiovascular function. The efficiency of the

conduit function, to act as pipes permitting adequate blood supply from the heart to distal tissues and organs, is a consequence of arterial diameter and resistance to flow [43]. Although arterial stiffening my increase resistance to flow somewhat, resistance is negligible in vessels larger than 0.5 mm [102]. Further any resistance to flow caused by arterial stiffening in large and medium sized conduit arteries is offset by concurrent arterial dilation [133]. Rather the presence of atherosclerotic plaques leading to lumen narrowing is the most common occlusive vascular disease [96]. However, arterial stiffening and subsequent vessel narrowing can impair blood flow and perfusion in the posterior and anterior tibial arteries (<0.3 mm in size), leading to ischaemia [33,34].

In contrast, through direct and in-direct mechanisms, arterial stiffening has a dramatic effect on the second role of arteries, to dampen pressure oscillations caused by the intermittent nature of LV ejection and permit continuous blood flow to the microcirculation - the cushioning function [43]. The 'direct' mechanism involves the generation of a higher-pressure wave by the LV ejecting into a stiffer arterial system. For each cardiac cycle, LV contraction ejects a bolus of blood into the aorta, generating a forward-travelling pulse pressure wave. To dampen this pulsatility (high pressure and flow), central arteries act as a '*Windkessel*', storing up to 50% of SV momentarily via a stretching of the arterial wall (**Figure 2.5**) [43,74]. During diastole, the elastic tensile energy 'stored' in the vessel wall during distension recoils, propelling the accumulated blood forward into the peripheral circulation [74]. This phenomenon is advantageous because it, i) keeps systolic blood pressure (SBP) low, limiting cardiac workload, ii) maintains diastolic pressure and flow, essential for coronary perfusion, and iii) ensures the peripheral micro-circulation receives a steady flow of blood [43,69]. However, increased arterial stiffness attenuates the ability of arteries to cyclically distend and store elastic energy [69,74]. If distension is limited, more blood has to be transported over a longer distance in systole, which requires a higher driving systolic pressure, elevating cardiac workload and widening pulse pressure, whilst also diminishing diastolic pressure and flow [43,102]. Blood flow and pressure remain intermittent, with exaggerated flow and pulsatility leading to short capillary transit time and reduced metabolic exchanges in the microcirculation and organs [104].

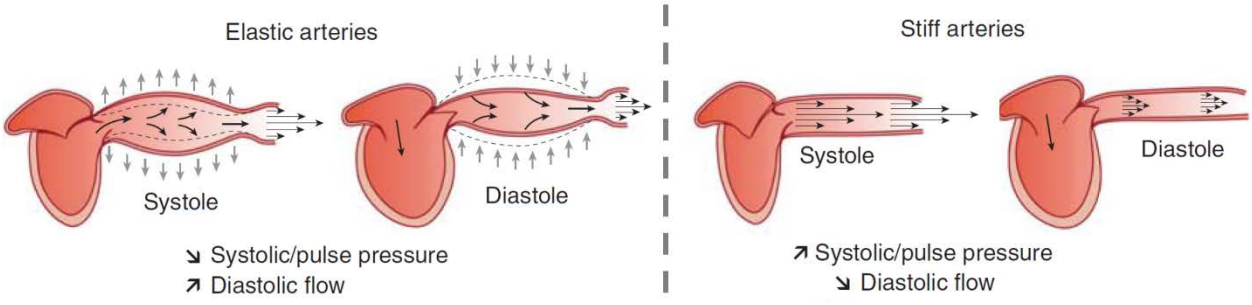

Figure 2.5 Schematic representation of the role of arterial stiffness in dampening blood pressure pulsatility and assuring adapted blood flow through the peripheral circulation (Briet *et al.* [74]).

The direct mechanism is complemented by an 'in-direct' mechanism. Arterial stiffening increases the velocity to which an arterial pulse wave propagates along the arterial wall (PWV), influencing the timing of reflected pressure waves. The pressure wave generated in the aorta (forward or incident wave) is propagated to arteries throughout the body [26]. In the healthy young, the arterial system is heterogenous, characterised by low aortic PWV and high peripheral PWV and thus by a significant 'stiffness gradient' [15,26,69]. This stiffness gradient, together with local arterial branching's and lumen narrowing, creates regions of impedance mismatch causing partial reflections of the incident pressure wave (**Figure 2.6**) [15,26,69]. These wave reflections help to diminish the incident pressure wave, preventing pulsatile energy being transmitted distally into the microcirculation [15,102,176]. Reflected waves also summate to form an aggregate backward-traveling reflected wave that returns to the aorta in late systole, producing a favourable early diastolic pressure rise that boosts coronary perfusion, without increasing the LV afterload [177]. The desirable timing is disrupted by an increased PWV due to arterial stiffening. With increases in PWV, the reflected waves return earlier impacting on the central arteries during systole, amplifying aortic and ventricular pressures during systole, and augmenting cardiac workload. As the reflected waves occur during systole, its role in maintaining diastolic pressure is reduced [15,26,69]. Further, as central arterial stiffness increases to a greater extent than peripheral artery stiffness with age, the stiffness gradient is dissipated [15,43]. This reduces partial reflection of the incident wave, increasing the transmission of the pulsatile energy into the peripheral microcirculation [102].

These mechanisms illustrate that increased arterial stiffness deleteriously impacts both the heart as well as the peripheral tissues and organs (**Figure 2.7**). The most typical clinical consequence of increased arterial stiffness is isolated systolic hypertension [21,54], a standalone contributor to CVD burden, characterised by elevated SBP and PP. From a cardiac perspective, arterial stiffening increases workload, reduces ventricular ejection efficiency and impairs perfusion of the heart itself [102]. Chronic ejection into stiffer vasculature leads to LV hypertrophy [178,179] and increased systolic load strongly predicts HF and atrial fibrillation in the general population [180,181]. Concomitant with reversal of the stiffness gradient, arterial stiffening leads to greater penetration of pulsatile energy into the microvasculature of target organs, particularly those that require high blood flow and therefore must operate at low arteriolar resistance [102]. In this respect, arterial stiffening can lead to reduced albuminuria and a reduced glomerular filtration rate due to damaged glomeruli, and eventually CKD

[74,182]. Similarly, excess pulsatility to the cerebral microvasculature can impair cerebral autoregulation [102,183] and increases the risk of brain infarction and incident stroke [10,102].

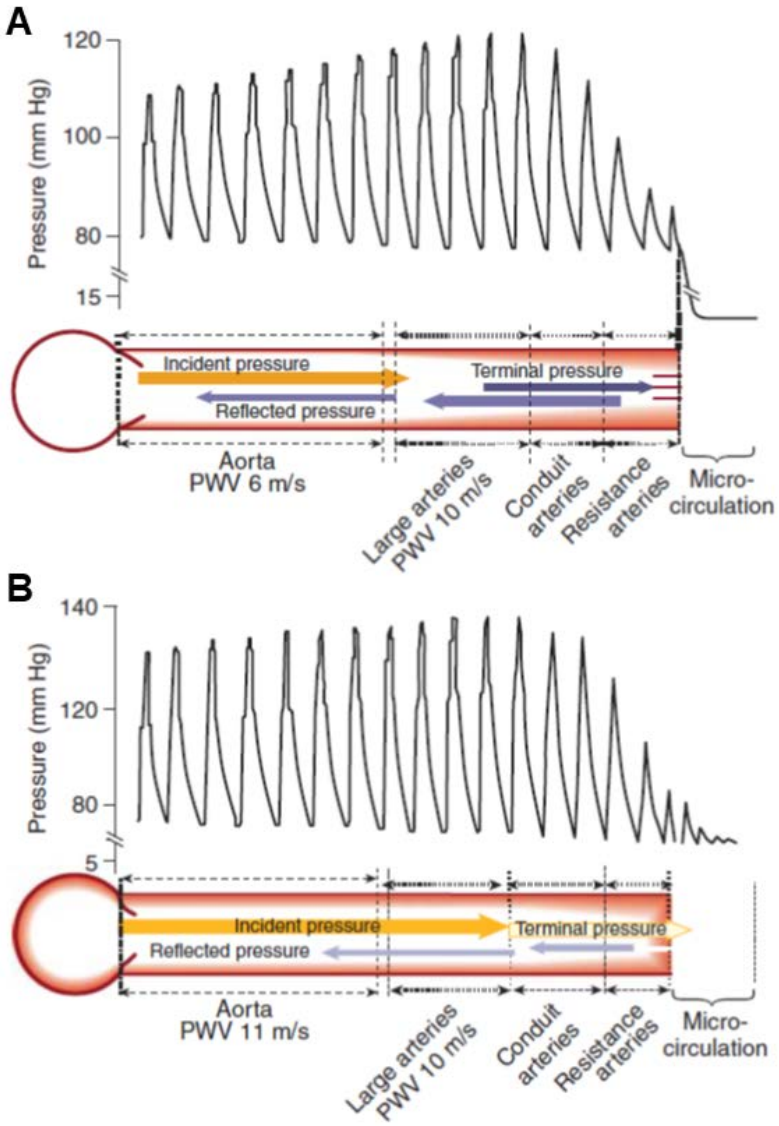

Figure 2.6. Haemodynamic impact of the central-to-peripheral arterial stiffness gradient. A. When aortic stiffness is lower than that of medium-sized conduit arteries, creating impedance mismatch, partial wave reflections attenuate pulse pressure transmission to the microcirculation. B. Increases in aortic stiffness with age, which is less marked in medium sized conduit arteries, diminishes the stiffness gradient and thus wave reflection, meaning pulsatile pressure is not sufficiently dampened, damaging the microcirculation (London *et al.* [43]).

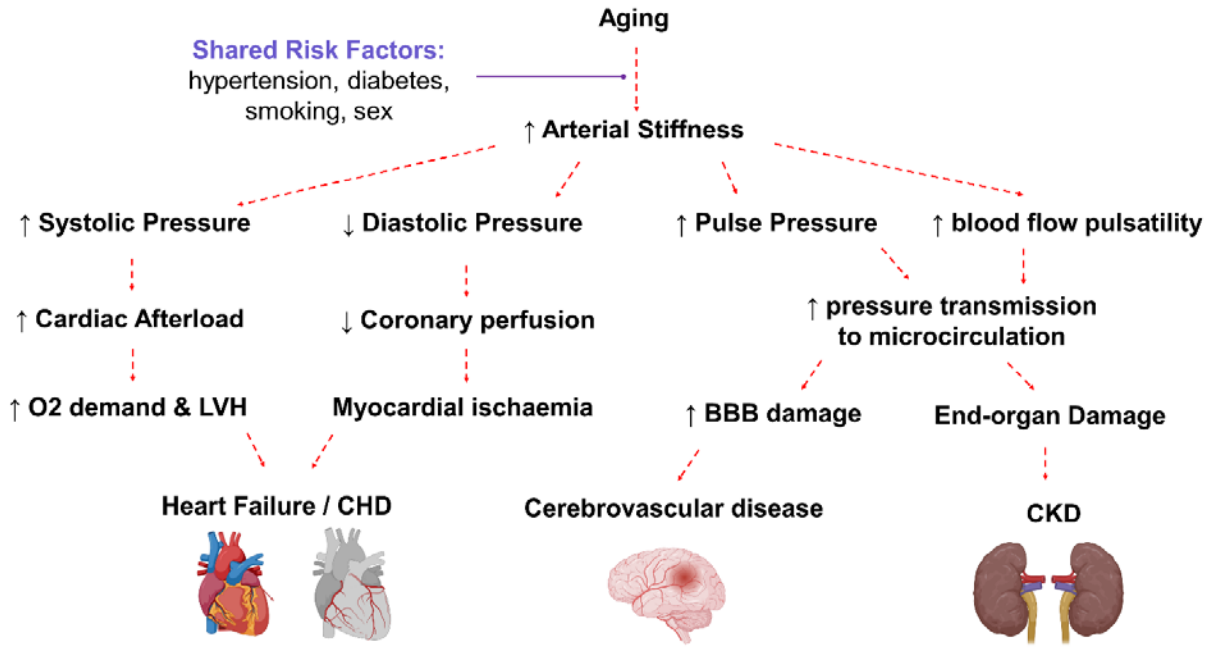

Figure 2.7 Potential mechanisms linking arterial stiffening with cardiovascular disease. **Abbreviations:** BBB, blood brain barrier; CHD, coronary heart disease; CKD, chronic kidney disease; LVH, left ventricular hypertrophy.

Consistent with its central role in cardiovascular function, arterial stiffness is now considered a key biomarker of vascular health, integrating the combined and accumulated effect of aging and arterial system insults. Indeed, regional measures of arterial stiffness (i.e. PWV) integrate the damage of risk factors on the arterial wall over a long period, whereas traditional risk factors, including BP, hyperglycaemia and dyslipidaemia can fluctuate. Evidence indicates that arterial stiffening is also one of the earliest markers of CVD, occurring before overt disease manifestation [107]. Importantly, measures of arterial stiffness have been shown to improve the accuracy of determining CVD risk [8,10], however their prognostic value is dependent on the region being assessed.

Central Arterial Stiffness and Cardiovascular Disease

Most published data on the clinical and prognostic significance of arterial stiffness is based upon cfPWV, a measure often cited as the gold standard and most clinically informative [8,9]. This is perhaps not surprising given that cfPWV is predominantly a measure of the stiffness of the aorta, a large proximal highly elastic vessel that provides by far the largest cushioning capacity in the arterial system, with the ascending aorta and arch providing a third of the total pressure cushioning capacity

alone [9,43]. Because of its continuous haemodynamic exposure to pulsatile mechanical stresses, elastin in the aorta is particularly susceptible to the degenerative effects of mechanical fatigue, inherently making the aorta prone to injury and disease [184]. Aortic arterial stiffness therefore increases markedly with aging and pathologies, and is a strong marker of vascular aging [13-16]. As previously discussed, this has multiple direct and in-direct consequences that have a major impact on cardiovascular health.

To this end, increased aortic stiffness, as measured by cfPWV, has been shown to be an important, independent determinant of CVD risk [6,8]. Indeed, cfPWV has predictive value for cardiovascular and all-cause mortality, fatal and non-fatal coronary events, and fatal strokes in clinical cohorts including, essential hypertension [17,185], diabetes [186,187], end stage renal disease [188], as well as the general population/community-based cohorts [18,189]. A participant level meta-analysis of prospective studies including 17,635 participants reported that a 1-standard deviation increase in cfPWV is associated with a 35%, 54% and 45% increase in CHD, stroke and overall CVD events, respectively [10] (**Table 2.3**). A recent meta-analysis update reported that a 1 m/s increase of cfPWV was associated with 1.12-fold increase for future CVD events [190]. Further, cfPWV demonstrated an independent predictive value for CVD events after adjustment for risk factors [17], and provided better discrimination and reclassification of CVD risk than traditional CVD risk factors alone [10] suggesting it can enhance risk assessment in primary prevention. As such the assessment of cfPWV to assist in the determination of CVD risk is now well established in epidemiological settings [6].

Table 2.3 Pooled adjusted Hazard Ratios (95% CIs) of a 1-SD Increase in aortic pulse wave velocity for all-cause Mortality, cardiovascular disease (CVD) mortality, coronary heart disease (CHD) events, stroke events, and CVD events (Ben Shlomo *et al.* [10])

Outcome	Model 1	Model 2	Model 3
CHD Events	1.35 (1.22-1.50)	1.32 (1.18-1.48)	1.23 (1.11-1.35)
CVD Events	1.45 (1.30-1.61)	1.37 (1.23-1.52)	1.30 (1.18-1.43)
Stroke Events	1.54 (1.34-1.78)	1.37 (1.21-1.54)	1.28 (1.16-1.42)
CVD Mortality	1.41 (1.27-1.56)	1.35 (1.20-1.53)	1.28 (1.15-1.43)
All-cause Mortality	1.22 (1.16-1.27)	1.20 (1.15-1.26)	1.17 (1.11-1.21)

*Model 1 adjusts for sex and age. Model 2 adjusts for sex, age-group, and systolic blood pressure. Model 3 adjusts for cardiovascular risk factors. **Abbreviations**: CHD, coronary heart disease; CVD cardiovascular disease.

Despite its strong prognostic value, cfPWV is still not routinely assessed in clinical practice, and for several reasons. As previously discussed, PWV is highly dependent on MAP [112,113]. Whilst PWV measures can be adjusted for MAP [115], the curvilinear nature and individual distinctiveness of the pressure-diameter relationship are persistent limitations [175]. Indeed, statistical adjustment cannot distinguish between changes in PWV due to acute pressure dependency and due to arterial remodelling [115]. This means comparing arterial stiffness-related outcomes between individuals, tracking changes over time, and determining optimal treatment strategies can be challenging [20]. Assessment of cfPWV using commonly employed applanation tonometry or ultrasound is also highly operator dependent, requiring a high-degree of technical precision to ensure high quality data, as well as being time-consuming [9]. Finally, regardless of the approach, assessment of the carotid artery can be challenging in-patient populations, including persons who are obese and those with advanced carotid artery atherosclerosis [109], with a potential to compromise accuracy.

Given the lack of uptake of cfPWV measures, there is significant interest in evaluating whether PWV determined on more accessible and convenient peripheral arterial segments, derived using simple operator-independent (automatic) oscillometric cuff-based methods, can demonstrate prognostic and diagnostic capabilities, and encourage clinical adoption of arterial stiffness phenotyping due to their ease of use. But few oscillometric devices have been validated for determining peripheral PWV, and compared to cfPWV, little is known about the prognostic utility of peripheral PWV. More broadly, a persistent focus on cfPWV has ignored the integrated role that medium-sized peripheral conduit arteries play in the cardiovascular system. In particular, there can be important pathophysiological changes within medium-sized conduit and muscular arteries of the lower-limbs that contribute to CVD risk [21].

Lower-limb Arterial Stiffness and Cardiovascular Disease

The femoral-tibial arterial pathways of the lower-limbs are prone to developing both arteriosclerosis and atherosclerosis, principal drivers for CVD [26]. Assessments of lower-limb arterial health provide clinicians with the opportunity to conveniently detect local disease pathology, but can also indicate overall CVD risk [27]. This is because poor lower-limb arterial health indicates systemic pathophysiology [23]. They can be particularly useful in identifying high-risk patients; adults with both poor peripheral vascular health and cardiac or cerebrovascular disease are at greater risk of incident

CVD events [25,191]. The greater accessibility of lower-limb arteries also means they are easier to directly assess than central arteries, making them a good candidate for clinical adoption.

Atherosclerosis in the lower-limbs is routinely assessed in epidemiological and clinical settings using ABI. Defined as the ratio of SBP measured at the ankle to SBP measured at the brachial artery (**Figure 2.8**), ABI was first employed as a non-invasive tool for the screening of occlusive peripheral artery disease (PAD) [8]. Whilst ABI indicates arterial stenosis in the lower-limbs, any occlusion is a manifestation of systemic atherosclerosis. [28,192]. It is therefore not surprising that ABI is strongly associated with traditional CVD risk factors [193-195]. A low ABI (<0.9) also serves as prognostic marker for CVD beyond that of traditional CVD risk factors alone [29-32]. However, despite its popularity, a recent review by the US Preventive Services Task Force concluded that there was no direct and limited indirect evidence on the benefits of patient ABI screening in unselected or asymptomatic populations [192,196]. ABI also changes little with risk factor modification or pharmacological intervention, limiting its use as a surrogate clinical outcome or monitoring tool at a population level [6,197].

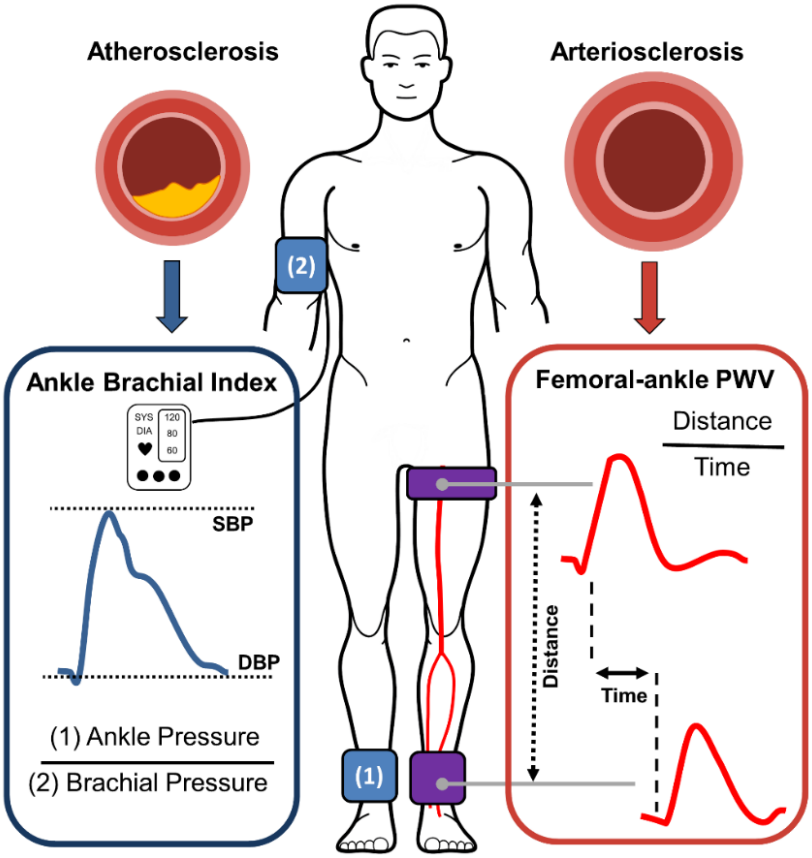

Figure 2.8 Determination of ankle brachial index and femoral-ankle pulse wave velocity (PWV).

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure.

Unlike atherosclerosis, arterial stiffness in the lower-limbs is not routinely assessed in clinical or epidemiological settings. This is largely due to the fact that age-related increases in peripheral artery stiffness generally are much less marked than central arteries, with its measurement therefore thought to be of limited prognostic value [11]. Lower-limb arteries are inherently stiffer than central arteries, which means they may be less susceptible to the age-related fracturing and depletion of elastin, and collagen deposition. But a number of studies have reported that lower-limb arterial stiffness, and faPWV, increases significantly with age [38,148,198], and markedly after 50 years of age, particularly in women [199]. Further, increased faPWV is also associated with traditional biological and lifestyle CVD risk factors, including male sex, diabetes, dyslipidaemia, and of course BP [37,38,148]. This suggests that faPWV may be a useful marker of vascular aging.

Despite this, few studies have sought to identify the association of lower-limb arterial stiffness with CVD or CVD risk, and those that have, reported contradictory findings. In 305 end-stage renal disease patients followed over an average of 70±49 months, faPWV could not predict cardiovascular mortality, unlike cfPWV [41]. Similarly, in 159 men with and without CVD, faPWV was not associated with CHD severity, whereas cfPWV demonstrated a strong independent association [42]. In contrast, in the Hoorn study, a population-based cohort of 579 elderly individuals, local femoral artery stiffness was independently associated with CVD events and mortality (**Figure 2.9**) [200]. A 1-SD decease in femoral artery distensibility and compliance (the inverse of stiffness) was associated with a 39% and 47% increase in risk of an incident event and all-cause mortality, independent of central arterial stiffness and cardiovascular risk factors [200]. The use of brachial BP to determine local femoral arterial stiffness indices may have confounded associations [99]. However, in support, essential hypertension patients with high faPWV (>13 m/s) had a significantly higher incidence of stroke than those with low faPWV, an association which was not observed for heart-femoral (aortic) PWV [40]. This suggests lower-limb arterial stiffness had a stronger clinical impact on the incidence of stroke than central arterial stiffness. But these studies highlight the contradictory findings of existing literature. One important consideration that may have given rise to these contradictory findings is that the greater cellularity of lower-limb arteries may lead to short-term alterations in smooth muscle tone, causing greater changes in stiffness within a given participant, than are seen between participants [96,201], although whether the autonomic nervous system has a pressure independent role in the regulation of arterial stiffness is uncertain [202,203]. Regardless, all of these studies were relatively small (n~500), and most were conducted in patient populations only, limiting

inference to the general population. Clearly further research is warranted in order to identify if faPWV is a robust biomarker for CVD, particularly in general community-dwelling populations where arguably CVD risk screening has the greatest impact.

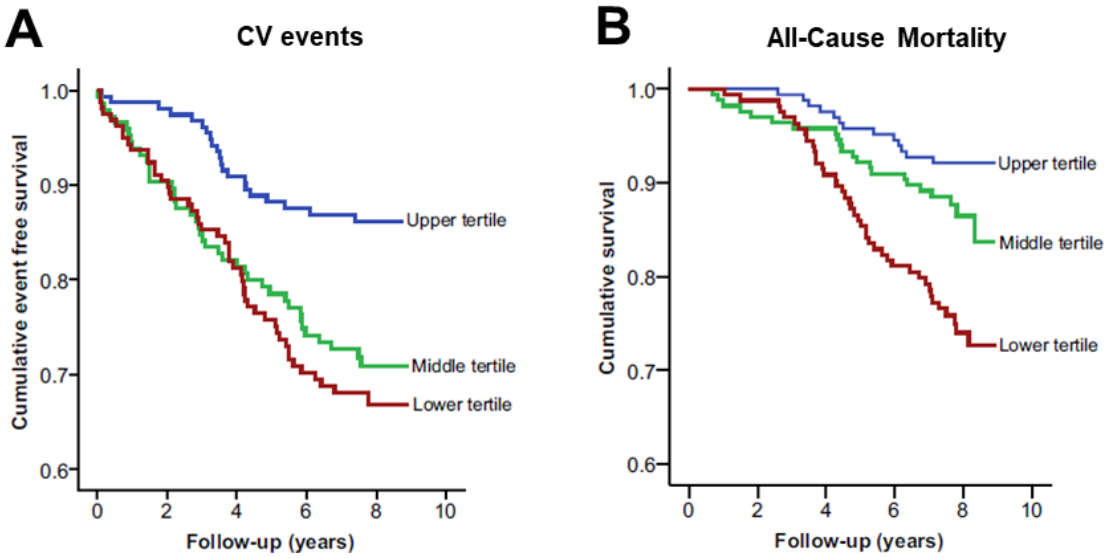

Figure 2.9 Probability of cardiovascular (CV) events (A) and all-cause mortality (B) in a population-based cohort according to local femoral distensibility tertiles. Cox regression analyses showed that lower femoral distensibility were associated with higher CVD incidence and greater all-cause mortality (Van Sloten *et al.* [200]).

As for its association with CVD, there is little existing literature describing how increased lower-limb arterial stiffness/PWV explicitly indicates or contributes to the development of CVD, but there are a few potential mechanisms. As for ABI and systemic atherosclerosis, femoral-tibial arterial stiffening may simply serve as a proxy for stiffening elsewhere in the arterial tree. Indeed, increased faPWV is associated with increased cfPWV [37]. Femoral stiffness may be particularly indicative of coronary artery stiffness given that these vessels demonstrate similar wall characteristics, with both exhibiting a high collagen/elastin ratio [200]. However, the independent associations between lower-limb arterial stiffness and CVD events/mortality [40,200] suggest there are distinct pathways. As for central arterial stiffness, increased faPWV may contribute to CVD directly and in-directly. Although of greater muscularity and positioned distally, the arteries in the femoral-tibial pathway (particularly the proximal femoral artery) still contribute to pulse wave cushioning [26]. Increased faPWV, with a concomitant reduction in the ability to store tensile energy, therefore likely contributes directly to increased SBP, PP and myocardial workload, as well as a continuation of intermittent exaggerated flow

described previously (**see Figure 2.4**). However, perhaps the most notable consequence of increased faPWV is a modification of wave reflections, an in-direct mechanism (**see Figure 2.5**). Wave reflections originate in various locations, including in peripheral bifurcations and smaller muscular arteries [8]. Increased lower-limb arterial stiffness results in reflection points being closer to the heart, and leads to reflected waves travelling more rapidly along the arterial tree, both of which contribute to earlier aortic wave reflections [204]. As discussed previously, this boosts SBP and PP, augmenting cardiac workload leading to ventricular hypertrophy [35], reducing coronary perfusion, and elevating pressure transmission to the microcirculation [15,22,65]. The increased central BP pulsatility is also, in turn, a factor in the damage of smaller arteries, (**Figure 2.10**), highlighting the 'cross-talk' between smaller peripheral and large central arteries [205,206].

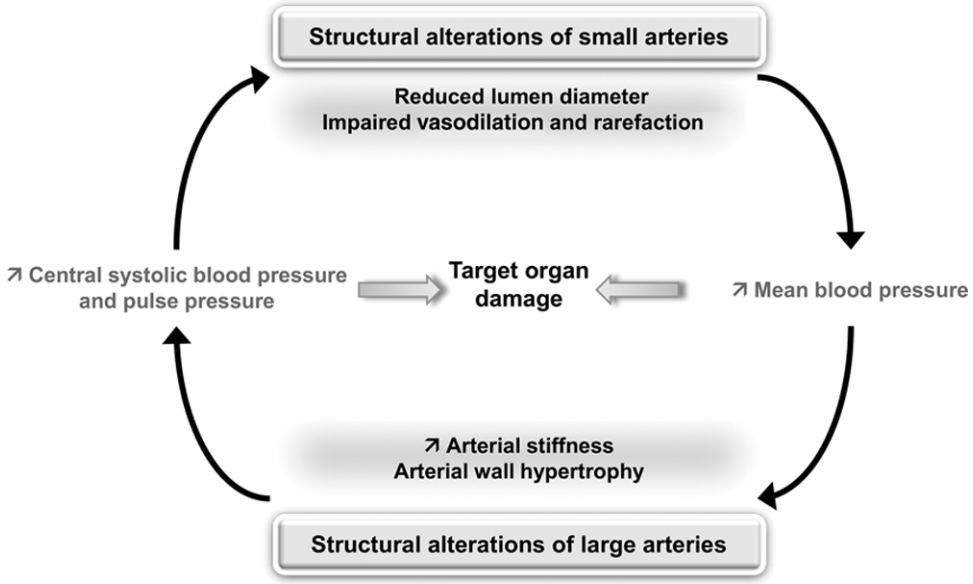

Figure 2.10 Small and large artery cross-talk: a vicious circle of aggravation between micro-circulation and macro-circulation. Alterations of smaller arteries increase mean blood pressure, which in turn contributes to stiffening of large arteries, leading to an increased central systolic and pulse pressure. The increased central blood pressure aggravates small artery damage (Safar *et al.* [206]).

One factor which may have contributed to the limited use of faPWV, clinically and epidemiologically, is that despite the plethora of methods and devices claiming to measure arterial stiffness, non-have been specifically validated for assessing faPWV [207], and there are few reliability studies [130]. To be of any clinical use in the screening of CVD risk, a measurement technique must reflect underlying (patho)physiology with reasonable accuracy and do so consistently, in order to permit objective monitoring over-time. Recently it has been demonstrated that cfPWV can be

measured simply and quickly when applanation tonometry is combined with oscillometry [105], with immediate measurement output. The SphygmoCor XCEL (XCEL) device, which is already widely used in epidemiological research [208], makes use of a volume displacement cuff placed around the upper leg to acquire the femoral pulse, and a tonometer to simultaneously record the carotid pulse. The cuff-based approach means that the arterial stiffness assessment requires less user expertise and makes the process less operator dependent. However, it is currently unknown whether this technique is suitable for investigating faPWV. Of note, it would also be easier if measurements of arterial stiffness could be performed in the sitting position, analogous with the routine measure of BP in clinical practice. If oscillometric-derived measurement of faPWV are shown to be both accurate (valid) and precise (reliable), this would represent a potentially viable tool for a range of larger-scale investigations of the association between faPWV and CVD.

The Arterial Stiffness Gradient and Cardiovascular Disease

One significant advantage of faPWV, over 'global regional' hfPWV or baPWV measures for example, is that it is exclusively a measure of peripheral arterial stiffness, and when combined with cfPWV, permits determination of the central-to-peripheral arterial stiffness gradient, a novel biomarker of promising prognostic utility [44].

To briefly recap, the normal stiffness gradient of a healthy cardiovascular system is physiologically advantageous permitting, i) the transformation of highly pulsatile SV into a smooth consistent blood flow, ii) a gradual attenuation of the forward pressure wave preventing the transmission of pulsatile forces to end-organs, and, iii) the moderation of wave reflection back towards the heart, boosting coronary perfusion [43,209]. However, aging and/or lifestyle factors disrupt this beneficial phenomenon. The aorta tends to stiffen whereas changes in lower-limb arterial stiffness, for example, are less marked (**Figure 2.11**) [8]. The subsequent equalisation or reversal of the stiffness gradient is responsible for, i) reduced organ perfusion and dysfunction, ii) the transmission of excessive forward pressure into the microcirculation, and iii) coronary hypoperfusion and increased myocardial workload [43,44,176]. The kidneys and the brain are densely vascularised and require high-flow, low resistance arterial hemodynamics, so they may be particularly susceptible to stiffness gradient loss. For example, Mitchell *et al.* [176] reported that aortic stiffening and reduced wave reflection were associated with excessive flow pulsatility in the brain, a risk factor for reduced brain volume, heightened infarction risk and poor cognitive function. The stiffness gradient hypothesis eloquently

explains the impact of increased arterial stiffness on both the heart and peripheral microcirculation, and theoretically is a more logical choice for CVD risk determination than aortic stiffness alone [209]. However, the use of the stiffness gradient as a biomarker of CVD risk is still at a preliminary stage.

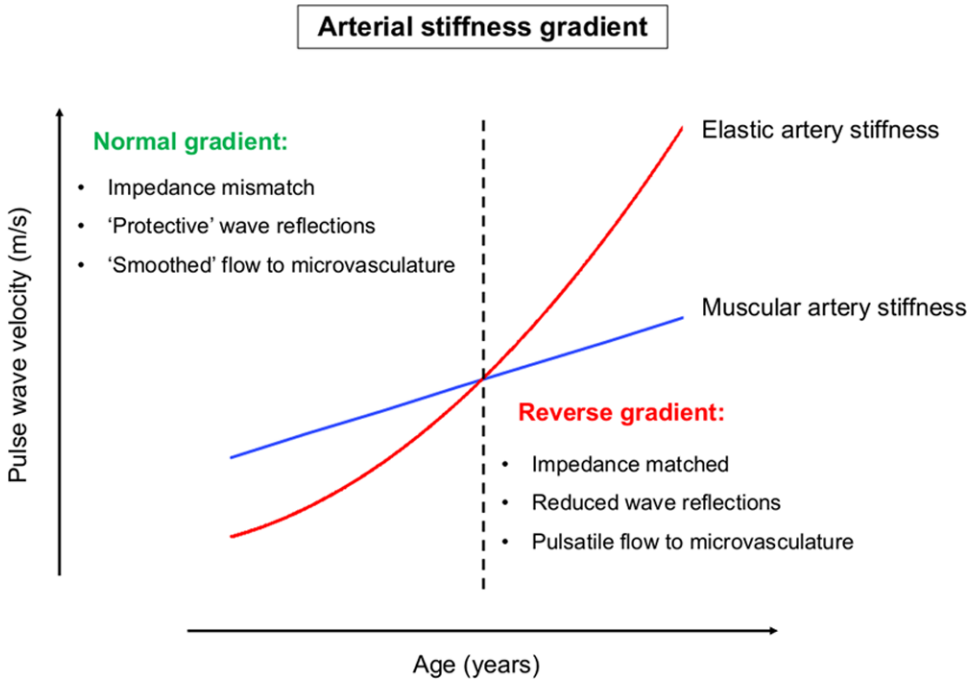

Figure 2.11 Reversal of the normal central-to-peripheral arterial stiffness gradient has a number of clinical consequences on both the myocardium and peripheral microcirculation. (Yu *et al.* [11]).

To date, the few studies that have investigated the prognostic utility of the stiffness gradient have focused on the aortic to brachial stiffness gradient (ab-SG), defined as carotid-radial PWV (crPWV) divided by cfPWV [44-49]. In 310 adults with hemo-dialysis, a decreased ab-SG was significantly associated with greater all-cause-mortality (hazard ratio 1.23), after adjustment for CVD and risk factors [44]. Interestingly, cfPWV was not associated with mortality. Similarly, in a study of 181 peritoneal dialysis patients, ab-SG was associated with both all-cause and CVD mortality following adjustment, whereas cfPWV was not associated with either outcome [46]. The ab-SG has also shown to stronger associations with kidney function in diabetes patients [45], as well as CHD and stroke [48], than cfPWV. Notwithstanding these promising findings, all of these studies were conducted in high-risk groups, limiting inference to the general population, in which CVD risk screening is arguably more efficacious. Indeed, in the Framingham Heart Study of 2,114 healthy (free of CVD) older adults [49],

the ab-SG demonstrated only equitable association with incident CVD events when compared to cfPWV. The authors concluded that the prognostic value of ab-SG is entirely attributable to cfPWV, which should remain the reference standard. However, ancillary analysis by Niiranen *et al.* [49] did reveal that the association between ab-SG and incident CVD events was stronger than cfPWV in adults ≥ 70 years of age, suggesting the stiffness gradient biomarker may be of most use in identifying CVD risk in older adults.

One limitation of the ab-SG is its use of the brachial artery as a representative of medium-sized muscular conduit vessels in the periphery. Typically, stiffness of the brachial artery changes little with age [210], and the upper extremities represent only a small portion of the vasculature. In contrast, the lower limbs make up a significant portion of the arterial tree, are more prone to athero- and arterio-sclerotic processes than the upper limbs [200,211], and are major sites of wave reflections [26]. Characterising the stiffness gradient using faPWV to determine the aortic-to-femoral stiffness gradient (af-SG), whereby faPWV is divided by cfPWV, may provide a more comprehensive picture of cardiovascular risk. However, no studies have investigated the association of the af-SG with CVD, and, like faPWV, its clinical and prognostic potential is unknown.

Of course, an important factor determining the clinical utility of any biomarker, is whether or not it can demonstrate acceptable reliability. In the only study to assess the reliability of an arterial stiffness gradient measure, Beltrami and colleagues [196] reported that the ab-SG demonstrated only moderate reliability (0.52) as indicated by intra-class-correlation coefficients (ICC's), less than the segmental cfPWV (0.89) and crPWV (0.66) PWV measures from which they were derived. The origins of the ab-SG's lower reliability are difficult to discern, but it is likely a consequence of the additional random and measurement error, as well as biological variability, that arises from combining PWV measures. Awareness of this phenomena is important, as although cfPWV and faPWV demonstrate good reliability in young healthy adults, their reliability has been reported to be poorer in older adults [212]. In this respect, an advantage of the af-SG over ab-SG measures is that faPWV has consistently reported greater reliability than crPWV [213,214]. This is likely due to the upper-extremity arteries being more susceptible to the impact of changes in sympathovagal balance on vascular tone [203], but also because the propagation time of the pulse-wave and distance travelled for crPWV is shorter than for faPWV, meaning the absolute error in determining transit time is greater [8]. However, like its ability to identify CVD risk, the reliability of the af-SG is unknown.

Finally, one potential advantage of arterial stiffness gradient measures over regional PWV is that expressing arterial stiffness in this manner is suggested to provide a BP independent index of

vascular aging, given that both central and peripheral arterial stiffness are similarly impacted by MAP [24]. Fortier *et al.* [215] tested this hypothesis in 417 patients with renal dysfunction, and reported that whilst cfPWV and crPWV both demonstrated positive associations with MAP, there was no correlation between ab-SG and MAP, even following adjustment for confounding variables (**Figure 2.12**). This observation is supported by others [45,46]. Picone *et al.* [45] reported that the ab-SG was not associated with MAP in both diabetic and non-diabetic control groups, whilst Bia *et al.* [46] did not find any association within 151 haemodialysis patients. However, whilst Armstrong *et al.* [216] confirmed that the ab-SG was MAP independent in hypertensive, renal dysfunction and diabetes patients groups, they reported a significant positive association in healthy subjects. The presence of disease may therefore impact the MAP dependence of the ab-SG and, as such, it may have limited potential as a screening tool for clinical assessment of arterial stiffness beyond cfPWV in otherwise healthy people. Whether this characteristic is also true for the af-SG is not known, as its dependence on MAP has not been investigated. Should the af-SG demonstrate MAP independence in both healthy and diseased populations, it may be of significant clinical value.

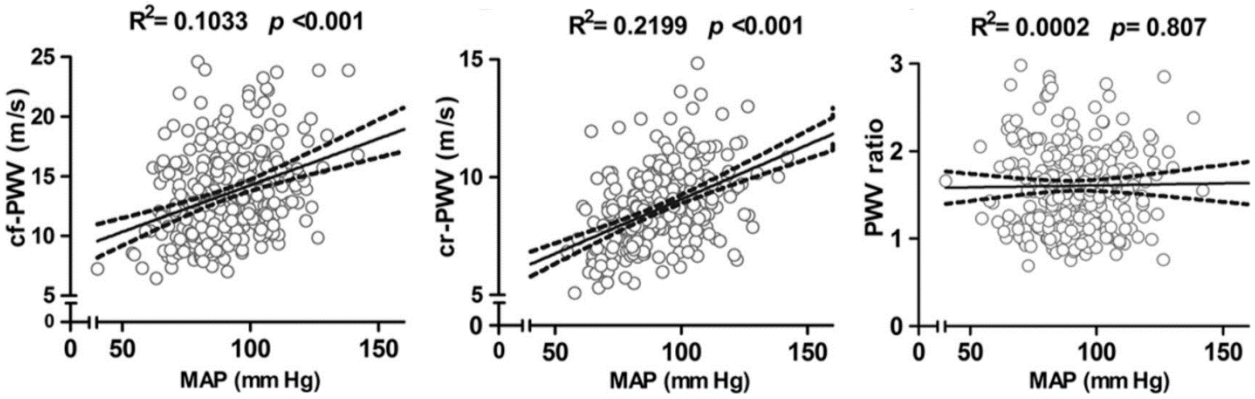

Figure 2.12 Relationship between mean arterial pressure (MAP) and carotid-femoral pulse wave velocity (cfPWV), carotid-radial pulse-wave velocity (crPWV) and the aortic-brachial arterial stiffness gradient (often referred to as PWV ratio) in 417 renal dysfunction patients (Fortier *et al.* [215]).

Determining Clinical Utility

There are a number of important considerations with regards to study design, including observing accepted guidelines [217] and controlling the balance between the internal and external validity of a study [218], that have informed the approaches used in this thesis. The most pertinent of these considerations with be discussed here. For clarity, in a research design context, internal validity concerns the rigor (degree of control) of the study design, particularly the controlling of confounding variables. External validity refers to whether study findings can be generalised to the population at large. There is a reciprocal relationship (trade-off) between internal and external validity [218].

Criteria 1: Measurement Validity and Reliability

The increasing interest in the use of arterial stiffness as a marker of CVD risk, coupled with the technological developments in biomedical engineering, has led to a plethora of simple non-invasive devices being developed for the assessment of PWV. But concerns about the highly variable research designs and methodologies adopted within validation studies, and its potential to detrimentally impact clinical and epidemiological adoption, led to the publication of 'guidelines for the validation of non-invasive haemodynamic devices' by the ARTERY Society [217]. These guidelines inform the experimental research designs and evaluative criteria employed within this thesis to determine PWV measurement accuracy (validity) and precision (reliability) in **chapters 3 and 6** *(Objectives 1.1 and 2.2)*.

Determining the validity of a novel tool or biomarker requires comparing the accuracy of its measurement with a reference standard [50]. For PWV, the reference standard is the invasive simultaneous recording of pressure waveforms using high-fidelity pressure sensors. But recognising the difficulties and risks for this approach, an acceptable minimum secondary reference is the sequential recording of proximal and distal ECG-gated pressure waveforms, whereby transit time is determined from the waveforms using the intersecting tangent method, as which can be (and is routinely) determined using applanation tonometry or ultrasound [50,217]. The order of device is randomised between subjects and, following protocols for standardisation of conditions, the average of three recordings (within 0.5 m/s) is used to compare devices. The accuracy of the test device is evaluated using both the mean difference from the reference and standard deviation (SD) of this difference (**Table 2.4**), with Bland and Altman Limits of Agreement analysis also permitting

identification of measurement bias (the tendency for the any difference to vary with changes in the mean) [219].

The guidelines set out by the ARTERY Society [163] can also be readily applied for the determination of measurement reliability. Three measurement time-points are recommended, with a minimum separation of 24 hours and a maximum completion time of 1 month to avoid bias due to the effect of ageing and variation in other confounding influences. Although the validation outcomes can be applied, it is more common to calculate ICC estimates of the three measures, whereby, < 0.5, 0.5 - 0.74, 0.75 - 0.9 and > 0.9 indicate poor, moderate, good and excellent reliability [220].

Table 2.4 The accuracy of a test device is determined by both the mean difference from the reference standard and the standard deviation of this difference (Wilkinson *et al.* [217]).

Outcome	Mean Difference	Standard Deviation
Excellent	≤ 0.5 m/s	≤ 0.8 m/s
Acceptable	< 1.0 m/s	< 1.5 m/s
Poor	> 1.0 m/s	> 1.5 m/s

With respect to validity and reliability studies, the research setting is typically constrained to maximise internal validity, i.e. does a particular PWV device truly reflect the arterial stiffness of the segment of interest. In this respect, there are two major threats to internal validity, i) the impact of extraneous variables e.g. time of day, and ii) the demographic characteristics of the sample population, both of which may confound PWV measures. The control of extraneous variables, chiefly BP, is achieved by strictly implementing practices for standardising conditions as already discussed (**Table 2.2**). But the presence of cardiovascular pathology can also impact the accuracy of PWV measures [109]. For example, PWV is underestimated in subjects with arterial stenosis of extremities, aortic aneurysm, or aortic stenosis, otherwise, it is overestimated in subjects with aortic regurgitation [221]. Prior to clinical use it is of high importance to determine whether any error, bias or variability inherent to a health test is caused by the technique itself and not a consequence of the presence of cardiovascular pathology. Threat to internal validity can be minimised using the application of exclusion criteria (**Table 2.5**) to ensure participants are free of conditions which give rise to 'false' PWV measures and/or the recruitment of a relatively young cohort. However, it must be recognised

that the latter reduces external validity, and therefore the generalisability of any findings to general or clinical populations.

Table 2.5 Participant exclusion criteria for the accurate determination of pulse wave velocity.

Exclusion Criteria
Not in sinus rhythm / or pacemaker dependent
Pregnancy or body mass index >30 kg/m^2
Known significant carotid or femoral stenosis
Impalpable artery/poor acoustic window at site of measurement
Under 18 years of age

Criteria 2: Associations with Cardiovascular Disease

In epidemiolocal and clinical research the relationships between arterial stiffness measures and CVD are routinely explored and characterised using multivariable linear regression models. These models can be used to describe relationships between independent (i.e. PWV) and dependent or outcome (i.e. CVD) variables, but more usefully can be used to predict the probability that a certain type of individual will be shown to have CVD [222]. Two of the most common models are linear regression and logistic regression. While both models provide a description of any relationship (e.g. direction and strength), linear regression is used when the outcome is continuous, whereas logistic regression is used when the outcome is dichotomous - permitting prediction of probability of belonging to a group, i.e. CVD or no disease [223]. Evaluating any relationship is undertaken through significance testing, but more importantly quantifying the magnitude of an effect size. For linear models, the effect size is simply the estimated coefficient, beta (β), whereas for logistic models the effect size is typically reported as an odds ratio (OR) with a 95% confidence interval (CIs). The OR (coupled with CIs) can be used to determine whether a particular exposure is a risk factor for a particular outcome, and will be the primary marker for evaluating the prognostic potential of faPWV and the af-SG in the present thesis.

In observational studies, multivariable modelling is valuable because it permits simultaneous control (held constant mathematically) of several confounding variables in a single model, so that the effect of an independent variable on an outcome can be more purely estimated [222]. But all multivariable models, and therefore estimated effects, are dependent on the assumption that there is no unknown/unmeasured confounding. Similarly, controlling for too many or unnecessary

confounders, termed over-adjustment, can obscure a true effect, create an apparent effect when none exists or lead to violations of linear model assumptions (e.g. non-collinearity) resulting in imprecise effects [224]. Careful consideration of true confounders is therefore necessary to maintain internal validity, and predominantly relies upon prior knowledge and existing literature. With respect to the association between PWV and CVD (or MAP), the adjustment for potential confounders is haphazard with little consensus about which confounders are most relevant. Whilst various approaches have been adopted, as previously highlighted the major parameters to be considered are age, BP, sex, and race, and to a lesser degree the classical risk factors, specifically diabetes and smoking [74,163].

Although internal validity is a priority for much research, in applied disciplines such as epidemiology where the aim is to ultimately improve public health, it is also important that external validity be emphasised and strengthened. Low external validity limits translation into public health practice [225]. With respect to this thesis, it is pertinent to identify the association between PWV and CVD in a population in which its use as a marker of CVD risk is targeted. Identification of CVD risk is typically undertaken beyond middle age (> 40 years) [2], whilst measures of lower-limb PWV are likely of greatest utility after the 6th decade, given that faPWV may change most markedly beyond this point [199]. In this thesis, the association between PWV measures and CVD in **Chapters 4 and 5**, as well as af-SG and MAP in **Chapter 7** will be explored through secondary analysis of a public use dataset obtained from a population of older adults (60 to 90 years) who are part of the atherosclerosis risk in communities' (ARIC) study. The ARIC study is a longitudinal cohort of 15,792 men and women aged 45–64 years enrolled between 1987 and 1989 in four US communities (Forsyth County, North Carolina; Jackson, Mississippi; Minneapolis, Minnesota; and Washington County, Maryland), designed to investigate the incidence, survival rate, and determinants of CVD [226].

Part 1

Femoral-Ankle Pulse Wave Velocity

Commentary…

The assessment of faPWV may well improve the accuracy of risk prediction by providing unique and additive CVD risk information [11]. However, to be of any clinical use in improving the screening of CVD risk, any biomarker measurement technique must reflect underlying (patho)physiology with reasonable accuracy, and do so reliably, in order to permit objective evaluation and monitoring over-time [6]. Oscillometric cuff-based devices, whereby a pressure cuff is place around the limb, offer automated and user-independent determination of PWV and may encourage clinical adoption of arterial stiffness phenotyping due to their ease of use. But whilst a number of oscillometric devices have demonstrated acceptable accuracy and reliability in the measurement of central arterial stiffness [130], whether they can also be used to accurately and reliably determine faPWV is unknown [207]. **Chapter 3** will address ***objective 1.1*** and seek to identify whether a simple oscillometric cuff-based device can determine faPWV with acceptable accuracy (validity) and precision (reliability) in supine and seated postures (European Society of Cardiology Criteria 1).

3

Validity and reliability of lower-limb pulse wave velocity assessments using an oscillometric technique

Stone, K, Fryer, S, Zieff G, Faulkner J, Credeur D, Lambrick D, Hanson E, & Stoner L

Experimental Physiology, 104(5):765-774, 2019

SUMMARY

There is a growing interest in the deleterious effects of sedentary behaviour on lower-limb arterial health. To permit further investigation, including in larger epidemiological studies, there is a need to identify lower-limb arterial health assessment tools that are valid and reliable, yet simple to administer. This study sought to determine the validity and between-day reliability of femoral-ankle pulse-wave velocity (faPWV) measures obtained using an oscillometric-based device (SphygmocCor XCEL) in supine and seated positions. Doppler ultrasound (US) was used as the criterion. A total of 47 healthy adults were recruited for validity (n=32) and reliability (n=15) analyses. Validity was determined by measuring faPWV in seated and supine positions using the XCEL and US devices, in a randomised order. Between-day reliability was determined by measuring seated and supine faPWV using the XCEL on 3 different mornings, separated by a maximum of 7 days. The validity criteria (absolute standard error of estimate ([aSEE] <1.0 m/s) was met in the supine (aSEE = 0.8 m/s, 95% CI: 0.4-1.0), but not the seated (aSEE = 1.2 m/s, 95 % CI: 1.1, 1.2) position. Intra-class correlation coefficient estimates revealed the XCEL demonstrated good reliability in the supine position (ICC=0.83, 95% CI: 0.65, 0.93), but poor reliability in the seated position (ICC = 0.29, 95% CI: 0.23, 0.63). The oscillometric XCEL device can be used to determine lower-limb PWV with acceptable validity and between-day reliability in the conventionally recommended supine position, but not the seated position.

Introduction

Epidemiological research supports the association between chronic sedentary behaviour and cardiovascular health [227,228], but the mechanisms by which acute sedentarism leads to impaired cardiovascular health are not well understood. Sedentary behaviour appears to target the athero- and arterio-sclerotic susceptible vasculature of the lower extremities [229]. For example, recent evidence indicates that the unique haemodynamic milieu created by prolonged sitting can acutely impair lower-extremity endothelial function, mediated by reductions in conduit artery antegrade blood flow and shear rate [230-233]. Arterial health in the leg is typically assessed using flow mediated dilation (FMD), a measure of endothelial function [232,233]. Whilst leg FMD is arguably valid [234], a highly trained operator is required to ensure precision (reliability) [235], and in spite of this, reliability may still be poor [236]. Secondly, leg FMD assessments have only been validated in the supine position [234,237] and unavoidable postural manoeuvres may confound study outcomes. Therefore, to assist further investigation of the deleterious effects of sedentary behaviour, there is a pressing need to identify valid (accurate) and reliable (precise), yet simple, techniques for evaluating lower-limb arterial health.

One of the most popular methods for measuring arterial health, and the gold-standard assessment of arterial stiffness, is pulse-wave velocity (PWV) [103]. Arterial stiffness, and thus PWV, is dependent on arterial structure as well as arterial function. For example, a reduction in the endothelium-derived vasodilator nitric oxide leads to a decrease in arterial elasticity [238], the reciprocal of arterial stiffness. Whilst lower-limb arterial structure is not expected to change following acute sedentary behaviour, arterial function can become impaired, as indicated by decreased FMD, i.e., impaired endothelial function [230-233]. The relationship between arterial function and arterial stiffness suggests that assessments of PWV may be a viable alternate to FMD. However, any measurement tool must first demonstrate accuracy and precision, and ideally should be user objective and simple to conduct. Measurements of PWV can be undertaken using several methodologies, including ultrasound [239] and applanation tonometry [13]. Doppler ultrasound (US) is often used in population-based studies [189] having been shown to be precise [239] and accurate [240,241]. But the most widely used technique is applanation tonometry, where typically a tonometer (a pen-like sensor) is used to obtain electrocardiogram gated proximal and distal arterial pulse waveforms [50]. However, the high skill requirements and sequential nature of both ultrasound and tonometry techniques make them unsuitable for large epidemiological studies.

Recently it has been demonstrated that carotid-femoral (aortic) PWV (cfPWV) can be measured simply and quickly when applanation tonometry is combined with oscillometry [110], permitting immediate measurement output. To assess cfPWV, the SphygmoCor XCEL (XCEL) device makes use of a volume displacement cuff placed around the upper leg to acquire the femoral pulse, and a tonometer to simultaneously record the carotid pulse. This simple and largely automated technique makes it an attractive tool for determining arterial health. However, it is currently unknown whether this technique is suitable for investigating arterial health in the legs. If XCEL-derived measurement of femoral-ankle PWV (faPWV) in the leg is shown to be both valid and reliable, in the supine and/or seated position, this would represent a potentially viable tool for a range of larger-scale investigations of the effects of sedentary behaviour on cardiovascular health.

Therefore, the primary aim of this study was to determine the validity and between-day reliability of faPWV measures obtained using the XCEL in a supine position, which is conventionally used for vascular assessments. The secondary aim was to determine the validity and between-day reliability of faPWV measures obtained using the XCEL in a seated position. When comparing against the criterion, US, the accuracy of the XCEL device was considered acceptable if the absolute standard error of estimate was <1.0 m/s [19] and the standardized standard error of estimate was moderate (0.6 – 1.2) or better [242]. Although there is no universal criterion, in general, intra-class correlation coefficient (ICC) estimates of < 0.5, 0.5-0.75, 0.75-0.9 and > 0.9 indicate poor, moderate, good and excellent reliability [220].

Methodology

This observation study is reported in accordance with STROBE (STrengthening the Reporting of OBservational studies in Epidemiology) guidelines [243].

Ethical Approval

The study conformed to the Declaration of Helsinki, except for registration in a database, and was approved by the University of North Carolina at Chapel Hill Office of Human Research Ethics (**Appendix 1**). Participants were informed of the methods and study design verbally and in writing before providing written informed consent.

Participants

To ascertain the upper limit of validity and reliability for XCEL measures of faPWV, a homogenous cohort of young (18 – 40 years), healthy participants were recruited. A total of 32 participants were recruited for validity analyses only, whilst a distinct group of 15 participants were recruited for reliability analyses only. Participants were excluded if they reported any known cardio-metabolic disorders, were taking medications known to effect cardiovascular function or reported cigarette smoking. To account for potential influences of hormonal status on study outcomes, premenopausal women were studied during the early follicular phase of their menstrual cycle or during placebo phase of oral contraceptive use.

Experimental Design

Following familiarisation, participants were tested in an environmentally controlled room (temperature: 22 ± 1°C, relative humidity: 51 ± 2%). All participants were 12h fasted and were asked to avoid strenuous physical activity, caffeine and alcohol for 24 h prior. Following a 20-min rest period in a supine or seated position, faPWV was determined in the non-dominant leg using the XCEL and the criterion, Doppler ultrasound, devices. The participant was then transferred into the alternate posture, and asked to rest quietly for a further 20-min, after which all assessments were repeated. At each posture oscillometric pressure waveforms were recorded on the left upper arm, from which central haemodynmic measures were derived. Stroke volume, cardiac output and peripheral vascular resistance were also determined. For reliability analyses, the same experimental protocol outlined above was subsequently repeated on three separated occasions (with all three days being included in the analyses), separated by a maximum of 7 days, whereby only faPWV and cfPWV measures were recorded in supine and seated positions using the XCEL. PWV assessment sites were marked and recorded to ensure consistency across test days. Measurement of cfPWV acted as a quality control for identifying the suitability of lower-limb PWV assessments using the XCEL. Positioning was randomised for the determination of validity and reliability, whereby participants were allocated to one of two conditions: i) supine first, or, ii) seated first. All measures were recorded in triplicate, with the average of the closest two recordings being used for analyses.

Experimental Measures

SphygmoCor XCEL Pulse Wave Velocity. The XCEL device (AtCor Medical, Sydney, New South Wales, Australia) enables simultaneous assessment of proximal and distal arterial waveforms using a tonometer and volume displacement cuff, respectively, to determine arterial pulse transit time (PTT). Femoral-ankle ($faPTT_{XL}$) and carotid-femoral (cfPTT) PTT was measured as the time between diastolic feet of the proximal (tonometer) and distal (cuff) arterial pulse waveforms (**Figure 3.1a**). PWV is calculated by dividing PTT by arterial path length, or PWV distance (*D*).

For cfPWV, the tonometer was placed on the left carotid artery and the oscillometric cuff was placed on the left thigh at the level of the femoral artery, following recommended manufacturer guidelines [110]. Using custom made callipers; the carotid-femoral *D* (cf*D*) was estimated by measuring the liner distance from the suprasternal notch to the top of the cuff at the centre line of the leg and subtracting the distance from the suprasternal notch to the carotid artery. Accordingly, cfPWV was calculated as: cfPWV = cf*D* / cfPTT.

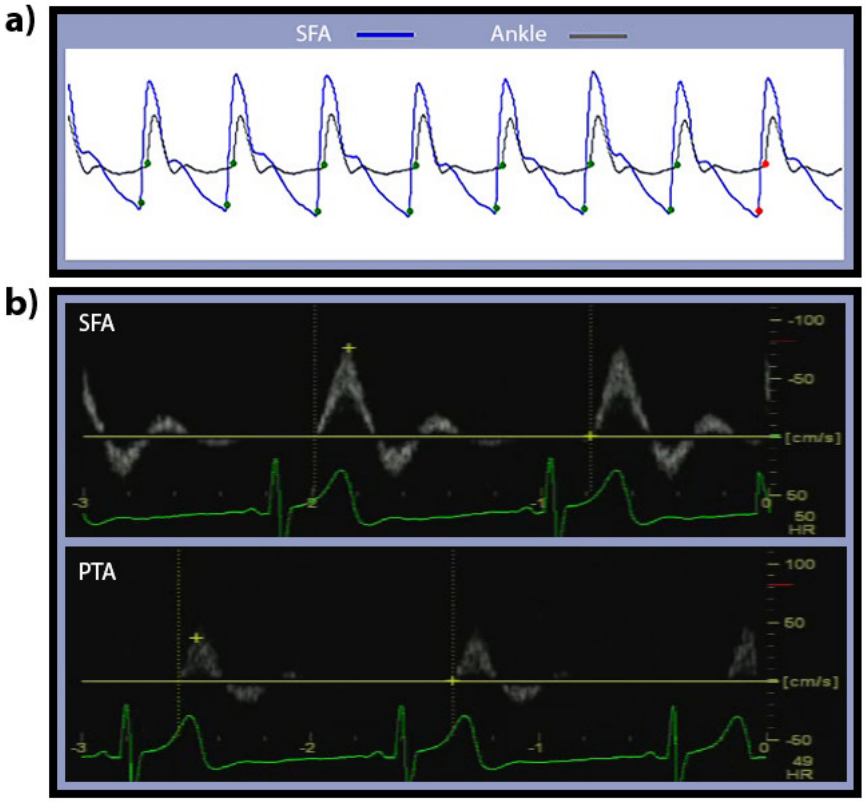

Figure 3.1 Examples of waveforms recorded at the superficial femoral artery (SFA) and ankle or posterior tibial artery (PTA) using SphygmoCor XCEL (a) and Doppler ultrasound (b) devices.

For faPWV, the tonometer was placed at the point indicating the top edge of the ultrasound probe at the level of the superficial femoral artery (SFA), whilst the ankle cuff (SC10, Hokanson) was positioned with the bottom edge proximal to the malleoli. Femoral-ankle *D* (faD_{XL}) was estimated by measuring the linear distance from the point of tonometric applanation to the top of the ankle cuff at the centre line of the leg. The XCEL device automatically corrects for sources of difference that exist when using an oscillometric rather than a tonometric only technique to determine its intended outcome measure, cfPWV [110]. Of relevance to the present study, the XCEL reduces both *D* and PTT in order to adjust for the inclusion of an extra segment of femoral artery due to the position of the cuff being below the femoral bifurcation. Accordingly, *D* is reduced by an operator-determined measurement, termed 'Femoral to Cuff' distance ($D_{F\text{-}C}$). PTT is reduced by a constant factor multiplied by $D_{F\text{-}C}$ in order to remove the time delay (PTT_{DELAY}) associated with the inclusion of the additional femoral artery segment. In order to eliminate these in-built adjustments and obtain a true or corrected faPTT_{XL}, the following formulas were applied to the PTT reported by the XCEL:

1. $PTT_{DELAY} = (D_{F\text{-}C}) \times 0.08449$ ms†
2. *Corrected* faPTT_{XL} = PTT_{DELAY} + faPTT_{XL}

faPWV was then calculated as:

3. faPWV = faD_{XL} / *Corrected* faPTT_{XL}

Technical Note: faPWV was measured using the 'Direct PWV Distance method' inherent to the XCEL. An arbitrary distance (e.g. 100 mm) can be entered for $D_{F\text{-}C}$ during data collection and subsequently used during the calculation of PWV. †This value represents an average femoral transit time per unit distance measured in a group of 15 individuals as indicated in the original validation paper [110] and subsequently provided by AtCor.

Doppler Ultrasound Pulse Wave Velocity. Ultrasound assessments of faPWV were used as the criterion. A LOGIQ P6 ultrasound device equipped with an 11-2 mHz linear array probe (GE Healthcare, Wauwatosa, USA) was used to sequentially scan and obtain ECG gated pulse-wave Doppler waveforms at the SFA and posterior tibial artery (PTA) sites. The SFA was imaged 2cm below the bifurcation from the common femoral artery; the PTA was imaged by placing the ultrasound probe directly over the line indicating the top of the ankle cuff used for XCEL assessments. Three

10s video recordings were obtained at each position. Images were analysed offline using ImageJ (Version 1.51q, National Institutes of Health, Bethesda, USA) [244] by a single blinded operator. The interval between the r-wave of the QRS complex and the foot of the systolic upstroke in the Doppler spectral envelope was measured and averaged over at least five consecutive cardiac cycles for each video (**Figure 3.1b**) [239,241]. Ultrasound PTT was defined as the difference between the intervals of time measured at each arterial segment ($faPTT_{US}$). Ultrasound arterial path length was estimated by measuring the linear distance from the mid-point of probe at the SFA to the mid-point of the probe at the PTA (faD_{US}). $faPWV_{US}$ was then calculated as: $faPWV = faD_{US} / faPTT_{US}$.

Pulse Wave Analysis. To aid in the interpretation of the effects of posture on faPWV, oscillometirc pressure waveforms were recorded on the left upper arm using pulse wave analysis (PWA) inherent to the XCEL device [245]. Each single measurement cycle consisted of a 60s brachial blood pressure recording followed by a 10-s sub-systolic recording. A corresponding aortic pressure waveform was then generated using a validated transfer function [246], from which central: systolic blood pressure (cSBP), augmentation index (AIx), augmentation index normalized to heart rate of 75 bpm (AIx@75), forward aortic pressure (Pf), backward aortic pressure (Pb) and reflection magnitude (RM) were derived.

Cardiac Output, Stroke Volume and Peripheral vascular resistance. Cardiac output (CO), stroke volume (SV) and systemic vascular resistance (SVR) were determined using a commercially available continuous-wave Doppler ultrasound device (USCOM 1A, Uscom, Sydeny, Austrailia). A single operator placed a 3.3MHz continuous-wave probe over the acoustic window at the level of the sternal notch to obtain six consecutive trans-pulmonary Doppler flow profiles.

Statistical Analysis

Statistical analyses were performed using Statistical Package for Social Sciences version 24 (SPSS, Inc., Chicago, Illinois). All data are reported as means and standard deviation (SD) unless otherwise stated. Statistical significance was defined as $P<0.05$ (two tailed). Two measures of validity were used to determine agreement between test and criterion devices: i) an absolute standard error of estimate (aSEE), and ii) a standardized standard error of estimate (sSEE). aSEE was calculated as: $aSEE = SD \times \sqrt{(1-r^2)}$ [242,247], whereby SD is the standard deviation of the criterion measure and r is the Pearson

product-moment correlation between test and criterion devices. To calculate a 95% confidence interval for the aSEE, Pearson's correlation and associated 95% confidence intervals were derived from regression analysis. sSEE was calculated by dividing aSEE by the standard deviation of the criterion, whereby <0.20 is considered a trivial difference, 0.2-0.6 small, 0.6-1.2 moderate, 1.2-2.0 large and >2.0 very large difference [242]. Relative standard of error (RSE) was also calculated by dividing the aSEE by the measurement device mean and multiplying it by 100. Bland-Altman plots [219] were generated to permit visual analysis of the uniformity of error over the range of participant measurement values. To test the effect of posture on faPWV, following verification of the normality of distribution, two-way repeated measures analysis of variance (ANOVA) was used. Effect sizes are reported using partial eta-squared (η^2_p), where 0.0099, 0.0588 and 0.1379 represent a small, medium and large effects, respectively [248]. Lastly, the effect of posture on all other central and peripheral haemodynamic variables was assessed using pair-wise t-tests. Effect sizes are reported using Cohen's *d*, where <0.20 is considered to be a small effect, >0.20 to <0.50 a moderate effect, and >0.60 a large effect.

For between-day reliability of the XCEL test device, intra-class correlation coefficient (ICC) estimates and their 95% confidence intervals were determined using a single-rating, absolute-agreement, 2-way mixed-effects model in SPSS. A mixed model was used as it is unaffected by sample size [249]. Although there is no universal standard for classifying the magnitude of ICC, for criterion-related assessments: values less than 0.50 are indicative of poor reliability, values between 0.50 and 0.75 indicate moderate reliability, values between 0.75 and 0.90 indicate good reliability, and values greater than 0.90 indicate excellent reliability [220]. A standard error or measurement (SEM) was also calculated according to the formula: SD* √(1-ICC) and a reliability coefficient (RC) was calculated according to the formula: 1.96*SEM*√2 [242,250].

Results

Of the 32 participants recruited for validation, only 31 were included in the final analyses (**Table 3.1**). For the excluded participant, PTA ultrasound images could not be analysed. The excluded participant did not differ in terms of demographics or XCEL PWV from the rest of the study population. All 15 participants completed three sessions for reliability analyses (**Table 3.1**).

Table 3.1 Participant demographic data for validity and reliability analyses.

	Validity		Reliability	
	mean	(SD)	mean	(SD)
N	31		15	
Age (years)	25.8	(5.8)	22.1	(6.4)
Female (%)	48		60	
Height (m)	1.72	(0.08)	1.69	(0.06)
Weight (kg)	73.9	(13.8)	65.2	(7.7)
BMI (kg/m^2)	24.7	(3.3)	22.8	(1.9)
PA Sessions (No./wk)	4.5	(2.0)	4.2	
PA (mins/session)	69	(41)	54	(16)

Abbreviations: BMI, body mass index; PA, physical activity; SD, standard deviation.

Table 3.2 indicates that posture had a significant effect on peripheral and central haemodynamic variables. SBP, DBP, MAP, cSBP, HR and SVR all increased (all P <.05) in the seated compared to supine position, whilst AIx, Pb, Pf, and CO decreased (all P < .05). AIx@75 and RM were not altered by posture (P > .05).

In the supine position, the XCEL faPWV demonstrated acceptable accuracy (aSEE <1.0 m/s), being moderately different when compared to the criterion (**Table 3.3**). In contrast, in the sitting position, the aSEE was greater than 1.0 m/s; however, the sSEE indicated that the XCEL faPWV was again only moderately different to the criterion. For supine faPWV measures the error was uniform (**Figure 3.2a**), but there was a bias for seated faPWV measures with the difference between devices being greater for the higher PWV values (**Figure 3.2b**). Values of faPWV obtained by the two devices were significantly correlated in the supine (P < 0.001, **Figure 3.2c**) but not the seated (P = 0.054, **Figure 3.2d**) position. Repeated-measures ANOVA analysis revealed that there was no interaction effect (P < .844, η^2_p = .001), but a significant main effect for device (P < .001, η^2_p = .892,

mean difference = 1.2, 95% CI [.08, 1.5]) and posture ($P < 0.001$, $\eta^2_p = .515$ mean difference 3.0, 95% CI [2.6, 3.4]).

Table 3.2 Mean values for peripheral and central haemodynamic responses to change in posture. Bold indicates significant at $P < 0.05$.

	Supine		Seated			
	X	(SD)	X	(SD)	*P*	*d*
SBP (mmHg)	115	(20)	118	(8)	0.028	-0.28
DBP (mmHg)	67	(7)	74	(7)	<0.001	-0.93
MAP (mmHg)	99	(8)	103	(7)	<0.001	-0.52
cSBP (mmHg)	101	(8)	103	(7)	0.042	-0.27
AIx (%)	4.9	(10.3)	0.0	(11.4)	0.022	0.45
AIx75 (%)	-3.5	(11)	-4.9	(13.4)	0.479	0.11
Pb (mmHg)	12	(2)	11	(2)	0.022	0.40
Pf (mmHg)	25	(3)	24	(3)	0.005	0.55
RM (%)	45.9	(8)	45.0	(5.5)	0.375	0.15
CO (L/min)	4.6	(1.0)	3.7	(0.9)	<0.001	0.89
HR (bpm)	55	(10)	61	(12)	<0.001	-0.53
SV (ml)	84.4	(18.4)	63.1	(17.9)	<0.001	1.17
SVR (d.s.cm^{-5})	1510	(285)	2029	(605.5)	<0.001	-1.10

Abbreviations*:* AIx, augmentation index; AIx@75; augmentation index normalized to a heart rate of 75 bpm; CO, cardiac output; cSBP, central systolic blood pressure; *d,* Cohen's d; DBP, diastolic blood pressure; HR, heart rate; MAP, mean arterial pressure; Pb, aortic backward wave pressure; Pf, aortic forward wave pressure; RM, reflection magnitude; SBP, systolic blood pressure; SV, stroke volume; SVR, systemic vascular resistance.

Table 3.3 Comparison of femoral-ankle pulse wave velocity determined by SphygmoCor XCEL and Doppler ultrasound devices.

	Ultrasound X	(SD)	XCEL X	(SD)	aSEE (95% CI) m/s		sSEE (95% CI) SD		RSE (95% CI) %	
Supine										
PTT (ms)	68.4	(8.6)	78.8	(9.0)	7.4	(4.3, 8.7)	0.9	(0.5, 1.0)	10.8	(6.3 12.7)
PWV (m/s)	9.8	(1.2)	8.6	(1.1)	0.8	(0.4, 1.0)	0.7	(0.3, 0.9)	8.4	(3.8, 10.3)
Seated										
PTT (ms)	52.5	(7.1)	58.6	(6.0)	5.83	(4.8, 5.9)	0.8	(0.7, 0.8)	11.1	(9.1, 11.4)
PWV (m/s)	12.8	(1.8)	11.6	(1.3)	1.21	(0.9, 1.3)	0.7	(0.5, 0.7)	9.4	(7.1, 10.1)
Δ										
PTT (ms)	15.9	(6.7)	20.2	(8.6)	8.2	(6.4, 8.5)	1.2	(0.9, 1.3	51.4	(40.4, 53.7)
PWV (m/s)	3.0	(1.5)	2.9	(1.2)	1.2	(1.0, 1.2)	0.8	(0.7, 0.8)	39.8	(33.4, 40.0)

Abbreviations*:* aSEE, absolute standard error of estimate; CI, confidence interval; PTT, pulse transit time; PWV, pulse wave velocity; RSE, relative standard error; sSEE, standardised standard error of estimate.

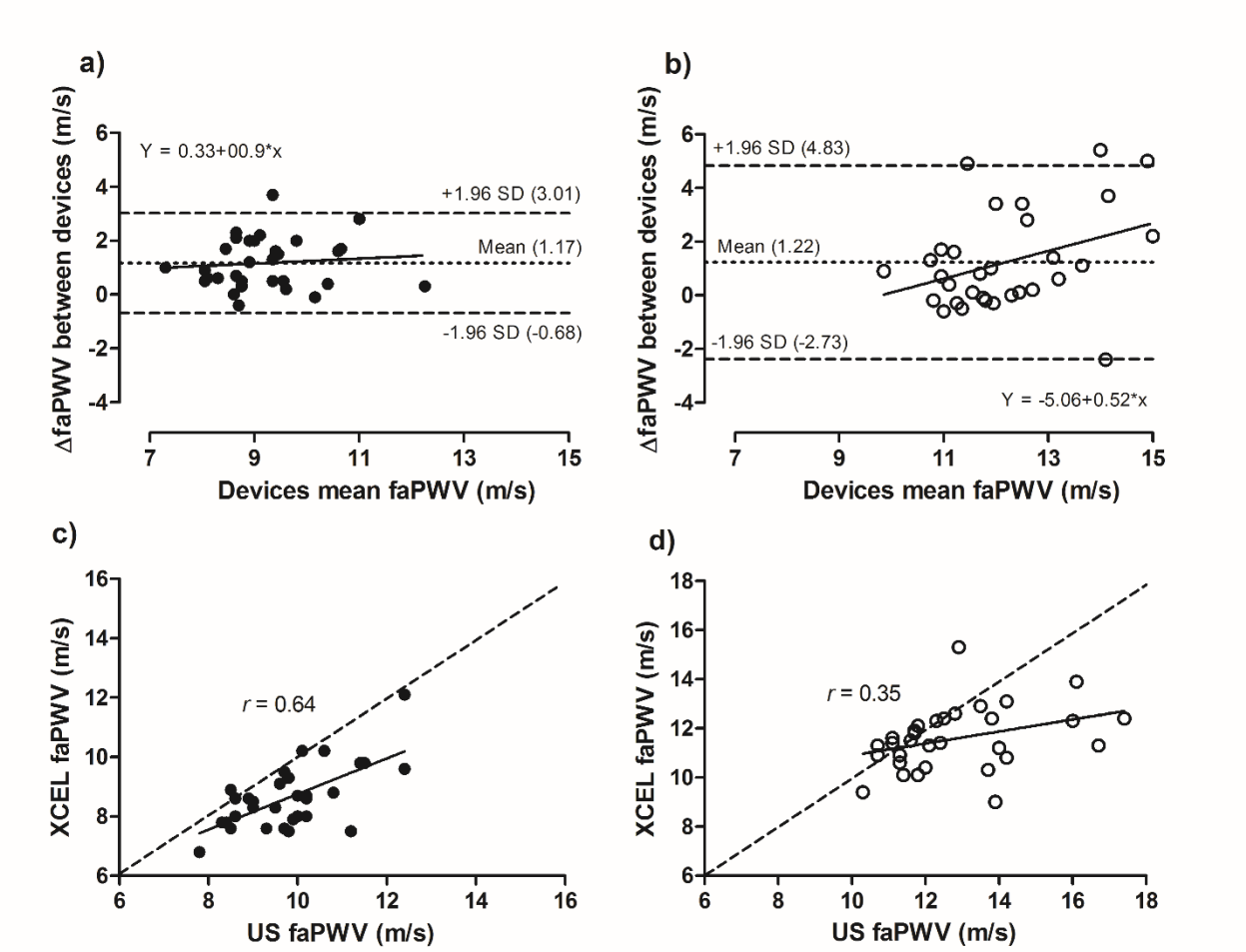

Figure 3.2 Bland-Altman plots (top panels) and correlation analysis (bottom panels) for femoral-ankle PWV (faPWV) obtained by SphygmoCor XCEL (XCEL) and Doppler ultrasound (US) devices in supine (closed circles) and seated (open circles) positions.

Estimates for reliability of measures of faPWV and cfPWV are presented in **Table 3.4**. The XCEL demonstrated good between-day reliability (ICC: 0.83, 95% CI: 0.65 – 0.93) when measuring faPWV in the supine position, but only moderate (ICC: 0.67, 95% CI: 0.40 – 0.86) reliability when measuring cfPWV. In the supine position, faPWV (ICC: 0.29, 95% CI: 0.23, 0.63) and cfPWV (ICC: 0.57, 95% CI: 0.25, 0.81) measures demonstrated poor and moderate reliability, respectively.

Table 3.4 Reliability of the SphygmoCor XCEL to determine central and peripheral PWV in supine and seated positions.

	ICC (95% CI)		SEM (95% CI)		RC (95% CI)	
Supine						
faPWV (m/s)	0.83	(0.65-0.93)	0.53	(0.33-0.75)	1.46	(0.92-2.09)
cfPWV (m/s)	0.67	(0.40-0.86)	0.48	(0.31-0.65)	1.34	(0.87-1.80)
Seated						
faPWV (m/s)	0.29	(0.23-0.63)	1.19	(0.86-1.39)	3.29	(2.37-3.86)
cfPWV (m/s)	0.57	(0.25-0.81)	0.54	(0.36-0.71)	1.51	(1.00-1.97)

Abbreviations*:* cfPWV, carotid-femoral pulse-wave velocity; CI, confidence interval; faPWV, femoral-ankle pulse-wave velocity; ICC, intra-class correlation coefficient; RC, repeatability coefficient; SEM, standard error of measurement.

Discussion

Given the growing interest in the effects of sedentary behaviour, particularly prolonged sitting, on cardiovascular health, there is a pressing need to identify tools capable of accurately and precisely measuring the athero- and arterio-sclerotic susceptible vasculature of the lower extremities. The primary finding of the current study is that in the supine position XCEL measures of faPWV demonstrate acceptable validity, when compared against the criterion, and good between-day reliability. The secondary finding is that XCEL faPWV measures did not achieve the validity criteria and demonstrated poor reliability in the seated position. It is therefore recommended that, as per conventional practice for many vascular assessments, XCEL-based lower-limb arterial health assessments be conducted in the supine position.

Study Limitations

In order to better contextualize the present findings, several limitations should first be addressed. A relatively homogenous group of young healthy participants were recruited in order to ascertain the upper-limit of validity and reliability for XCEL-based leg PWV assessments. Prior to clinical use it is of high importance to determine whether any error, bias or variability inherent to a health test is caused by the technique itself and not a consequence of the presence of cardiovascular pathology. Further studies are therefore required in order to generalize any findings of the present study in clinical

populations of varying age and health states. Second, whilst every effort was made to match PWV distance (arterial path length) between test and criterion techniques, due to variance in participant anatomy this was not always possible. Mean arterial path length (PWV distance) was 672 ± 46.5 mm for XCEL and 662 ± 51.9 mm for US. Consequently, this resulted in small differences in PTT between PWV assessment techniques (**Table 3.3**). Although PWV was calculated relative to an independent path length, this may have resulted in the introduction of small measurement error.

Supine Pulse Wave Velocity

The current study found that there was acceptable agreement (aSEE: 0.8 m/s, 95% CI: 0.4, 1.0) between the XCEL device and a criterion, US, for measuring faPWV in the supine position. Relatively few studies have compared electro-mechanical (pressure) and US techniques when determining PWV, and existing research has naturally focused on the assessment of cfPWV as a primary measure. Calabia and colleagues [240] reported very good agreement (mean difference [MD] = 0.13±0.19 m/s) between US and the tonometer-like mechano-transducer based Complior system, when determining cfPWV in the supine position. Similarly, Jiang et al. [241] compared US and the standard tonometer only SphygmoCor device for measuring cfPWV and reported good correlation and agreement (MD = 0.3 m/s). Whilst these findings indicate agreement between mechanical (pressure) and US techniques, neither methods employed by Calabia [240] and Jiang [241] incorporated the use of a volume displacement cuff to detect the femoral or distal arterial waveform. Therefore, prior to this study it was unclear whether a device which combines applanation tonometry and oscillometric arterial waveform detection technologies could be used to accurately measure leg PWV.

It is recognised that although the validity criteria were met, a significant main effect of device was observed. This difference may originate from; i) continued difference in waveform detection between mechanical and acoustic techniques, or, ii) the use of a standard assumed constant when applying the femoral segment correction. The in-built XCEL correction assumes the same PTT when calculating the PTT delay associated with the inclusion of the additional femoral artery segment. The PTT of 0.08449 ms for every 10 mm of arterial length is an average derived from only 15 individuals [110], but comparable to published values [198]. This constant is thought to be independent of sex, age, BP and ethnicity. However, the use of individualised or population specific constants may improve correlation and minimize differences between US and oscillometric techniques. A caveat is that this approach would likely make the simple XCEL technology more technically and logistically

challenging. Finally, despite a significant main effect for device, faPWV changes induced by the postural shift between supine and seated postures were similar between XCEL and US devices (**Table 3.3**) and no interaction effect was observed.

The observed ICC of 0.83 (95%CI: 0.65, 0.93) indicates that the XCEL demonstrates good between-day reliability when measuring faPWV. Perhaps surprisingly, the ICC for faPWV was greater than the quality control measure, cfPWV, a measure previously shown to be highly precise [251]. This finding further indicates the reproducibility of XCEL-based faPWV measures in the supine position. While clinically relevant data is not available for the lower-limbs, the typical error of XCEL derived faPWV (0.8 m/s, 95% CI: 0.4, 1.0) is also less than the clinically meaningful mean difference of 1.0 m/s observed for the quality control measure, cfPWV [19].

Seated Pulse Wave Velocity

Unlike supine measures, seated XCEL faPWV values did not meet the validity criteria, with the absolute SEE (1.2 m/s, 95% CI: 0.9, 1.3) being above the clinically meaningful difference of 1.0 m/s set *a priori*. While these findings indicate that the accuracy of the XCEL may not be adequate in the seated position, there may be a number of reasons for this. The algorithm presented by Butlin and colleagues [110] was only validated in the supine position and may be impacted by postural shift and/or greater vascular tortuosity (hip and knee bend) in the seated position. Additionally, the contrasting technologies used to obtain and analyse arterial pressure waveforms may simply demonstrate greater divergence when arterial geometry is changed. ICC values for faPWV in the seated position also demonstrated poor reliability (ICC: 0.29, 95% CI: 0.23, 0.63). Interestingly, XCEL measures of cfPWV only demonstrated moderate reliability; a measure previously observed to be both highly accurate and reliable in the supine position [110,251].

The present findings indicate that postural change may have a significant impact on the ability of XCEL to accurately and precisely measure central and lower-limb PWV. During orthostasis there is a propensity for blood to pool in the sub-diaphragmatic venous system [252]. This likely occurred in the current study, as indicated by the reduction in SV, CO, and Pb [253]. To compensate for blood pooling, and attempt to ensure adequate venous return, the baroreflex response results in lower extremity vasoconstriction and increased SVR, as evident in the current study [254]. Accordingly, both faPWV and cfPWV measures are likely to be influenced by the interaction of the variability of the test (XCEL) device and the response of the autonomic nervous system to orthostatic induced venous

pooling. Accordingly, blood pooling during sitting may lead to greater variability and divergence between the XCEL and US PWV techniques with regards to the timing and characteristics of the recorded pulse waveform.

Implications and future direction

The growth in sedentary behaviours in today's society necessitates the need for the identification of novel, simple tools to help unmask the link between sedentary behaviour and cardiovascular health, as well as enable the early detection and monitoring of CVD development. Whilst it is acknowledged that further research determining the validity and reliability in populations of varying age and health states is needed, the findings of the current study indicate that the oscillometric XCEL device can be used to simply, accurately and precisely measure lower-limb PWV in the healthy young, in a supine position. Although faPWV assessments in a seated position did not meet the validity or reliability criteria, cardiovascular assessments are routinely conducted in a supine position, including FMD [237], due to the potential for orthostatic induced haemodynamic shifts to confound measures. Whilst postural manoeuvres are unavoidable, this technique does confer several advantages over traditional vascular health assessments. Specifically, measurement of PWV via oscillometry is easier to perform, less operator dependent and more reliable than FMD, particularly in the legs. For example, FMD of the popliteal artery demonstrated extremely poor between-day reliability (ICC = 0.25) in the only study to report lower-limb FMD reliability, to the authors knowledge [236]. To give a clinical context, the absolute SEM for the current study was 0.53 m/s, meaning that a change of greater than 6.1%, from a mean of 8.6 m/s, is required to infer a true effect, In contrast, using an average baseline FMD of 4.1% and an absolute SEM of 1.74% (calculated from data) [236], a change of greater than 42% would have to be observed to be determined true when the FMD technique is used on the leg.

Although it is still unclear what role functional and structural changes in lower-limb vasculature may have on the heart or the development of central CVD, it is recognised that arterial stiffness in the lower extremities is an important determinant of cardiac afterload. Recently, lower-leg vasodilatory function was observed to decrease and central arterial stiffness (cfPWV) to concomitantly increase in response to 3-hrs of prolonged sitting [255]. Whilst a direct link could not be made, increased PWV in the legs has been reported to be linked to greater aortic stiffness [38] and left ventricular mass [35]. Accordingly, assessments of faPWV, as an adjunct to cfPWV (aortic), may be useful in further understanding the effects of sedentary behaviour on CVD risk and, more specifically,

understanding how the lower-limb vasculature interacts with central vasculature and cardiac properties. Given that central and peripheral arterial health can be assessed simply, accurately and precisely in the supine position using the oscillometric XCEL device, this tool may be useful in helping to further understand the pathological mechanisms linking CVD to sedentarism.

CONCLUSION

The aim of this study was to determine the accuracy (validity) and precision (reliability) of faPWV measurements obtained using the oscillometric-based XCEL device in supine and seated positions. Findings suggest that faPWV can be determined with acceptable accuracy and precision, but only in the supine position. The use of the XCEL device may provide research scientists with a practical, accurate and precise way of investigating the impact of sedentary behaviour, including prolonged sitting, on lower-limb vascular health. Importantly, this technology may also be useful in helping to understand how the lower-limb vasculature acutely and chronically interacts with central vascular and cardiac properties.

Commentary…

Chapter 3 addressed ***objective 1.1*** and demonstrated that faPWV can be determined with acceptable accuracy and good precision using a simple oscillometric cuff-based device, thereby satisfying European Society of Cardiology Criteria 1 [6]. The second requirement for any novel risk marker is that in order to justify its measurement, it must also improve risk prediction beyond biomarkers that are already routinely used; that is, new markers should provide independent incremental prognostic information. An existing biomarker of arterial health in the lower-limbs, used in clinical and epidemiological practice, is ABI [28]. As well as indicating arterial stenosis in the lower-limbs or PAD, a low ABI also serves as a strong prognostic marker or heightened global atherosclerotic CVD risk [29-32]. Whether the assessment of lower-limb arterial stiffening, an arteriosclerotic process, can provide additional CVD risk information is unknown. The few studies which have examined the association of faPWV with CVD risk have reported inconsistent findings [39-42], but all of these studies were relatively small and conducted in patient populations only. **Chapter 4** will address ***objective 1.2*** and seek to identify whether the assessment of faPWV can provide additional CVD risk information beyond ABI, an existing marker of lower-limb arterial health (European Society of Cardiology criteria 2).

4

Associations of lower-limb atherosclerosis and arteriosclerosis with cardiovascular risk factors and disease in older adults

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Atherosclerosis, in press

SUMMARY

Atherosclerosis and arteriosclerosis contribute to vascular aging and cardiovascular disease (CVD) risk. Both processes can be assessed simply in the lower-limbs and reflect systemic pathology. However, only atherosclerosis is routinely assessed, typically via ankle-brachial index (ABI). Arteriosclerosis can be assessed using femoral-ankle pulse wave velocity (faPWV), but no studies have identified whether ABI and faPWV similarly associate with overt CVD and risk factors, nor whether faPWV confers additional information. The aims of this study were, (i) To compare the independent associations of ABI and faPWV with traditional CVD risk factors, including age, sex, systolic blood pressure (SBP), high-density lipoprotein (HDL), total cholesterol (TC), smoking, and diabetes; and, ii) determine the independent and additive associations of ABI and faPWV with a composite measure of prevalent CVD. We evaluated ABI and faPWV in 4,330 older-aged (75.3±5.0 years) adults using an oscillometric screening device. Associations between ABI and faPWV with CVD risk factors and CVD were determined using mixed-model linear- and logistic-regression. ABI and faPWV were associated with age, HDL, and smoking. ABI was associated with sex, TC, diabetes. faPWV was associated with SBP. Both ABI and faPWV were inversely associated with CVD. Low ABI (≤0.9 vs. >0.9) and low faPWV (≤9.94 vs. >9.94) increased the odds of CVD by 2.41-fold (95% CI:1.85,3.17) and 1.46-fold (95% CI:1.23,1.74), respectively. The inverse association between faPWV and CVD was independent of ABI and CVD risk factors. ABI and faPWV, measures of lower-limb atherosclerosis and arteriosclerosis, are independently associated with CVD risk factors and prevalent CVD. Assessment of faPWV may confer additional risk information beyond ABI.

Introduction

The determination of traditional cardiovascular disease (CVD) risk factors including age, blood pressure (BP) and diabetes, enables clinicians and epidemiological researchers to estimate an individual's risk of developing CVD [83,256,257], including coronary heart disease (CHD) [258], heart failure (HF) [84] and stroke [85]. However, traditional CVD risk factors do not fully explain CVD burden [259]. Novel biomarkers have been shown to improve patient risk stratification, including those indicating lower-limb arterial health [260]. The assessment of lower-limb arterial health provides clinicians with the opportunity to conveniently detect local disease pathology, but also determine general CVD risk, as poor lower-limb arterial health is a manifestation of systemic pathophysiology [27]. Importantly, such assessments also permit identification of high-risk patients, given that individuals with peripheral vascular diseases are at a higher risk of cardiovascular events and death, than those with isolated cardiac or cerebrovascular disease [25,191].

Ideally, lower-limb arterial health assessment would screen for both atherosclerosis and arteriosclerosis as both processes contribute to vascular aging, and therefore, CVD risk [26,80]. Atherosclerosis, the narrowing of the artery by the deposition of atheromatous plaque, typically occurs in the intima layer, and is principally characterized by the accumulation of lipids and fibrous elements, smooth muscle cell migration and foam cell development [68]. In contrast, arteriosclerosis, the stiffening and thickening of the arterial wall, reflects degenerative changes of the extra-cellular matrix in the media layer, and is principally characterized by elastin fatigue fracture, and collagen deposition and cross-linking [80]. Although these phenomena are typically viewed as distinct pathways, they do share common pathophysiological mechanisms. For example, increased luminal pressure induced by arterial stiffening impairs endothelial function and augments collagen production and deposition in the arterial wall, accelerating the formation of atheromatous plaque [80,128]. But whilst they typically co-exist, and may interact, their relative contribution to vascular aging, and therefore cardiovascular risk, may differ by arterial territory [128]. Whether atherosclerosis and arteriosclerosis of the femoral-tibial pathway equally associate with CVD risk factors and disease, or whether their combined assessment confers additional cardiovascular risk information is unknown.

The ankle-brachial index (ABI) is a simple biomarker of atherosclerosis in the legs [28]. Defined as the ratio of systolic blood pressure (SBP) measured at the ankle to SBP measured at the brachial artery, ABI was first employed as a non-invasive tool for the screening of occlusive peripheral artery disease (PAD) [8]. Whilst ABI indicates arterial stenosis in the lower-limbs, any occlusion

typically indicates the presence of systemic atherosclerosis [28,192]. As such low resting ABI (<0.9) typically serves as an independent prognostic marker for all CVD [29-32]. Despite its popularity, evidence supporting the use of ABI as a screening or monitoring tool at a population level, particularly in asymptomatic adults, remains limited [192,260].

A simple and potentially complimentary measure of lower-limb arterial health, and a measure of arteriosclerosis, is arterial stiffness. Emerging evidence indicates that arterial stiffening is one of the earliest detectable markers of changes in vascular structure and function of the arterial wall [102]. Lower-limb arterial stiffness can be determined using femoral-ankle pulse wave velocity (faPWV). Importantly, faPWV is clinically viable as it can be assessed with accuracy and precision using simple oscillometry [261], and faPWV can be determined concurrently with ABI. Lower-limb(s) arterial stiffness is associated with CVD risk factors, including BP, diabetes and dyslipidemia [36-38] and also reflects systemic CVD burden [36,39,40,148,262]. However, it is unknown whether ABI and faPWV are similarly associated with prevalent CVD, nor whether faPWV confers incremental information over and above ABI and established CVD risk factors [6].

As a proof of concept [6], the aims of the current study were: (i) to compare the independent associations of ABI and faPWV with traditional CVD risk factors, including age, sex, systolic blood pressure (SBP), high-density lipoprotein (HDL), total cholesterol (TC), smoking, and diabetes; and, ii) determine the independent and additive associations of ABI and faPWV with a composite measure of prevalent CVD status that includes CHD, HF, and stroke. These aims were undertaken using a well characterized community-dwelling population of older men and women from the Atherosclerosis Risk in Communities (ARIC) Study cohort.

Methodology

This observational study is reported in accordance with STROBE (STrengthening the Reporting of OBservational studies in Epidemiology) guidelines [243]. Participants provided written informed consent, and the study was approved by the Institutional Review Boards at all field centers, coordinating center, and central labs and reading centers. Confirmation of study approval is presented in **Appendix 2**.

Study Population

The ARIC Study is a population-based, longitudinal study of 15,792 men and women aged 45–64 years enrolled between 1987 and 1989 from 4 US communities (Forsyth County, North Carolina; Jackson, Mississippi; Minneapolis, Minnesota; and Washington County, Maryland). Details of the baseline visit have been previously described [226]. Prior to exclusions, the current analysis includes 6,538 participants who attended visit 5 between 2011 and 2013 and had ABI and PWV measures completed (5,683 total participants at visit 5).

We excluded participants with the following conditions due to concerns of ABI and PWV data quality: BMI ≥40 kg/m^2, major arrhythmias (atrial or ventricular premature beats, atrial fibrillation or flutter), major arrhythmias with evidence of biased PWV waveforms, abdominal aorta ≥5 cm, history of aortic or peripheral revascularization or aortic graft, aortic stenosis, moderate or greater aortic regurgitation and an ABI ≥ 1.5, which indicates incompressible arteries. Additionally, we excluded participants whose race was other than white or African American (due to small sample size) as well as those with missing ABI, PWV or vascular risk factor data.

Participants were asked not to consume food or drink, and refrain from tobacco and vigorous physical activity after midnight prior to the clinic visit or for 8 hours prior to the visit. Visit 5 study examination included interviewer-administered questionnaires to obtain demographic data, medical history and lifestyle information, blood and urine collection, and assessment of vascular risk factors and cardiovascular phenotypes, including ABI and PWV.

Experimental Measures

Ankle-brachial index and pulse wave velocity. Technicians measured both ABI and faPWV following a standardized protocol using the automated cardiovascular screening device VP-1000 Plus (Omron, Kyoto, Japan) [111], after participants were supine for 5–10 minutes. The validity and reliability of the automatic device for measuring ABI [111,212] and PWV [130] have previously been described. Quality assurance for PWV included central training and recertification, quarterly equipment calibration, and ongoing quality control reviews by one of the authors (H.T.) on a stratified random sample of 40 records per month with feedback provided to technicians. Approximately 78% of records were considered optimal quality, 17% were good quality, 3% were acceptable, and none were poor or unacceptable.

Ankle-brachial index. Assessments were completed with the participant in a supine position with both arms resting along his/her side while bent to 90 degrees at the elbows. Two electrocardiogram clips were attached on the inner side of both wrists, and BP cuffs were placed on both arms and ankles. BP was measured simultaneously in the four limbs at least twice at an interval of 5 minutes. Using the higher value of the right or left brachial SBP as the denominator, the ABI, the ratio of ankle SBP to brachial SBP [28], was calculated for right and left legs. The mean ABI of the two measurements was recorded for each leg. The minimum ABI, of left or right leg, was used for analyses.

Femoral-ankle pulse-wave velocity. Bilateral femoral arterial waveforms were acquired by applanation tonometry sensors on the common femoral arterials (via elastic tape around the hip). Bilateral posterior-tibial arterial pressure waveforms were detected by extremities cuffs connected to plethysmographic and oscillometric pressure sensors wrapped on both ankles. Distance for faPWV was automatically calculated by the VP-1000 Plus using height-based formulas, as previously described [263]. A minimum of two PWV measurements were taken per participant and the last two measurements were averaged. The maximum of left and right faPWV measures was included for analysis.

Measurement of CVD Risk Factors and Covariates

Demographics. Age was calculated from date of birth. Sex and race were self-reported. History of smoking was self-reported and analyzed as dichotomous (current versus noncurrent).

Anthropometrics. Body weight was measured to the nearest 0.1 kg, and height was recorded to the nearest centimeter (cm). Body mass index (BMI) was calculated using weight (kg) divided by height squared (m^2).

Blood Pressure. Three seated BP measurements were obtained after a 5-minute rest using an oscillometric automated sphygmomanometer (Omron HEM-907 XL, Omron, Kyoto, Japan), and the average of the last two measurements was used. Hypertension was defined as at least one of: SBP ≥140 mm Hg, diastolic BP (DBP) ≥90 mm Hg, or antihypertensive medication use.

Blood Markers. Blood samples were obtained following a standardized venipuncture protocol and shipped weekly to ARIC central laboratories where assays for total cholesterol (TC) and high-density

lipoprotein (HDL), and fasting glucose concentration were performed. Total plasma cholesterol concentrations were determined enzymatically using a Cobas-Bio analyzer with reagents purchased from Boehringer Mannheim Biochemicals, (Indianapolis, IN). Plasma HDL concentrations were measured using the method of Warnick et al. [264]. Diabetes was defined as fasting glucose ≥126 mg/dl, non-fasting glucose ≥200 mg/dl, antidiabetic medication use, or self-reported diagnosis of diabetes by a physician.

Medications. Participants were asked to bring to the clinical visit all prescription and non-prescription medications taken within the two preceding weeks. That information was transcribed and categorized using MediSPAN prescription codes and classified into medication categories. Participants also self-reported medication use. Medications used included β-blockers, α-blockers, calcium channel blockers, diuretics, angiotensin-converting enzyme inhibitors, and angiotensin II receptor blockers.

Prevalent Cardiovascular Disease

Prevalent CVD was defined as composite measure of CHD, stroke and HF [83]. Prevalent CHD was defined by self-reported prior physician diagnosis of myocardial infarction or coronary revascularization, or prevalent myocardial infarction according to adjudicated electrocardiogram. Prevalent HF was classified by having at least one of the following: an adjudicated diagnosis of a HF event, International Classification of Disease, 9th Revision, Clinical Modification (ICD-9-CM) discharge code of 428.X in first position not overruled by a physician, any physician report of HF, self-reported HF or self-report of HF medication with pro-BNP greater than 125 pg/mL, or subsequent self-report of HF or HF medication (defined as medications participants reported taking for the treatment of HF). Prevalent stroke was defined by self-reported prior physician diagnosis of stroke or trans ischemic attack and whether they had ever experienced the sudden onset of specific stroke symptoms (weakness, numbness, loss of vision, loss of understanding, inability to express).

Statistical Analyses

Statistical analyses were performed using R Statistical Software. The α-level was set *a-priori* for all statistical procedures at $\alpha = 0.05$. Cumulative frequency and Q-Q plots were used to compare the distributions of ABI and faPWV. Participant characteristics were stratified by faPWV quartiles and were estimated as mean and standard deviation (SD), or frequencies and percent. Descriptive data across faPWV quartiles were compared using one-way analysis of variance (ANOVA) for continuous

outcomes and Kruskal-Wallis for categorical outcomes, with Bonferroni correction for multiple comparisons. We initially explored associations between ABI and faPWV with 5-year age groups using Spearman correlations (r). For linear regression we report unstandardized and standardized β coefficient estimates and 95% confidence intervals (95% CI), and the R^2 values for model fit.

For aim 1, ABI and faPWV associations with traditional CVD risk factors were evaluated using mixed model linear regression, with race and field center specified as random intercepts. Independent variables included age, sex, SBP, HDL, TC, smoking, and diabetes. All independent variables were initially entered into the model (model 1), and variables significantly associated with ABI and faPWV ($P<0.1$) were retained using a backward step-wise method (final model). For Aim 2, we investigated the associations between ABI and faPWV with a composite measure of CVD status that included CHD, HF and stroke using univariable and multi-variable binomial logistic regression. Univariable models included ABI or faPWV only. Multivariable model 1 included all CVD risk factors only. Multivariable model 2 included CVD risk factors and ABI or faPWV independently. Multivariable model 3 included all CVD risk factors, ABI, and faPWV.

Initially, ABI and faPWV were entered as continuous variables. Comparisons were also made whereby ABI and faPWV were entered as categorical variables. For ABI, data was categorized according to the recognized criteria for classifying PAD (ABI: ≤0.9 *vs.* >0.9). Given that no clinical threshold for faPWV has been identified, faPWV was categorized by quartiles, with the reference level and direction of association being determined following continuous analysis findings. For logistical regression, we report odds ratios as well as indicators of overall model fit including: Akaike's Information Criteria (AIC), McFadden's Pseudo R^2 (R^2McF) and Chi-square statistic (X^2). Likelihood ratio tests were used to compare the goodness-of-fit between nested logistic regression models. The Vuong likelihood ratio test was used to compare non-nested logistic regression models, within the *nonnest2* package in R [265]. All multivariable logistical regression models were adjusted for antihypertensive medication and race-field center. Assumptions of linearity, collinearity, homoscedasticity, and outliers were assessed for every model.

Results

Characteristics of the Study Population

Descriptive characteristics are reported in **Table 4.1**. Following exclusions, the sample included 4,330 cohort participants between the ages of 66 and 90 years, of which 59.5% were women and 22.3% were African American. Of the 5,683 participants who attended visit 5 and underwent ABI and PWV measurements: 1353 were excluded using the following criteria: pre-existing condition (n=579), race other than white or African American due to low numbers (n=15), missing ABI data (n=13), missing PWV data (n=433), missing risk factor data (n=83), and missing covariates (n=230).

Aim 1: Associations of ABI and faPWV with CVD Risk Factors

Linear regression analysis revealed a positive association between faPWV and ABI (R^2=0.039). Non-linearity between faPWV and ABI was explored by specifying the faPWV quadratic term. The quadratic term was significant (P<0.001), and the change in R^2 due to the quadratic term was significant (ΔR^2=0.04, P<0.001). **Figure 4.1** presents ABI and faPWV values stratified by age categories. ABI had a negative correlation with age *(r* = -0.08, 95% CI: -0.11, -0.05, *P* < 0.001) and faPWV had a positive correlation with age *(r* = 0.04, 95% CI: 0.01, 0.07, *P* = 0.004).

Table 4.1 Descriptive characteristics of ARIC visit 5 participants, overall and stratified by femoral-ankle pulse wave velocity (faPWV) quartiles.

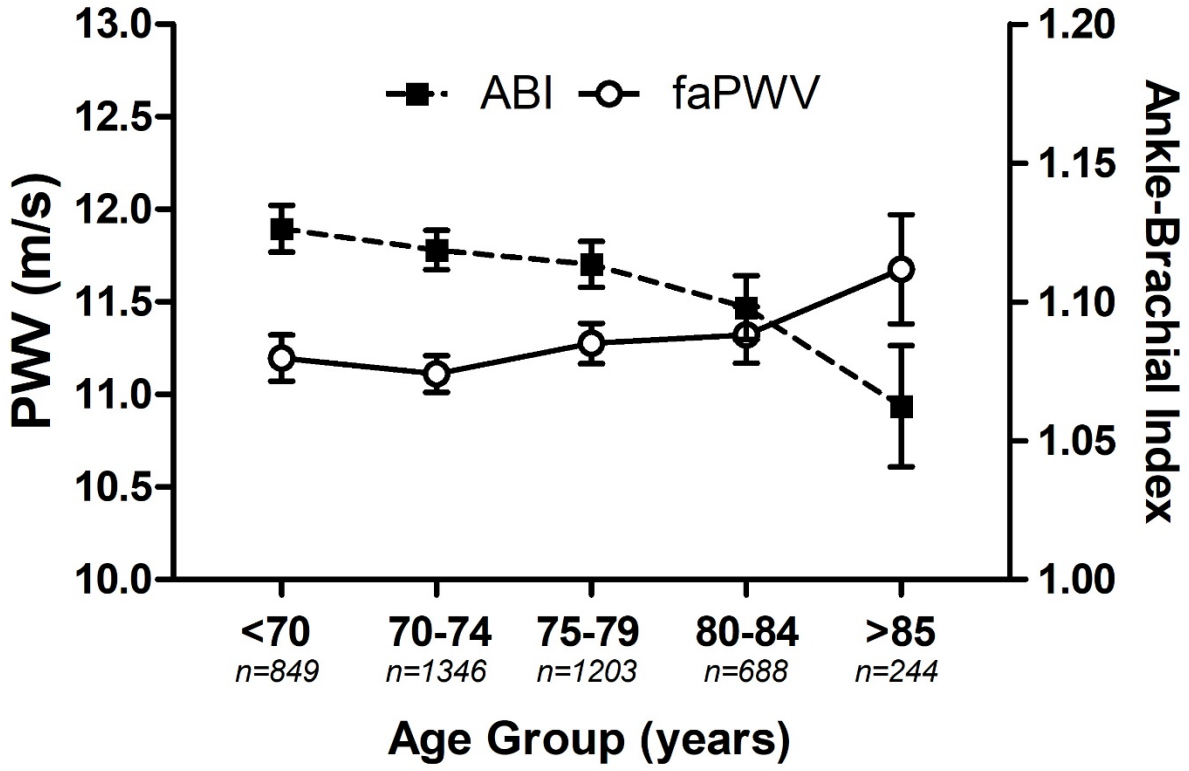
	Overall n = 4330		Q1 n = 1081		Q2 n = 1045		Q3 n = 1138		Q4 n = 1066		*P* Value
Continuous Variables (Mean, SD)											
Age (years)	75.3	(5.0)	75.2	(5.0)	74.9	(4.88)§	75.2	(5.02)§	75.7	(5.2)	0.002
Body Mass Index (kg/m^2)	29.2	(4.7)	28.1	(4.5)	27.4	(4.3)	26.7	(4.1)	28.2	(4.6)	<0.001*
Diastolic blood pressure (mm Hg)	66	(10)	62	(10)	65	(10)	67	(10)	70	(10.7)	<0.001*
Systolic blood pressure (mm Hg)	130	(18)	126	(17)	128	(17)	130	(16)	135	(18)	<0.001*
Heart rate (bpm)	65	(11)	63	(10.1)‡§	64	(10)‡§	65	(11.2)§	67	(11)	<0.001*
Fasting glucose (mg/dL)	6.2	(1.5)	6.2	(1.4)	6.3	(1.5)	6.2	(1.5)	6.2	(1.5)	<0.001
Total Cholesterol (mg/dL)	183	(41)	181	(40)§	182	(41)§	183	(41)	187	(43)	0.001
High-density lipoproteins (mg/dL)	53	(14)	51	(13)†‡§	53	(14)	54	(15)	54	(15)	<0.001
Ankle Brachial Index	1.11	(0.13)	1.06	(0.17)†‡§	1.11	(0.12)‡§	1.12	(0.11)	1.13	(0.11)	<0.001
Femoral-ankle PWV (m/s)	11.2	(1.9)	9.0	(0.8)	10.5	(0.3)	11.6	(0.4)	13.8	(1.5)	-
Categorical Variables (No., %)											
Sex											
Men	1750	(41)	443	(41)	417	(40)	468	(41)	422	(40)	0.821
Women	2580	(59)	638	(59)	628	(60)	670	(59)	644	(60)	
Race			†‡§		§						
African American	967	(22)	333	(31)	241	(23)	219	(19)	174	(16)	<0.001
White	3363	(78)	748	(69)	804	(77)	919	(81)	892	(84)	
Current smoker	248	(6)	82	(8)§	57	(5)	57	(5)	52	(5)	0.021
Prevalent Cardiovascular Disease											
Coronary heart disease	459	(11)	184	(17)‡§	147	(14)	147	(13)	131	(12)	<0.011
Heart failure	125	(3)	170	(16)†‡§	100	(10)	102	(9)	87	(8)	<0.001
Stroke	2835	(66)	32	(3)	38	(4)	26	(2)	29	(3)	0.296

Cardiovascular Disease Risk Factors											
Hypertension	610	(14)	751	(70)‡	685	(66)	741	(65)	654	(61)	<0.001
Diabetes	1276	(29)	358	(33)‡§	327	(31)	306	(27)	283	(27)	<0.001
Ankle Brachial Index ≤ 0.9	298	(6.9)	158	(14.8)†‡§	64	(6.1) §	44	(3.9)	32	(3.0)	<0.001
Medication use											
β-Blocker	1311	(30)	410	(38)‡§	328	31§	333	(29)§	240	(23)	<0.001
α-Blocker	140	(3)	60	(6)†‡§	27	(3)	30	(3)	23	(2)	<0.001
Diuretic	1666	(38)	499	(46)†‡§	418	40§	406	(36)	343	(32)	<0.001
ACE Inhibitor	986	(23)	245	(23)	231	(22)	281	(25)	227	(21)	0.278
ANG II receptor blocker	443	(10)	125	(12)	105	(10)	108	(9)	103	(10)	0.327
Calcium channel blocker	1068	(25)	345	(32)†‡§	267	(26)§	256	(23)	199	(19)	0.327

Abbreviations: PWV, pulse-wave velocity. **faPWV quartiles** Q1, <9.94 m/s; Q2, 9.94 m/s to 11.0 m/s; Q3, >11.0 m/s to 12.3 m/s Q4 >12.3 m/s.

**Comparisons: ***All groups significantly different;† vs. Q2; ‡ vs. Q3; § vs. Q4.

Figure 4.1 Mean ankle-brachial index (ABI) and femoral-ankle pulse wave velocity (faPWV) in 5-year age groups, with 95% confidence intervals. n = 4,330.



Backwards stepwise regression analysis was used to identify CVD risk factors associated with ABI and faPWV (**Table 4.2**). For ABI we observed positive associations with sex and HDL, and negative associations with age, TC, smoking and diabetes. Overall, faPWV was associated with fewer CVD risk factors, demonstrating positive associations with age, SBP, and HDL, and a negative association with smoking. Accordingly, only age, HDL, and smoking status were mutual covariates. The highest standardized beta coefficients were also different between measures, with ABI demonstrating the strongest association with smoking, sex and age and faPWV demonstrating the strongest association with SBP, HDL and smoking.

Table 4.2 Linear regression models for association of ankle-brachial index (ABI) and femoral-ankle pulse-wave velocity (faPWV) with traditional cardiovascular disease risk factors at visit 5.

	ABI					faPWV				
	β	Std. β	95% CI		*P Value*	β	Std. β	95% CI		*P Value*
Model 1										
Age (years)	0.00	-0.14	-0.17	-0.11	<0.001	0.01	0.03	0.00	0.06	0.045
Sex	0.04	0.16	0.13	0.19	<0.001	0.11	0.03	0.00	0.06	0.083
SBP (mm Hg)	0.00	0.01	-0.02	0.03	0.724	0.02	0.21	0.18	0.24	<0.001
HDL (mg/dL)	0.00	0.07	0.03	0.10	<0.001	0.01	0.07	0.03	0.10	<0.001
TC (mg/dL)	0.00	-0.07	-0.10	-0.04	<0.001	0.00	0.00	-0.03	0.04	0.869
Smoker	-0.10	-0.17	-0.20	-0.15	<0.001	-0.31	-0.04	-0.07	-0.01	0.011
Diabetes	-0.01	-0.03	-0.06	0.00	0.039	-0.10	-0.02	-0.05	0.01	0.128
Overall Model Fit	mR^2=	.07	cR^2=	.19		mR^2=	.06	cR^2=	.10	
Final Model*										
Age (years)	0.00	-0.14	-0.17	-0.11	<0.001	0.01	0.03	0.00	0.06	0.042
Sex	0.04	0.16	0.13	0.19	<0.001	0.11	0.03	0.00	0.06	0.089
SBP (mm Hg)						0.02	0.21	0.18	0.24	<0.001
HDL (mg/dL)	0.00	0.07	0.03	0.10	<0.001	0.01	0.07	0.04	0.10	<0.001
TC (mg/dL)	0.00	-0.07	-0.10	-0.04	<0.001					
Smoker	-0.10	-0.17	-0.20	-0.15	<0.001	-0.31	-0.04	-0.07	-0.01	0.012
Diabetes	-0.01	-0.03	-0.06	0.00	0.039					
Overall Model Fit	mR^2 =	.07	cR^2=	.19		mR^2=	.06	cR^2=	.10	

Abbreviations: β, beta coefficient; CI, confidence interval; std. β, standardized beta coefficient; mR2, marginal R squared coefficient; cR2, conditional R squared coefficient; SBP, systolic blood pressure; HDL, high-density lipoproteins; TC, total cholesterol. **Adjustments*: antihypertensive medication, race/field center.

AIM 2: Associations of ABI and faPWV with Prevalent CVD

Table 4.3 presents associations for ABI and faPWV with prevalent CVD (CHD, HF and stroke) following univariable and multivariable logistic regression analyses. When specified as continuous variables, both ABI and faPWV were negatively associated with prevalent CVD (univariable) and were negligibly impacted with adjustment for traditional CVD risk factors (multivariable 2). Similarly, when specified as categorical variables, both a low ABI (<0.9) and a low faPWV (<9.94 m/s) were associated with prevalent CVD (univariable), and these associations remained significant after adjustment for CVD risk factors (multivariable 2). After adjustment for CVD risk factors, a low ABI increased the odds of CVD by 2.41-fold, whilst a low faPWV increased the odds of CVD by 1.46-fold. Comparison of non-nested models, whereby ABI or faPWV were entered independently (univariable and

multivariable), showed that ABI explained a significantly greater portion of the variance in prevalent CVD than faPWV.

For continuous and categorical analyses, the inverse relationships with CVD remained significant when ABI and faPWV were included in the model together (multivariable 3). Goodness of fit comparisons of nested models showed that when ABI and faPWV were entered together, the model explained significantly more variation in prevalent CVD than when ABI was entered independently with CVD risk factors. This indicates that faPWV does explain variation in prevalent CVD beyond ABI and CVD risk factors.

Sensitivity and Ancillary Analysis

Associations of ABI and faPWV with CVD risk factors and prevalent CVD were repeated following exclusion of participants with low ABI ≤ 0.9 (n=298) and high ABI≥1.4 (n=12), as the presence of PAD or arterial calcification, respectively, do have the potential to impact faPWV measures. This included logistical regression analysis where ABI was entered as a categorical variable comparing quartiles (Q1 vs. Q2, Q3 and Q4) rather than using the clinically used cut-off of ≤0.9. The were no notable differences when compared to the primary analysis. Analysis of mean faPWV, and left and right faPWV measures independently had no impact on findings when compared to those determined in primary analysis. Finally, given that high rather than low PWV is typically associated with CVD, the association between high faPWV (Q1-Q3: ≤ 12.3 m/s *vs.* Q4: > 12.3 m/s) and the composite measure of CVD was explored, but no significant associations were observed.

Table 4.3 Logistic regression models for association of ankle-brachial index (ABI) and femoral-ankle pulse-wave velocity (faPWV) with cardiovascular disease at visit 5.

	Model Coefficients				Overall Model Fit			
Continuous	**OR**	**95% CI**		**P Value**	**AIC**	**R^2McF**	**X^2 (df)**	**P Value**
Univariable								
ABI	0.12	0.07	0.20	<0.001	4407	.015	64.30	<0.001
faPWV	0.91	0.87	0.94	<0.001	4447	.005	23.81*	<0.001
Model 1								
CVD risk factors					3971	.117	522.00	<0.001
Model 2								
ABI	0.12	0.07	0.21	<0.001	3921	.129	574.00†	<0.001
faPWV	0.92	0.88	0.96	<0.001	3960	.120	534.52*†	<0.001
Model 3								
ABI	0.13	0.07	0.24	<0.001	3917	.130	579.41‡	<0.001
faPWV	0.95	0.91	0.99	0.021				
Categorical								
Univariable								
ABI	3.12	2.45	3.98	<0.001	4393	.018	78.20	<0.001
faPWV	1.57	1.34	1.84	0.008	4441	.006	30.02 *	<0.001
Model 1								
CVD risk factors					3971	.117	522.00	<0.001
Model 2								
ABI	2.41	1.85	3.17	<0.001	3932	.126	562.46†	<0.001
faPWV	1.46	1.23	1.74	<0.001	3955	.121	539.89*†	<0.001
Model 3								
ABI	2.25	1.71	2.96	<0.001	3924	.128	572.86‡	<0.001
faPWV	1.35	1.12	1.61	0.001				

Categorical comparisons: categorical analyses, comparisons represent ≤0.9 vs. >0.9 for ABI, and Q1 (≤9.94 m/s) vs. Q2-Q4 (>9.94 m/s) for faPWV, whereby higher values were set as the reference level following continuous analysis. **Model descriptions**: Model 1 - CVD risk factors only including: age, sex, systolic blood pressure, HDL cholesterol, total cholesterol, smoking status and diabetes. Model 2 - CVD risk factors plus ABI or faPWV separately. Model 3 - CVD risk factors plus ABI and faPWV concurrently. *Adjustments: All multivariable models were adjusted for antihypertensive medication use, and race/field center. Comparisons: *vs univariable and multivariable 2 ABI models (non-nested); †vs. multivariable 1(P<0.001). ‡vs. multivariable 2~ABI (P<0.001). **Abbreviations**: OR, odds ratio; CI, confidence interval; R2McF, McFadden's Pseudo R2; X2, Chi square statistic, df, degrees of freedom.

Discussion

The aims of the current study were: (i) to compare the independent associations of ABI and faPWV with traditional CVD risk factors, and ii) determine the independent and additive associations of ABI and faPWV with a composite measure of prevalent CVD. Our findings indicate that in older adults, ABI is associated with age, sex, HDL, TC, diabetes and smoking, whilst faPWV was associated with age, HDL, smoking and uniquely SBP, but any mutual associations were more strongly associated with ABI. However, both ABI and faPWV were independently associated with CVD beyond traditional risk factors. Further, the association between faPWV and CVD persisted beyond ABI and CVD risk factors, indicating that faPWV may confer unique and additive clinical CVD risk information.

Limitations and Strengths

The strengths and limitations of this study need to be addressed to contextualize the findings and better facilitate comparisons to the existing literature. Firstly, the cross-sectional design precludes the assessment of causality in the observed associations. Additionally, as with any observational study, we cannot rule out the possibility of residual confounding - though we did include several important confounders in our models. Secondly, the generalizability of our findings is limited to older populations and cannot be extended to younger, healthier cohorts. Further, the predominate inclusion of participants who had survived from baseline (1987-1989) and attended the Visit 5 examination (2011-2013), and were thus likely healthier compared to those who did not participate in the visit, may have generated a bias within the study population. Finally, the use of height-based formulas to calculate faPWV were validated in a Japanese population and may not be applicable to other racial or ethnic groups. A major strength is that this is the first study to directly compare the associations ABI and faPWV, two bio-markers of lower-extremity arterial health, with traditional CVD risk factors and prevalent CVD, and does so using a large community-dwelling population.

Comparison to the Literature: CVD Risk Factors

Consistent with previous findings, both ABI [194,195,266] and faPWV [39,148,267] were associated with age. Aged vessels demonstrate elevated expression of pro-inflammatory molecules, encouraging the accumulation of atherogenic lipoproteins, and thus, plaque development [268]. It is also expected that distensible elastin fibres in the arterial wall become fragmented and discontinuous with age, this, coupled with an attenuation of the elastin-collagen ratio, promotes arterial stiffening [20,39]. However, the stronger association between age and ABI compared to faPWV may indicate a greater propensity

for atherosclerotic, rather than arteriosclerotic, processes with age in the lower-limb vasculature. Lower susceptibility to arteriosclerotic pathology in the lower limbs may, in part, be due to peripheral muscular arteries inherently exhibiting higher stiffness, compromising chiefly of vascular smooth muscle cells and low elastin [96].

Vascular aging may also be differentially accelerated by lifestyle behaviours or the presence of disease. For example, there is strong evidence linking smoking with atherosclerosis, particularly of the lower-limb [269-271]. But the weaker association of smoking with faPWV compared to ABI in the present study is indicative of the weaker association between smoking and arterial stiffness more broadly [157]. Interestingly, consistent with previous observations in the ARIC study [158], we report a negative association between faPWV and smoking. Smoking-induced dysregulation of the metalloproteinase system, leading to the breakdown of collagen, and thus arterial wall hemodynamic properties, has been presented as a cause of this phenomenon [272]. Both faPWV and ABI were equally associated with HDL, with the analogous positive association highlighting the key role of this antiatherogenic lipoprotein. But faPWV was not associated with TC, which is perhaps not surprising given that broadly cross-sectional studies have typically found weak [37] or no relationship [148,267,273] between single lipid parameters and arterial stiffness. In contrast, the link between dyslipidemia and atherosclerosis is well established, with elevated LDLs stimulating endothelial cells to secrete atherogenic cytokines, encouraging foam cell formation in the intima, a first step in the formation of an atherosclerotic lesion [274]. Dyslipidemia is also exasperated by hyperglycemia, further promoting atherogenesis in diabetic individuals [274], particularly of the lower-limb [275], supporting the positive association between ABI and diabetes in this study. Although prevalent diabetes can also accelerate arterial remodelling [150], we did not observe an association between faPWV and diabetes. This may be because diabetes preferentially leads to stiffening of central over peripheral arteries [148].

Unlike ABI, faPWV was associated with SBP. The strong positive association between faPWV and SBP is consisted with previous literature [27,32,49,52] and reflects the inter-dependency of BP and arterial stiffness. Our failure to observe an association between ABI and brachial SBP has also been reported by others [276] and likely indicates that the variation in ABI is principally driven by ankle SBP. However, like faPWV [267], ABI has reliably been associated with hypertension [193,194], including in ancillary analysis of the present study, reflecting the systemic impact of both arteriosclerosis and atherosclerosis pathology in the lower-limb. Collectively, our findings suggest that ABI and faPWV may be complimentary indexes of vascular aging in older adults, with some unique

risk factors associations, however, ABI may be more sensitive to both fixed (age, sex) and modifiable (smoking, diabetes) CVD risk factors that accelerate vascular aging.

Comparison to the Literature: Prevalent CVD

Both ABI and faPWV were negatively associated with prevalent CVD, a composite measure that included HF, CHD, and stroke, beyond traditional CVD risk factors. Low ABI (≤0.9) and low faPWV (≤9.94 m/s) increased the odds of CVD by 141% and 46%, respectively. Further, low faPWV remained associated with CVD in fully adjusted models, indicating that faPWV may provide independent incremental prognostic value beyond both traditional CVD risk factors and ABI. Low ABI has reliably been associated with CVD [29-31], but the link between lower-limb arterial stiffness and CVD is less clear. Arteriosclerosis, the stiffening and thickening of the arterial wall, is typically indicated by high PWV and has a positive association with disease. For example, Kawai [40] reported that high faPWV was associated with higher stroke incidence, whilst faPWV is also found to be higher in type 2 diabetes patients than healthy controls [148], both intuitive, positive, associations. But, aligned with our, perhaps unexpected, findings, low faPWV has been associated with myocardial stress [36] and coronary artery disease [262] in older adults, as well as ischemic heart disease in diabetes patients [39]. The association between faPWV and CVD may therefore be differentially linked to CVD type and severity.

Although a few studies have reported inverse relationships between faPWV and CVD [36,39,262], the underlying pathophysiological mechanism(s) are unclear. Residual confounding may have contributed to the inverse relationship, given that some CVD risk factors (e.g. age *vs.* smoking) were differentially associated with faPWV. However, our multivariable models were adjusted for known confounders and widely recognized CVD risk factors. Alternatively, reduced lower-limb arterial stiffness may chiefly be due to the presence of significant arterial atherosclerosis leading to arterial stenosis [38], or PAD, which is prevalent in older adults [28]. Significant stenosis reduces BP downstream, on which PWV is dependent, suppressing pulse wave propagation [277]. To account for this possibility, we excluded participants with ABI ≤ 0.9, however, an inverse association with CVD persisted. Finally, it has been suggested that loss of elasticity and recoil may not necessarily translate to arterial stiffening, and may occur in the absence of stenosis [278]. But whether through atherosclerotic-dependent or -independent means, low faPWV may contribute to a reduction in the central to peripheral arterial stiffness gradient, augmenting the transmission of excessive forward

pressure into the microcirculation, a pathophysiological basis for cardiovascular events and target organ damage [44,279].

Implications and Conclusions

The assessment of lower-limb arterial health permits clinicians to accurately assess CVD risk, and identify individuals at higher risk of cardiovascular events [260]. However, CVD screening has typically focused on the determination of atherosclerosis in the lower-limbs using ABI [6,28], and likely ignores the contribution that lower-limb arteriosclerosis may contribute to CVD risk. Prior to this study it was known that arteriosclerosis in the lower-limbs is associated with CVD risk factors [36-38] and is associated with CVD [36,39,40,148,262]. The current study extends the literature by reporting that whilst lower-limb atherosclerosis may be a more sensitive marker of CVD, faPWV, an assessment of lower-limb arteriosclerosis, is independently associated CVD beyond ABI, and therefore may confer unique and additive CVD risk information. But of note, it is low faPWV, perhaps representing a loss of arterial elasticity and breakdown in wall structure with disease, rather than arteriosclerosis per se, that is more strongly associated with CVD. A regression of peripheral arterial stiffness may contribute to a reduction in the physiologically advantageous central to peripheral arterial stiffness gradient, a pathophysiological basis for cardiovascular events and target organ damage [56,57]. Importantly, faPWV can be measured accurately and reliably using simple oscillometry, and undertaken concurrently with ABI, meaning it is viable for time-sensitive clinical and epidemiological research settings. However, future work is warranted to; i) confirm if combined faPWV and ABI measures improve prediction of CVD outcome beyond ABI alone, and ii) identify the mechanisms leading to low faPWV and its role in heightening CVD risk.

Commentary…

Chapter 4 addressed ***objective 1.2*** and demonstrated that faPWV provides independent additional CVD risk information beyond an existing biomarker of lower-limb arterial health, ABI, thereby satisfying European Society of Cardiology Criteria 2 [6]. The findings of **chapters 4 and 5** indicate that the standalone measure faPWV satisfies both ESC criteria 1 and 2, and is therefore a promising biomarker for clinical integration. One advantage of faPWV is that, when combined with cfPWV, it permits determination of the central to peripheral arterial stiffness gradient, a novel biomarker of promising clinical utility [44]. To date, the available literature has only employed upper-limb PWV to derive the stiffness gradient, demonstrating that it can predict CVD better than cfPWV [44-49], but in patient populations only. Characterising the stiffness gradient using faPWV to determine the af-SG, may provide a more comprehensive picture of CVD risk [200,211], however, the clinical and prognostic potential of the af-SG is unknown. Again, to be of clinical utility the af-SG needs to satisfy criteria 1 and 2 listed by the European Society of Cardiology Criteria. **Chapter 5** will address ***objective 2.1*** and firstly seek to identify whether the assessment af-SG can provide additional CVD risk information beyond the criterion measure of arterial stiffness, cfPWV (European Society of Cardiology Criteria 2).

Part 2

Aortic-Femoral Arterial Stiffness Gradient

5

The Aortic-Femoral Arterial Stiffness Gradient

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SUMMARY

The aortic to femoral arterial stiffness gradient (af-SG) may be a novel measure of arterial health and cardiovascular disease (CVD) risk, but its association with CVD risk factors and CVD status, and whether or not they differ from the referent measure, carotid-femoral pulse-wave velocity (cfPWV), is not known. Accordingly, we compared the associations of the af-SG and cfPWV with, (1) age and traditional CVD risk factors, and (2) CVD status. We evaluated 4,183 older-aged (75.2±5.0 years) men and women in the community-based Atherosclerosis Risk in Communities (ARIC) Study. cfPWV and femoral-ankle PWV (faPWV) were measured using an automated cardiovascular screening device. The af-SG was calculated as faPWV divided by cfPWV. Associations of af-SG and cfPWV with age, CVD risk factors (age, body mass index, blood pressure, heart rate, glucose and blood lipid levels) and CVD status (hypertension, diabetes, coronary heart disease, heart failure, stroke) were determined using linear and logistic regression analyses. (1) The af-SG and cfPWV demonstrated comparable associations with age and CVD risk factors, except body mass index. (2) A low af-SG was associated with diabetes, coronary heart disease, heart failure and stroke, whilst a high cfPWV was only associated with diabetes. Although future studies are necessary to confirm clinical utility, the af-SG is a promising tool that may provide a unique picture of hemodynamic integration and identification of CVD risk when compared to cfPWV.

Introduction

Carotid-femoral pulse-wave velocity (cfPWV), a measure of central aortic stiffness, predicts cardiovascular disease (CVD) risk in both general [10,19] and patient populations [41] independent of traditional risk factors. In contrast, upper extremity (arms) and lower-extremity (legs) peripheral measures of arterial stiffness are used infrequently because of their limited or inconsistent prognostic value [18,41,200]. However, the central to peripheral arterial stiffness gradient (SG) may reflect the hemodynamic integration of the cardiovascular system and confer unique and prognostic information [44]. To date, the few studies that have investigated the utility of the SG have focused on the aortic to brachial SG (ab-SG), defined as the ratio of carotid-radial PWV (crPWV) and cfPWV [44-49]. However, the aortic to femoral SG (af-SG), defined as the ratio of femoral-ankle PWV (faPWV) and cfPWV, incorporates the lower extremities in the assessment of vascular stiffness and may thus provide a more comprehensive picture of hemodynamic integration.

In a healthy cardiovascular system, the arterial vasculature progressively stiffens between the elastic ascending aorta and the muscular conduit arteries of the periphery [198,280]. This gradient, or impedance mismatch, is physiologically advantageous, permitting the transformation of the highly pulsatile stroke volume into a smooth consistent blood flow, including during diastole [43]. The gradual attenuation of the forward pressure wave prevents the transmission of pulsatile forces to the micro-circulation and moderates wave reflections back towards the myocardium [15,43,74]. Although no clinical threshold has been identified, reversal of the SG can increase pressure transmission, leading to end-organ damage, and augment reflected wave pressure, increasing myocardial load [43,44,176]. The available literature suggests that most of the change in the SG is attributable to the aortic-iliac pathway, and not the peripheral vasculature [49,281]. Aortic stiffness increases with age and can be accelerated by lifestyle factors [282] and disease [278]. Age or disease related changes in the upper extremities are less marked [200], and the upper extremities represent only a small portion of the vasculature. In contrast, the lower extremities make up a significant portion of the arterial tree, are more prone to athero- and arterio-sclerotic processes than the upper extremities [200,211], and are major sites of wave reflections [26]. However, the association of the af-SG with age, traditional CVD risk factors and CVD status, and whether these associations differ to those of cfPWV, is not known.

The primary aims of the current study were to compare the associations of af-SG and cfPWV with; 1) age and traditional CVD risk factors, and, (2) CVD status. These aims were undertaken using

a well characterized population of older men and women from the Atherosclerosis Risk in Communities (ARIC) Study cohort.

Methodology

This observational study is reported in accordance with STROBE (STrengthening the Reporting of OBservational studies in Epidemiology) guidelines [243]. Participants provided written informed consent, and the study was approved by the Institutional Review Boards at all field centers, coordinating center, and central labs and reading centers. Confirmation of study approval is presented in **Appendix 2.**

Study Population

The ARIC Study is a population-based, longitudinal study of 15,792 men and women aged 45–64 years enrolled between 1987 and 1989 from 4 US communities (Forsyth County, North Carolina; Jackson, Mississippi; Minneapolis, Minnesota; and Washington County, Maryland). Details of the baseline visit have been previously described [226]. Prior to exclusions, the current analysis includes 6,538 participants who attended visit 5 between 2011 and 2013 and had PWV measures completed (5,683 total participants at visit 5).

We excluded participants with the following conditions due to concerns of PWV data quality: BMI ≥40 kg/m^2, major arrhythmias (Minnesota codes 8-1-3, 8-3-1, and 8-3-2), Minnesota code 8-1-2 with evidence of biased PWV waveforms, aortic aneurysms, abdominal aorta ≥5 cm, history of aortic or peripheral revascularization or aortic graft, aortic stenosis, and moderate or greater aortic regurgitation. Additionally, we excluded participants whose race was other than white or African American (due to small sample size), with missing PWV or vascular risk factor data, as well as those with outlying PWV values, defined as PWV values 3 standard deviations above or below the mean.

Participants were asked not to consume food or drink, and refrain from tobacco and vigorous physical activity after midnight prior to the clinic visit or for 8 hours prior to the visit. Visit 5 study examination included interviewer-administered questionnaires to obtain demographic data, medical history and lifestyle information, blood and urine collection, and assessment of vascular risk factors and cardiovascular phenotypes, including PWV.

Experimental Measures

Pulse wave velocity. After participants were supine for 5–10 minutes, technicians measured cfPWV and faPWV following a standardized protocol, using the automated cardiovascular screening device VP-1000 Plus (Omron, Kyoto, Japan) [111]. The device simultaneously measured bilateral brachial blood pressures, and carotid, femoral and posterior tibial arterial pulse waves. PWV was estimated as the distance between two arterial recording sites divided by transit time (TT): distance/TT. For cfPWV assessments, arterial waveforms were simultaneously acquired for 30 seconds by applanation tonometry sensors attached on the left common carotid artery (via neck collar) and left common femoral artery. The distance from the carotid to the femoral artery was directly measured with a segmometer (Rosscraft, Surrey, Canada) and calculated as the carotid to femoral distance minus the distance between the suprasternal notch to the carotid applanation site. For faPWV assessments, bilateral posterior-tibial arterial pressure waveforms were detected over 10 seconds by extremities cuffs connected to plethysmographic and oscillometric pressure sensors wrapped on both ankles. Distance for faPWV was automatically calculated by the VP-1000 Plus using height-based formulas, as previously described [263]. A minimum of two PWV measurements were taken per participant and the last two measurements were averaged. The average of left and right faPWV measures was included for analysis.

The validity and reliability of the automatic device for measuring PWV have previously been described [111,130]. Quality assurance for PWV included central training and recertification, quarterly equipment calibration, and ongoing quality control reviews by one of the authors (H.T.) on a stratified random sample of 40 records per month with feedback provided to technicians. Approximately 78% of records were considered optimal quality, 17% were good quality, 3% were acceptable, and none were poor or unacceptable.

Aortic-Femoral Arterial Stiffness gradient. The af-SG was calculated by dividing femoral-ankle PWV (faPWV) by carotid-femoral PWV (cfPWV). This method emphasizes the model arterial system, whereby in a healthy cardiovascular system arterial stiffness increases between central and distal arteries [43]. Although no clinical threshold has been identified, to give greater context, an af-SG greater than 1.0 (i.e. faPWV>cfPWV) can be considered physiologically normal, whereas an af-SG of 1.0 or less (i.e. cfPWV ≥ faPWV) can be considered pathological [49].

Covariate Measurements

Demographics. Age was calculated from date of birth. Sex and race were self-reported. History of smoking was self-reported and analyzed as dichotomous (current versus noncurrent).

Anthropometrics. Body weight was measured to the nearest 0.1 kg, and height was recorded to the nearest centimeter. Body mass index (BMI) was calculated as body mass (kg) divided by height squared (m^2).

Blood Pressure. Three seated blood pressure (BP) measurements were obtained after a 5-minute rest using an oscillometric automated sphygmomanometer (Omron HEM-907 XL, Omron, Kyoto, Japan), and the average of the last two measurements was used. Hypertension was defined as systolic BP (SBP) ≥140 mm Hg, diastolic BP (DBP) ≥90 mm Hg, or antihypertensive medication use. Mean arterial pressure (MAP) was calculated as: (SBP+(2*DBP))/3.

Blood Markers. Blood samples were obtained following a standardized venipuncture protocol and shipped weekly to ARIC central laboratories where assays for total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, and fasting glucose concentration were performed. Total plasma cholesterol concentrations were determined enzymatically [283] using a Cobas-Bio analyzer with reagents purchased from Boehringer Mannheim Biochemicals, (Indianapolis, IN). Plasma low-density lipoprotein (LDL) cholesterol, concentration was calculated using the Friedewald equation, [284] and HDL concentrations were measured using the method of Warnick et al. [264]. Diabetes was defined as fasting glucose ≥126 mg/dl, non-fasting glucose ≥200 mg/dl, antidiabetic medication use, or self-reported diagnosis of diabetes by a physician.

Medications. Participants were asked to bring to the clinical visit all prescription and non-prescription medications taken within the two preceding weeks. That information was transcribed and categorized using MediSPAN prescription codes and classified into medication categories. Participants also self-reported medication use. Medications used included β-blockers, α-blockers, calcium channel blockers, diuretics, angiotensin-converting enzyme inhibitors, and angiotensin II receptor blockers.

Prevalent Cardiovascular Diseases. Prevalent CHD was defined by self-reported prior physician diagnosis of myocardial infarction or coronary revascularization, or prevalent myocardial infarction

according to adjudicated electrocardiogram. Prevalent HF was classified by having at least one of the following: an adjudicated diagnosis of a HF event, International Classification of Disease, 9th Revision, Clinical Modification (ICD-9-CM) discharge code of 428.X in first position not overruled by a physician, any physician report of HF, self-reported HF or self-report of HF medication with pro-BNP greater than 125 pg/mL, or subsequent self-report of HF or HF medication (defined as medications participants reported taking for the treatment of HF). Prevalent stroke was defined by self-reported prior physician diagnosis of stroke or trans ischemic attack and whether they had ever experienced the sudden onset of specific stroke symptoms (weakness, numbness, loss of vision, loss of understanding, inability to express).

Statistical Analysis

Statistical analyses were performed using R Statistical Software. The α-level was set *a-priori* for all statistical procedures at $\alpha = 0.05$. Cumulative frequency and Q-Q plots were used to compare the distributions of cfPWV, faPWV, and af-SG. Participant characteristics were stratified by af-SG quartiles and were estimated as means and standard deviation (SD), or frequencies and percent. Descriptive data across quartiles were compared using one-way analysis of variance (ANOVA) for continuous outcomes and Kruskal-Wallis for categorical outcomes, with Bonferroni correction for multiple comparisons. For linear regression we report unstandardized and standardized β coefficient estimates and 95% confidence intervals (95% CI), and the R^2 values for model fit. When possible, partial R^2 values for dependent variables were determined using semi-partial correlation analysis inherent to the *ppcor* package in R [285]. For logistical regression we report odds ratios and 95% CI.

For the first part of aim 1, linear regression was used explore whether there were sex or race interactions with age for af-SG, cfPWV, and faPWV. In the event of significant sex or race interactions ($P<0.05$), stratified analysis was used. Subsequently, associations between af-SG, cfPWV, and faPWV with 5-year age groups was determined using Spearman correlations (r). For the second part of aim 1, cfPWV, faPWV and af-SG associations with traditional CVD risk factors were evaluated using multivariable linear regression. Independent variables included age, BMI, current smoking, DBP, SBP, heart rate, glucose, HDL cholesterol, LDL cholesterol, and triglycerides. Variables significantly associated with cfPWV and af-SG ($P<0.1$) were retained using a backward step-wise method. Linear regression models were adjusted for race, field center, sex, current smoker, medication count and prevalent CVD. For aim 2, we investigated the associations between cfPWV, faPWV and af-SG as continuous variables with CVD status, including CHD, HF, stroke, hypertension and diabetes, using

multi-variable binomial logistic regression. To further interrogate associations with disease status, given that no clinical threshold for af-SG has been identified, comparisons were also made whereby cfPWV, faPWV and af-SG measures were entered as categorical variables (quartile 1 to quartile 3 *vs.* quartile 4 for cfPWV and faPWV, quartile 1 *vs.* quartile 2 to quartile 4 for af-SG). Logistic regression models were adjusted for age, race, field center, sex, MAP, current smoker and medication count. Assumption of linearity, collinearity, homoscedasticity, and outliers were assessed for every model.

Results

Characteristics of the Study Population

Descriptive characteristics, overall and stratified by af-SG quartiles, are reported in **Table 5.1** Following exclusions, the sample included 4,183 cohort participants between the ages of 66 and 90 years, of which 59.5% were women and 22.3% were African American. Of the 5,683 participants who attended visit 5 and underwent PWV measurements: 1500 were excluded using the following criteria: pre-existing condition (n=579), race other than white or African American (n=15), missing PWV data (n=529), PWV values 3 SDs above or below the mean (n=76), missing risk factor data (n=81), and missing covariates (n=220).

Association between Carotid-Femoral Pulse Wave Velocity and Femoral-Ankle Pulse Wave Velocity

The linear association between cfPWV and faPWV was explored and this was non-significant (R^2=0.0002, β= -0.03, 95% CI [-0.07, 0.04], P=0.35). Subsequently, linearity was explored by specifying the faPWV quadratic term. The quadratic term was significant (β= 0.02, 95% CI [0.003, 0.04], P=0.03), but the change in R2 was marginal (ΔR2 = 0.001). Accordingly, linear models were used for subsequent analysis. In a model regressing cfPWV and faPWV, the age, (P= 0.23), race (P =0.12) and sex (P = 0.76) interaction terms were non-significant. There was a non-significant correlation between cfPWV and faPWV (r = 0.02 [95% CI: -0.05, 0.02], P=0.35).

Table 5.1 Descriptive characteristics of ARIC visit 5 participants, overall and stratified by aortic-femoral arterial stiffness gradient (af-SG) quartiles.

	Overall n = 4183		Q1 n = 1057		Q2 n = 1038		Q3 n = 1041		Q4 n = 1047		*P* Value
Continuous Variables (Mean, SD)											
Age (years)	75.2	(5.0)	76.7	(5.2)	75.4	(5.0)	74.7	(4.8)	74	(4.7)	<0.001*
Body Mass Index (kg/m^2)	27.9	(4.4)	28.2	(4.6)†	28.1	(4.54)†	27.8	(4.3)	27.3	(4.2)	<0.001
Diastolic blood pressure (mm Hg)	66	(10)	65	(10.5)†,‡	66	(10)	67	(10)	67	(10)	<0.001
Systolic blood pressure (mm Hg)	130	(17)	134	(18)	131	(17)	128	(16)	126	(17)	<0.001*
Heart rate (bpm)	65	(11)	66	(11)†	65	(11)†	65	(11)†	63	(10)	<0.001
Fasting glucose (mg/dL)	6.2	(1.5)	6.5	(1.7)†,‡,§	6.3	(1.6)†,‡	6.1	(1.3)	6.0	(1.1)	<0.001
LDL (mg/dL)	2.7	(0.9)	2.7	(0.9)†,‡	2.7	(0.9)	2.8	(0.9)	2.8	(0.9)	0.013
HDL (mg/dL)	1.4	(0.4)	1.3	(0.4)†,‡	1.4	(0.4)†	1.4	(0.4)†	1.4	(0.4)	<0.001
Triglycerides (mg/dL)	1.4	(0.6)	1.4	(0.7)†	1.4	(0.6)†	1.4	(0.6)†	1.3	(0.6)	<0.001
cfPWV (m/s)	11.6	(3.0)	15.1	(2.6)	12.1	(1.7)	10.6	(1.4)	8.7	(1.6)	<0.001*
faPWV (m/s)	10.8	(1.7)	9.7	(1.5)	10.5	(1.4)	11.2	(1.5)	11.9	(1.7)	<0.001*
af-SG	0.998	(0.3)	0.653	(0.1)	0.873	(0.1)	1.050	(0.1)	1.410	(0.3)	-
Categorical Variables (No., %)											
Sex			†,‡		‡						
Men	1692	(40)	591	(56)	449	(43)	388	(37)	399	(38)	0.002
Women	2491	(60)	456	(44)	592	(57)	650	(63)	658	(62)	
Race			†,‡,§		†						
African American	934	(22)	362	(35)	215	(21)	183	(18)	174	(16)	<0.001
White	3249	(78)	685	(65)	826	(79)	855	(82)	883	(84)	
Prevalent Cardiovascular Disease											
Hypertension	3012	(72)	867	(83) †,‡,§	745	(71.6)†	731	(70) †	687	(65)	<0.001
Coronary heart disease	586	(14)	204	(20)†,‡,§	152	(14.6)†	117	(11)	113	(11)	<0.001
Heart failure	432	(10)	175	(17)†,‡,§	86	(8)	85	(8)	86	(8)	<0.001

Stroke	114	(3)	50	(5)†,‡,§	25	(2)	18	(2)	21	(2)	<0.001
Diabetes	1230	(29)	437	(42)†,‡,§	318	(31)‡	261	(25)	214	(20)	<0.001
Medication use											
β-Blocker	1172	(28)	346	(33) †,‡	310	(30)†,‡	269	(26)	247	(23)	<0.001
α-Blocker	135	(3)	52	(5)†,‡	40	(4)†	22	(2)	21	(2)	<0.001
Diuretic	1611	(39)	488	(47)†,‡,§	387	(37)	387	(37)	349	(33)	<0.001
ACE Inhibitor	1274	(31)	369	(35)†,‡	298	(29)	301	(29)	307	(29)	0.012
ANG II receptor blocker	687	(16)	211	(20)†	174	(17)†	166	(16)	136	(13)	<0.001
Calcium channel blocker	1032	(25)	388	(37)†,‡,§	257	(25)†	215	(21)†	172	(16)	<0.001
Current smoker	239	(6)	64	(6)	52	(5)	55	(5)	68	(6)	0.448

Abbreviations: HDL, high-density lipoprotein cholesterol; LDL, Low-density lipoprotein cholesterol; cfPWV, carotid-femoral pulse-wave velocity; faPWV, femoral-ankle pulse-wave velocity. af-SG quartiles Q1, <0.784; Q2, 0.784 to 0.956; Q3, 0.957 to 1.160, Q4 >1.160. *Comparisons*: *All groups significantly different; † vs. Q4; ‡ vs. Q3; § vs. Q2.

Aim 1: Associations with Age and CVD Risk Factors

There was no significant sex or race by age interactions for cfPWV, faPWV or af-SG (all P > 0.05). **Figure 5.1** presents cfPWV, faPWV, and af-SG values stratified by age categories. Mean cfPWV increased and mean af-SG decreased across 5-year age groups from <70 to >85 years, whilst faPWV did not differ across age groups. Age was positively correlated (Spearman) with cfPWV *(r* = 0.22, 95% CI: 0.19, 0.25, *P* < 0.01) and negatively correlated with af-SG *(r* = -0.18, 95% CI: -0.21, -0.15, *P* < 0.01), but not correlated with faPWV *(r* = 0.03, 95% CI: 0.00, 0.06, *P* = 0.09).

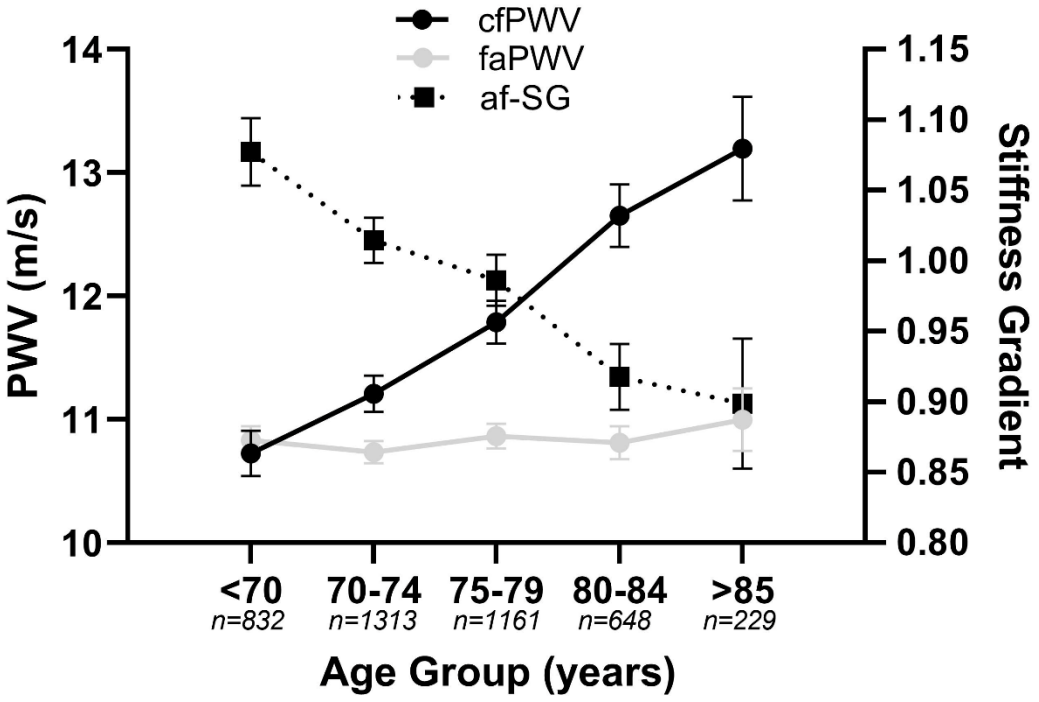

Figure 5.1 Mean carotid-femoral pulse-wave velocity (cfPWV), femoral-ankle pulse-wave velocity (faPWV) and aortic-femoral arterial stiffness gradient (af-SG) in 5-year age groups, with 95% confidence intervals. n = 4,183.

Backwards stepwise regression analysis was used to identify CVD risk factors that were associated with af-SG and cfPWV (**Table 5.2**). For cfPWV, there was a positive association with age, SBP, HR, and fasting glucose, and a negative association with BMI, DBP, and HDL. With the exception of BMI, CVD risk factor associations for af-SG were consistent, albeit in opposing directions due to the nature of the measure, with cfPWV. The highest standardized regression coefficients were observed for the same CVD risk factors (SBP, HR, age, DBP). For faPWV, there was a positive association with age, DBP and fasting glucose, and a negative association with BMI.

Aim 2: Associations with Age and CVD Status

Table 5.3 presents associations for cfPWV, faPWV and af-SG with CVD status following multivariable logistic regression analyses. For final model logistic regression analyses, when specified as a continuous variable, cfPWV was positively associated with CHD, stroke and diabetes, but a high cfPWV was only associated with diabetes. For af-SG as a continuous variable, there were negative associations with CHD, HF, stroke and diabetes, and a low af-SG was also associated with CHD, HF, stroke and diabetes. For faPWV as a continuous variable, there were negative associations with CHD and HF, but a low faPWV was only associated with HF in categorical analysis.

Sensitivity and Ancillary Analysis

Data analysis conducted with the exclusion of peripheral arterial disease (PAD) patients, as identified by an ankle-brachial index (ABI) below 0.9, revealed no notable differences. Further, analysis of af-SG derived using left and right faPWV measures separately had no impact on findings when compared to those determined using a mean of left and right faPWV measures.

Table 5.2 Linear regression models for association of carotid-femoral pulse wave velocity (cfPWV), femoral-ankle pulse wave velocity (faPWV) and aortic-femoral arterial stiffness gradient (af-SG) with cardiovascular disease risk factors at visit 5.

	cfPWV						faPWV						af-SG					
	β	Std. β	95% CI		*P*	†R2	β	Std. β	95% CI		*P*	†R2	β	Std. β	95% CI		*P*	†R2
Model 1		R^2 =	0.22				R^2 =	0.16						R^2 =	0.10			
Age (years)	0.11	0.19	0.17	0.20	<0.001	0.030	0.02	0.05	0.04	0.06	<0.001	0.00	-0.01	-0.12	-0.12	-0.12	<0.001	0.012
BMI (kg/m2)	-0.04	-0.05	-0.07	-0.03	<0.001	0.002	-0.10	-0.25	-0.27	-0.24	<0.01	0.05	-0.01	-0.08	-0.08	-0.08	<0.001	0.006
DBP (mm Hg)	-0.03	-0.10	-0.11	-0.09	<0.001	0.005	0.05	0.30	0.29	0.31	<0.001	0.05	0.01	0.20	0.20	0.20	<0.001	0.022
SBP (mm Hg)	0.06	0.35	0.35	0.36	<0.001	0.074	0.00	0.02	0.02	0.03	0.22	0.00	0.00	-0.25	-0.25	-0.25	<0.001	0.037
HR (bpm)	0.06	0.22	0.21	0.23	<0.001	0.044	0.02	0.11	0.11	0.12	<0.001	0.01	0.00	-0.12	-0.12	-0.12	<0.001	0.013
FBG (mg/dL)	0.21	0.10	0.04	0.16	<0.001	0.009	0.03	0.03	-0.01	0.06	0.07	0.00	-0.01	-0.06	-0.07	-0.06	<0.001	0.003
LDL (mmol/l)	-0.04	-0.01	-0.11	0.09	0.382	0.000	-0.05	-0.03	-0.09	0.03	0.07	0.00	0.00	-0.01	-0.02	0.00	0.492	0.007
HDL (mmol/l)	-0.79	-0.10	-0.35	0.16	<0.001	0.007	0.11	0.02	-0.13	0.17	0.17	0.00	0.09	0.10	0.07	0.13	<0.001	0.000
TG (mmol/l)	0.09	0.02	-0.12	0.16	0.226	0.000	0.14	0.05	-0.03	0.14	<0.001	0.00	0.01	0.01	0.00	0.03	0.400	0.000
Final Model*		R^2 =	0.24				R2 =	0.18						R^2 =	0.13			
Age (years)	0.11	0.18	0.16	0.20	<0.001	0.028	0.02	0.05	0.05	0.06	<0.001	0.00	-0.01	-0.11	-0.12	-0.11	<0.001	0.011
BMI (kg/m2)	-0.05	-0.08	-0.10	-0.06	<0.001	0.005	-0.08	-0.22	-0.23	-0.21	<0.001	-0.04	0.00	-0.05	-0.05	-0.04	0.004	0.002
DBP (mm Hg)	-0.03	-0.11	-0.12	-0.10	<0.001	0.007	0.05	0.33	0.32	0.33	<0.001	0.05	0.01	0.20	0.20	0.21	<0.001	0.022
SBP (mm Hg)	0.06	0.35	0.34	0.36	<0.001	0.069							0.00	-0.24	-0.24	-0.24	<0.001	0.032
HR (bpm)	0.07	0.24	0.23	0.24	<0.001	0.048	0.02	0.11	0.10	0.11	<0.001	0.01	0.00	-0.13	-0.13	-0.13	<0.001	0.016
FBG (mg/dL)	0.18	0.09	0.03	0.14	<0.001	0.005	0.06	0.05	0.02	0.08	<0.001	0.00	-0.01	-0.04	-0.05	-0.03	0.008	0.001
HDL (mmol/l)	-0.57	-0.07	-0.33	0.19	<0.001	0.001							0.06	0.06	0.03	0.09	<0.001	0.002
TG (mmol/l)							0.03	0.01	-0.07	0.09	0.52	0.00						

***Final Model Adjustments:** race, field center, sex, current smoker, prevalent cardiovascular diseases (coronary heart disease, stroke, heart failure), and number of medications (β-blockers, α-blockers, calcium channel, blockers, diuretics). *Abbreviations*: β, beta coefficient; BMI, body mass index; DBP, diastolic blood pressure; SBP; systolic blood pressure; HR, heart rate; FBG, fasting blood glucose; LDL, Low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; TG, triglycerides ; std. β, standardized beta coefficient; 95% CI, 95% confidence interval; †, partial R2.

Table 5.3 Logistic regression models for association of carotid-femoral pulse wave velocity (cfPWV), femoral-ankle pulse wave velocity (faPWV) and aortic-femoral stiffness gradient (afSG) with CVD status at visit 5.

	CHD				Heart Failure				Stroke				Hypertension				Diabetes			
Continuous	**OR**	**95% CI**		**P**	**OR**	**95% CI**		**P**	**OR**	**95% CI**		**P**	**OR**	**95% CI**		**P**	**OR**	**95% CI**		**P**
Model 1																				
cfPWV	1.07	1.04	1.10	<0.001	1.08	1.05	1.11	<0.001	1.12	1.06	1.19	<0.001	1.15	1.12	1.18	<0.001	1.12	1.10	1.15	<0.001
faPWV	0.87	0.83	0.92	<0.001	0.81	0.77	0.87	<0.001	0.90	0.81	1.01	0.079	0.99	0.95	1.03	0.648	0.90	0.87	0.94	<0.001
af-SG	0.38	0.28	0.52	<0.001	0.31	0.22	0.46	<0.001	0.27	0.13	0.55	<0.001	0.41	0.33	0.50	<0.001	0.29	0.23	0.37	<0.001
Final Model*																				
cfPWV	1.04	1.01	1.07	0.014	1.03	0.99	1.06	0.139	1.08	1.02	1.15	0.014	1.03	0.99	1.06	0.206	1.12	1.09	1.15	<0.001
faPWV	0.92	0.87	0.98	0.008	0.90	0.84	0.96	0.002	0.93	0.83	1.05	0.245	1.02	0.96	1.09	0.457	0.99	0.95	1.04	0.710
af-SG	0.57	0.41	0.80	0.000	0.44	0.21	0.90	0.024	0.57	0.41	0.80	0.001	0.89	0.65	1.21	0.457	0.41	0.32	0.54	0.000
Categorical	**OR**	**95% CI**		**P**	**OR**	**95% CI**		**P**	**OR**	**95% CI**		**P**	**OR**	**95% CI**		**P**	**OR**	**95% CI**		**P**
Model 1																				
cfPWV	1.36	1.12	1.65	0.002	1.69	1.36	2.09	0.059	1.81	1.23	2.67	0.003	1.94	1.63	2.31	<0.001	1.93	1.67	2.24	<0.001
faPWV	1.20	0.98	1.47	0.082	1.76	1.41	2.21	<0.001	0.98	0.64	1.50	0.925	0.96	0.82	1.13	0.618	1.29	1.10	1.51	0.001
af-SG	0.57	0.48	0.69	<0.001	0.44	0.36	0.55	<0.001	0.40	0.27	0.58	<0.001	0.46	0.39	0.55	<0.001	0.47	0.41	0.55	<0.001
Final Model*																				
cfPWV	1.15	0.93	1.43	0.200	1.26	1.00	1.59	0.053	1.42	0.94	2.13	0.098	1.03	0.79	1.34	0.829	1.85	1.57	2.18	<0.001
faPWV	1.18	0.95	1.47	0.130	1.38	1.09	1.75	0.007	0.90	0.58	1.39	0.624	0.52	0.42	0.64	<0.001	1.07	0.91	1.26	0.396
af-SG	0.72	0.58	0.88	0.002	0.67	0.53	0.83	<0.001	0.51	0.34	0.76	0.001	0.97	0.74	1.27	0.815	0.58	0.49	0.68	<0.001

***Final Model Adjustments:** age, race, field center, sex, mean arterial pressure, current smoker, and number of medications (β-blockers, α-blockers, calcium channel, blockers, diuretics). *Abbreviations*: CHD, coronary heart disease; β, beta coefficient; 95% CI, 95% confidence interval; OR, odds ratio. af-SG quartile comparisons: Q1: <=0.784 vs. Q2-Q4: >0.784; cfPWV quartile comparisons: Q1-Q3: <=13.3 m/s vs. Q4: >13.3 m/s; faPWV quartile comparisons: Q1: <=9.68 m/s vs. Q2-Q4: >9.68 m/s.

Discussion

The primary aims of the current study were to compare the associations of af-SG and cfPWV with, 1) age and traditional CVD risk factors, and, (2) CVD status. Our findings suggest that the af-SG and cfPWV demonstrate similar associations with age and traditional cardiovascular risk factors including DBP, SBP, HR, fasting glucose, and HDL, but do contrast in their associations with BMI. However, the af-SG demonstrates a unique association with CVD status; specifically, a low af-SG was associated with coronary heart disease, heart failure and stroke, but a high cfPWV was not. Accordingly, the af-SG may be a clinically useful marker of arterial stiffness and confer a unique picture of hemodynamic integration, vascular pathophysiology, and the identification of CVD risk

Limitations and Strengths

The strengths and limitations of this study need to be addressed to best contextualize the findings. Firstly, the generalizability of our findings is limited to older populations and cannot be extended to younger, healthier cohorts. Further, the predominate inclusion of participants who had survived from baseline (1987-1989) and attended the Visit 5 examination (2011-2013), and were thus likely healthier compared to those who did not participate in the visit, may have generated a bias within the study population. Secondly, the use of height-based formulas to calculate faPWV were validated in a Japanese population and may not be applicable to other racial or ethnic groups. Finally, we did not exclude patients based upon peripheral arterial disease (PAD) diagnosis which has the potential to impact measures of faPWV. However, our sensitivity analysis excluding PAD patients (ABI < 0.9) and using af-SG derived from left and right faPWV measures independently did not impact findings. A major strength is that this is the first study to explore af-SG, an index of central to peripheral arterial stiffness gradient, derived using the lower extremity, and does so using a large community-dwelling population.

Comparison to the Literature: Age and CVD Risk Factors

Both af-SG and cfPWV were significantly associated with age, whilst faPWV was stable across age-groups. This finding supports the previous assertion that age-related changes in the SG are chiefly driven by cfPWV [49,281], and therefore the af-SG may confer limited prognostic value over cfPWV. However, stratification of participants into af-SG quartiles revealed that both cfPWV and faPWV contribute towards af-SG measures, with a decrease in the af-SG (**Table 5.1**) appearing to be a

consequence of decreased faPWV as well as increased cfPWV. Indeed, faPWV was significantly different between af-SG quartiles, with individuals in Q1 (an af-SG of <0.784) displaying the lowest faPWV and most adverse CVD risk factor profile**.** Although the cross-sectional nature of the present study limits inference, collectively these findings suggest that reductions in the af-SG are likely to be pathological and are impacted by the central and peripheral vasculature. Although femorotibial arterial stiffness is thought to change little with age [273,286], faPWV has been shown to regress in the presence of CVD risk factors and disease, including diabetes [278] and in hemodialysis patients [287], respectively. A low peripheral arterial stiffness has been presented as a novel consequence of increased aortic arterial stiffness [44,209,287]. This reduction in peripheral muscular artery stiffness is thought to shift the site of pressure wave reflection distally, attenuating wave reflection and its influence on central BP and cardiac workload [15]. Although preserving cardiac function and aortic pressure, this could lead to greater transmission of the forward pressure wave to the microcirculation and cause end-organ damage [74,176,177]. Accordingly, variations in faPWV may have clinically important consequences and integration of faPWV in the af-SG may be a relevant complimentary approach to cfPWV, providing an alternative picture of hemodynamic integration and prognostic information beyond aortic stiffness.

The af-SG and cfPWV were associated with similar CVD risk factors, albeit in opposing directions due to the nature of measures, with an increase in age and a worsening of risk factors (DBP, SBP, HR, fasting glucose, and HDL) associated with a worsening of af-SG and cfPWV. However, cfPWV and af-SG did contrast in their association with BMI. In the Framingham Heart Study of 2,114 older adults [49], the upper extremity ab-SG demonstrated equitable prognostic value when compared to cfPWV and was significantly associated with age, BMI, HDL, BP, and HR, all recognized correlates of cfPWV. These risk factors were all found to be correlated with both af-SG and cfPWV in the present study. The finding of a negative association with BMI and cfPWV is consistent with existing literature [37,273]. In cross-sectional studies, a lower aortic PWV in obese individuals has been attributed to higher cardiac output and lower peripheral vascular resistance [288,289]. However, longitudinal studies report a robust positive relationship between adiposity and central PWV progression [154,290]. This is consistent with the association between af-SG and BMI in the present study. These findings suggest that elevated adiposity may be associated with a lower central PWV at baseline, but normal age changes in central PWV are accelerated with greater adiposity. The af-SG may permit the identification of a novel association between adiposity and arterial stiffness.

Comparison to the Literature: CVD Status

The af-SG and cfPWV demonstrated unique associations with CVD status. Consistent with previous literature, cfPWV was associated with CHD [107], stroke [48], and diabetes [278] but in contrast to previous findings, was not associated with heart failure [71] or hypertension [291]. Further, a high cfPWV was only associated with diabetes. Comparatively, the af-SG was associated with CHD, HF, stroke and diabetes, and a low af-SG was also associated with diabetes, CHD, HF and stroke. A high af-SG reduced the odds of having diabetes by 42% and similarly a high cfPWV increased the odds of having diabetes by 85% reflecting the significant impact diabetes has on systemic arterial stiffness. Although there are multiple pathways, diabetes can accelerate arterial remodelling by augmenting production of advanced glycation end products that cross-link with collagen and elastin [150]. Interestingly, diabetes has been associated with both a higher cfPWV [45,278] and a lower faPWV [278], which would contribute to a low af-SG. A high af-SG reduced the odds of having CHD, HF and stroke by 28%, 33% and 49%, respectively, but none were significant for cfPWV when arterial stiffness measures were entered as categorical predictors. This suggests that the af-SG may demonstrate greater sensitivity with certain disease pathologies than segmental PWV alone.

In contrast to those mechanisms previously described, a reduced af-SG may actually *augment* wave reflection amplitude and increase pulse and central systolic pressure [281]. Systolic pressure elevation increases myocardial oxygen demand and induces left ventricular hypertrophy, a marker of HF, elevating CVD risk [43]. Pulse pressure elevation induces arterial remodelling, increasing arterial wall stiffness and thickness, and promoting atherosclerotic plaque development [292], characteristics of CHD. In this scenario, reversal of the SG and the concomitant increase in distance to wave reflection sites is still expected to increase the transmission of pulsatile flow to the periphery and lead to tissue and target organ damage [293], perhaps explaining the association between low af-SG and stroke. However, these contrasting theories highlight that the mechanism(s) for how a low af-SG (i.e. worsening) may contribute to both myocardial and end-organ pathology, and whether or not cfPWV is the sole determinant [49,281], is still unclear.

Implications

A low central to peripheral SG may augment the transmission of excessive forward pressure into the microcirculation, a pathophysiological basis for cardiovascular events and target organ damage [15,18,43]. To date, the literature has focused on the upper-extremity derived ab-SG. The ab-SG has

been reported to be a *better* prognostic indicator of CVD outcome than classical cfPWV in diseased populations [44,45,47,48] and comparable in healthy populations [49]. But it has been argued that the prognostic value, and therefore clinical utility, of the ab-SG is principally attributable to increases in cfPWV [49]. However, the upper extremities represent only a small portion of the arterial tree and the absolute hemodynamic load is likely to be limited. In contrast, the lower extremities make up a considerable portion of the arterial tree and contribute significantly to wave reflection morphology and myocardial workload [26]. The current study extends the scant SG literature by being the first to report that the lower-extremity derived af-SG demonstrates comparable association with CVD risk factors when compared to cfPWV, but importantly, a unique association with CVD status, specifically coronary heart disease, heart failure and stroke. Collectively, these findings indicate that the af-SG may be clinically useful for the non-invasive assessment of arterial health and CVD risk. However, to confirm utility, future studies should seek to: i) identify the association of af-SG with CVD outcomes and end-organ damage, and ii) identify the mechanisms by which low af-SG contributes to disease progression.

Conclusions

Future studies are necessary to confirm the clinical utility of the af-SG, including whether the af-SG can predict CVD outcomes. However, the present findings indicate that the af-SG is a promising tool that may provide a unique picture of hemodynamic integration, vascular pathophysiology, and the identification of CVD risk.

Commentary…

Chapter 5 addressed ***objective 2.1*** and demonstrated that the af-SG provides independent additional CVD risk information beyond cfPWV, thereby satisfying European Society of Cardiology Criteria 2 [6]. However, as for faPWV, to be of any clinical use in improving the screening of CVD risk, any biomarker measurement technique must be able to determine underlying (patho)physiology with acceptable reliability in order to permit objective monitoring over-time. **Chapter 3** demonstrated that a simple oscillometric cuff-based device can measure faPWV with good reliability, and previously Hwang *et al.* [251] showed that it can also measure cfPWV with excellent reliability. However, combining segmental PWV measures to determine a stiffness gradient can augment random and measurement error, and reduce measurement precision [213]. Whether the af-SG can demonstrate acceptable precision is unknown. **Chapter 6** will address ***objective 2.2*** and seek to identify whether a simple oscillometric cuff-based device can determine af-SG with acceptable precision (reliability) in a supine posture (European Society of Cardiology Criteria 1).

6

The Aortic-Femoral Arterial Stiffness Gradient Demonstrates Good Between-Day Reliability

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SUMMARY

The aortic-femoral arterial stiffness gradient (af-SG), defined as the ratio of femoral-ankle pulse-wave velocity (faPWV) to carotid-femoral pulse-wave velocity (cfPWV), is a promising marker of cardiovascular disease (CVD) risk. Yet to be of value in clinical and research settings, an arterial health assessment tool must be reliable (precise). This study sought to determine the between-day reliability of the af-SG. Twenty-five, non-smoking, young healthy adults (40% female, age 22.6±2.7 years, body mass index 23.9±2.8 kg/m2) were tested under standardized conditions on three different mornings in a fasted state, separated by a maximum of seven days. In a supine position, measures of cfPWV and faPWV were recorded in triplicate. The af-SG was calculated as faPWV divided by cfPWV. Intra-class correlation coefficient (ICC), standard error of measurement (SEM), and minimal detectable change (MDC) were calculated. The af-SG (ICC = 0.77, SEM = 0.08 m/s), cfPWV (ICC = 0.84, SEM = 0.29 m/s) and faPWV (ICC = 0.84, SEM = 0.38 m/s) measures all demonstrated good between-day reliability, according to accepted ICC criteria. The MDC (MDC%) between repeat measures within an individual was 0.22 m/s (13.8%) for af-SG, 0.79 m/s (14.2%) for cfPWV, and 1.05 m/s (13.8) for faPWV. These findings indicate that the af-SG demonstrates good reliability in young healthy adults. Further research is needed to identify if af-SG measurement variability is affected by age or disease.

Introduction

In a healthy cardiovascular system arterial stiffness progressively increases from the highly elastic aorta to the muscular conduit arteries of the periphery [294]. This stiffness gradient is physiologically advantageous, permitting a gradual attenuation of the forward pressure wave into a smooth consistent blood flow to prevent the transmission of highly pulsatile forces to the micro-circulation [15,43]. However, stiffness of the aorta increases with age [15] and is accelerated by lifestyle factors, such as smoking and physical inactivity [282]. In contrast, age-related changes in the stiffness of the peripheral vasculature are less marked; typically remaining stable [15], but may decrease in the presence of disease [287]. These phenomena lead to a reversal of the stiffness gradient, increasing forward pressure transmission and potentially leading to end-organ damage [43,44]. Indeed, recent studies have emphasized the importance of the stiffness gradient to clinical outcomes, and its ability to provide prognostic information beyond regional arterial segment stiffness measures alone, including the criterion measure of arterial health, carotid-femoral pulse wave velocity (cfPWV) [44,45]. Although several indexes of the central to peripheral arterial stiffness gradient have emerged as promising screening tools, in order to be of use in clinical and epidemiological settings, they must demonstrate acceptable reliability (precision).

The most widely used measure of the central to peripheral stiffness gradient to date is the aortic-brachial stiffness gradient (ab-SG), defined as the ratio of carotid-radial PWV (crPWV) to cfPWV [44,46,49]. The ab-SG has been reported to be a stronger prognostic indicator of cardiovascular disease (CVD) than cfPWV in diseased [44], and comparable in healthy adults [49]. Importantly, the ab-SG has demonstrated acceptable reliability (intra-class correlation coefficient [ICC: 0.52]) [213]. Recently, the less explored lower-extremity derived aortic-femoral stiffness gradient (af-SG), defined as the ratio of femoral-ankle PWV (faPWV) to cfPWV, demonstrated paralleled associations with traditional CVD risk factors when compared to cfPWV, but unique associations with CVD status, specifically coronary heart disease, heart failure and stroke [279]. Inclusion of the lower-extremities, which make up a significant portion of the arterial tree, contribute significantly to wave reflection morphology [26], and are prone to athero- and arterio-sclerotic processes [200], likely permits the af-SG to provide a more comprehensive picture of hemodynamic integration. But although the af-SG has demonstrated promising utility, the between-day reliability is not known. If the af-SG is shown to be reliable, this would represent a potentially viable tool for use in clinical and epidemiological settings.

The aim of this study was to determine the upper-limit of between-day reliability of the af-SG in a sample of healthy individuals. Although there is no universal criterion, in general, intra-class correlation coefficient estimates of < 0.5, 0.5-0.75, 0.75-0.9 and > 0.9 indicate poor, moderate, good and excellent reliability [220].

Methods

This observational study is reported in accordance with STROBE (STrengthening the Reporting of OBservational studies in Epidemiology) guidelines [243]. The study conformed to the Declaration of Helsinki, except for registration in a database, and was approved by the University of North Carolina at Chapel Hill Office of Human Research Ethics. Participants were informed of the methods and study design verbally and in writing before providing written informed consent.

Participants

To ascertain the upper limit of reliability for the af-SG, a relatively homogenous cohort of young healthy adults were recruited. Participants were free of known cardio-metabolic disorders, were not taking medications known to effect cardiovascular function and were not cigarette smokers. To account for potential influences of hormonal status on study outcomes, premenopausal women were studied during the early follicular phase of their menstrual cycle or during the placebo phase of oral contraceptive use.

Experimental Design and Measures

Following familiarization, participants were tested in an environmentally-controlled room (temperature: 22 ± 1°C, relative humidity: 51 ± 2%). All participants were 12h fasted and were asked to avoid strenuous physical activity, caffeine and alcohol for 24 h prior. Following a 20-min rest period in a supine position, cfPWV and faPWV were determined using the SphygmoCor XCEL (AtCor Medical, Sydney, New South Wales, Australia) on three separated days, separated by a maximum of 7 days.

PWV measurements were calculated by dividing arterial path length (D) by the pulse transit time (PTT) between a proximal and distal arterial segment. Carotid-femoral and femoral-ankle PTT were measured as the time between diastolic feet of the proximal (tonometer) and distal (cuff) arterial

pulse using the XCEL. For cfPWV, the tonometer was placed on the left carotid artery and the oscillometric cuff was placed on the left thigh at the level of the femoral artery, following recommended manufacturer guidelines. For faPWV, the tonometer was placed on left superficial femoral artery at the point of maximal pulsation determined via palpation, and the ankle cuff (SC10, Hokanson) was positioned proximal to the malleoli [261]. Using custom made calipers to overcome body contours; the carotid-femoral *D* was estimated by measuring the linear distance from the suprasternal notch to the top (proximal end) of the cuff at the center line of the leg and subtracting the distance from the suprasternal notch to the carotid artery. Femoral-ankle *D* was estimated by measuring the linear distance from the point of tonometric applanation to the top of the ankle cuff at the centre line of the leg. Femoral-ankle PTT was corrected following manufacturer guidelines, as previously described [261]. All PWV measures were recorded by the same trained technician, in triplicate, with the average of the closest two recordings being used for analyses.

The af-SG was calculated by dividing faPWV by cfPWV. This method emphasizes the model arterial system, whereby in a healthy cardiovascular system arterial stiffness increases between central and distal arteries [43]. Although no clinical threshold has yet been identified, to give greater context, an af-SG greater than 1.0 (i.e. faPWV>cfPWV) can be considered physiologically normal, whereas an af-SG of 1.0 or less (i.e. cfPWV≥faPWV) can be considered pathological [49]. An alternative way to estimate the mismatch between central and peripheral arteries that has recently been presented [213], is to calculate the absolute difference between cfPWV and faPWV segments (af-SG_{ABS} = faPWV-cfPWV). It is pertinent to identify if the calculation of af-SG_{ABS} impacts reliability.

Oscillometirc pressure waveforms were also recorded on the left upper arm using pulse wave analysis (PWA) inherent to the XCEL device [245]. Each single measurement cycle consisted of a 60s brachial blood pressure recording from which systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial pressure (MAP) were derived.

Statistical Analysis

For cfPWV, faPWV, and af-SG measures, values over the three visits are reported as pooled averages and standard deviations (SD). Between-day reliability of PWV measures was assessed by calculating the intra-class correlation coefficient (ICC), standard error of measurement (SEM), and minimal detectable change (MDC). The ICC was calculated according to the formula: SDb^2 / [SDb^2+SDw^2], where SDb^2 and SDw^2 are the between and within-subject variance, respectively. Although there is no universal standard for classifying the magnitude of ICC, for criterion-related assessments: values less

than 0.50 are indicative of poor reliability, values between 0.50 and 0.75 indicate moderate reliability, values between 0.75 and 0.90 indicate good reliability, and values greater than 0.90 indicate excellent reliability [220]. The SEM was calculated according to the formula: SD* √(1-ICC) and the MDC calculated according to the formula: 1.96*SEM*√2 [242,247]. To calculate MDC independent of the units of measurement, the MDC% was calculated as (MDC/mean) x 100, where the mean is the mean score of all trials. All statistical analysis was performed in RK Ward, a front end to the R statistical software, or Microsoft Excel (Microsoft, Redmond, WA) with significance set at $P < .05$.

Results

Twenty-five participants were recruited, of which 40% (n = 10) were female, with a mean age of 22.6 ± 2.7 years, mean height of 172.2 ± 7.3 cm, mean weight of 71.1 ± 11.2 kg and a mean body mass index of 23.9 ± 2.8 kg/m^2. Participants completed an average of 4.1 ± 1.7 physical activity sessions per week for an average of 51.3 ± 13.2 minutes. There was no missing participant data. Descriptive statistics for PWV and af-SG measures by visit and between-visits are presented in **Table 6.1**. Reliability estimates for PWV and af-SG measures are presented in **Table 6.2**. Although cfPWV and faPWV measures demonstrated greater reliability than both af-SG and af-SG$_{ABS}$, all PWV measures demonstrated good between-day reliability (ICC: 0.77-0.84). However, whilst cfPWV, faPWV and af-SG demonstrated comparable MDC%, the af-SG$_{ABS}$ demonstrated an MDC% that was more than twice as large as the other arterial stiffness markers.

Table 6.1 Descriptive statistics for PWV and stiffness gradient measures by visit and between-visits.

	Visit 1		Visit 2		Visit 3		Between-visit		Pooled	
	Mean	SD	Mean	SD	Mean	SD	MD	SD	Mean	SD
cfPWV (m/s)	5.72	0.92	5.51	0.68	5.54	0.72	0.12	0.36	5.59	0.72
faPWV (m/s)	8.84	1.02	8.59	1.08	8.71	1.03	0.09	0.38	8.71	0.96
af-SG	1.57	0.22	1.56	0.15	1.59	0.19	-0.01	0.10	1.57	0.16
af-SG_{ABS}	3.13	0.94	3.08	0.81	3.17	0.83	-0.03	0.43	3.12	0.77

Abbreviations: cfPWV, carotid-femoral pulse-wave velocity; faPWV, femoral-ankle pulse-eave velocity; af-SG, aortic-femoral arterial stiffness gradient, af-SG_{ABS}; absolute aortic-femoral arterial stiffness gradient.

Table 6.2 Between-day reliability of PWV and arterial stiffness gradient measures.

	ICC	(95% CI)		SEM	(95% CI)		MDC	(95% CI)		MDC%	(95% CI)	
cfPWV (m/s)	0.84	(0.67-	0.93)	0.29	(0.19-	0.41)	0.79	(0.53-	1.15)	14.19	(9.56-	20.51)
faPWV (m/s)	0.84	(0.68-	0.93)	0.38	(0.26-	0.55)	1.05	(0.71-	1.52)	12.08	(8.13-	17.48)
af-SG	0.77	(0.54-	0.89)	0.08	(0.05-	0.11)	0.22	(0.15-	0.31)	13.79	(9.39-	19.54)
af-SG_{ABS}	0.78	(0.56-	0.90)	0.36	(0.24-	0.51)	0.99	(0.68-	1.41)	31.78	(21.60-	45.14)
SBP (mmHg)	0.83	(0.64-	0.92)	3.73	(2.52-	5.37)	10.34	(6.98-	14.88)	8.93	(6.03-	12.84)
DBP (mmHg)	0.74	(0.48-	0.88)	2.72	(1.86-	3.81)	7.53	(5.16-	10.57)	11.33	(7.76-	15.91)
MAP (mmHg)	0.80	(0.56-	0.91)	2.55	(1.73-	3.64)	7.07	(4.79-	10.08)	8.53	(5.78-	12.16)

Abbreviations: cfPWV, carotid-femoral pulse-wave velocity; faPWV, femoral-ankle pulse-eave velocity; af-SG, aortic-femoral arterial stiffness gradient, af-SGABS; absolute aortic-femoral arterial stiffness gradient; CI, confidence interval; ICC, intra-class correlation coefficient; MDC, minimum detectable change; SEM, standard error of measurement.

Discussion

This study is the first to report the between-day reliability of af-SG, a novel marker of systemic arterial health. Our analysis showed that the reliability of af-SG is good according to the current recommended ICC interpretation guidelines [220]. However, af-SG demonstrated lower-reliability than that of the single segment PWV measures, cfPWV and faPWV, from which the af-SG was derived.

In the only other study to assess the reliability of an arterial stiffness gradient measure, Beltrami and colleagues [213] reported a moderate ICC of 0.52 for the upper-extremity derived ab-SG, much lower than the ICC of 0.77 observed for the af-SG in the present study. This disparity between the

aforementioned study and ours is likely due to the use of different peripheral arterial segments to calculate ab-SG and af-SG measures, crPWV and faPWV respectively, and their inherent differences in measurement error. Individually, our central arterial stiffness measure, cfPWV (ICC: 0.84), and our peripheral arterial stiffness measure, faPWV (ICC: 0.84), both demonstrated good reliability, which is consistent with that reported in existing literature [130,214], including that of Beltrami et al. [213] (cfPWV ICC: 0.89). Further, although an inappropriate approach for ratios, the mean difference (MD) and SD of this difference for both cfPWV (MD ≤0.12 m/s, SD≤0.36) and faPWV (MD ≤0.09 m/s, SD≤0.38) in our study would fall into the excellent (mean difference ≤0.5 m/s, SD≤0.8) category of the ARTERY Society guidelines [217]. In contrast the upper-extremity marker of peripheral arterial stiffness used by Beltrami et al. [213], crPWV, has consistently reported lower reliability (ICC: 0.56-0.66) than both cfPWV and faPWV [213,214]. The greater variability of crPWV compared to faPWV may be due to the upper-extremity arteries being more susceptible to the impact of changes in sympathovagal balance on vascular tone [295], but also because the propagation time of the pulse-wave and distance travelled is shorter than for faPWV, meaning the absolute error in determining carotid-radial transit time is greater [8].

Estimates of MDC for PWV measures can aid in the calculation of appropriate study sample size and, importantly, permit clinicians and researchers to evaluate whether changes in PWV for an individual are true, or beyond that of the measurement variation. In our study, the MDC for af-SG was 0.22, suggesting that a change of more than 0.22 may be necessary in order to determine whether a change in af-SG exceeds measurement variation. Importantly, the relative change required (MDC%) for af-SG of 13.8% in the present study was similar to that of cfPWV (14.2%), a criterion PWV measure, and was less than half than that reported for ab-SG (29%) by Beltrami et al. [213]. Although future studies should undertake direct comparisons, collectively, these findings suggest that the lower-extremity derived af-SG may be a more reliable marker of the arterial stiffness gradient than the upper-extremity derived ab-SG. As a final consideration, the arterial stiffness gradient can be derived in different ways, with calculations of the relative [44,279] and absolute [213,296] difference between central and peripheral arterial stiffness being presented in the literature. In this study, whilst the absolute measure, af-SG_{ABS} (faPWV minus cfPWV), demonstrated a comparable ICC, it had an MDC% more than two-fold larger than that of the relative measure, af-SG (faPWV divided by cfPWV). This finding is consistent to that reported for the ab-SG [213] and suggests that relative measures of the arterial stiffness gradient are therefore preferred to absolute measures.

Regardless of approach, both the present study and that of Beltrami *et al.* [213] reported lower reliability coefficients for arterial stiffness gradient measures than for the segmental PWV measures from which they were derived. Although the current research does not permit conclusive assertions on the origin of the af-SG's lower reliability, it is likely a consequence of the additional random and measurement error, as well as biological variability, that arises from combining cfPWV and faPWV measures. Awareness of this phenomena is important, as although cfPWV and faPWV demonstrate good reliability in young healthy adults, their reliability has been reported to be poorer in older adults [212]. Indeed, a limitation of this study is the recruitment of a relatively homogeneous group of young, healthy participants, limiting the overall generalizability of our findings to populations of varying age and health states. However, prior to clinical use, it is imperative to identify whether any inherent variability is caused by the technique itself and not a consequence cardiovascular pathology. A major strength is that this is the first study to determine the between-day reliability of the af-SG, a novel marker of arterial health.

CONCLUSION

High reliability of arterial health assessment tools is critical for their analysis and interpretation, as well as permit accurate CVD risk assessment and stratification. The current study found that the af-SG, a novel marker of arterial health, demonstrates good reliability (precision). Whilst further research is needed to identify if the reliability is impacted by age or health states, the af-SG is a promising tool that may assist clinicians and epidemiological researchers in the identification and stratification of CVD risk.

Commentary…

Chapter 6 addressed ***objective 2.2*** and demonstrated that the af-SG can be determined with acceptable precision using a simple oscillometric cuff-based device, thereby satisfying European Society of Cardiology Criteria 1 [6]. Therefore, as for faPWV, the findings of **chapters 5 and 6** indicate that the composite measure af-SG satisfies both ESC criteria 1 and 2, and is therefore a promising biomarker for clinical integration. One further potential, but unconfirmed, advantage of the af-SG is a purported independence to BP. This BP independence is thought to arise because central and peripheral arterial stiffness are similarly impacted by MAP [24]. Should the af-SG demonstrate MAP independence, it may be of significant clinical value, allowing clinicians to optimise treatment strategies and making the tracking of changes over-time more straightforward. Although the af-SG has demonstrated promising utility, the dependence of the af-SG on BP is not known. **Chapter 7** will address ***objective 2.3*** and seek to identify whether the composite biomarker, af-SG, can demonstrate an independence to blood pressure.

7

The Aortic-Femoral Arterial Stiffness Gradient is Blood Pressure Independent in Older Adults

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SUMMARY

Aortic arterial stiffness is a strong independent predictor of cardiovascular disease (CVD), however its dependence on mean arterial pressure (MAP) limits its clinical utility. The aortic-femoral arterial stiffness gradient (af-SG), a novel marker of CVD risk, may be a promising alternative, but its dependence on MAP is not known. The aim of this study was to determine the relationship between MAP and the af-SG in healthy older adults and those with established disease, including hypertension and diabetes. We evaluated the dependency of the af-SG on MAP in healthy older adults (n = 694, aged 74 ± 5 years), and adults with hypertension (n = 2040, aged 76 ± 5 years), and diabetes (n = 1405, aged 75 ± 5 years) as part of the community-based Atherosclerosis Risk in Communities (ARIC) Study. Carotid-femoral pulse-wave velocity (cfPWV), femoral-ankle PWV (faPWV) and blood pressure were measured using standardized protocols. The af-SG was calculated as faPWV divided by cfPWV. Multivariable regression analysis was performed to test the independent association of MAP with af-SG, with adjustments for known confounders including age, sex, body mass index, blood glucose and heart rate. There was no significant relationship between the af-SG and MAP in healthy (β = 0.002, p = .301), hypertension (β = -0.001, p = .298) or diabetes (β = -0.001, p = .063) population groups, with MAP explaining <0.1, <0.1 and 0.2% of the variance in the af-SG, respectively. These findings suggest that the af-SG may be regarded as a MAP independent index of arterial health and CVD risk in older adults.

Introduction

Arterial stiffness measures are commonly used to investigate arterial health and assist in the evaluation of cardiovascular disease (CVD) risk [8,297]. Pulse wave velocity (PWV) is the referent standard measure of arterial stiffness, of which, carotid-femoral PWV, a measure of central aortic stiffness, is the most prominent, and a strong independent predictor of CVD [10,19]. However, an inherent limitation of arterial stiffness measures, including cfPWV, is that they are highly dependent on the operational mean arterial pressure (MAP) [112,113]. In turn, MAP is known to be affected by a range of physiological, mechanical, and psychological factors [21-23]. Whilst arterial stiffness measures can be adjusted for MAP, the curvilinear nature and individual distinctiveness of the pressure-diameter relationship are persistent limitations [24]. In particular, comparing arterial stiffness-related outcomes between individuals, tracking changes over time, and determining optimal treatment strategies can be challenging. This likely limits the widespread adoption of arterial stiffness measures in clinical practice [20]. A MAP independent measure of arterial stiffness may therefore be of significant clinical value. One promising biomarker, that has demonstrated MAP independence, is the central to peripheral arterial stiffness gradient [215].

The central to peripheral arterial stiffness gradient is typically characterized as the ratio of upper- or lower-extremity arterial stiffness to central arterial stiffness [44,279]. Expressing arterial health in this manner is suggested to provide a MAP independent index of vascular aging, given that both central and peripheral arterial stiffness are similarly impacted by MAP [24]. The most widely explored measure is the aortic-brachial stiffness gradient (ab-SG), defined as the ratio of carotid-radial PWV (crPWV) to cfPWV [44,49]. The ab-SG has been shown to predict incident CVD and all-cause mortality in dialysis patients [44], as well as healthy older adults [49]. But of relevance, whilst the ab-SG was shown to be MAP independent in populations with prevalent renal disease, hypertension and diabetes [215,216], it was not among healthy adults [216]. The presence of disease may therefore impact the MAP dependence of the ab-SG and, as such, its clinical value. Recently, our research group reported that the aortic-femoral arterial stiffness gradient (af-SG), defined as the ratio of femoral-ankle PWV (faPWV) to cfPWV, was strongly associated with prevalent CVD in older adults [279]. Specifically, a low af-SG, as that which might occur with age or in the presence of disease[11], was associated with coronary heart disease, heart failure and stroke, whilst a high cfPWV was not [279]. Inclusion of the lower-extremities, which make up a significant portion of the arterial tree, may permit the af-SG to provide a more comprehensive picture of hemodynamic integration than the ab-SG. But although the af-SG has demonstrated promising utility, the dependence of the af-SG on MAP, and

whether or not this relationship is influenced by disease status, is not known. Should the af-SG demonstrate MAP independence in both healthy and diseased populations, it may be of significant clinical value.

The aim of this study was to determine the relationship between MAP and the af-SG in healthy subjects and those with established disease, specifically hypertension and diabetes patients. This aim was undertaken using a well characterized population of older men and women from the Atherosclerosis Risk in Communities (ARIC) Study cohort.

Methodology

This observational study is reported in accordance with STROBE (STrengthening the Reporting of OBservational studies in Epidemiology) guidelines [243]. Participants provided written informed consent, and the study was approved by the Institutional Review Boards at all field centers, coordinating center, and central labs and reading centers. Confirmation of study approval is presented in **Appendix 2**.

Study Population

The ARIC Study is a population-based, longitudinal study of 15,792 men and women aged 45–64 years enrolled between 1987 and 1989 from 4 US communities (Forsyth County, North Carolina; Jackson, Mississippi; Minneapolis, Minnesota; and Washington County, Maryland). Details of the baseline visit have been previously described [226]. Prior to exclusions, the current analysis includes 6,538 participants who attended visit 5 between 2011 and 2013, 5,683 of whom had PWV measures completed.

We excluded participants with the following conditions due to concerns of PWV data quality: BMI ≥40 kg/m^2, major arrhythmias (Minnesota codes 8-1-3, 8-3-1, and 8-3-2), Minnesota code 8-1-2 with evidence of biased PWV waveforms, aortic aneurysms, abdominal aorta ≥5 cm, history of aortic or peripheral revascularization or aortic graft, aortic stenosis, and moderate or greater aortic regurgitation. Additionally, we excluded participants whose race was other than white or African American (due to small sample size), with missing PWV or vascular risk factor data, as well as those with outlying PWV values, defined as PWV values 3 standard deviations above or below the mean.

We categorized the remaining 4,139 participants into the following groups: (i) Apparently healthy: participants who were free of hypertension, diabetes, prevalent CVD and were not using medications for those conditions; (ii) Participants with hypertension: a systolic blood pressure (SBP) ≥140 mm Hg, diastolic blood pressure (DBP) ≥90 mm Hg, or antihypertensive medication use; (iii) Participants with diabetes: fasting glucose ≥126 mg/dl, non-fasting glucose ≥200 mg/dl, antidiabetic medication use, or self-reported diagnosis of diabetes by a physician.

Participants were asked not to consume food or drink, and refrain from tobacco and vigorous physical activity after midnight prior to the clinic visit or for 8 hours prior to the visit. The visit 5 study examination included interviewer-administered questionnaires to obtain demographic data, medical history and lifestyle information, blood and urine collection, and assessment of vascular risk factors and cardiovascular phenotypes, including PWV.

Experimental Measures

Blood pressure and pulse wave velocity. After participants were supine for 5–10 minutes, technicians measured blood pressure, cfPWV and faPWV following a standardized protocol, using the automated cardiovascular screening device VP-1000 Plus (Omron, Kyoto, Japan) [111]. The device simultaneously measured bilateral brachial blood pressures, and carotid, femoral and posterior tibial arterial pulse waves in the supine position. PWV was estimated as the distance between two arterial recording sites divided by transit time (TT): distance/TT (**Figure 7.1**). For cfPWV assessments, arterial waveforms were simultaneously acquired for 30 seconds by applanation tonometry sensors attached on the left common carotid artery (via neck collar) and left common femoral artery. The distance from the carotid to the femoral artery was directly measured with a segmometer (Rosscraft, Surrey, Canada) and calculated as the carotid to femoral distance minus the distance between the suprasternal notch to the carotid applanation site. For faPWV assessments, bilateral posterior-tibial arterial pressure waveforms were detected over 10 seconds by extremities cuffs connected to plethysmographic and oscillometric pressure sensors wrapped on both ankles. Distance for faPWV was automatically calculated by the VP-1000 Plus using height-based formulas, as previously described [263]. A minimum of two PWV measurements were taken per participant and the last two measurements were averaged. The average of left and right faPWV measures was included for analysis.

The validity and reliability of the automatic device for measuring PWV have previously been described [111,130]. The device has been widely used in prospective observational studies and for

independently predicting CVD and all-cause mortality [298,299], and is recommended by the American Heart Association as criterion device for the non-invasive validation studies [50]. Quality assurance for PWV included central training and recertification, quarterly equipment calibration, and ongoing quality control reviews by one of the authors (H.T.) on a stratified random sample of 40 records per month with feedback provided to technicians. Approximately 78% of records were considered optimal quality, 17% were good quality, 3% were acceptable, and none were poor or unacceptable.

Aortic-femoral arterial stiffness gradient. The af-SG was calculated by dividing the femoral-ankle PWV (faPWV) by carotid-femoral PWV (cfPWV). This method emphasizes the model arterial system, whereby in a healthy cardiovascular system arterial stiffness increases between central and distal arteries [43]. Although no clinical threshold has been identified, to give greater context, an af-SG greater than 1.0 (i.e. faPWV>cfPWV) can be considered physiologically normal, whereas an af-SG of 1.0 or less (i.e. faPWV < cfPWV) can be considered pathological [49].

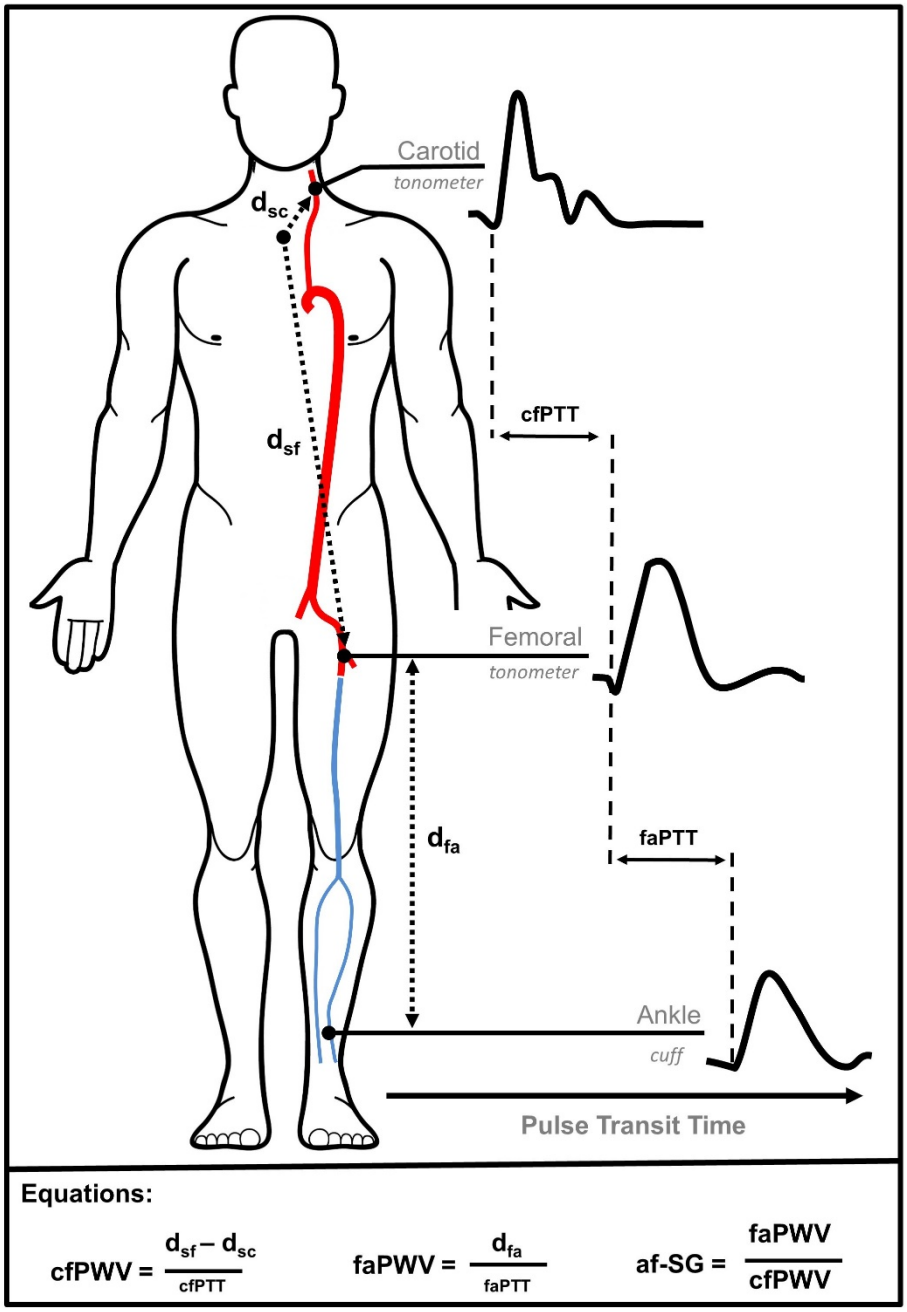

Figure 7.1 The aortic-femoral arterial stiffness gradient (af-SG) was calculated as femoral-ankle pulse wave velocity (faPWV) divided by carotid-femoral pulse wave velocity (cfPWV). Applanation tonometry was used to sequentially obtain waveforms at the left carotid and left femoral arteries, with cfPWV being estimated as the distance between the sternal notch and the femoral sampling site (dsf) minus the sternal notch to carotid sampling site (dsc), divided by the time delay (pulse transit time) between carotid and femoral waveforms (cfPTT). Simultaneously, bilateral posterior-tibial arterial pressure waveforms were detected using oscillometric cuffs at the ankles, with faPWV being estimated as the distance between femoral and ankle sampling sites determined using height-based formulas, divided by the time delay between femoral and ankle waveforms (faPTT).

Demographic and Covariate Measurements

Demographics. Age was calculated from date of birth. Sex and race were self-reported. History of smoking was self-reported and analyzed as dichotomous (current versus noncurrent).

Anthropometrics. Body weight was measured to the nearest 0.1 kg, and height was recorded to the nearest centimeter. Body mass index (BMI) was calculated body mass (kg) divided by height squared (m^2).

Blood markers. Blood samples were obtained following a standardized venipuncture protocol and shipped weekly to ARIC central laboratories where assays for total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, and fasting glucose concentration were performed. Total plasma cholesterol concentrations were determined enzymatically [283] using a Cobas-Bio analyzer with reagents purchased from Boehringer Mannheim Biochemicals, (Indianapolis, IN). Plasma low-density lipoprotein (LDL) cholesterol, concentration was calculated using the Friedewald equation, [284] and HDL concentrations were measured using the method of Warnick et al. [264].

Medications. Participants were asked to bring to the clinical visit all prescription and non-prescription medications taken within the four preceding weeks. That information was transcribed and categorized using MediSPAN prescription codes and classified into medication categories. Participants also self-reported medication use. Medications used included β-blockers, α-blockers, calcium channel blockers, diuretics, angiotensin-converting enzyme inhibitors, and angiotensin II receptor blockers.

Prevalent Cardiovascular Diseases. Prevalent CHD was defined by self-reported prior physician diagnosis of myocardial infarction or coronary revascularization, or prevalent myocardial infarction according to adjudicated ECG. Prevalent HF was classified by having at least one of the following: an adjudicated diagnosis of a HF event, International Classification of Disease, 9th Revision, Clinical Modification (ICD-9-CM) discharge code of 428.X in first position not overruled by a physician, any physician report of HF, self-reported HF or self-report of HF medication with pro-BNP greater than 125 pg/mL, or subsequent self-report of HF or HF medication (defined as medications participants reported taking for the treatment of HF). Prevalent stroke was defined by self-reported prior physician diagnosis of stroke or TIA and whether they had ever experienced the sudden onset of specific stroke symptoms (weakness, numbness, loss of vision, loss of understanding, inability to express). An ankle-

brachial index (ABI) of less than or equal to 0.9 was used to indicate peripheral artery disease (PAD). An ABI was determined for each leg and calculated as the highest ankle SBP divided by the highest of the right or left brachial SBP [28].The lower value of right and left ABI was used for our analysis

Statistical Analysis

Statistical analyses were performed using R Statistical Software (R Foundation for Statistical Computing, Vienna, Austria). The α-level was set *a-priori* for all statistical procedures at $\alpha = 0.05$. Cumulative frequency and Q-Q plots were used to compare the distributions of cfPWV, faPWV, and af-SG. Participant characteristics were estimated as means and standard deviation (SD), or frequencies and percent. Descriptive data across quartiles were compared using one-way analysis of variance (ANOVA) or Kruskal-Wallis for continuous outcomes, and Pearson's chi-squared for categorical outcomes, with Bonferroni correction for multiple comparisons. Univariable and multivariable linear regression was used to assess the relationships between MAP and cfPWV, faPWV and af-SG. The impact of age, sex and race on the relationship between MAP and cfPWV, faPWV and af-SG was examined by introducing an interaction term between MAP and the variable under consideration, and assessed using the significance of the interaction term in univariable and multivariable models. Multivariable regression models were adjusted for known or potential confounders including age, sex, BMI, fasting blood glucose, heart rate, race and field center. For linear regression we report unstandardized and standardized β coefficient estimates and 95% confidence intervals (95% CI), and the R^2 values for model fit. Partial R^2 values for dependent variables were determined using semi-partial correlation analysis within the *ppcor* package in R [285]. Assumption of linearity, collinearity, homoscedasticity, and outliers were assessed for every model.

Results

Characteristics of the Study Population

Of the 5,683 participants who attended visit 5 and underwent PWV measurements: 1500 were excluded using the following criteria: pre-existing condition (n=579), race other than white or African American (n=15), missing PWV data (n=529), PWV values 3 SDs above or below the mean (n=76), missing risk factor data (n=81), and missing covariates (n=220). Finally, 50 healthy (defined as above) participants were excluded due to prevalent CVD. Following exclusions, the sample included 4,139 cohort participants between the ages of 66 and 90 years, with 694, 2040, 1405 participants being categorized into apparently healthy, hypertension and diabetes groups, respectively.

Descriptive characteristics, stratified by patient group, are reported in **Table 7.1**. Healthy subjects were significantly younger, had lower BMI and systolic BP, and displayed a more favourable blood lipid profile than disease groups. cfPWV was significantly lower and the af-SG was significantly higher in the healthy when compared to disease groups, and both were significantly different between hypertension and diabetes groups. faPWV was significantly lower in the diabetes group than that of healthy and hypertension groups only.

Table 7.1 Descriptive characteristics of ARIC visit 5 participants, stratified by healthy and disease groups.

	Healthy n = 694		Hypertension n = 2040		Diabetes n = 1405		*P* Value
Continuous Variables (Mean, SD)							
Age (years)	74	(4.6)	75.5	(5.04)‡	75.2	(5.08)‡	<0.001
Body Mass Index (kg/m^2)	26.1	(4.0)	27.6	(4.3)	29.2	(4.4)	<0.001†
Systolic blood pressure (mm Hg)	122	(11)	133	(18)	130	(18)	<0.001†
Diastolic blood pressure (mm Hg)	65	(8)	67	(10.9)‡	65	(10.1)§	<0.001
Heart rate (bpm)	65	(10)	64	(10.7)	66	(11.2)‡,§	<0.001
Fasting glucose (mg/dL)	5.6	(0.5)	5.7	(0.5)	7.3	(2.0)	<0.001†
LDL (mg/dL)	3.1	(0.8)	2.8	(0.8)	2.4	(0.9)	<0.001†
HDL (mg/dL)	1.5	(0.4)	1.4	(0.6)	1.5	(0.7)	<0.001†
Triglycerides (mg/dL)	1.3	(0.5)	1.4	(0.6)‡	1.4	(0.6)‡	<0.001
Ankle-brachial index	1.14	(0.10)	1.10	(0.13)‡	1.09	(0.15)‡	<0.001
Femoral-ankle PWV (m/s)	11.0	(1.6)	10.9	(1.7)	10.6	(1.69)‡,§	<0.001
Carotid-femoral PWV (m/s)	10.5	(2.4)	11.5	(3.0)	12.3	(3.2)	<0.001†
Aortic-femoral stiffness gradient	1.10	(0.3)	1.01	(0.3)	0.93	(0.3)	<0.001†
Categorical Variables (No., %)							
Sex						‡,§	
Male	261	(38)	771	(38)	635	(45)	<0.001
Female	433	(62)	1269	(62)	770	(55)	
Race							
African American	62	(9)	459	(22)	410	(29)	<0.001†
White	632	(91)	1581	(78)	995	(71)	
Prevalent Cardiovascular Disease							
Coronary heart disease	0	(0)	294	(14)	269	(19)	<0.001†
Heart failure	0	(0)	206	(10)	210	(15)	<0.001†

Stroke	0	(0)	46	(2)	60	(4)	<0.001†
Ankle-brachial index <0.9	0	(0)	135	(7)	133	(6)	<0.001†
Medication use							
β-Blocker	0	(0)	703	(34)‡	464	(33)‡	<0.001
α-Blocker	0	(0)	71	(3)‡	64	(5)‡	<0.001
Diuretic	0	(0)	916	(45)	695	(49)	<0.001†
ACE Inhibitor	0	(0)	517	(25)	430	(31)	<0.001†
ANG II receptor blocker	0	(0)	221	(11)	203	(14)	<0.001†
Calcium channel blocker	0	(0)	583	(29)‡	448	(32)‡	<0.001†
Current smoker	45	(6)	114	(6)	76	(5)	0.588

Abbreviations: PWV, pulse wave velocity; HDL, high-density lipoprotein cholesterol; LDL, Low-density lipoprotein cholesterol. *Comparisons*: †for the comparison between all groups; ‡ vs. healthy; § vs. hypertension.

Associations with Mean Arterial Pressure

Within the diabetes group only, there were significant sex by MAP interactions for af-SG and faPWV, and a race by MAP interaction for faPWV in univariable analyses (all $P < 0.05$), but none were significant following covariate adjustment in multivariable models. Univariable associations of the af-SG, cfPWV and faPWV with MAP by participant group are presented in **Figure 7.2**. The af-SG was not associated with MAP in healthy and hypertension groups, but was significantly associated with MAP in the diabetes group ($P<0.05$). MAP explained 0.1%, 0.1% and 0.4% of the variation in the af-SG within healthy, hypertension and diabetes groups, respectively. Both cfPWV (2.5 - 5.1%) and faPWV (6.6-9.3%) were significantly associated with MAP in all population groups. Multivariable associations of the af-SG, cfPWV and faPWV by group are presented in **Table 7.2**. Overall, multivariable adjustment had a small effect on MAP estimates; however, the significant association between af-SG and MAP in diabetes participants was no longer significant following covariate adjustment. In multivariable models MAP explained <0.1%, <0.1% and 0.2% of the variation in the af-SG within healthy, hypertension and diabetes groups, respectively.

Sensitivity and Ancillary Analysis

Analysis of af-SG determined using left and right faPWV measures separately revealed no notable differences to those determined using a mean of left and right faPWV measures. Independently, the exclusion of those participants with an ankle brachial index (ABI) ≤0.9 and all participants with prevalent CVD (hypertension and diabetes groups only) did not impact the key findings. Finally, compared to the primary findings, stratification of all subjects by quartiles of age (<71,71-74,75-79,>79 years) did not reveal any contrasting associations for the af-SG with MAP (**Table 7.3**).

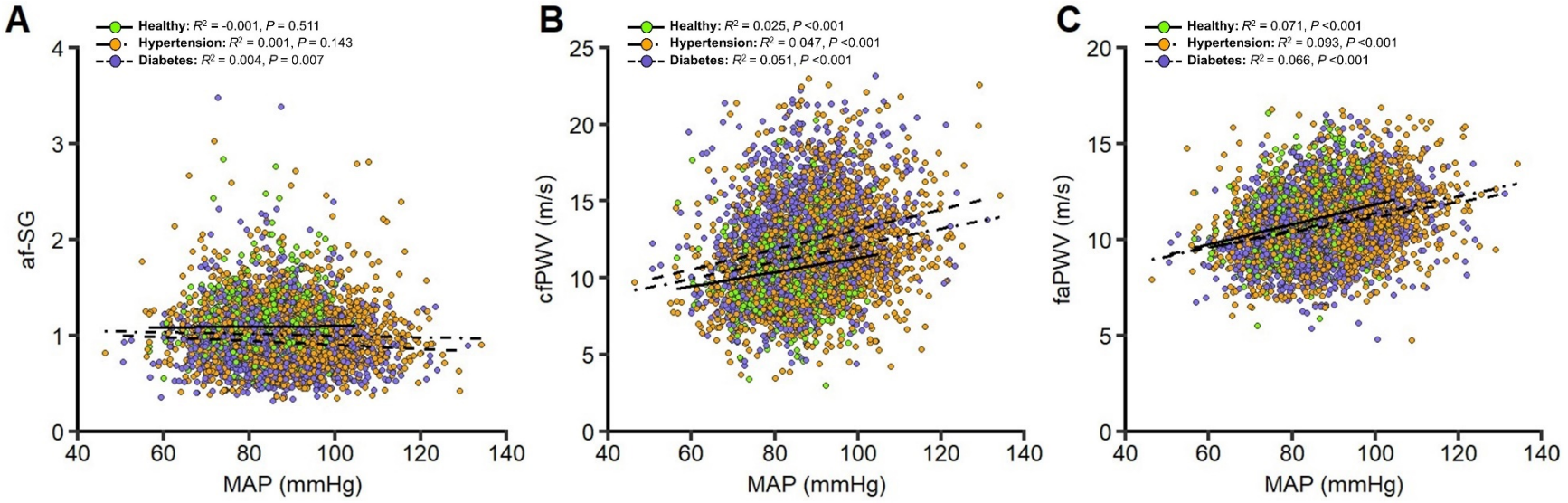

Figure 7.2 Relationship between mean arterial pressure and the aortic-femoral stiffness gradient, carotid-femoral pulse wave velocity, and femoral-ankle pulse-wave velocity in healthy (n=694), hypertension (n=2040), and diabetes (n=1405) population groups. Abbreviations: cfPWV, carotid-femoral pulse-wave velocity; faPWV, femoral-ankle pulse-wave velocity; af-SG, aortic-femoral arterial stiffness gradient; MAP, mean arterial pressure.

Table 7.2 Multivariable linear regression models for the association between mean arterial pressure and the aortic-femoral stiffness gradient, carotid-femoral pulse wave velocity, and femoral-ankle pulse-wave velocity in healthy (n=694), hypertension (n=2040), and diabetes (n=1405) groups.

	HEALTHY						HYPERTENSION						DIABETES					
	β	Std. β	95% CI		*P*	$^{b}R^2$	β	Std. β	95% CI		*P*	$^{b}R^2$	β	Std. β	95% CI		*P*	$^{b}R^2$
cfPWV	$^{a}R^2$=	0.211					$^{a}R^2$=	0.169					$^{a}R^2$=	0.143				
MAP	**0.051**	**0.178**	**0.158**	**0.198**	**<0.001**	**0.029**	**0.052**	**0.208**	**0.198**	**0.218**	**<0.001**	**0.042**	**0.061**	**0.214**	**0.200**	**0.228**	**<0.001**	**0.043**
Age	0.163	0.314	0.279	0.349	<0.001	0.095	0.150	0.255	0.231	0.279	<0.001	0.062	0.143	0.230	0.200	0.261	0.000	0.051
Sex	0.759	0.154	-0.200	0.509	<0.001	0.020	0.423	0.069	-0.182	0.320	0.001	0.004	0.490	0.077	-0.236	0.391	0.002	0.006
BMI	-0.062	-0.104	-0.146	-0.062	0.004	0.009	-0.083	-0.121	-0.150	-0.093	<0.001	0.013	-0.031	-0.044	-0.080	-0.008	0.090	0.002
FBG	0.407	0.085	-0.248	0.417	0.016	0.007	0.128	0.024	-0.200	0.248	0.264	0.001	0.125	0.079	0.001	0.157	0.002	0.006
HR	0.056	0.233	0.216	0.250	<0.001	0.048	0.050	0.179	0.167	0.190	<0.001	0.030	0.050	0.175	0.161	0.189	0.000	0.029
faPWV	$^{a}R^2$=	0.113					$^{a}R^2$=	0.190					$^{a}R^2$=	0.182				
MAP	**0.061**	**0.313**	**0.299**	**0.327**	**0.000**	**0.089**	**0.046**	**0.316**	**0.310**	**0.322**	**<0.001**	**0.096**	**0.043**	**0.282**	**0.274**	**0.289**	**<0.001**	**0.074**
Age	0.034	0.095	0.070	0.121	0.009	0.009	0.001	0.003	-0.010	0.017	0.869	0.000	-0.006	-0.017	-0.033	0.000	0.501	0.000
Sex	-0.184	-0.055	-0.311	0.202	0.160	0.002	-0.083	-0.023	-0.168	0.122	0.263	0.000	0.122	0.036	-0.128	0.200	0.143	0.001
BMI	-0.050	-0.125	-0.155	-0.094	0.001	0.014	-0.077	-0.191	-0.207	-0.174	<0.001	0.033	-0.093	-0.243	-0.262	-0.224	0.000	0.054
FBG	-0.018	-0.005	-0.246	0.235	0.884	0.000	0.102	0.032	-0.097	0.162	0.123	0.001	0.045	0.054	0.013	0.095	0.029	0.003
HR	0.015	0.091	0.079	0.104	0.016	0.007	0.024	0.145	0.138	0.151	0.000	0.020	0.022	0.144	0.137	0.151	0.000	0.003
af-SG	$^{a}R^2$=	0.075					$^{a}R^2$=	0.057					$^{a}R^2$=	0.054				
MAP	**0.002**	**0.039**	**0.036**	**0.042**	**0.301**	**0.001**	**-0.001**	**-0.023**	**-0.024**	**-0.022**	**0.298**	**0.001**	**-0.001**	**-0.050**	**-0.051**	**-0.048**	**0.063**	**0.002**
Age	-0.014	-0.193	-0.199	-0.188	<0.001	0.036	-0.012	-0.186	-0.189	-0.183	<0.001	0.033	-0.010	-0.164	-0.167	-0.161	<0.001	0.026
Sex	-0.099	-0.142	-0.196	-0.087	<0.001	0.017	-0.043	-0.066	-0.095	-0.038	0.003	0.004	-0.028	-0.046	-0.078	-0.014	0.084	0.002
BMI	0.000	-0.005	-0.011	0.001	0.897	0.000	-0.001	-0.012	-0.015	-0.009	0.591	0.000	-0.006	-0.089	-0.092	-0.085	0.001	0.007
FBG	-0.047	-0.069	-0.120	-0.018	0.070	0.004	0.004	0.006	-0.019	0.032	0.775	0.000	-0.006	-0.042	-0.050	-0.034	0.112	0.002
HR	-0.005	-0.142	-0.144	-0.139	<0.001	0.018	-0.002	-0.072	-0.073	-0.070	0.001	0.005	-0.002	-0.067	-0.069	-0.066	0.012	0.004

Abbreviations: cfPWV, carotid-femoral pulse-wave velocity; faPWV, femoral-ankle pulse-wave velocity; afPWV ratio, aortic-femoral pulse-wave velocity ratio; MAP, mean arterial pressure, BMI, body mass index; FBG, fasting blood glucose; HR, heart rate, β, beta coefficient; std. β, standardized beta coefficient; ^{a}R2, Model adjusted R squared coefficient; ^{b}R2, partial R squared coefficient. *Adjustments*: age, sex, body mass index, fasting blood glucose, heart rate, race and field center.

Table 7.3 Relationship between mean arterial pressure and the aortic-femoral stiffness gradient, carotid-femoral pulse wave velocity, and femoral-ankle pulse-wave velocity stratified by quartiles of age.

	cfPWV					faPWV					af-SG				
	β	Std. β	95% CI		*P*	β	Std. β	95% CI		*P*	β	Std. β	95% CI		*P*
Univariable															
<71	0.068	0.276	0.260	0.293	<0.001	0.045	0.297	0.287	0.307	<0.001	-0.002	-0.074	-0.076	-0.072	0.033
71-74	0.059	0.248	0.236	0.261	<0.001	0.045	0.308	0.300	0.315	<0.001	-0.001	-0.047	-0.048	-0.045	0.093
75-79	0.072	0.264	0.249	0.280	<0.001	0.045	0.288	0.280	0.297	<0.001	-0.002	-0.065	-0.067	-0.064	0.027
>79	0.051	0.181	0.162	0.199	<0.001	0.035	0.223	0.213	0.233	<0.001	-0.001	-0.035	-0.037	-0.033	0.303
Multivariable*															
<71	0.045	0.297	0.287	0.307	<0.001	0.051	0.331	0.321	0.341	<0.001	0.000	-0.014	-0.016	-0.011	0.697
71-74	0.054	0.227	0.215	0.239	<0.001	0.050	0.339	0.332	0.347	<0.001	0.000	-0.016	-0.017	-0.014	0.567
75-79	0.063	0.231	0.216	0.246	<0.001	0.047	0.304	0.296	0.312	<0.001	-0.001	-0.042	-0.044	-0.040	0.156
>79	0.047	0.165	0.146	0.183	<0.001	0.037	0.236	0.226	0.246	<0.001	0.000	-0.016	-0.018	-0.014	0.645

Abbreviations: cfPWV, carotid-femoral pulse-wave velocity; faPWV, femoral-ankle pulse-wave velocity; afSG, aortic-femoral arterial stiffness gradient; β, beta coefficient; std. β, standardized beta coefficient. **Adjustments*: age, sex, body mass index, fasting blood glucose, heart rate, race and field center. Age groups: <71 years: n = 825; 71-74 years: n = 1303; 75-79 years: n = 1150; >79 years: n = 861.

Discussion

The aim of this study was to determine the relationship between MAP and the af-SG in healthy older adults as well as those with established disease, specifically hypertension and diabetes. The principal finding was that, unlike cfPWV and faPWV, the af-SG was found not to be dependent on MAP in healthy, hypertension or diabetes population groups. This finding suggests that the af-SG may be regarded as a MAP independent index of arterial health and CVD risk in older adults.

Limitations and Strengths

The strengths and limitations of this study need to be addressed to best contextualize the findings and better facilitate comparisons to the existing literature. Firstly, the generalizability of our findings is limited to older populations and cannot be extended to younger, healthier cohorts. Further, the predominate inclusion of participants who had survived from baseline (1987-1989) and attended the Visit 5 examination (2011-2013), and were thus likely healthier compared to those who did not participate in the visit, may have generated a bias within the study population. Secondly, the use of height-based formulas to calculate faPWV were validated in a Japanese population and may not be applicable to other racial or ethnic groups. A major strength is that this is the first study to examine the association between the af-SG and MAP among different patient and healthy populations.

Comparison to The Literature

A major finding of this study is that unlike cfPWV and faPWV, MAP was not associated with af-SG in a large population of community-dwelling older adults, regardless of health status. Following adjustment for known confounders in multivariable models, MAP explained <0.1, <0.1 and 0.2% of the variation in the af-SG in healthy, hypertension and diabetes groups, respectively. Whilst no other studies have investigated the MAP dependence of the af-SG, the present findings are consistent with the majority of previous literature investigating the MAP dependency of the upper-extremity derived ab-SG [45,46,215,216]. The ab-SG has demonstrated independence to MAP in dialysis and renal dysfunction patients [46,215], and comparable to the present study, diabetes and hypertension patient groups [45,216]. Of those reported, the variation in af-SG explained by MAP in the present study across populations (~0.2%) is lower than that reported for the ab-SG (0.4-9.6%) [45,46,215,216], supporting our inference of MAP independence. However, unlike the af-SG in the present study, the ab-SG was shown not to be MAP independent among healthy adults [216]. The divergent findings between the dependence of ab-SG and af-SG on MAP in healthy populations is likely due to several

factors, including: i) the inherent difference in the structural characteristics of the peripheral vascular segments used to determine the respective arterial stiffness gradients, and, ii) the contrasting demographic characteristics of the populations in which MAP dependence was explored.

Arterial wall stiffness is dependent on both MAP and the intrinsic structure of the arterial wall. Distending tension is primarily borne by elastin-distensible fibres at low pressure, but an increase in MAP increases vessel diameter and transfers the distending load to the less extensible collagen fibres, leading to an augmentation in arterial stiffness [43]. Increased arterial stiffness shifts the pressure-diameter relationship upwards, meaning a higher pressure is required to induce a similar change in diameter [24]. Consequently, an arterial segment of higher arterial stiffness will inherently be impacted by MAP to a lesser degree. Relative to the upper-extremities, the lower-extremities incorporate a greater proportion of inelastic muscular conduit arteries. Additionally, lower-extremity arterial stiffness is typically higher than that of the upper extremities, in order to manage the greater hydrostatic load induced during orthostasis [148]. It is therefore plausible that the inclusion of a greater proportion, of intrinsically stiffer, arterial segments (femoro-tibial) may lessen the effect of MAP on the af-SG. However, no studies have directly compared the MAP dependence of ab-SG and af-SG measures.

The pressure-diameter relationship is also influenced by age and disease. With normal aging, the elastin-distensible fibres become fragmented and discontinuous, this, coupled with a reduction in elastin expression attenuating the elastin-collagen ratio, shifts the mechanical load to the stiffer collagen fibres [43,100]. The stiffness of elastin and collagen fibres is also increased via additional cross-linking by advanced glycation end-products (AGE) [100]. This degeneration is accelerated by the presence of disease, with calcification of the elastic lamellae and the cross-linking by AGEs occurring at an advanced rate with diabetes for example [150,300]. The progression of vascular dysfunction is partly offset by arterial dilation, with arterial cross-sectional area increasing with age [20]. However, these phenomena steepen the slope of the pressure-diameter relationship, lessening the effect of MAP on arterial stiffness measures. To illustrate, compared to healthy adults, hypertensive adults demonstrate an augmented aortic PWV [216,301], and an attenuated dependence of aortic stiffness on transmural pressure has been reported in hypertensive versus normotensive patients [302]. Therefore, as for the ab-SG [45,46,215,216], the consequences of vascular aging and disease likely contribute to the independence of the af-SG to MAP among older adults in the present study, particularly within hypertension and diabetes patient groups. Further, although it is difficult to discern the impact of age and disease, vascular aging also likely explains the contrasting findings

between the pressure-dependence of ab-SG and af-SG measures in healthy adults. Whilst free from hypertension, diabetes and prevalent CVD, the healthy adults in the present study were significantly older (74 ± 5 years *vs* 51 ± 8 years) and, expectantly, had a ~30% greater cfPWV (10.5 ± 2.4 m/s *vs* 7.5 ± 1.8 m/s years), than the healthy adults in the study conducted by Armstrong et al. [216] to investigate the MAP dependence of the ab-SG. This may suggest that the observed independence of the af-SG to MAP in the present study could in-part be due to use of older adults who are further along the vascular aging pathway. However, our healthy adults demonstrated aortic stiffness measures which closely reflect age-specific reference values (10.4-11.7 m/s) for normotensive adults [303], and are therefore representative of healthy older adults in the general population. As such the af-SG may be regarded as a MAP independent index of arterial health in older adults.

Implications and Conclusions

The assessment of aortic arterial stiffness, typically as cfPWV, to assist in the determination of CVD risk is now well established in epidemiological and clinical research settings [6]. However, notwithstanding the strong association of aortic stiffness with clinical outcomes [10,19], this persistent focus ignores the integrated role that peripheral muscular arteries play in the cardiovascular system. Although the peripheral vasculature is less impacted by age and disease compared to the central vasculature [11], there can be important pathophysiological changes within this region that may contribute to CVD risk [200]. In this respect, incorporation of peripheral arterial stiffness into risk prediction in the form of the ab-SG or af-SG has been shown to confer unique and prognostic information beyond cfPWV alone, with particular use in older-age and diseased populations [44,49,279], and may better explain the impact of pathophysiological changes in arterial stiffness on both myocardium and peripheral circulation [209]. For example, our research group demonstrated that the af-SG was associated with coronary heart disease, heart failure and stroke in community-dwelling older adults, whilst cfPWV was not [279]. The current study extends the scant arterial stiffness gradient literature by being the first to report that, unlike cfPWV, the af-SG is not dependent on MAP in older adults, regardless of health status. The independence of af-SG to MAP is a significant advantage, and overcomes a likely barrier to the widespread adoption of arterial stiffness measures into clinical practice. Collectively, these findings indicate that the af-SG may be of clinical utility as a simple non-invasive assessment of arterial health and identification of CVD risk. However, a number of gaps in the literature remain and need to be addressed in order to ascertain whether the af-SG is a clinically viable surrogate endpoint, including whether the af-SG predicts CVD events and mortality,

and if it is sensitive to risk factor modification or pharmacological intervention [6]. Further, to confirm utility, future research should seek to identify if age or disease, in younger adults, impacts the dependency of the af-SG on MAP.

8

Summary & Future Directions

There is an urgent need to identify novel cardiovascular biomarkers which can improve CVD risk prediction and permit timely-efficacious treatment; necessary to combat the unabated rise in CVD [6]. Clinical and epidemiological evidence attests that arterial stiffness is a key early biomarker of cardiovascular health, and can be assessed non-invasively and simply using PWV. Numerous empirical studies and meta-analyses have demonstrated that cfPWV improves CVD risk prediction beyond traditional risk factors [8,10], but clinical adoption is poor, likely because its BP dependence makes clinical decision challenging [20]. A persistent focus on cfPWV has also meant the prognostic significance of arterial stiffness in the lower-limbs has received little attention, yet it can be simply assessed using faPWV and its measurement may well improve the determination of CVD risk [11]. An advantage of faPWV assessment is that it permits determination of the af-SG, a biomarker of promising prognostic utility, that may also be BP independent. However, whether faPWV and af-SG are robust biomarkers for CVD is unknown. Further, to be of clinical utility, the European Society of Cardiology asserts that any novel biomarker must i) be determined with accuracy and precision - criteria 1, and, ii) provide additional CVD risk information beyond existing biomarkers - criteria 2 [50]. The principal aim of thesis was to determine the clinical utility of lower-limb arterial stiffness, for which two overarching objectives were identified, 1) the clinical utility of the standalone biomarker faPWV, and 2) the clinical utility of the composite biomarker af-SG (**Objective & Approach**).

The first series of objectives were to **identify whether the standalone biomarker, faPWV, 1.1) can be determined with acceptable accuracy (validity) and precision (reliability), and, 1.2) can provide additional CVD risk information beyond ABI, an existing marker of lower-limb arterial health.** In **Chapter 3** ***(objective 1.1)*** faPWV measures determined using a simple oscillometric cuff-based device were compared to a criterion, and subsequently repeated on three separate days. In a homogenous cohort of young adults, the oscillometric device demonstrated acceptable accuracy (**Table 3.3**) and good precision (**Table 3.4**) in a supine posture, according to recognised evaluative outcomes [217]. Whilst it would be clinically advantageous if arterial stiffness could be assessed whilst sitting, analogous with routine BP measurement, the oscillometric device could not accurately nor precisely determine faPWV in the seated posture. Although perhaps an impediment to clinical uptake, generally arterial stiffness assessments are routinely conducted in a supine position due to the potential for orthostatic haemodynamic shifts to confound measures [50,304]. Regardless, oscillometric cuff-based assessment of faPWV is a good candidate to encourage clinical adoption of arterial stiffness phenotyping due to its simplicity and ease of use. In **Chapter 4**

(objective 1.2) the association between faPWV and ABI with a composite measure of CVD, including CHD, HF and stroke, were determined in a population of community-dwelling older adults. Using multivariable logistic regression analyses, faPWV was found to be independently associated with CVD, beyond both traditional Framingham cardiovascular risk factors and ABI (**Table 4.3**). But paradoxically, it was low faPWV (≤9.94 m/s), perhaps representing a loss of arterial elasticity through arteriosclerotic independent means such as a breakdown in arterial wall structural matrix with disease [278], that increased the odds of CVD by 46%. The assessment of faPWV may therefore provide novel pathophysiological insight into the consequences of alterations in peripheral arterial stiffness and CVD risk. Although the mechanisms have yet to be fully elucidated, low peripheral arterial stiffness has been presented as novel consequence of increased aortic arterial stiffness and a mechanism for preventing myocardial damage [44,209,287]. Although cardiac function is preserved, this reduces the physiologically advantageous central to peripheral arterial stiffness gradient, a pathophysiological basis for cardiovascular events and target organ damage [56,57]. Collectively, the findings of **Chapter 3** and **Chapter 4**, indicate that standalone measure faPWV satisfies criteria 1 and 2 for a clinically viable biomarker, as listed by European Society of Cardiology [6], given that it can be determined simply, accurately and precisely, and provides independent additional CVD risk information beyond existing cardiovascular health measures.

The second series of objectives was to **identify whether the composite biomarker af-SG, 1.1) can provide additional CVD risk information beyond the criterion measure of arterial stiffness, cfPWV, 1.2) can be determined with acceptable precision (reliability), and, 1.3) can demonstrate an independence to blood pressure.** In **Chapter 5** *(objective 2.1)* the association of af-SG and cfPWV with CVD was determined in a population of community-dwelling older adults. Extending the scant stiffness gradient literature, the af-SG was uniquely and independently associated with heart failure, beyond cfPWV and traditional cardiovascular risk factors. Moreover, whilst a low af-SG (<0.784) increased the odds of CHD, HF stroke, hypertension and diabetes prevalence, high cfPWV increased the odds of having diabetes only (**Table 5.3**). This suggests lower-extremity derived af-SG provides a unique and additive picture of CVD risk when compared to the criterion arterial stiffness measure, cfPWV. Further, use of the af-SG is a more logical choice for risk determination than aortic stiffness as it better explains the impact of pathophysiological changes in arterial stiffness on both myocardium and peripheral micro-circulation [209]. Current evidence suggests that stiffness gradient measures may be of greatest use in improving CVD risk prediction accuracy, beyond cfPWV,

in older adults [49,209]. In **Chapter 6** ***(objective 2.2)*** the precision of the af-SG was determined by assessing faPWV and cfPWV on three separate days using a simple oscillometric device. The af-SG demonstrated good precision in a supine posture, according to recognised validation and reliability outcomes (**Table 6.2**) [217]. However, combining segmental PWV measures likely augments random and measurement error, reducing measurement precision. This may impact the ability of the af-SG to accurately assess CVD risk in elderly or patient populations where PWV measures inherently demonstrate poorer precision [212]. But collectively, like faPWV, the findings of **Chapter 5** and **Chapter 6** indicate that the composite measure af-SG can satisfy criteria 1 and 2 for a clinically viable biomarker, as listed by European Society of Cardiology [6], given that it can be determined simply and precisely, and provides independent additional prognostic value beyond existing cardiovascular health measures. Finally, in **Chapter 7** ***(objective 2.3)*** the dependence of the af-SG on BP was determined in older adults. Unlike existing stiffness gradient measures [215,216], the af-SG was found to be MAP independent in healthy as well as hypertensive and diabetic population groups (**Table 7.2**), overcoming a significant barrier to the widespread adoption of arterial stiffness measures into clinical practice. An independence to BP permits researchers to disentangle arterial stiffness as a separate prognostic biomarker, whilst allowing clinicians to optimise treatment strategies and making the tracking of changes over-time more straightforward [24,123].

Collectively, the findings of this thesis indicate that the assessment of lower-limb arterial stiffness could be of high clinical utility particularly in older adults, however, a number of gaps in the literature remain, and may be considered as general limitations of this thesis. *Firstly*, prior to clinical use, it is of high importance to determine whether any error, bias or variability inherent to a biomarker is caused by the technique used to determine it and not a consequence of the presence of cardiovascular pathology. As such, we recruited a homogenous group of young healthy adults to determine the upper-limit of validity and reliability of faPWV and af-SG measures. Whilst maximising internal validity, this approach reduced external validity and the generalisation of findings to populations who more likely undergo CVD risk assessment. To counter, the oscillometric cuff-based technique used in this thesis has been reported to measure cfPWV with good accuracy and precision in older adults [110,251], and it is reasonable to assume this would also be the case for faPWV. However, future studies should confirm accuracy and reliability of faPWV and af-SG in clinical populations of varying age and health states. *Secondly*, our studies were not designed to address the mechanisms for how faPWV or the af-SG regression contributes to disease progression. Specifically, to our knowledge, no data exist on the longitudinal changes in the intrinsic structure of femoral-tibial

arteries. The use of faPWV and the af-SG as parameters for risk prediction is still at its preliminary stages, but understanding femoral-tibial stiffness regression will likely be important from an academic point of view but also from a therapeutic one, and will be a valuable field to explore in the future. *Thirdly*, although the studies presented in this thesis show a clear association of faPWV and af-SG with CVD, their cross-sectional nature means that it is not possible to conclude explicitly that these measures directly predicted incident CVD events because the studies were not longitudinal i.e. patients were not followed-up. In this respect, a biomarker only deserves clinical integration if it can satisfy all criteria outlined by the European Society of Cardiology [6] (**Table 1.1**), the next step of which is prospective validation ~ **Does a biomarker predict future outcomes? – Criteria 3.**

To plug these gaps in the literature and advance the case for clinical adoption, our research group has already planned and obtained approval to conduct a study that seeks to determine the ability of faPWV and the af-SG to predict future CVD outcomes (**Appendix 3**), as well as compare to existing measures, the study design for which is fully described in **Appendix 4**. This study design will also include determination of between-day reliability of faPWV and af-SG in older adults. Briefly, secondary analysis of longitudinal data (~8 years) from a cohort of community-based healthy (free of prevalent CVD, n= ~5000) older adults who are part of Atherosclerosis Risk in Communities (ARIC) study will be used to determine whether faPWV, cfPWV and af-SG can predict incident CVD, including CHD, HF and stroke, as well as all-cause mortality. Reliability coefficients with be determined using a subgroup (n=79) of participants whom agreed to return for repeat visits 4 to 8 weeks apart.

To conclude, the findings of this thesis indicate that the assessment of lower-limb arterial stiffness, either as a standalone biomarker faPWV or its inclusion within the composite biomarker af-SG, could be of high clinical utility given that they can be assessed simply, accurately and precisely using oscillometric technologies and may improve our ability to accurately predict CVD risk given that they can provide additional CVD risk information beyond exiting risk markers. Accurate assessment of CVD risk is a vital preventative tool for reducing the global burden of CVD. However, a number of gaps in the literature remain before the assessment of lower-limb arterial stiffness can be considered for clinical integration, the foremost of which is identifying, i) whether these novel biomarkers can predict CVD events and mortality, ii) whether they can be used as surrogate therapeutic end-points, and, iii) if they are sensitive to risk factor modification or pharmacological intervention [6].

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APPENDICIES

Appendix 1: Chapter 3 and Chapter 6 Ethics Approval

THE UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL
Individual Investigator Agreement (Independent)

A. This Agreement is entered into by and between The University of North Carolina at Chapel Hill ("UNC-Chapel Hill") for its Office of Human Research Ethics and Institutional Review Boards ("IRB") and **Keeron Stone** (hereafter designated as "**INVESTIGATOR**").

UNC Chapel Hill holds a Federalwide Assurance (FWA#4801) approved by the federal Office for Human Research Protections (OHRP) and has established one or more IRBs (the "UNC-Chapel Hill IRB") pursuant to the federal regulations at 45 CFR 46 governing human subjects research.

The INVESTIGATOR named above desires to collaborate with UNC-Chapel Hill in the conduct of the research described in **Section B**, is not covered by an assured institution's FWA, and is not acting as an employee of any institution in the conduct of this research. Therefore, UNC-Chapel Hill has agreed to extend its FWA to cover this INVESTIGATOR for the purposes of this research.

B. Both UNC-Chapel Hill and **INVESTIGATOR** agree that the UNC-Chapel Hill IRB will provide initial review and continuing oversight of the human subjects research protocol described below pursuant to 45 CFR 46 and the terms of UNC- Chapel Hill's FWA:

Name of Research Project: The validity and reliability of lower limb pulse-wave velocity assessments using a novel cuff-based technique
IRB Study #: 17-0745
Principal Investigator at UNC-Chapel Hill: Lee Stoner
Sponsor or Funding Agency: n/a

C. **INVESTIGATOR** agrees that:

(1) The above-named **INVESTIGATOR** has reviewed: 1) *The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research* (or other internationally recognized equivalent; see section B.1. of the Terms of the Federalwide Assurance (FWA) for International (Non-U.S.) Institutions); 2) the U.S. Department of Health and Human Services (HHS) regulations for the protection of human subjects at 45 CFR 46 (or other procedural standards; see section B.3. of the Terms of the FWA for International (Non-U.S.) Institutions); 3) UNC-Chapel Hill's FWA (FWA #4801) (copies available upon request); and 4) the relevant policies and procedures of UNC-Chapel Hill for the protection of human subjects (copies available online and upon request).

(2) The **INVESTIGATOR** understands and hereby accepts the responsibility to comply with the standards and requirements stipulated in the above- referenced documents and to protect the rights and welfare of human subjects involved in research conducted under this Agreement. The **INVESTIGATOR** will personally conduct or supervise performance of the research conducted under this Agreement. The **INVESTIGATOR** will ensure that all collaborators, students, and employees conducting research under this Agreement will comply with the terms of this Agreement.

(3) The **INVESTIGATOR** will comply with all other applicable federal, international, state, and local laws, regulations, and policies that may provide additional protection for human subjects participating in research conducted under this Agreement.

(4) The **INVESTIGATOR** will abide by all determinations of the UNC-Chapel Hill IRB and will accept the final authority and decisions of the UNC-Chapel Hill IRB including, but not limited to, directives to terminate performance of designated research activities.

(5) The **INVESTIGATOR** and all individuals who will have contact with human subjects during the performance of the research will complete any educational training required by UNC-Chapel Hill and/or the UNC-Chapel Hill IRB prior to initiating research covered under this Agreement.

(6) The **INVESTIGATOR** shall provide accurate and complete information to the UNC-Chapel Hill IRB in all applications for review and other communication with the UNC-Chapel Hill IRB. The **INVESTIGATOR** will report promptly to the UNC-Chapel Hill IRB any proposed changes in the research conducted under this Agreement. **INVESTIGATOR** will not initiate changes in the research without prior UNC-Chapel Hill IRB review and approval, except where necessary to eliminate apparent immediate hazards to subjects.

(7) The **INVESTIGATOR** will report immediately to the UNC-Chapel Hill IRB any unanticipated problems involving risks to subjects or others in research covered under this Agreement.

(8) The **INVESTIGATOR**, when responsible for enrolling subjects, will obtain, document, and maintain records of informed consent for each such subject or each subject's legally authorized representative as required under HHS regulations at 45 CFR 46 or stipulated by the UNC-Chapel Hill IRB.

(9) The **INVESTIGATOR** acknowledges and agrees to cooperate with the UNC-Chapel Hill IRB for initial and continuing review, record keeping, reporting,

and certification for the research referenced above. The **INVESTIGATOR** will provide all information requested by the UNC-Chapel Hill IRB in a timely fashion.

(10) In conducting research involving FDA-regulated products, the **INVESTIGATOR** will comply with all applicable FDA regulations (including 21 CFR 50 & 56) and fulfill all investigator responsibilities (or investigator-sponsor responsibilities), where appropriate.

(11) The **INVESTIGATOR** will not enroll subjects in research under this Agreement prior to its review and approval by the UNC-Chapel Hill IRB.

(12) Emergency medical care may be delivered without UNC-Chapel Hill IRB review and approval to the extent permitted under applicable federal and state law. However, data and information obtained as a result of emergency medical care may not be included as part of federally-supported or-conducted research.

(13) This Agreement does not preclude the **INVESTIGATOR** from taking part in research not covered by this Agreement. The **INVESTIGATOR** understands that the purview of the UNC-Chapel Hill IRB extends only to the research protocol(s) specified in **Section B** and not to any other research protocol(s).

(14) The **INVESTIGATOR** acknowledges that he/she is primarily responsible for safeguarding the rights and welfare of each research subject and that the subject's rights and welfare must take precedence over the goals and requirements of the research.

(15) The **INVESTIGATOR** will not use, nor authorize others to use, the name, symbols, or marks of UNC-Chapel Hill in any advertising or publicity material or make any form of representation or statement in relation to the research protocol(s) specified in **Section B** which would constitute an expressed or implied endorsement by UNC-Chapel Hill except for factual representation of UNC-Chapel Hill's performance of research pursuant to this Agreement.

D. Both UNC-Chapel Hill and **INVESTIGATOR** agree to the following general provisions:

(1) The term of this Agreement shall begin upon full execution by the parties and shall continue in effect until expiration or termination of UNC-Chapel Hill IRB approval of the research covered under this Agreement.

(2) Each party will be responsible for its own negligence in connection with its performance of this Agreement and the research protocol(s) specified in **Section B**.

(3) This document must be kept on file by both parties and provided to OHRP or other regulatory agencies upon request.

(4) This Agreement shall be governed by North Carolina law.

(5) Correspondence for the parties shall be sent to the individuals listed below.

Signature of Signatory Official (or authorized designee) at UNC-Chapel Hill:

Date: 5/23/17

Name: Elizabeth Kipp Campbell, Ph.D., CIP
Institutional Title: Director, Office of Human Research Ethics
Phone:
Email:

Signature of Investigator:

K Stone

Name: Keeron Stone Title: Co-Investigator
Phone:
Email:

Date: 23/05/2017

Appendix 2: Chapters 4, 5 & 7 ARIC Secondary Analysis Approval

ARIC™ Research with Heart.

Lee Stoner
University of North Carolina
USA

Dear Lee Stoner

On behalf of the Publications Committee, I am notifying you that your ARIC manuscript proposal has been approved.

Manuscript Proposal #: 3272

Manuscript Proposal Title: Associations between measures of regional pulse wave velocity: The Atherosclerosis Risk in Communities (ARIC) Study

Note that manuscript proposals are approved for a three-year period. If at the end of this time a paper has not been submitted, the authors will be notified and the manuscript proposal will be considered withdrawn. Three years was chosen as a period in which most papers can be completed. However, authors can request an extension.

Keep in mind that lead authors are responsible for circulating each draft of their manuscript to all co-authors for review, comments, and suggestions as the paper progresses. Policies for ARIC paper and meeting abstract submissions are posted at http://www.cscc.unc.edu/aric/policy/.

Best wishes to you and your writing group in the preparation of this paper.

Sincerely yours,
Josef Coresh, MD, PhD
ARIC Publications

Committee JC/dc

cc: Aric Publications
Committee ARIC
Coordinating Center

Josef Coresh, MD, PhD Director
George W Comstock Center for Public Health Research and Prevention
2024 East Monument Street,Baltimore, MD 21287

Appendix 3: ARIC Secondary Analysis Approval

ARIC™ Research with Heart.

Keeron Stone
School of Sport and Exercise
University of Gloucestershire

Wednesday, April 21, 2021

Dear Keeron Stone

On behalf of the Publications Committee, I am notifying you that your ARIC manuscript proposal has been approved.

Manuscript Proposal #: 3812

Manuscript Proposal Title: Short-term prognostic impact of the aortic-femoral arterial stiffness gradient in older adults without prevalent cardiovascular disease: The Atherosclerosis Risk in Communities (ARIC) Study

Note that manuscript proposals are approved for a three-year period. If at the end of this time a paper has not been submitted, the authors will be notified and the manuscript proposal will be considered withdrawn. Three years was chosen as a period in which most papers can be completed. However, authors can request an extension.

Keep in mind that lead authors are responsible for circulating each draft of their manuscript to all co-authors for review, comments, and suggestions as the paper progresses. Policies for ARIC paper and meeting abstract submissions are posted at https://sites.cscc.unc.edu/aric/pubs-policies-and-forms-pg.

Best wishes to you and your writing group in the preparation of this paper.

Sincerely yours,
Josef Coresh, MD, PhD
Chair, ARIC Publications

Committee JC/dc

cc: Aric Publications
Committee ARIC
Coordinating Center

Josef Coresh, MD, PhD Director
George W Comstock Center for Public Health Research and Prevention
2024 East Monument Street,Baltimore, MD 21287

Appendix 4: ARIC Manuscript Proposal

ARIC Manuscript Proposal #3812

PC Reviewed: ___/___/¬¬21___ Status: _____ Priority: ____
SC Reviewed: _____ Status: _____ Priority: ____

1.a. Full Title: Short-term prognostic impact of the aortic-femoral arterial stiffness gradient in older adults without prevalent cardiovascular disease: The Atherosclerosis Risk in Communities (ARIC) Study

b. Abbreviated Title (Length 26 characters): Arterial stiffness gradient

2. Writing Group:

Writing group members:
Keeron Stone*
Lee Stoner
Michelle Meyer
Gerardo Heiss
Anna Kucharska-Newton
Kevin Heffernan
Simon Fryer
James Faulkner
Kunihiro Matsushita
Hirofumi Tanaka

*I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. __KS___ **[please confirm with your initials electronically or in writing]**

First author: Keeron Stone
Address: University of Gloucestershire, School of Sport and Exercise, Gloucester, UK, GL2 9HW.
Phone: 01242715192 Fax: n/a
E-mail: [redacted]/[redacted]

ARIC author to be contacted if there are questions about the manuscript and the first author does not respond or cannot be located (this must be an ARIC investigator).
Michelle Meyer

Address: UNC-Chapel Hill
Department of Emeregcy Medicine
170 Manning Dr., CB# 7594
Chapel Hill, NC 27599
Phone: [Redacted] , E-mail: [Redacted]

3. Timeline: We plan to complete the manuscript(s) within 3 years from approval.

Appendix 4: ARIC Manuscript Proposal

4. Rationale:

Increased arterial stiffness is an independent predictor of cardiovascular disease (CVD) [1]. Although several measures of arterial stiffness have been established, carotid-femoral pulse-wave velocity (cfPWV) is the most widely used in clinical and epidemiological studies given its strong association with cardiovascular events and mortality [2]. In contrast, upper extremity (arms) and lower-extremity (legs) peripheral measures of arterial stiffness are used infrequently because of their limited or inconsistent prognostic value [3]. However, assessments of the central to peripheral arterial stiffness gradient have emerged as promising screening tools, with recent studies demonstrating their ability to provide prognostic information beyond regional arterial segment stiffness measures alone [4,5].

To date, the few studies that have investigated the utility of the arterial stiffness gradient measures have focused on the upper extremity derived aortic to brachial SG (ab-SG), defined as the ratio of carotid-radial PWV (crPWV) and cfPWV. An increased ab-SG has been found to be a better a predictor of all-cause mortality than cfPWV in dialysis patients [4], but conferred no unique or additive value in community-dwelling older adults [6]. However, the gradient between the aorta and the lower extremities may provide a more comprehensive picture of hemodynamic integration and be a more sensitive marker of arterial health and CVD risk. Indeed, compared to the upper extremities, the lower extremities make up a significant portion of the arterial tree, are more prone to athero- and arterio-sclerotic processes, and are major sites of wave reflections [7]. As far as we are aware, our group has published the only study to explore the utility of the lower-extremity derived aortic to femoral SG (af-SG), defined as the ratio of femoral-ankle PWV (faPWV) and cfPWV. In the Atherosclerosis Risk in Communities (ARIC) study, we reported a comparable association of af-SG with traditional CVD risk factors when compared to cfPWV[5] . However, we found a unique association with CVD status. Indeed, whilst a high cfPWV was only associated with diabetes, a low af-SG was associated with coronary heart disease, heart failure, stroke and diabetes [5]. However, to confirm its clinical utility the ability of the af-SG to predict incident CVD events and mortality must be identified.

Finally, in order to be of utility, clinical assessments must demonstrate reliability (precision). Indeed, quantifying PWV measurement variation is critical for applications to risk assessment and stratification, and eventual translation to clinical practice. We recently reported that the novel af-SG exhibits good (intra-class correlation coefficient = 0.77) reliability in young healthy adults [8]. But both age and CVD are known to deleteriously impact the precision of PWV measures [9]. The reliability of the af-SG in older adults is not known.

Summary: The ARIC Study cohort is a community-based study, with measures of PWV on over 6,000 older adults. Using data from ARIC Visit 5 and longitudinal data of CVD events and mortality, we plan to address the following questions, which will allow us to generate hypotheses regarding the prognostic implications of a pathological aortic to femoral arterial stiffness gradient (af-SG).

5. Main Hypothesis/Study Questions:

i. A. Does the af-SG predict incident cardiovascular disease or mortality?
B. Does the af-SG predict incident cardiovascular disease or mortality beyond that of cfPWV?
C. Does the af-SG, characterised using hfPWV predict incident cardiovascular disease or mortality

ii. What is the short-term repeatability of the af-SG in older adults?

6. Design and analysis (study design, inclusion/exclusion, outcome and other variables of interest with specific reference to the time of their collection, summary of data analysis, and any anticipated methodologic limitations or challenges if present).

Study design: Longitudinal of data from ARIC visit 5, and longitudinal data of incident CVD events and mortality from Visit 5 assessed through to December 2019.

Inclusions: All white and black ARIC participants with PWV data obtained at visit 5. For repeatability analysis the subgroup (n=79) of visit 5 participants whom agreed to return for a repeat visit 4-8 weeks are the initial visit.

Exclusions: Missing information on PWV, blood pressure, and antihypertensive medication use or other covariates of interest; not white or African-American; and exclusions recommended by the ARIC ABI/PWV Working group: participants with BMI>=40, participants with major arrhythmias (based on ECG data), reported use of antiarrhythmic or vasoactive medications per the ARIC medication survey use (MSR Item 33.g) and/or specific medication codes in the ARIC database. To examine incident outcomes, we will exclude those with prevalent coronary heart disease, heart failure, or stroke as well as an ankle-brachial index <0.9.

Exposures: Carotid-femoral PWV (cfPWV), femoral-ankle PWV (faPWV), heart-femoral PWV (hfPWV) and blood pressure (MAP) indices determined using the Omron VP-1000 plus system (Omron Healthcare, Kyoto, Japan) A minimum of two measurements was taken per participant and the last two usable measurements (i.e. non-zero values) were averaged. The af-SG is calculated as faPWV / cfPWV.

Outcomes: The primary outcome of interest will be a composite cardiovascular disease that comprised coronary heart disease, heart failure, and stroke. Secondary outcomes will include coronary heart disease, heart failure, stroke, all examined separately. We will also investigate all-cause mortality since cardiovascular disease is a leading cause of death in the United States. All cardiovascular outcomes (except coronary revascularization) will be adjudicated by physician reviewers [10]. Coronary heart disease will be defined as definite or probable myocardial infarction, coronary heart disease death, or coronary revascularization procedure. Heart failure will be adjudicated as definite or probable acute decompensated heart failure based on hospitalization record review [11]. Stroke will be defined as definite or probable cases of ischemic or hemorrhagic strokes based on stroke hospitalization and death records. All-cause mortality will be defined as death due to any cause and will be ascertained through annual cohort follow-up, hospital surveillance, and linkage to the National Death Index [10]. All outcomes were assessed through December 31, 2019.

Covariate Measurements:

Demographic variables: age, sex, race, field center, education level, body mass index, history of hypertension (prevalent hypertension and/or blood pressure medication use), history of diabetes, history of smoking, history of alcohol consumption, fasting glucose, triglycerides, total cholesterol, HDL-cholesterol, LDL-cholesterol, measures of kidney disease (serum creatinine, crystatin C, urine albumin-creatinine ratio).

Hemodynamic variables: resting heart rate, systolic blood pressure, diastolic blood pressure, pulse pressure, mean arterial pressure, central augmentation index, carotid-augmentation index, ankle-brachial index.

Appendix 4: ARIC Manuscript Proposal

Statistical Analysis for predictive value of cfPWV and af-SG.

We will present participant characteristics as means and standard deviations, as medians and inter-quartile ranges (IQR), or as frequencies and percent, where appropriate. If lack of normality is not a concern and transformation is not required then conventional statistics will be used. If normality is a concern we will use non-parametric methods.

The relationship between arterial stiffness measures and the cumulative incidence of the outcomes will be visually assessed using Kaplan-Meier curves. Log-rank tests will be used to compare the survival distributions. Cox proportional hazards models will be used to estimate the associations of cfPWV and derived af-SG measures with cardiovascular events and all-cause mortality after adjusting for confounders based upon their known associations with arterial stiffness, CVD, and mortality. To allow for potential non-linear associations, PWV and derived af-SG measures will be categorized into quartiles. The second lowest quartile (Q2) will serve as the refence category in all analyses.

Sensitivity analyses. For models examining cardiovascular events as the outcome we will conduct a competing risk analysis using sub distribution hazard models that consider death from non-cardiovascular causes as a competing event. Sub-group analysis will be used to examine whether associations with cardiovascular events are modified by sex or race.

Statistical Analysis for Repeatability of af-SG

The analysis will include participants (n=79; mean age 75.7 years) from a repeatability study nested within the ARIC study visit 5 (2011-2013) who underwent two standardized visits, four to eight weeks apart. Trained technicians obtained two PWV measurements at each visit using the VP-1000 Plus system. For af-SG, we will calculate intra-class correlation coefficient (ICC), standard error of measurement (SEM) and the minimal detectable change (MDC) with 95% confidence. The ICC will be calculated according to the formula: $SDb^2/ (SDb^2+SDw^2)$, where SDb^2 and SDw^2 are the between and within-subject variance. In general, ICC values above 0.75 are considered to indicate excellent reproducibility. The SEM will be calculated according to the formula: $SD* \sqrt{(1-ICC)}$. The MDC will be calculated according to the formula: $1.96*SEM*\sqrt{2}$. The MDC is defined as the critical difference in a parameter that must be exceeded between two sequential results for a statistically significant change to occur in an individual.

Limitations: Some PWV measurements were not collected due to technical errors, participant factors and scheduling conflicts. Despite adjusting for heart rate, some residual confounding cannot be excluded.

7.a. Will the data be used for non-ARIC analysis or by a for-profit organization in this manuscript? ____ No

b. If Yes, is the author aware that the current derived consent file ICTDER05 must be used to exclude persons with a value RES_OTH and/or RES_DNA = "ARIC only" and/or "Not for Profit" ? ____ Yes ____ No

(The file ICTDER has been distributed to ARIC PIs, and contains the responses to consent updates related to stored sample use for research.)

8.a. Will the DNA data be used in this manuscript? No

8.b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the current derived consent file ICTDER05 must be used to exclude those with value RES_DNA = "No use/storage DNA"? ____ Yes ____ No

9. The lead author of this manuscript proposal has reviewed the list of existing ARIC Study manuscript proposals and has found no overlap between this proposal and previously approved manuscript proposals either published or still in active status. ARIC Investigators have access to the publications lists under the Study Members Area of the web site at: http://www.cscc.unc.edu/aric/mantrack/maintain/search/dtSearch.html

Yes

10. What are the most related manuscript proposals in ARIC (authors are encouraged to contact lead authors of these proposals for comments on the new proposal or collaboration)?

Stoner *et al.* (2020). Associations between measures of regional pulse wave velocity: The Atherosclerosis Risk in Communities (ARIC) Study.

MP#3661: Agreement between estimated pulse wave velocity and carotid-femoral pulse wave velocity: The Atherosclerosis Risk in Communities (ARIC) Study (Lee Stoner)

MP#2694: Short-term prognostic impact of cardio-ankle vascular index (CAVI) in community-dwelling older adults (Kunihiro Matsushita)

11.a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data? ____ ____ No

11.b. If yes, is the proposal

___ A. primarily the result of an ancillary study (list number* _____)

___ B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* _____ _____ _____)

*ancillary studies are listed by number https://sites.cscc.unc.edu/aric/approved-ancillary-studies

12a. Manuscript preparation is expected to be completed in one to three years. If a manuscript is not submitted for ARIC review at the end of the 3-years from the date of the approval, the manuscript proposal will expire.

12b. The NIH instituted a Public Access Policy in April, 2008 which ensures that the public has access to the published results of NIH funded research. It is **your responsibility to upload manuscripts to PubMed Central** whenever the journal does not and be in compliance with this policy. Four files about the public access policy from http://publicaccess.nih.gov/ are posted in http://www.cscc.unc.edu/aric/index.php, under Publications, Policies & Forms.

http://publicaccess.nih.gov/submit_process_journals.htm shows you which journals automatically upload articles to PubMed central.

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